VERNALIZATION IN *CHEIRANTHUS ALLIONII* HORT.

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635.936.832:631.589

VERNALIZATION IN *CHEIRANTHUS ALLIONII* HORT.

(Samenvatting: Vernalisatie in *Cheiranthus Allionii* Hort.)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP WOENSDAG 25 NOVEMBER 1964 TE 16 UUR

DOOR

G. W. M. BARENDSE

H. VEENMAN & ZONEN N.V. – WAGENINGEN – 1964

STELLINGEN

I

De tegenstrijdige resultaten in de literatuur in verband met de invloed van de daglengte tijdens de vernalisatie, in het bijzonder de invloed van korte dag, kunnen worden toegeschreven aan verschillen in lichtintensiteit.

II

De kwalificatie *absolute* c.q. *kwalitatieve* koubehoefte bij planten vereist een nadere definiëring van de temperatuur en lichtomstandigheden.

III

Bij de in de literatuur vermelde deelprocessen, die als gevolg van vernalisatie zouden plaats vinden, wordt onvoldoende onderscheid gemaakt tussen inductie en realisatie van de bloemknopvorming.

IV

De opvatting van BORTHWICK en HENDRICKS dat in groene plantendelen in het donker phytochroomconversie van P_{730} in P_{660} plaats vindt, heeft onvoldoende bewijsgrond.

V

De relatieve combinatiegeschiktheid binnen een groep genotypen hoeft niet zozeer een eigenschap te zijn van deze genotypen dan wel van de populatie tegen welke getoetst wordt.

VI

Het inzicht van NAPP-ZINN, dat voor het zg. late bloeien van *Arabidopsis thaliana* 'Stockholm' 4 tot 5 genen verantwoordelijk zouden zijn, is onvoldoende gefundeerd.

K. NAPP-ZINN. Zeitschr. Vererbungslehre **93**, 1962: 154–163.

VII

Een evenwichtige ontwikkeling van de welvaart in de ontwikkelingslanden kan slechts dan worden bereikt, indien verbetering van de landbouwproduktie gepaard gaat met een relatief snelle toename van het aantal niet-agrariërs.

J. H. L. JOOSTEN. Landbouwk. tijdschr. **76**, 1964: 122–124.

VIII

Economisch bezien, is het ter beschikking stellen van landbouwoverschotten aan ontwikkelingslanden veelal overbodig en mogelijk zelfs schadelijk voor de economie van het ontvangende land.

J. H. L. JOOSTEN. Landbouwvoorl. **20**, 1963: 69–76.

G. W. M. BARENDSE

Wageningen, 25 november 1964.

IX

De huidige ontwikkeling in de detailhandel vooral met betrekking tot de groei naar grotere eenheden en de verkoop via zelfbediening vereist een aanpassing van het veilingwezen.

X

Voor snellere vorderingen in het wetenschappelijk onderzoek in Nederland is het werken in groepsverband noodzakelijk en moeten weerstanden hiertegen worden overwonnen.

VOORWOORD

Gaarne betuig ik op deze plaats mijn dank aan allen die aan het tot stand komen van dit proefschrift hebben bijgedragen.

U, hooggeleerde WELLENSIEK, hooggeleerde promotor, ben ik dankbaar voor het vertrouwen dat U blijkbaar in mij stelde, waardoor ik mijn studie aan de Landbouwhogeschool met een promotie heb mogen voltooien. Ik hoop dit vertrouwen niet te hebben beschaamd.

U, hooggeleerde BOLT, hooggeleerde SCHUFFELEN en weledelgestrenge JUSTESEN dank ik voor de voortreffelijke inleiding op het gebied van de algemene bodemkunde en bemestingsleer, en de proefveldtechniek.

Mijn collega's ben ik erkentelijk voor hun belangstelling in mijn werk en voor hun vriendschap.

Op het Laboratorium voor Tuinbouwplantenteelt zijn velen mij behulpzaam geweest. Een bijzonder woord van dank geldt echter JEANNE en JANSJE voor hun veelzijdige hulp, MIRJAM voor het snelle en accurate typen van het manuscript, SABARTE BELACORTU, VAN DE PEPPEL en medewerkers voor hun technische hulp, mej. WOLDA en ANKE voor hun hulpvaardigheid, REIJER en HENK voor het foto- en tekenwerk.

CONTENTS

This thesis will also be published as Mededelingen van de Landbouwhogeschool, Wageningen, No. 64-14 (1964). (Communication of the Agricultural University).

CHAPTER 1

GENERAL

1.1. INTRODUCTION

This paper deals with the flower formation in *Cheiranthus allionii*. In general, the control of flowering, besides being of academic interest, is of crucial importance in agricultural and horticultural practice. For this reason man has since long tried to alter the onset to flowering at will. However, no real progress in this field can be expected if the fundamental processes leading to flowering are not understood. From the several stages of flowering the actual switch from vegetative to reproductive development of plants has attracted first attention in the research, which deals with the physiology of flowering.

The genetical constitution of a plant determines which external factors will affect its development. Particularly low temperature and day length control the floral initiation in a specific manner.

We speak of vernalization, when low temperature is required to induce or hasten the development of the capacity for flower formation, while the flower formation as such takes place afterwards. However, in literature other meanings of vernalization have been adopted, which are not in accordance with this definition. In the context of other meanings of vernalization the breaking of dormancy as well as the stimulation of flower formation by high temperature, long days, short days, light or dark, nutrition etc. are included. The author will confine himself to the definition of vernalization in the restricted sense, which is accepted implicitly or explicitly by CHOUARD (17), GREGORY and PURVIS (38, 114), LANG and MELCHERS (72, 98) and WELLENSIEK, DOORENBOS and ZEEVAART (145).

No attempt will be made to present a complete review of literature on this subject as many more or less extensive reviews or treatises are available (14, 17, 18, 38, 58, 72, 73, 85, 86, 87, 90, 98, 103, 109, 114, 121, 142, 145, 149). A brief historical outline will suffice.

The necessity of low temperature for the development of certain plants was probably known long before any mention was made of it in the literature. Some of the earliest known records on the effect of low temperature on cereals are those from ANONYMOUS (2, 1837) and from ALLEN (1, 1850). KLIPPART (65, 1857) was the first to undertake some systematic research and to describe this phenomenon more exactly, when he wrote that germination as well as low temperature are essential to make winter wheat behave like spring wheat. But he and those before him still did not seem to understand that freezing temperatures are less effective than temperatures slightly above freezing point. Some systematic research extended to other plants was undertaken as early as 1898 by VON SEELHORST (127). The notion 'cold requirement' was introduced by GASSNER (34, 1910) and it was finally his work published in 1918 (35) that initiated more systematic research by others as well. Particularly around this time a great

interest in the effect of low temperature, specially on cereals, awakened in the USSR as the study of this phenomenon resulted in important practical implications in that country. From the many Russian workers on this subject the names of MURINOV (102), MAXIMOV (91, 92, 93), POJARKOVA (93, 112), and LYSENKO (84, 85) should be mentioned.

LYSENKO (85, p. 16) introduced the notion 'vernalization' in the middle of 1929, when seeds of the winter wheat Ukrainka, following a suitable low temperature treatment, developed into plants which eared fully and uniformly after being sown in the spring under practical farming conditions. This method of pretreating seeds of winter cereal varieties for spring sowing was called yarovizatsia (from 'Jar': meaning formerly fire, or god of the spring), which has come to be known by its latinized equivalent 'vernalization' (from Latin 'vernum', meaning spring). The term vernalization has since been extended to other plants as well. It was also from his studies on vernalization that LYSENKO and his followers developed their well known theory of the stadial development of plants. Ever since a tremendous number of investigations, particularly in cereals, has been undertaken in many countries. By far the greatest number of these investigations, however, were merely aimed at the practical results to be obtained from its use. A few investigations only were concerned with the more fundamental processes underlying the vernalization.

Often contradictory results were obtained from experiments on vernalization. This was mainly due to the fact that at that time the importance of the photoperiodical behaviour of many plants after vernalization was not understood. Even after the discovery of the crucial importance of day length for the flower formation in many plants by GARNER and ALLARD in 1920 (33) it still took a number of years before the interrelation between vernalization and photoperiodism was generally realized

Particularly GREGORY and PURVIS et al (31, 32, 36, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 113, 115, 116) in their studies on rye have been primarily concerned with the mechanism of vernalization from the very beginning in 1934.

Also well known is the work on *Hyoscyamus niger* started by MELCHERS (95, 1936), who was later joined by LANG (78, 1943). Various incidental investigations on many plants have since appeared, but did not add much principally new information. Even today accurate experiments aimed at elucidating the mechanism of vernalization are rather scanty and thus present-day theories on vernalization are mainly based on experimental evidence from a few plants only. Most of these theories, however, are merely descriptive interpretations of experimental results obtained from a single plant such as formulated for rye by GOTT, GREGORY and PURVIS (37, 115, 116), for *Hyoscyamus niger* by LANG and MELCHERS (78), for wheat by VAN DE SANDE BAKHUIJSEN (121) and for *Arabidopsis thaliana* by NAPP-ZINN (104, 105).

In the present investigations the principle of studying one species only, but on a relatively extensive scale, has been followed.

1.2. Scope of the investigations

Cheiranthus allionii was introduced in the literature by Wellensiek and Barendse (143), who have shown that it is a useful plant for studies on flowering. Its flowering is primarily controlled by vernalization and secondarily by photoperiodical conditions. Therefore, photoperiodism will be dealt with only in as far as it is related to vernalization.

In the present investigations with *Cheiranthus allionii* various aspects of vernalization are studied to obtain more insight into its underlying principles. Primarily the relation between vernalization, age and day length has been studied in detail, before more specific aspects of the various extemal conditions could be tackled. Particularly the effect of temperature besides the vernalizing ones and day length both before, during the course of, or after the vernalization have been studied extensively. Some investigations were done on the effect of gibberellic acid and of 5-fluorouracil, on the localization and translocation of the vernalization effect, whereas hardly any work could be done on the genetical basis of the vernalization requirement of *Cheiranthus allionii*, because of limitations of the material itself.

CHAPTER 2

MATERIAL AND METHODS

2.1. Plant material and growth conditions

Commercial seeds of *Cheiranthus allionii* cv. 'Orange Queen' proved to be very heterogeneous in earlier experiments. Sufficiently uniform material has been obtained by inbreeding. Apart from a selected non-cold requiring strain, *Cheiranthus allionii* is absolutely cold requiring and sensitive to both seed and plant vernalization.

All experiments were carried out in greenhouses which were heated during winter and kept at about 20°C. In summer, the temperature occasionally reached higher values.

The plants were sown, transplanted and subsequently grown individually in pots with fertile soil and dug in benches with peat. Before receiving any treatment, the plants were grown at 20°C under long-day conditions, where they remain vegetative indefinitely.

Vernalization took place in cold rooms which were kept automatically at a constant temperature approaching 5°C. The cold rooms were illuminated with fluorescent light of low intensity during 16 hours per day.

Continuous-darkness treatments were given in ventilated lightproof compartments installed in a greenhouse.

Short days consisted always of 8 hours of day light, which were obtained by covering a bench in the greenhouse with lightproof curtains from 4.30 p.m. until 8.30 a.m.

Long days consisted of 16 hours of fluorescent light of rather high intensity during day-time, whereas long days obtained by extending the natural days to 16 hours with incandescent light of low intensity were used for more specific photoperiodic treatments.

Continuous-light treatments, principally in winter, consisted of fluorescent light of rather high intensity, whereas otherwise natural days were supplemented with incandescent light of low intensity according to purpose of experiments.

The equipment for high temperature treatments consisted of a glass compartment, installed in a greenhouse, in which the temperature was controlled automatically at 35°C.

2.2. Vernalization techniques

Two methods of seed vernalization have been practiced. In specific seed vernalization experiments the seeds were soaked with water in petri-dishes on wetted filter-paper at room temperature for 24 hours before being vernalized at 5°C for different durations. In the remaining experiments the seeds were sown in wet soil at greenhouse temperature 24 hours before the start of the vernalization. In case different durations of seed vernalization were given, these

vernalization treatments were started on such dates that all ended on the same day.

Plant vernalization was applied by placing plants in the cold rooms at 5°C for different durations. To avoid excessive drying up, the pots with plants were dug in boxes filled with wetted peat. In case plants of different ages had to be vernalized during different periods, the sowing and the vernalization were started on such dates that the treatments ended simultaneously.

In this manner the plants, after seed as well as plant vernalization, received their after-treatment under exactly the same external conditions, so that the results became comparable.

2.3. Abbreviations

The abbreviations which will be used throughout are listed below, whereas others will be explained as they occur.

CD: continuous darkness.
CL: continuous light.
GA_3: gibberellic acid.
LD: long day(s).
LDP: long-day plant(s).
PV: plant vernalization.
SD: short day(s).
SV: seed vernalization.

2.4. Observations

The dates of appearance of first macroscopically visible flower buds have been recorded. This stage is indicated as 'flowering'. In order to obtain a quantitative analysis, the results are judged by the percentages of flowering plants and by the mean numbers of days to flowering, i.e. the average numbers of days from the end of the vernalization until the appearance of the first visible flower buds. Other data have accidentally been recorded such as numbers of leaves per plant or lengths of main stems, but they will not be used as they did not open up new aspects.

CHAPTER 3

FACTORS INTERACTING WITH THE EFFECT OF VERNALIZATION

3.1. Stage of development

Wellensiek and Barendse (143) have shown that, although SV as well as PV is possible in *Cheiranthus allionii*, the sensitivity to vernalization varies in consecutive stages of the plant, whereas also the influence of external conditions, such as day length, on flower formation after vernalization varies in consecutive stages. According to this different behaviour of the plants in the consecutive stages three groups have been distinguished in each of which specific aspects were studied. No experimental results will be presented in this section as they will appear later on. Only a brief description of the characteristics of these groups are put forward.

3.1.1. *Seeds*

Soaked seeds are the most sensitive to vernalization. However, flowering after SV is highly dependent on external conditions such as temperature, day length and light intensity. One week of SV, when followed by favourable external conditions may result in a small percentage of flowering plants, which increases with increasing duration of SV, until after 5 or 6 weeks of SV maximal flowering is reached. Under less favourable conditions the maximum percentage of flowering is low, whereas under unfavourable conditions no flowering at all occurs.

3.1.2. *Young plants*

In the stages immediately following the seed stage, the sensitivity to vernalization decreases rapidly until a minimum vernalizability is reached with plants of 12 to 16 days old, after which in the following stages the vernalizability gradually increases until maximum vernalizability is reached again. The level of the percentages of flowering and the mean numbers of days to budding after vernalization are determined by the duration of PV. The flowering after PV of young plants also depends on external conditions, however. This dependence decreases with the age of the plants.

3.1.3. *Grown-up plants*

Plants of about 10 weeks or older possess a maximum sensitivity to vernalization. However, they are not as sensitive as seeds, because at least 4 weeks of PV are necessary in order to obtain a small percentage of flowering plants, which increases with an increasing duration of PV until after 6 weeks of PV 100% flowering is reached. The flowering of grown-up plants after PV is hardly influenced by the external conditions.

3.2. TEMPERATURE

3.2.1. *Effect of temperature after SV*

SV was applied for 6 weeks, followed by LD at different temperatures. The LD consisted of natural LD or artificial LD obtained by strong fluorescent light of approximately 16000 lux on plant level. Temperature treatments of 40 plants per treatment were given in a new and not yet completely controlled phytotron. The mean measured temperatures were generally somewhat higher than the temperatures aimed at, but in few cases only a seriously deviating temperature was observed for several days in succession.

The results presented in table 1 show that at temperatures of about 18°C or

TABLE 1. The effect of different temperatures (°C) during natural LD (n LD) or artificial LD (a LD) after 6 weeks of SV.

Measured mean temperatures		% flowering		Days to budding	
n LD	a LD	n LD	a LD	n LD	a LD
12.9	14.4	100	100	77	76
16.9	18.4	100	93	45	57
18.7	16.4	95	93	49	47
20.4	19.0	89	70	42	47
25.7	22.2	50	65	44	45
25.8	26.6	33	8	43	32

higher the percentages of flowering decrease with an increasing temperature after SV in natural LD as well as in artificial LD, whereas there are no consistent differences between natural or artificial LD. At the mean temperatures of 12.9°C and 14.4°C in natural LD and artificial LD respectively, the mean numbers of days to budding are considerably higher than with the remaining temperature treatments. Particularly in artificial LD after SV the mean numbers of days to budding tend to decrease with an increasing temperature. These results demonstrate that a temperature of approximately 18°C or higher has a devernalizing effect and that this devernalizing effect increases with increasing temperature. At a temperature of approximately 15°C or lower, the growth is considerably delayed, which causes the delay in the flower formation. With these light conditions the so-called neutral temperature lies somewhere around 17°C at which maximal flowering takes place most rapidly.

3.2.2. *Effect of temperature after PV*

Since it was found that temperature within certain limits did not affect flower formation, only three temperatures have been used after PV namely 5°, 20° and 35°C. Some data, selected from one experiment in which plants received 6 weeks of PV, are presented in table 2. Each treatment consisted of 20 plants. The mean numbers of days to budding have been counted from the end of the PV treat-

TABLE 2. The effect of temperature (°C) after 6 weeks of PV, during following LD

Temperature	% flowering	Days to budding
5	100	51
20	100	15
35	45	22

ment, which for 5°C means from the date on which the other treatments were removed to 20° or 35°C.

The results show that at the vernalizing temperature of 5°C both flower induction as well as flower formation can take place, although flower formation takes place very slowly. The most rapid flower formation takes place at 20°C and therefore this temperature has been used in most following experiments. The high temperature of 35°C causes a decrease in the percentage of flowering plants and a delay in flower formation, known as devernalization. We shall return to this specific effect of high temperature in chapter 4, section 4.2.1. p. 25.

3.3. LIGHT

3.3.1. *Effect of light quality*

The effect of light quality during PV was studied by comparing fluorescent light obtained from one Philips TL 40 W/29 of 1.2 m length per 0.5 m^2 and incandescent light obtained from one Philinea tube of 120 W and 1 m length per 0.45 m^2. Both light sources were installed approximately 40 cm above plant level. The intensity of the incandescent light was lower than that of the fluorescent light. Treatments of 20 plants consisted of 8 or 16 hrs of fluorescent light and 8, 16 or 24 hrs of incandescent light per day during PV. In table 3 the results of two experiments are compiled. In the first experiment 6 weeks of PV, in the second experiment 4 or 5 weeks of PV were given, whereas the light treatments were the same in both experiments.

TABLE 3. The effect of light quality and day length during 4, 5 or 6 weeks of PV. The light qualities consisted of fluorescent light (fl.) or incandescent light (inc.)

Treatment	% flowering			Days to budding		
	4 w. PV	5 w. PV	6 w. PV	4 w. PV	5 w. PV	6 w. PV
8 hrs fl.	81	94	100	15	13	15
16 hrs fl.	13	69	100	14	13	15
8 hrs inc.	0	13	63	∞	13	18
16 hrs inc.	19	31	79	17	15	16
24 hrs inc.	29	81	100	17	15	15

Curiously, the percentages of flowering after 4 or 5 weeks of PV with 16 hrs of fluorescent light are lower than those after 4 or 5 weeks of PV with 8 hrs of fluorescent light. These results indicate that too much light during suboptimal

PV may be disadvantageous. This has been confirmed by a third experiment which will not be presented. Incandescent light during PV shows that the percentages of flowering increase with increasing day lenghts at all durations of PV, thus demonstrating an increasing effectiveness of PV with increasing day lengths.

After fluorescent light during PV the percentages of flowering are higher and the mean numbers of days to budding are lower, both with one exception, than those after incandescent light during PV. It is therefore obvious that fluorescent light during PV is more effective than incandescent light.

In conclusion, the two experiments have shown that (a) light of sufficient intensity and duration is necessary for optimal PV, (b) the effectiveness of PV is influenced by the light quality during PV, although at least a part of the effect has to be ascribed to the different light intensities of fluorescent and incandescent light, and (c) incandescent light has a day-length effect, whereas the day-length effect of fluorescent light is opposite to that of incandescent light and occurs only with suboptimal PV.

The effect of light quality after SV has been studied in connection with day length, whereas inevitably the effect of light intensity was included as well. Earlier experiments had shown that extending the natural SD with fluorescent or incandescent light did not lead to flower formation after SV. Two factors were assumed to be possibly responsible for this behaviour, namely the low light intensity of the natural winter days and/or a too slight formative effect of fluorescent as well as incandescent light of low intensity. Indications that light quality would play a role after vernalization were derived from the facts (a) that after PV better flowering was observed in LD from extension of the natural day with fluorescent light than from extension by means of incandescent light, and (b) that light quality does play a role during PV.

Earlier experiments, which will be treated in chapter 4 p. 35, had shown that the effect of SD or CD i.e. day length was most decisive between 4 and 6 weeks after the SV i.e. just before flower initiation takes place. For this reason and to reduce the handling of the plants the effect of the light quality has been studied after 4 weeks or later after the end of the SV. In the first experiment the plants were grown in natural LD for 4 weeks after 6 weeks of SV, after which they received the following treatments: natural LD, 24 hrs of fluorescent light, 12 hrs of fluorescent light extended by 0, 4, 6, 8, 10 or 12 hrs of incandescent light. Both fluorescent and incandescent light were of rather high intensity in comparison with the intensities normally used for day extension. The above treatments have been continued for three weeks, whereafter the plants were removed to natural LD again, because only accidental flowering occurred in all treatments except in natural LD in which 84% of the plants flowered after 41 days on the average. Even in the following natural LD, however, no further flowering occurred in the remaining treatments.

In a second experiment the plants were grown for 6 weeks under natural LD after 6 weeks of SV, whereafter they received the following treatments: natural LD, 24 hrs of fluorescent light, or 12 hrs of fluorescent light extended by 0, 6 or

12 hrs of incandescent light. Each treatment consisted of 15 plants. The results are presented in table 4.

TABLE 4. The effect of light quality and day length after 6 weeks of SV. The light treatments consisted of natural LD (n LD), fluorescent light (fl.) or incandescent light (inc.)

Treatment	% flowering	Days to budding
n LD	81	51
24 hrs fl.	73	50
12 hrs fl.	47	51
12 hrs fl. + 6 hrs inc.	53	51
12 hrs fl. + 12 hrs inc.	73	50

The percentage of flowering in LD is the highest, demonstrating that natural LD is optimal for flowering. The percentages of flowering in 24 hrs of fluorescent light and in 12 hrs of fluorescent light, followed by 12 hrs incandescent light are equal, thus demonstrating that there is no difference in light quality at the intensities used. The increasing percentages of flowering at the treatments of 12 hrs of fluorescent light extended by 0, 6 or 12 hrs show a day-length effect. The mean numbers of days to budding do not differ significantly at the different light treatments.

In a third experiment the plants were grown for 5 weeks under natural LD after 6 weeks of SV, whereafter they received: 8 hrs of natural day light, 16 hrs of natural day light, 8 hrs of natural day light extended by 16 hrs of fluorescent light or by 8 or 16 hrs of incandescent light. Each treatment consisted of 15 plants. The results are presented in table 5.

TABLE 5. The effect of light quality and day length after 6 weeks of SV. The light treatments consisted of natural day light (n DL) supplemented with fluorescent light (fl.) or incandescent light (inc.)

Treatment	% flowering	Days to budding
8 hrs n DL	60	43
16 hrs n DL	95	42
8 hrs n DL + 16 hrs fl.	78	41
8 hrs n DL + 8 hrs inc.	65	42
8 hrs n DL + 16 hrs inc.	68	40

The percentage of flowering in natural LD is the highest, which demonstrates that natural LD is the best condition for flowering. The percentage of flowering after day extension with fluorescent light is somewhat higher than those after day extension with incandescent light. The mean numbers of days to budding are not influenced by the different light treatments.

Altogether the results of the three experiments show (a) that irrespective of the day length or light quality, the days consisting of artificial light only are

insufficient for flower formation, which has to be ascribed to the low light intensities used, and (b) that extension of the natural day may have a small effect, fluorescent light being somewhat better than incandescent light, which can be ascribed to the higher intensity of the fluorescent light. Finally, it should be remembered that flower formation has been obtained under LD obtained by 16 hrs of fluorescent light of high intensity (p. 7).

In conclusion, it is apparent that all the different light effects can be carried back to one factor, namely the light intensity, low light intensity being disadvantageous for flower formation.

3.3.2. *The effect of light intensity*

Since the effect of light intensity after vernalization was clearly demonstrated, albeit unintentionally, in the former section, no more experiments have been carried out on this specific subject. The general conclusion which can be derived from the experiments treated in the sections 3.2.1. (p. 7) and 3.3.1 (p. 8–11) is that, particularly after SV, both long days and high light intensity are required for optimal flowering.

3.4. Day length

3.4.1. *Effect of day length before PV*

Wellensiek and Barendse (143) already demonstrated that SD before PV of plants of different ages results in a disadvantageous influence on the effectiveness of subsequent vernalization. However, it could not be concluded whether this disadvantageous effect of SD was due to a direct effect on the subsequent vernalization itself or to the fact that SD retards growth. Thus these SD-plants could have been physiologically 'younger' at the beginning of the vernalization in comparison with plants grown in LD, causing a different vernalizability.

An experiment has been carried out in which grown-up plants were used to exclude the 'age' effect on vernalization. The plants, pretreated in LD, received 0, 1, 2, 3 or 4 weeks of SD, immediately followed by PV during 4 or 5 weeks. Each treatment consisted of 20 plants. The results presented in table 6 show that

Table 6. The effect of limited SD-periods before PV

SD before PV in weeks	% flowering		Days to budding	
	4 w. PV	5 w. PV	4 w. PV	5 w. PV
0	64	88	13	11
1	78	81	13	11
2	78	100	13	11
3	39	41	13	11
4	10	19	14	12

the percentages of flowering decrease rapidly, when 3 or 4 weeks of SD are given before PV.

It is obvious from these results that indeed SD before PV exerts a direct disadvantageous effect on the subsequent PV itself, which has to be called anti-vernalization.

3.4.2. *Effect of day length during PV*

The effect of day length during PV has been studied in connection with the effect of light quality during PV. For the results reference should be made to table 3 on p. 8, whereas for the conclusions reference should be made to the last paragraph of the section concerned.

3.4.3. *Effect of day length after SV*

In continuous SD after SV no flowering occurs. WELLENSIEK and BARENDSE (143) described experiments in which the effect of increasing LD-periods, followed by SD, and the effect of increasing SD-periods, followed by LD, were studied. It was found that at least a LD-period of 5 weeks after SV was necessary in order to obtain any flowering, whereas at a LD-period of 6 weeks after SV nearly all plants flowered within this LD-period. SD-periods after SV delayed flowering in all cases, but the percentage of flowering was decreased only after a SD-period of 5 weeks or longer. These results demonstrated that optimal flowering is only possible under permanent LD-conditions.

The above described experiments initiated some other experiments, particularly on the disadvantageous effect of SD, which, however, will be treated in chapter 4, section 4.2.2.3 (p. 34) as they were more concerned with the devernalizing effect of SD.

Furthermore, the effect of day length after SV has been studied in relation with the effect of light quality after SV. For the results of those experiments reference should be made to section 3.3.1 (p. 8) and tables 4 and 5 (p. 10).

3.4.4. *Effect of day length after PV*

The day length after PV, particularly of young plants, is of crucial importance for flower formation, but it has already been put forward on p. 16 that the influence of external conditions such as day length depends to a great extend on the age of the plants at the beginning of the vernalization and on the duration of the vernalization. The interrelation between these factors is treated in the following section.

3.5. RELATION BETWEEN AGE, DURATION OF VERNALIZATION AND DAY LENGTH AFTER VERNALIZATION

During the course of experimentation the relation between age, duration of vernalization and day length after vernalization became apparent. This relation was studied extensively before more specific aspects of the external conditions such as temperature or day length could be tackled. Several experiments concerning the above relation have been carried out in consecutive years, later experiments always being extensions and/or supplements of former ones. A choice of experimental results will be presented which enables a complete picture to be formed.

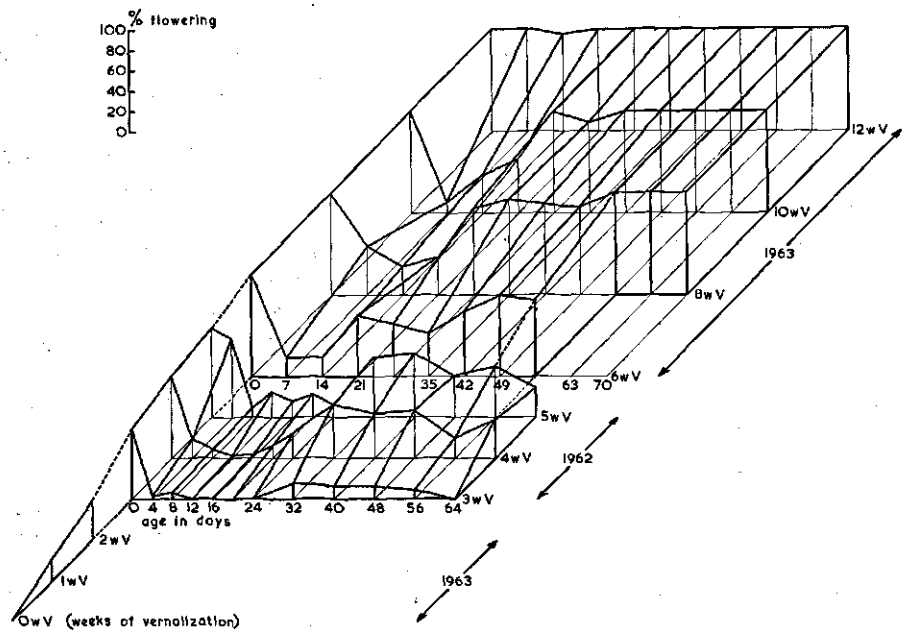
3.5.1. *Effect of LD after vernalization*

In an experiment, carried out in the spring of 1962, three series of plants of 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56 and 64 days of age were vernalized for 3, 4 or 5 weeks, followed by LD. Each treatment consisted of 32 plants.

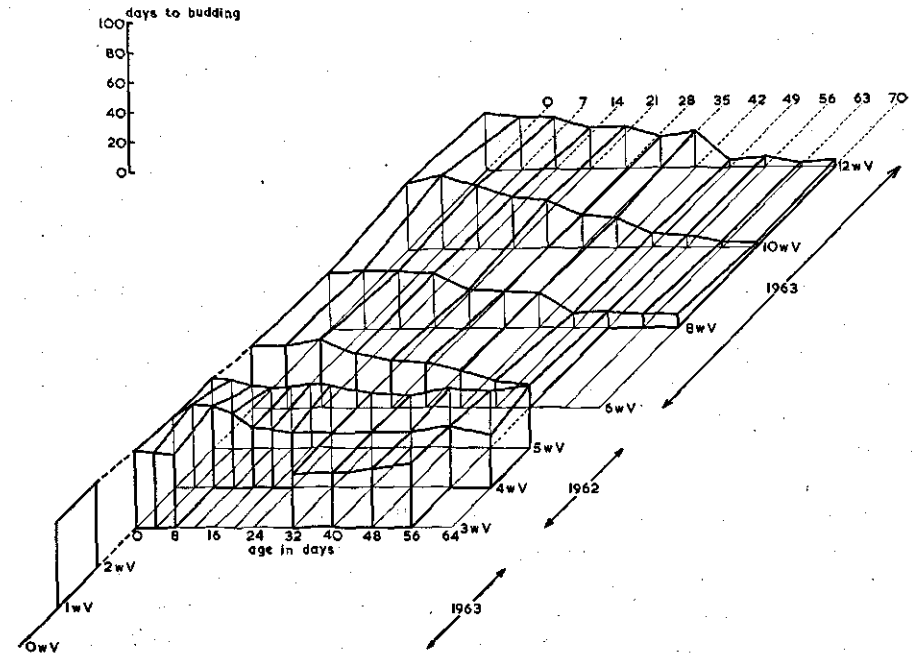
A supplemental experiment has been carried out in the spring of 1963. Four series of plants of 0, 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days were vernalized for 6, 8, 10 or 12 weeks, followed by LD. Each treatment consisted of 24 plants. In both experiments LD was obtained by extending the natural day with incandescent light of low light intensity. The results of the two experiments supplemented with some data of another SV experiment, carried out in the spring of 1963, are presented in the graphs 1A and 1B. Reference should also be made to the photos 1, 2 and 3, in which by a choice of 10 representative plants per treatment a picture of the results of the experiment of 1962 is given, and to photo 4 which shows the results of an experiment in 1962 which is not included in the graphs.

Considering first the age series, which received 3 weeks of vernalization, it is seen in graph 1A that vernalization at an age of 0 days, i.e. SV results in a relatively high percentage of flowering plants. However, a strongly decreased percentage of flowering is found after PV of 4 day old plants in comparison with SV, whereas in the following stages the percentages of flowering remain low and even decrease to zero after PV of 12 to 24 day old plants. PV of 32 day old plants results in a small percentage of flowering plants, after which the percentages of flowering gradually decrease with increasing age until it is again zero with 64 day old plants. It should be mentioned here that in other experiments with grown-up plants, i.e. plants older than 64 days, no flowering was obtained after 3 weeks of PV. Those varying percentages of flowering in relation to age thus demonstrate that seeds are the most sensitive to vernalization and that in stages immediately following the seed stage the sensitivity to vernalization decreases rapidly until a minimum sensitivity is reached, after which the sensitivity again reaches a maximum in the following stages, which, however, is lower than after SV. Thus is demonstrated that plants are not as sensitive as seeds to vernalization, which can also be seen from the fact that after 1 or 2 weeks of SV a small percentage of flowering is already obtained.

Considering the age series, which received 4 weeks of vernalization, it is seen that the course of the percentages of flowering up to a certain age shows a great similarity with that after 3 weeks of vernalization. The percentages of flowering after 4 weeks of vernalization, however, are higher than after 3 weeks of vernalization at all ages, which means that the percentages of flowering increase with an increasing duration of the vernalization. With plants of 56 days after 4 weeks of vernalization a decreased percentage of flowering is found, after which the percentage of flowering shows a tendency to increase again in 64 day old plants. A decreased sensitivity after 4 weeks of vernalization with plants of about 56 days seems to be rather consistent as it was also found in an earlier experiment with plants of 56 days and in a later experiment with 64 day old plants.



GRAPH 1A. The relation between age and duration of vernalization, in LD after vernalization. The influence on the percentage of flowering.



GRAPH 1B. The relation between age and duration of vernalization, in LD after vernalization. The influence on the mean number of days to budding.

It is known from other experiments with grown-up plants that the percentages of flowering after 4 weeks of PV may vary considerably, but never reach 100%.

The course of the percentages of flowering after 5 weeks of vernalization resembles those after 3 or 4 weeks of vernalization. Again the percentages of flowering are generally higher than after 4 weeks of vernalization and again after a maximum for PV a decrease of the percentage of flowering is observed towards 64 day old plants. As it is known from other experiments with older plants that the percentage of flowering after 5 weeks of PV is normally higher than found in this age series and may sometimes amount to 100%, it may finally be concluded that with plants of 56 to 64 days of age another minimum sensitivity to vernalization really exists. The age series after 6 weeks of vernalization of the experiment of 1962 shows that 100% flowering occurred after SV, so that the optimum duration for SV is reached. Although it is not clear from this experiment, there is no significant decrease in the percentage of flowering in 56 day old plants, but as has been shown in other experiments the percentages of flowering increase gradually with increasing age until with grown-up plants 100% flowering is obtained. As usually seeds or grown-up plants are used in vernalization experiments, 6 weeks of vernalization is considered optimal.

With a further increase in the duration of the vernalization from 6 to 8, 10 or 12 weeks, the percentages of flowering at all ages increase until maximum i.e. 100% flowering is reached. After 12 weeks of vernalization the consistent minimum of sensitivity to vernalization of young plants between 4 and 24 days was not demonstrated any more. However, in another experiment this was already the case after 10 weeks of vernalization as can be seen on photo 4.

The overall picture of the mean numbers of days to budding in graph 1B shows that they decrease with increasing age, but that this decrease is slow after suboptimal vernalization, whereas it is more rapid after longer durations of vernalization. The mean numbers of days to budding decrease with age until a more or less constant value is reached, which value will be lower, the longer the duration of vernalization used. Furthermore, when the sensitivity to vernalization decreases, the mean numbers of days to budding may tend to increase. This is the case in the age series after 4 weeks of vernalization. They may also remain high as in other durations of vernalization. Thus a low sensitivity to vernalization is expressed by a low percentage of flowering and a high mean number of days to budding. The opposite holds true for a high sensitivity to vernalization.

After SV the mean numbers of days to budding decrease slowly with increasing duration of the SV until a constant value is reached. Also after vernalization of plants up to about 40 days a more or less constant value for the mean numbers of days to budding is reached with an increasing duration of PV. These constant values are lower after PV of older plants.

The mean numbers of days to budding gradually decrease in plants of about 40 days or older with increasing duration of vernalization until the plants will even flower during the vernalization treatment itself, as has already been demonstrated by WELLENSIEK and BARENDSE (143). This decrease proceeds more rapidly with older plants. That plants younger than 6 weeks do not reach

flower formation during a permanent cold treatment is due to the fact that the development at 5 °C is extremely slow and no appreciable growth is observed at this temperature in these young plants.

It is worth while to note that normally flower initiation after SV does not take place earlier than 5 to 6 weeks after the SV and this is exactly the initial age at which plants may reach flower formation at a permanent temperature of 5 °C. This means that the plants have to reach a certain size in order to make flower initiation possible. It is therefore obvious that plants younger than 6 weeks will first have to reach the required size. However, the plants succumb before this size is ever reached.

3.5.2. *Effect of SD after vernalization*

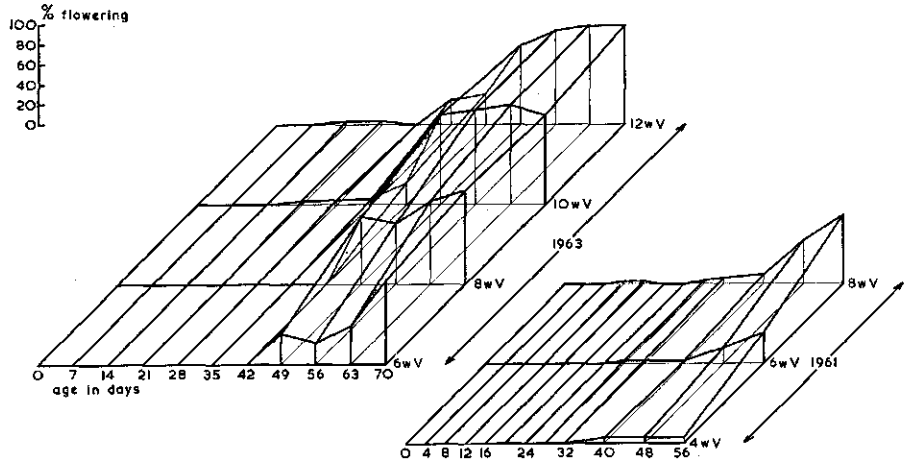
An experiment carried out in the spring of 1961 consisted of three age series of plants of 0, 4, 8, 12, 16, 20, 24, 28, 32, 40, 48 or 56 days, which were vernalized for 4, 6 or 8 weeks, followed by SD. Each treatment consisted of 32 plants. An extended experiment, which overlapped the former, was carried out in the spring of 1963. Four age series of plants of 0, 7, 14, 21, 28, 35, 42, 49, 56, 63 or 70 days were vernalized for 6, 8, 10 or 12 weeks, followed by SD. Each treatment consisted of 24 plants. The results of the two experiments are presented in the graphs 2A and 2B.

The percentages of flowering, presented in graph 2A, show that after SV or PV of young plants no flowering at all occurred in SD and that flowering starts at a certain age. This age is younger as the duration of the PV is longer. The percentages of flowering increase with increasing age in all age series. Furthermore, the percentages of flowering increase with an increasing duration of PV at the different ages. The comparable vernalization treatments of 6 and 8 weeks of PV show that the values of the experiment of 1961 are lower than those of the experiment of 1963, but that in 1961 flowering started at a somewhat earlier age. These differences must be due to other external conditions than the day length.

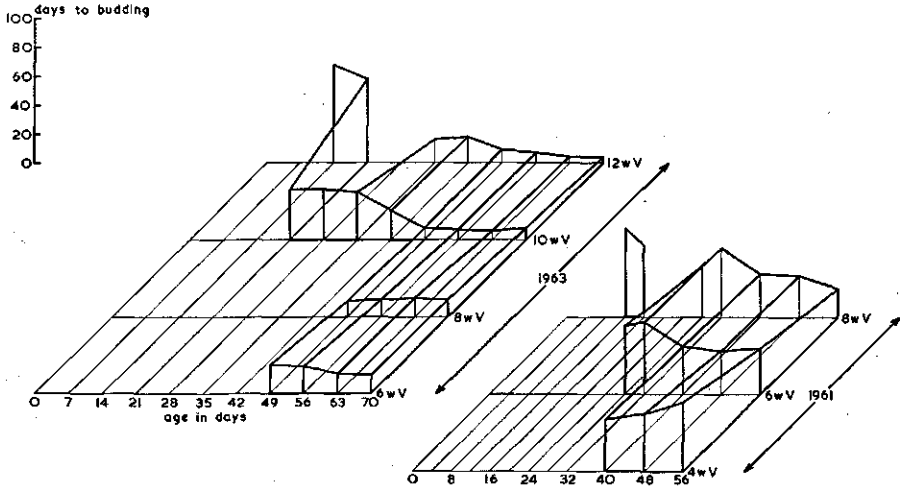
The overall picture of the mean numbers of days to budding, presented in graph 2B, show a decrease with increasing age and also a decrease with increasing duration of the vernalization. It is obvious from the above results that SD after vernalization has a disadvantageous effect on flower formation which decreases with increasing age of the vernalized plants and with increasing duration of vernalization.

A comparison of the results obtained in LD and SD after vernalization shows that LD is optimal for flower formation as particularly the comparable percentages of flowering are generally higher and the comparable mean days to budding generally a little lower in LD. These differences become smaller with increasing age until at an age of 70 days and after a vernalization lasting 8 weeks flower formation is no longer influenced by the day length. Thus day neutrality depends on age and on the duration of vernalization and this is in accordance with the former conclusion of WELLENSIEK and BARENDSE (143). For this reason most further experiments on PV have been carried out with

grown-up plants of 12 weeks or older, of which had been established as day neutral after suboptimal vernalization as well.



GRAPH 2A. The relation between age and duration of vernalization, in SD after vernalization. The influence on the percentage of flowering.



GRAPH 2B. The relation between age and duration of vernalization, in SD after vernalization. The influence on the mean number of days to budding.

3.6. CHEMICALS

From the many chemicals which have been used in relation to vernalization according to the literature, the gibberellins have proven to be most apt for promoting flower formation in several plants. Moreover, the results obtained with gibberellins are generally more consistent than numerous reports on other chemicals which are often uncertain or contradictory. Gibberellin-like substances are shown to be present in several higher plants and they may appear to be common substances present in most higher plants. For this reason the effect of GA_3 has been studied in *Cheiranthus allionii*.

The use of 5-fluorouracil (5-FU) in vernalization experiments was initiated by the concept that cell divisions are a prerequisite for vernalization (139, 141) and it was therefore assumed that, if the cell division could be blocked by means of 5-FU (4), the effectiveness of vernalization could possibly be influenced.

3.6.1. *Effect of GA_3 on vernalization*

In the first experiment SV was applied for 0, 1, 2, 3, 4 or 5 weeks, followed by LD. After SV half of the plants of each SV treatment received a 25 ppm GA_3 treatment three times per week, applied by means of a pipette, 3 drops at a time. The ultimate number of plants was 24 per treatment.

The results in table 7 show that GA_3 alone, without SV, results in a small

TABLE 7. The effect of GA_3 after SV

SV in weeks	% flowering		Days to budding	
	$-GA_3$	$+GA_3$	$-GA_3$	$+GA_3$
0	0	8	∞	59
1	4	17	61	57
2	38	75	50	50
3	67	92	49	51
4	83	96	49	49
5	71	79	51	50

percentage of flowering. By a prolonged application of GA_3 on the remaining vegetative plants, it became obvious that GA_3 may induce flower formation in some plants without vernalization, but it cannot be considered as an effective inducive agent as only prolonged applications of high concentration exert a reasonable effect.

The results further show that the percentages of flowering after GA_3 are higher in all cases in comparison with $-GA_3$. Considerable differences in the percentages of flowering are found after 2 and 3 weeks of SV. The mean numbers of days to budding are not influenced by GA_3.

To investigate a possible effect of GA_3 before or during SV, a following experiment has been carried out in which seeds were soaked in water or 100 ppm GA_3 for 24 hours at room temperature before being vernalized for 0, 1, 2, 3, 4 or 6 weeks, followed by LD. After SV half of the plants of each group were treated with 100 ppm GA_3 three times per week. In this manner the following combinations of GA_3 treatments were obtained:

– GA/ – GA: no GA_3 before and during, no GA_3 after SV
– GA/+GA: no GA_3 before and during, GA_3 after SV
+GA/ – GA: GA_3 before and during, no GA_3 after SV
+GA/+GA: GA_3 before and during, GA_3 after SV

The results of this experiment are presented in table 8.

TABLE 8. The effect of GA_3 during SV and after SV

1	2	3	4	5	6	7	8	9
SV in weeks	% flowering				Days to budding			
	– GA/ – GA	– GA/ +GA	+GA/ – GA	+GA/ +GA	– GA/ – GA	– GA/ +GA	+GA/ – GA	+GA/ +GA
0	0	9	0	0	∞	63	∞	∞
1	22	54	25	58	56	54	57	55
2	38	52	34	65	55	54	55	55
3	50	91	56	71	53	54	53	54
4	67	75	83	74	55	53	53	51
6	96	86	71	83	52	57	53	53

A comparison of the percentages of flowering of the columns 2 and 3 confirms the conclusion of the former experiment that GA_3 promotes flower formation, particularly after suboptimal SV. A comparison of the percentages of flowering of the columns 2 and 4 shows that after 0, 1, 2 or 3 weeks of SV no significant differences occur, whereas the percentage of flowering of column 4 after 4 weeks of SV is higher and after 6 weeks of SV is lower than that of column 2. It seems therefore that GA_3 before and during SV has no consistent effect. The above conclusions are further supported by a comparison of the percentages of flowering of column 3 and 5 on the one hand and the columns 4 and 5 on the other hand, which by subtraction give the netto effect of GA_3 before and during SV, and after SV respectively. This experiment also shows that the mean numbers of days to budding, columns 6, 7, 8 and 9, after SV are not influenced by GA_3.

Another experiment has been carried out in which after 6 weeks of SV, followed by LD, GA_3 treatments were applied during 1 or 2 weeks of SD, given at different moments after the end of the SV, in order to check whether the disadvantageous effect of SD could be prevented by GA_3. However, as this experiment was carried out in connection with other experiments, to be described on p. 34 until 36, the results will be presented and discussed later.

Finally, an experiment has been carried out on the effect of GA_3 before PV. PV for 4 weeks was preceded by 3 weeks of applications of 100 ppm GA_3 three times per week, whereas the control plants remained untreated. From the 20 plants per treatment in both cases 11 plants i.e. 55% came into flower. The mean days to budding after PV amounted to 21.6 days for the control and to 19.6 days for the GA_3 treated plants. It is obvious from this experiment that GA_3 has no effect on subsequent PV as well.

3.6.2. *Effect of 5-FU on vernalization*

In order to obtain a suitable concentration of 5-FU, a germination test has been carried out with a range of concentrations of 5-FU varying from 10^{-4} molar to 5.10^{-3} molar. A concentration of 5.10^{-4} molar was chosen for further experimentation as at this concentration germination is visibly inhibited, whereas the majority of the treated seeds kept their viability.

In an experiment seeds or plants were treated before, during or after vernalization with 5-FU, by soaking the seeds in the 5-FU solution or by dripping the plants three times per week. Small growth delays have been observed, particularly when 5-FU was applied after the vernalization. The results of the experiment were irregular, but nothing of a direct influence of 5-FU on vernalization appeared.

3.7. Discussion

Vernalization of soaked seeds and of plants may lead to flower formation in *Cheiranthus allionii*. However, the ability to be vernalized is not the same in consecutive stages of the plant. After SV and after PV of young plants, the plants have to reach a certain size before flower formation is possible, whereas older plants are able to initiate flowers soon after PV. The optimum temperature for flower formation after SV as well as PV, particularly of young plants, has to be a compromise between the optimum temperature for growth and the neutral temperature i.e. the temperature at which devernalization does not take place yet. The optimum temperature for flower formation, being the temperature at which complete flowering takes place most rapidly, however, is difficult to determine exactly as it is modified by other external conditions after vernalization, particularly by the light conditions. The better the light conditions after vernalization, the higher the temperature which may be maintained after vernalization.

The realization of flowering after SV and PV of young plants takes considerably more time and thus the light conditions have more time to influence the flower formation. Hence it is obvious that the light conditions are relatively more important after SV and PV of young plants. These facts link up with the results obtained by Lang and Melchers (78), who showed that the critical day length of the annual strain of *Hyoscyamus niger* increases with increasing temperatures, whereas Friend and Purvis (32) demonstrated in Petkus winter rye that the devernalization by high temperature could be diminished or prevented among others by weak light instead of darkness during the high temperature treatment.

Light quality. Fluorescent light during PV increases its effectiveness in comparison with incandescent light at all durations of PV. The effect of light quality, fluorescent light versus incandescent light, in as much as it was studied in *Cheiranthus allionii*, seems to be largely due to differences in the light intensity of the light sources used, whereas it interacts with the day length.

The effect of the light intensity, particularly of importance after SV or PV of young plants, is obvious, low light intensity being inhibitory to flower formation. In literature, only little direct evidence on the effect of light intensity after

vernalization is presented, but it is generally concluded from indirect evidence that low light intensity inhibits flower formation. This effect has been explicitly reported by NAPP-ZINN (107) for *Arabidopsis thaliana*.

Short days before PV have a disadvantageous effect on subsequent vernalization, called anti-vernalization. In literature such cases are not known. No influence of the day length before PV has been reported for *Hyoscyamus niger* by LANG and MELCHERS (78) and for species of *Beta vulgaris*, *Brassica oleracea*, *Apium graveolens*, and *Daucus carota* by JUNGES (62).

Day-length effects during PV depend on the light quality and the duration of PV, whereas the light intensity will undoubtedly be of importance as well. Incandescent light during PV causes an increasing effectiveness of PV with increasing day length. Fluorescent light during PV probably causes a decreased effectiveness of PV in SD in comparison with LD at suboptimal PV, whereas no difference between SD and LD was observed at optimal PV.

As the influence of the above factors is generally overlooked, this might probably explain the contradictory results in literature. According to several authors (20, 21, 28, 29, 30, 54, 67, 148) and others listed by MATHON and STROUN (90, p. 61), SD during PV has a favourable effect on vernalization of cereals and several perennial grasses. Certain authors (81, 82, 111), however, could not confirm this and even claimed a favourable effect of LD during vernalization.

Considering the data in the literature more closely, it appears that favourable effects of SD during vernalization were generally found under field conditions, whereas favourable effects of LD were found under artificial light conditions in which a weak illumination was applied. Such a case has been explicitly reported by KREKULE (67). He also found that the day-length effect under artificial light of low intensity was obliterated with increasing light intensity. Another case is mentioned by MATHON and STROUN (90, p. 63) for *Campanula persicaefolia*. In plants other than cereals or grasses a promotive effect of long days during PV has generally been reported, such as *Beta vulgaris* (19), *Raphanus sativus* (64, 129), and *Silene armeria* by WELLENSIEK (unpublished). JUNGES (61) reported that weak CL during PV of spinach up to 15 days acted inhibitorily on PV in comparison with CD, whereas at longer durations of PV this inhibitory effect of CL is overcome. NAPP-ZINN (107) showed that light during PV of young plants of *Arabidopsis thaliana* promoted vernalization in comparison with darkness, whereas he found no effect during PV of older plants.

The vernalization temperature seems to be another factor which interacts with the effect of day length during vernalization, as has been reported for wheat by NIKIFOROV (111), who found that the higher the vernalization temperature, the shorter the day length necessary for optimal flowering after vernalization. A similar case has been reported by CHUJO (21), who found that the effect of SD was more pronounced at higher temperatures, including SD-induction at higher than vernalizing temperatures. From all these varying results in literature and those obtained in *Cheiranthus allionii*, it is obvious that light during PV plays an important role, but that its effect in either direction, positive or negative, does not show a general picture even within the same species. More experimen-

tation under strictly controlled conditions is needed in order to obtain more decisive results.

Day length after vernalization. Day-length effects during PV are linked up with the effect of limited periods of SD immediately after vernalization. In *Cheiranthus allionii* SD, including limited SD-periods, is always found to have a disadvantageous effect after vernalization. In literature a promotive effect of limited SD-periods after PV is reported for winter rye (113, 115), winter barley (20), perennial grasses (22) and for endive (53). However, an advantageous effect of SD seems to be found after suboptimal vernalization only. The data in literature suggest that induction by SD or by vernalization are independent processes. This is explicitly shown by WELLENSIEK (135) in *Campanula medium*, by CHOUARD (15) in *Scabiosa succisa*, by VAN DER VEEN and MEIJER (132, p. 44) in *Pelargonium grandiflorum*, by MATHON (89) in *Symphyandra hoffmani*, and by COOPER and CALDER (10, 11, 22) in some perennial grass species.

Plant age at the beginning of vernalization, duration of vernalization and day length after vernalization showed a close relation in *Cheiranthus allionii*. In LD after vernalization the vernalizability varies with age and plants of 12 to 16 days old possess a consistent minimum for vernalizability. A similar varying vernalizability with age has been reported by NAPP-ZINN (104, 105, 106, 107, 108) for strains of *Arabidopsis thaliana*, by MÜLLER-STOLL and HARTMANN (101) for *Oenothera* of Berlin, and by the author (unpublished) in two strains of *Lolium perenne* and one strain of *Lolium temulentum*. Indications for a varying vernalizability with age have been reported by HIGHKIN (56) and MOORE and BONDE (100) for peas. Cases in which the vernalizability starts at a certain age and increases with age until optimal vernalizability is reached, have been known longer and are reported for *Hyoscyamus niger* (122), *Campanula medium* and *Lunaria biennis* (135, 136, 137), *Dianthus barbatus* (134), and crops such as red garden beet (144).

Short days after vernalization result in a completely different flowering behaviour of *Cheiranthus allionii* in comparison with LD. SD has a disadvantageous effect on flower formation after SV as well as PV. However, the disadvantageous effect of SD decreases with age of the plants, grown-up plants being day neutral.

The disadvantageous effect of SD after PV of older plants can be partly overcome by an increasing duration of the PV. It is well known in the literature that most cold requiring plants require or prefer LD after vernalization for optimal flowering, whereas others are day neutral. So far no case is known of a plant which requires continuous SD after vernalization. Several LDP, which come into flower in LD without vernalization and do not flower in SD at all or very much retarded, are able to flower in SD or flowering is enhanced when these plants are vernalized as is the case for annual *Hyoscyamus niger* (78), *Silene armeria* (WELLENSIEK, unpublished), strains of *Arabidopsis thaliana* (70, 150), spinach (66, 133), *Sinapis alba*, *Agrostemma githago* and annual *Beta vulgaris* (51, 52), *Trifolium subterraneum* species (27), and for *Campanula rotundifolia* and *Campanula cespitora* (89). Some authors call this effect a decrease of the critical day length by vernalization.

Other cases are known of plants which normally will not flower in LD as well as SD without vernalization, but may do so in CL as is reported for species of winter rye, winter barley and winter vetch (8) and for *Melitotus* species (63), thus showing that plants which have an absolute cold requirement under natural conditions do not show this requirement under conditions which do not occur in nature. It is obvious from these results that even the notion absolute cold requirement is a relative one.

Gibberellic acid may substitute for the vernalization requirement in *Cheiranthus allionii* only partially. But as a prolonged application of GA_3 of a high concentration is necessary, GA_3 cannot be considered as an effective inducive agent. The fact that GA_3 induces flowering in a few plants only, whereas the flowering times are spread over a long period, is probably due to the different cold requirements of the different plants, determined by different genetical constitutions. Probably only plants with a relatively small cold requirement are induced by GA_3. Relatively few cases are known in literature in which GA substitutes for an absolute cold requirement. Such cases are *Hyoscyamus niger*, *Daucus carota*, *Brassica napus*, *Petroselinum sativum* (74, 75, 76), *Centaurium minus* MOENCH (12), winter rye (5), and some vegetable crops (6). Particularly in quantitative cold requiring plants GA may substitute for vernalization as has been reported for lettuce (7), endive (55, 117), varieties of *Chrysanthemum* (24), and strains of *Arabidopsis thaliana* (122).

Several LDP are enabled to flower or flowering is enhanced in SD, e.g. annual *Hyoscyamus niger*, *Samolus parviflorus*, *Crepis tectorum*, and *Silene armeria* (74, 75, 76), and some vegetable crops (147).

However, in recent years at least 9 different gibberellins have been identified, each of which may have different responses in different plants as has been shown by MICHNIEWICZ and LANG (99), or even within the same species, but different strains, as has been demonstrated by NAPP-ZINN (110) in *Arabidopsis thaliana*. It is apparent from these data that former negative results will probably have to be revised when other gibberellins than GA_3 will be used.

Gibberellin-like substances are present in several higher plants (9, 77, 80, 146). CAJLACHJAN and LOZHNIKOVA (9) showed that high concentrations of gibberellin-like substances were found in summer varieties of wheat, rape and rye, particularly in LD, whereas they were undetectable in the unvernalized winter varieties. They assumed that a high level of gibberellins was necessary for flower formation. If this is true, one could assume that GA-applications after vernalization merely increase the level of GA in the plant and thus may enhance the possibility of flowering. This idea fits well to the fact that e.g. in *Cheiranthus allionii* GA_3 exerts its strongest effect after suboptimal SV. However, in *Cheiranthus allionii* no effect of GA_3 has been found before and during SV or before PV. This has also been reported by TSUKAMOTO and KONISHI (131) for radish.

5-Fluorouracil has no direct influence on the vernalization process in *Cheiranthus allionii*, as has also been found in endive (55) and *Lunaria biennis* (WELLENSIEK, unpublished).

CHAPTER 4

FACTORS INVOLVED IN FIXATION AND REVERSION OF THE VERNALIZATION

4.1. Accumulation of the vernalization effect

Vernalization is an accumulative process which is initiated by and proceeds under a relatively low temperature until a 'vernalized condition' is reached in which the plant is able to flower after vernalization. The accumulative character of vernalization is, for example, demonstrated by the fact that the vernalization period may be interrupted with periods of non-vernalizing temperatures, resulting in an ultimate effect which is about equal to the effect of an uninterrupted vernalization period of the same length. The extent of the accumulation of a vernalization effect is influenced by several external conditions of which, however, the temperature is the most decisive.

4.1.1. *Low temperature*

Low temperatures (50) between the limits of -6 and $+14$°C, but normally between $+1$ and $+5$°C, are the fundamental agent of the vernalization process. In *Cheiranthus allionii* 5°C has been used. In the former chapter it has already been noted that the percentages of flowering after SV as well as after PV increase with an increasing duration of the vernalization, indicating that different plants reach their vernalized condition consecutively. This implies that the different plants possess different cold requirements, due to different genetical constitutions.

Furthermore, it was observed (143) that plants may come into flower during a prolonged treatment at 5°C, demonstrating that induction as well as realization of flower formation may take place at 5°C, which implies that it will be difficult to establish at which moment the vernalization process ends and the realization of flowering starts. They are probably overlapping events.

4.1.2. *Moderate temperature*

Moderate temperatures, lying somewhere between 15° and 25°C, are considered as temperatures which on the one hand maintain the already achieved vernalized condition, while on the other hand allowing a rapid realization of flowering. Such temperatures are also called neutral temperatures. However, whether a certain temperature is neutral or not is to a great extent determined by other factors such as the duration of the preceding vernalization and the day length.

4.1.3. *High temperature*

High temperature, such as 35°C used with *Cheiranthus allionii*, may cause a reversion of the vernalization process. Since revernalization is possible after devernalization by high temperature, a real reversion must have taken place. The extent to which devernalization by high temperature is possible, depends on

other factors such as duration of the preceding vernalization and duration of the high temperature treatment itself.

4.2. Devernalization

All processes leading to a reversion of the vernalized condition are considered as devernalization. The particular conditions which may lead to devernalization in *Cheiranthus allionii* are high temperature and SD or CD.

4.2.1. *Devernalization by high temperature*

4.2.1.1. Effect of high temperature before PV. Two experiments have been carried out in each of which the PV was preceded by periods of 0, 3, 7, 14, 21 or 28 days at the high temperature of 35°C. In the first experiment only 5 weeks of PV, in the second experiment 4 as well as 5 weeks of PV were applied. The number of plants per treatment varied from 15 to 20 plants. The results of both experiments are compiled in table 9.

Table 9. The effect of limited periods of high temperature before PV

Days at 35°C before PV	% flowering			Days to budding		
	exp. 1	exp. 2		exp. 1	exp. 2	
	5 w. PV	4 w. PV	5 w. PV	5 w. PV	4 w. PV	5 w. PV
0	93	69	88	14	13	11
3	–	75	95	–	13	11
7	100	63	88	15	15	12
14	60	65	100	18	14	11
21	61	40	92	22	14	11
28	25	25	66	31	15	12

In experiment 1 periods at 35°C of 14 days or longer before PV show a consistent decrease in the percentages of flowering in comparison with the control. In experiment 2 the same decrease in the percentages of flowering occurs, but now after periods of 21 days or longer before 4 weeks of PV and after a period of 28 days before 5 weeks of PV. In experiment 1 the mean days to budding increase with increasing periods of high temperature before PV. This is not clearly the case in experiment 2.

The decreasing percentages of flowering and the increasing mean days to budding demonstrate that long periods of high temperature have a disadvantageous effect on subsequent vernalization, which has to be called anti-vernalization. In other words: the vernalizability of the plants is decreased by preceding long durations of high temperature.

4.2.1.2. Effect of high temperature during interruptions of PV. In an earlier experiment (3) PV was interrupted with LD and SD at 20°C or natural day at 35°C at different moments after the beginning of the vernalization, whereas the total duration of the vernalization period was kept constant. However, the design of this experiment did not allow a clear separation between

the effects of vernalization, interruption and revernalization. In the experiment, which will be described in the following, this difficulty has been met with by constant revernalization periods.

PV treatments of 0, 1, 2, 3, 5 or 7 weeks were followed by interruptions with LD at 20°C or 35°C for 3, 7, 14 or 21 days. The interruptions were followed by revernalization for 3 or 5 weeks, being in itself insufficient for PV and sufficient for suboptimal PV respectively. The controls remained uninterrupted. The duration of the vernalization of the controls is equal to the total duration of the interrupted vernalization treatments, being 3, 4, 5, 6, 8 and 10 weeks respectively. Each treatment initially consisted of 20 plants but during the treatments incidentally one or more plants died, resulting in a varying number of plants, between 15 and 20, per treatment. The results for 3 weeks of revernalization are compiled in table 10.

With one exception only, the vernalization treatments, interrupted at 20°C for 3, 7, 14 or 21 days yield lower percentages of flowering than the corresponding controls, when the interruptions take place after 5 weeks of PV or earlier. The percentages of flowering decrease with an increasing duration of the interruption. It is therefore obvious that interruptions at 20°C have a disadvantageous effect, when given after suboptimal vernalization. As the revernalization period of 3 weeks is in itself insufficient for vernalization, it appears that the effect of these interruptions at 20°C consist of a nullifying effect on the previous vernalization, i.e. devernalization, which must be due to the relatively high temperature during the interruption in comparison with the vernalization temperature.

The percentages of flowering after interruptions at 35°C after 5 weeks of PV or earlier are generally higher than those after corresponding interruptions at 20°C, but still lower than those of the corresponding controls. The percentages of flowering decrease with increasing durations of interruptions at 35°C. After 7 weeks of PV short interruptions of 3 or 7 days at 35°C have no effect, whereas after interruptions of 14 or 21 days the percentages of flowering are lower than the corresponding controls.

The mean numbers of days to budding appear to be somewhat irregular, but nevertheless it is obvious that they decrease with an increasing duration of the vernalization, whereas no consistent effect of the temperature during the interruption is observed.

The overall picture of the percentages of flowering in table 11 shows the same trends as those of table 10. However, the percentages in table 11 are higher than in table 10 due to the longer revernalization period.

Particularly the percentages of flowering in table 11 show that the differences between interruptions at 20°C and at 35°C are most pronounced with interruptions of short duration, namely 3 or 7 days. At longer durations, 14 or 21 days, the differences more or less disappear, whereas the percentages of flowering after interruptions at 35°C are lower than those after interruptions at 20°C, particularly after 5 or 7 weeks of PV. The mean numbers of days to budding rather clearly tend to decrease with an increasing duration of the vernalization,

TABLE 10. The effect of 20° or 35 °C during interruptions of PV, followed by revernalization (V_2) for 3 weeks. $V_1 \rightarrow I \rightarrow V_2$: vernalization in weeks, followed by an interruption in days, followed by revernalization in weeks. - - -/- - -: percentage of flowering subsidiary mean days to budding of plants which came into flower during the interruption/total percentage of flowering subsidiary mean days to budding of plants which came into flower after the revernalization

Treatments			% flowering			Days to budding		
V_1	→ I →	V_2	Controls	20 °C	35 °C	Controls	20 °C	35 °C
3			0			∾		
4			15			17		
5			72			17		
6			93			16		
8			100			13		
10			100			12		
0	3	3		0	0		∾	∾
1	3	3		10	6		20	16
2	3	3		56	75		21	19
3	3	3		50	89		18	17
5	3	3		100	100		15	14
7	3	3		100	100		7	8
0	7	3		0	0		∾	∾
1	7	3		0	5		∾	22
2	7	3		–	47		–	20
3	7	3		–	67		–	19
5	7	3		62	83		11	17
7	7	3		100	100		7	8
0	14	3		0	0		∾	∾
1	14	3		0	25		∾	20
2	14	3		0	47		∾	20
3	14	3		40	47		19	19
5	14	3		75	78		9	17
7	14	3		81/100	18/59		10/ 5	10/11
0	21	3		0	0		∾	∾
1	21	3		0	11		∾	21
2	21	3		0	13		∾	22
3	21	3		0	22		∾	22
5	21	3		70/80	0/100		16/9	–/15
7	21	3		100/100	90/90		13/–	17/–

whereas no consistent differences occur between the interrupting treatments at 20 °C and 35 °C.

Unexpectedly, short interruptions at 35 °C after 5 weeks of PV or earlier have thus shown to be less disadvantageous than interruptions at 20 °C. As will be shown furtheron, high temperature normally exerts a devernalizing effect after PV. The only explanation which can be offered is that interruptions at 35 °C exert two effects: a devernalizing effect on the preceding vernalization and

TABLE 11. The effect of 20° or 35 °C during interruptions of PV, followed by revernalization (V_2) for 5 weeks. For details see legend of table 10

Treatments			% flowering			Days to budding		
V_1	→ I →	V_2	Controls	20 °C	35 °C	Controls	20 °C	35 °C
5			72			17		
6			93			16		
7			100			15		
8			100			13		
10			100			12		
12			100			11		
0	3	5		71	87		17	19
1	3	5		71	94		16	17
2	3	5		100	100		15	14
3	3	5		100	100		14	14
5	3	5		100	100		10	12
7	3	5		100	100		5	8
0	7	5		69	91		18	18
1	7	5		70	100		17	18
2	7	5		88	100		17	15
3	7	5		93	100		16	14
5	7	5		100	100		11	12
7	7	5		100	100		7	10
0	14	5		73	72		18	18
1	14	5		67	62		22	18
2	14	5		86	100		19	17
3	14	5		89	88		17	15
5	14	5		100	6/88		9	14/15
7	14	5		80/100	33/83		14/8	14/15
0	21	5		69	72		18	19
1	21	5		75	71		17	19
2	21	5		100	53		16	17
3	21	5		86	94		14	17
5	21	5		72/100	60		16/15	18
7	21	5		100/100	33/93		12/–	16/18

simultaneously an advantageous effect on the subsequent vernalization i.e. an increase of the vernalizability of the plants. The same conclusion has been reached earlier (3).

4.2.1.3. Effect of high temperature after SV. SV for 1, 2, 3, 4, 6 or 8 weeks was immediately followed by a period of 0, 3, 7, 10, 14 or 21 days at 35°C followed by about 20°C in LD. Each treatment consisted initially of 27 plants. The results are presented in table 12.

With several exceptions, the percentages of flowering after SV followed by high temperature treatments are lower than those of the corresponding controls, whereas they tend to decrease with an increasing duration of the high temperature

TABLE 12. The effect of limited periods of 35 °C, indicated in days (d.), after different durations of SV

Vernalization in weeks	0 d.	3 d.	7 d.	10 d.	14 d.	21 d.
	% flowering					
1	3	11	8	–	–	–
2	15	11	15	4	0	–
3	21	28	3	3	0	0
4	33	23	11	22	8	0
6	63	34	29	29	15	3
8	64	23	11	11	11	8
	Days to budding					
1	60	63	72	–	–	–
2	58	63	63	61	∞	–
3	57	60	61	71	∞	∞
4	52	55	67	68	61	∞
6	55	61	61	64	68	68
8	51	58	64	67	69	70

treatment. The mean numbers of days to budding are somewhat irregular, but nevertheless it is apparent that they are higher than those of the corresponding controls in all cases, whereas, with several exceptions, they show the tendency to increase with an increasing duration of the high temperature treatments. In spite of the somewhat irregular results it is obvious that devernalization by high temperature remains possible irrespective of the duration of SV and that the devernalizing effect more or less increases with increasing duration of the high temperature treatment.

In a subsequent experiment, after 6 weeks of SV, periods of 7 or 14 days at 35 °C were given at different moments after the SV, whereas the control remained permanently in LD at about 20 °C after SV. Each treatment consisted initially of 24 plants. The results are presented in table 13.

TABLE 13. The effect of 7 or 14 days at 35 °C at different moments after 6 weeks of SV. Between the end of the SV and the 35 °C treatments different numbers of LD at 20 °C are intercalated

Number of LD at 20 °C	% flowering			Days to budding		
	Control	7 d. 35 °C	14 d. 35 °C	Control	7 d. 35 °C	14 d. 35 °C
Control	56			61		
0		59	28		69	72
7		49	11		71	75
14		19	14		67	72
21		30	0		71	∞
28		17	0		71	∞
35		19	7		69	94
42		8	0		75	∞
49		8	0		64	∞

The percentages of flowering as well as the mean numbers of days to budding are again somewhat irregular. Nevertheless, it appears that the percentages of flowering after high temperature treatments, with one exception, are lower than the control, whereas they decrease as the high temperature treatments are given at later moments. Furthermore, the percentages of flowering after treatments of 14 days at 35 °C are lower than after treatments of 7 days at 35 °C.

The mean numbers of days to budding after treatments with high temperature are higher than the control in all cases. The results demonstrate that devernalization by high temperature becomes easier when it is applied later after SV, whereas the devernalizing effect increases with increasing duration of the high temperature treatment.

Lack of plants made it impossible to extend the above experiment further than 49 days. However, it is reasonable to assume that as soon as differentiation of flower buds has taken place, the effect of high temperature will only consist of a retardation of flowering.

4.2.1.4. Effect of high temperature after PV. PV during 4 or 6 weeks was immediately followed by a period of 0, 3, 7, 10, 14 or 21 days at 35 °C. The number of plants per treatment varied from 16 to 20. The results are presented in table 14.

TABLE 14. The effect of limited periods at 35 °C immediately after 4 or 6 weeks of PV

Days at 35 °C	% flowering		Days to budding	
	4 w. PV	6 w. PV	4 w. PV	6 w. PV
0	57	100	16	12
3	56	100	16	12
7	25	100	21	15
10	6	69	34	15
14	0	81	∞	18
21	6	69	34	18

The percentages of flowering after 4 weeks of PV decrease after a high temperature treatment of 7 days or longer, whereas the mean numbers of days to budding tend to increase with an increasing duration of the high temperature treatment. After 6 weeks of PV, followed by 10, 14 or 21 days of high temperature, a decreased percentage of flowering is found, whereas the mean numbers of days increase with increasing duration of the high temperature treatment. It is obvious from these results that after suboptimal PV an almost complete devernalization is possible by sufficiently long high temperature treatments, whereas after optimal PV only a limited devernalization is possible by rather long durations of the high temperature treatments.

In a following experiment 0, 2, 4, 6, 8, 10 or 15 days at 20 °C in LD were intercalated between 4 or 6 weeks of PV and 1 or 2 weeks of high temperature. The number of plants per treatment varied from 16 to 20. The results are presented in table 15.

TABLE 15. The effect of 1 or 2 weeks (w.) at 35 °C at different moments after 4 or 6 weeks of PV. Between the end of PV and the 35 °C treatments different numbers of LD at 20 °C are intercalated

Number of LD at 20 °C	% flowering				Days to budding			
	4 w. PV		6 w. PV		4 w. PV		6 w. PV	
	1 w.	2 w.	1 w.	2 w.	1 w.	2 w.	1 w.	2 w.
Control	57		100		18		12	
0	19	0	88	81	19	∞	17	15
2	13	19	93	75	24	23	13	13
4	31	0	81	94	23	∞	13	13
6	13	33	100	93	18	22	12	14
8	25	8	100	100	20	19	12	12
10	7	19	100	100	21	20	12	12
15	33	35	–	–	19	19	–	–

The percentages of flowering as well as the mean numbers of days to budding after 4 weeks of PV are irregular. However, the percentages of flowering after the high temperature treatments during 1 or 2 weeks are all lower than the control, whereas, with one exception, the mean numbers of days are somewhat higher than the control. No consistent differences are found between 1 or 2 weeks of high temperature.

After 6 weeks of PV a more or less decreased percentage of flowering is found only up to 6 days after PV, followed by 1 week of high temperature and up to 8 days after PV, followed by 2 weeks of high temperature. The mean numbers of days to budding after 6 weeks of PV tend to decrease as the high temperature treatments are given on later moments after the PV.

The results of this experiment indicate that after 4 weeks of PV, i.e. suboptimal PV, a limited devernalization remains possible up to 15 days after PV, whereas after 6 weeks of PV such a limited devernalization is only possible up to 6 or 8 days after PV.

4.2.2. *Devernalization in SD or CD*

4.2.2.1. Effect of SD before PV. The effect of SD before PV has already been demonstrated (p. 11). It was shown that SD exerts a disadvantageous effect on subsequent PV, hence acts as anti-vernalization.

4.2.2.2. Effect of SD or CD during interruptions of PV. PV treatments of 0, 1, 2, 3, 5 or 7 weeks were followed by interruptions with CL, LD, SD or CD at approximately 20 °C for 3, 7, 14 or 21 days. The interruptions were followed by revernalization for 3 or 5 weeks. The numbers of plants per treatment varied from 15 to 20. The results for 3 weeks of revernalization are compiled in table 16.

All percentages of flowering after the interrupted PV treatments are lower than those of the corresponding controls, when PV is interrupted after 3 weeks or earlier for 3 or 7 days, after 5 weeks or earlier for 14 or 21 days. The differ-

TABLE 16. The effect of day length i.e. CL, LD, SD or CD during interruptions of PV, followed by revernalization (V_2) for 3 weeks. For details see legend of table 10

Treatments $V_1 \rightarrow I \rightarrow V_2$	% flowering					Days to budding				
	Controls	CL	LD	SD	CD	Controls	CL	LD	SD	CD
3	0					∞				
4	15					17				
5	72					17				
6	93					16				
8	100					13				
10	100					12				
0 3 3		0	0	0	0		∞	∞	∞	∞
1 3 3		5	10	0	15		22	20	∞	21
2 3 3		64	56	44	45		18	21	18	19
3 3 3		69	50	40	47		19	18	20	19
5 3 3		100	100	100	100		16	15	14	16
7 3 3		100	100	100	100		9	7	9	12
0 7 3		0	0	0	0		∞	∞	∞	∞
1 7 3		5	0	0	0		22	∞	∞	∞
2 7 3		35	–	21	15		21	–	22	21
3 7 3		57	–	26	30		19	–	22	17
5 7 3		92	62	100	100		10	11	10	14
7 7 3		100	100	100	100		6	7	6	15
0 14 3		0	0	0			∞	∞	∞	
1 14 3		0	0	0			∞	∞	∞	
2 14 3		0	0	0			∞	∞	∞	
3 14 3		–	40	5			∞	19	22	
5 14 3		25/63	75	44			14/5	9	9	
7 14 3		81/100	81/100	44/89			12/3	10/5	14/5	
0 21 3		0	0	0			∞	∞	∞	
1 21 3		0	0	0			∞	∞	∞	
2 21 3		0	0	0			∞	∞	∞	
3 21 3		0	0	0			∞	∞	∞	
5 21 3		73/73	70/80	39/72			14/–	16/9	18/20	
7 21 3		100/100	100/100	100/100			12/–	13/–	14/–	

ences with corresponding controls increase with increasing duration of the interruptions.

The percentages of flowering after interruptions with CL in comparison with corresponding interruptions with LD do not show consistent differences. After interruptions during 3 or 7 days after 3 weeks of PV or earlier or during 14 or 21 days after 5 weeks of PV or earlier, the percentages of flowering after interruptions with SD are consistently lower than after corresponding interruptions with CL or LD. With one exception the same holds true for corresponding interruptions with CD. There are no consistent differences in the percentages of

flowering after interruptions with SD in comparison with those after interruptions with CD.

The mean numbers of days to budding of the controls as well as those after the interrupted PV treatments decrease with increasing duration of PV, irrespective of day length during or duration of the interruptions. There are no consistent differences in the mean numbers of days to budding after corresponding interruptions with the different day lengths.

In conclusion, the results of table 16 demonstrate that any interruption at 20 °C after 3 weeks of PV or earlier with short interruptions or after 5 weeks of PV or earlier with long interruptions has a devernalizing effect, irrespective of

TABLE 17. The effect of day length i.e. CL, LD, SD or CD during interruptions of PV, followed by revernalization (V_2) for 5 weeks. For details see legend of table 10

Treatments $V_1 \rightarrow I \rightarrow V_2$	% flowering					Days to budding				
	Controls	CL	LD	SD	CD	Controls	CL	LD	SD	CD
5	72					17				
6	93					16				
7	100					15				
8	100					13				
10	100					12				
12	100					11				
0 3 5		77	71	68	53		19	17	17	19
1 3 5		84	71	79	64		17	16	16	17
2 3 5		100	100	100	93		16	15	15	17
3 3 5		100	100	100	100		15	14	13	15
5 3 5		100	100	100	100		11	10	16	12
7 3 5		100	100	100	100		9	5	10	11
0 7 5		80	69	56	29		17	18	18	21
1 7 5		80	70	45	40		17	17	21	20
2 7 5		89	88	80	67		17	17	19	19
3 7 5		94	93	100	50		15	16	16	12
5 7 5		100	100	100	100		10	11	12	12
7 7 5		100	100	100	100		6	7	9	7
0 14 5		72	73	63			20	18	19	
1 14 5		100	67	55			18	22	19	
2 14 5		86	86	67			18	19	18	
3 14 5		100	89	71			16	17	19	
5 14 5		30/100	100	82			14/8	9	13	
7 14 5		70/100	80/100	14/100			13/3	14/8	14/13	
0 21 5		71	69	55			19	18	19	
1 21 5		67	75	60			16	17	17	
2 21 5		74	100	50			17	16	16	
3 21 5		71	86	60			14	14	19	
5 21 5		89/89	72/100	53/73			15/–	16/15	15/15	
7 21 5		100/100	100/100	69/88			11/–	12/–	13/15	

the day length during the interruptions. The devernalization by interruptions with SD or CD is somewhat stronger than by interruptions with CL or LD.

The percentages of flowering and the mean numbers of days to budding in table 17 show the same tendencies as those in table 16. However, the percentages of flowering in table 17 are higher which is due to the longer revernalization period.

Several percentages of flowering after interruptions with SD, particularly long interruptions, are lower than 72%, which means that they are lower than could be expected on account of the revernalization period of 5 weeks only. These results demonstrate that SD, particularly during long interruptions, may not only exert a devernalizing effect on the preceding vernalization, but also exerts anti-vernalization, hence influences the subsequent revernalization. This is in accordance with the effect of SD before PV as noted under section 4.2.2.1 (p. 31) of this chapter. Interruptions with CD show the same effect as SD, but more pronounced.

4.2.2.3. Effect of SD or CD after SV. SV during 6 weeks was immediately followed by SD-periods of 0, 14, 21, 28, 35, 42, 45, 49, 52, 56 or 70 days, whereas two other treatments were kept permanently in LD or SD. The limited SD-periods were followed by LD. Each treatment consisted initially of 32 plants.

The results presented in table 18 show that the percentages of flowering after

TABLE 18. The effect of limited SD-periods immediately following 6 weeks of SV

SD in days	% flowering	Days to budding	SD in days	% flowering	Days to budding
0	72	53	45	41	67
14	48	63	49	34	75
21	47	64	52	16	87
28	50	64	56	28	82
35	53	66	70	0	∞
42	44	70	∞	0	∞

the SD treatments are lower than the control, whereas they decrease slowly and somewhat irregularly after SD periods of 35 days or longer until at a SD-period of 70 days and in permanent SD no flowering occurs at all. The mean numbers of days to budding increase somewhat irregularly with increasing duration of the SD-periods. The results of this experiment indicate that only rather long SD-periods cause a reasonable devernalization after SV. However, in following experiments the effect of SD will be more closely defined.

SD-periods of 1, 2 or 3 weeks, interrupting the LD, were given at different moments after SV for 6 weeks. Each treatment consisted initially of 32 plants. The results are given in table 19.

A considerably decreased percentage of flowering as well as a considerably increased mean number of days to budding, in comparison with the controls, is found in the following treatments after SV: 5 w. LD, 1 w. SD, LD; 5 w. LD,

TABLE 19. The effect of 1, 2 or 3 weeks of SD, interrupting the LD after 6 weeks of SV at different moments

Interruption after (weeks)	% flowering			Days to budding		
	1 w. SD	2 w. SD	3 w. SD	1 w. SD	2 w. SD	3 w. SD
Control	72			53		
2	65	69	72	58	57	62
3	78	78	23	54	57	68
4	63	65	22	58	65	81
5	31	27	29	63	73	76
6	35	66	50	59	58	52
7	70	71	61	55	54	56

2 w. SD, LD; 3 w. LD, 3 w. SD, LD; 4 w. LD, 3 w. SD, LD; 5 w. LD, 3 w. SD, LD.

All the above treatments have in common the period of the 5th until the 6th week after SV during which SD was applied.

In general the first signs of flower bud differentiation, as seen under a binocular, are usually observed between the 6th and the 8th day before macroscopical flower buds are visible. Thus in the case of this experiment the flower bud differentiation will have started before 53 (control) minus 6 to 8 days i.e. 47 to 45 days after SV. The results of this experiment therefore suggest that the disadvantageous effect of SD after SV is most pronounced just before flower bud differentiation, i.e. when flower initiation takes place.

In a similar experiment, with SD-periods of 1 or 2 weeks at different moments after SV for 6 weeks, 100 ppm GA_3 was applied daily during the SD-treatments, except on the controls. Each treatment consisted initially of 24 plants. The results are presented in table 20.

TABLE 20. The effect of GA_3 applied during 1 or 2 weeks of SD, interrupting the LD after 6 weeks of SV at different moments

Interruption after (weeks)	% flowering				Days to budding			
	1 w. SD		2 w. SD		1 w. SD		2 w. SD	
	$-GA_3$	$+GA_3$	$-GA_3$	$+GA_3$	$-GA_3$	$+GA_3$	$-GA_3$	$+GA_3$
Control	84				41			
2	63	71	73	67	42	43	45	45
3	57	59	6	13	45	45	47	48
4	29	43	9	34	38	44	39	39
5	75	83	92	87	40	40	40	40
6	88	92	82	85	40	41	41	42

Particularly the percentages of flowering in table 20 show the same overall picture as those in table 19. However, flowering occurred sooner after the SV. Irrespective of the GA_3 treatments, a considerably decreased percentage of flowering in comparison with the control is found with the following treatments after SV: 4 w. LD, 1 w. SD, LD; 3 w. LD, 2 w. SD, LD and 4 w. LD, 2 w. SD,

LD. These treatments have in common the period in which SD was applied, i.e. during the 4th until the 5th week after SV which is again just before flower bud differentiation takes place. Thus the results of this experiment confirm those of the former. It is seen that in the above mentioned treatments the percentages of flowering after GA_3 treatments during the SD are higher than those of the non-treated ones. Thus GA_3 may overcome the disadvantageous effect of SD to a certain extent, but not completely.

The purpose of a subsequent experiment with CD was to establish whether the disadvantageous effect of SD is a real day-length effect or whether it is due to the effect of darkness only. The LD after 6 weeks of SV was interrupted after 0, 1, 2, 3, 4, 5, 6 or 7 weeks with 5 days of CD.

TABLE 21. The effect of 5 days of CD, interrupting the LD after 6 weeks of SV at different moments

Interruption after (weeks)	% flowering	Days to budding	Interruption after (weeks)	% flowering	Days to budding
Control	95	42	4	0	∞
0	50	48	5	23	43
1	81	49	6	54	43
2	18	53	7	82	42
3	30	50			

The results in table 21 show that both the percentages of flowering and the mean numbers of days to budding are irregular, which must be ascribed to the treatments with CD, being detrimental to the growth of the plants, whereas some plants even died. Nevertheless, it appears that the percentage of flowering is most decreased after CD given 4 weeks after SV, which is again just before flower bud differentiation. This experiment demonstrates that the effect of CD is principally the same as that of SD, but more pronounced. In conclusion it is apparent that darkness is the agent which causes the disadvantageous effect. Thus the effect of SD is caused by the relatively long dark periods of SD and has probably nothing to do with a day-length effect.

4.2.2.4. Effect of SD after PV. Plants of 0, 1, 2, 3, 4, 5 or 6 weeks old were vernalized for 6, 8, 10 or 12 weeks, immediately followed by the day-length treatments: LD; 3 weeks of SD → LD; 5 weeks of SD → LD; SD. Each treatment consisted initially of 24 plants. The results are compiled in table 22.

The percentages of flowering of column 2 show a marked decrease, particularly after vernalization of 1 or 2 week old plants at all durations of the vernalization besides 12 weeks, thus confirming the minimum of sensitivity to vernalization, which has been dealt with on p. 12-16. The percentages of flowering after vernalization at corresponding ages in column 2 increase, albeit with some irregularities, with increasing duration of the vernalization until maximum flowering is reached.

The percentages of flowering in column 3 show the same overall picture as

TABLE 22. The effect of SD after PV at different ages

1	2	3	4	5	6	7	8	9
Age in weeks	% flowering				Days to budding			
	Treatments after vernalization							
	LD	3 w. SD →LD	5 w. SD →LD	SD	LD	3 w. SD →LD[3])	5 w. SD →LD[3])	SD
	6 weeks of vernalization							
0	100	38	41	0	44	39	39	∞
1	19	0	0	0	43	∞	∞	∞
2	19	5	0	0	46	41	∞	∞
3	60	0	6	0	37	∞	22	∞
4	52	5	0	0	32	41	∞	∞
5	42	4	0	0	30	22	∞	∞
6	63	0	0	0	25	∞	∞	∞
	8 weeks of vernalization							
0	100	36	28	0	38	41	29	∞
1	48	0	4	0	40	∞	30	∞
2	28	0	9	0	39	∞	30	∞
3	37	0	5	0	37	∞	31	∞
4	85	8	0	0	26	35	∞	∞
5	94	21	0	0	25	14	∞	∞
6	89	26	0	0	23	13	∞	∞
	10 weeks of vernalization							
0	100[1])	43	30	0	45[1])	40	44	∞
1	11[1])	4	9	0	50[1])	45	28	∞
2	38[1])	17	0	0	43[1])	40	∞	∞
3	53	18	7[2])	4	35	33	22	35
4	100	25	9[2])	5	32	28	32	35
5	89	46	0[2])	6	22	17	∞	33
6	100	61	0[2])	22	20	13	∞	20
	12 weeks of vernalization							
0	100	43	7[1])	0	40	38	39	∞
1	100	17	10	0	36	33	40	∞
2	95	48	9	4	35	35	35	68
3	100	60	0	4	28	26	∞	58
4	100	21	7	0	29	27	32	∞
5	100[1])	71	0[2])	25	21[1])	15	∞	16
6	100[1])	70	20[2])	31	25[1])	14	27	18

[1]) Some plants died owing to mold, whereas the growth of the remaining plants was retarded.

[2]) As flowering of some plants occurred in SD, only vegetative plants were removed from SD to LD.

[3]) Days to budding were counted from the end of the SD-treatment instead of from the end of the vernalization.

those of column 2, however, they are much lower. These results indicate that the devernalizing effect of a limited SD-period after vernalization is connected with the age at which the plants are vernalized. A comparison of the percentages of flowering of columns 2, 3, 4 and 5 reveals that they decrease with increasing duration of the SD after vernalization, thus demonstrating that devernalization increases with increasing duration of SD. Particularly the percentages of flowering in continuous SD after 10 or 12 weeks of vernalization, column 5, indicate that the effect of SD decreases with increasing age. This was more clearly demonstrated on p. 16-18.

There is another factor, however, which interferes with the devernalizing effect of SD. After SV as well as after PV of rather young plants, the plants have to reach a certain size before flower initiation can take place and, as has been shown on p. 35, the effect of SD is most disadvantageous just before flower bud differentiation, whereas the effect of SD is rather small during the time which is necessary to reach the required size for flower initiation.

The mean numbers of days to budding of columns 6, 7, 8 and 9, with some irregularities, decrease with increasing age of the vernalized plants.

When the columns 6, 7 and 8 are compared, the mean numbers of days to budding tend to decrease with increasing duration of the SD after the vernalization, but this tendency is not very consistent. However, we have to keep in mind that the mean numbers of days to budding in columns 7 and 8 are counted from the end of the SD treatments, whereas the mean numbers of days to budding in column 6 are counted from the end of the vernalization. These results indicate that, when plants manage to flower after the SD treatments, they seem to have hardly developed towards flowering during this SD. The mean numbers of days to budding in permanent SD after vernalization in column 9 do not show consistent differences with those in LD in column 6.

4.2.3 *Absence of devernalization by means of desiccation of vernalized seeds*

4.2.3.1. Effect of temperature during desiccation. Only very few seeds germinate during SV, whereas most seeds remain in a swollen condition. Desiccation of such swollen seeds proved to be unharmful as far as germination is concerned, whereas the few germinated seeds were killed. Seeds, which have been vernalized for 6 weeks, were desiccated and kept at 20°C permanently, at 35°C for 1, 3 or 7 days or at 50°C for 1 or 3 days, after which the seeds of all treatments were preserved for 14 days at 20°C before being sown. The seeds of the control were sown immediately after SV. The number of plants per treatment varied from 16 to 32. The results are presented in table 23.

After desiccation of the seeds after SV at 20°C as well as at 35°C the germination was normal. The germination percentage was considerably decreased by the very high temperature of 50°C, particularly after 3 days at 50°C, but nevertheless about 25% of the seeds finally germinated and developed, albeit somewhat retarded.

The percentages of flowering do not differ considerably from the control,

which demonstrates that the vernalized condition of the seeds is neither affected by the desiccation itself nor by the temperature during desiccation. The somewhat increased mean number of days to budding after desiccation for 3 days at 50°C can be ascribed to the delayed germination.

TABLE 23. The effect of desiccation of vernalized seeds

Treatment after SV	% flowering	Days to budding
Control	63	51
20°C	63	52
1 day at 35°C	68	51
3 days at 35°C	59	51
7 days at 35°C	65	51
1 day at 50°C	63	52
3 days at 50°C	56	55

4.2.3.2. Effect of preservation of desiccated seeds. Seeds, which were vernalized for 6 weeks and desiccated at 20°C, were preserved at this same temperature for 1, 2, 3, ..., 15 or 46 weeks. The control was sown immediately after SV, whereas all treatments had taken place at such times that they could be sown simultaneously. All treatments germinated normally. The results are given in table 24.

TABLE 24. The effect of preservation of seeds, desiccated at 20°C after SV

Preservation in weeks	% flowering	Days to budding	Preservation in weeks	% flowering	Days to budding
0	65	60	8	–	–
1	50	60	9	86	60
2	73	60	10	75	56
3	–	–	11	74	57
4	73	59	12	69	59
5	62	61	13	–	–
6	64	59	14	62	60
7	59	62	15	96	55
			46	76	59

The percentage of flowering as well as the mean numbers of days to budding vary considerably. However, it is apparent that the vernalized condition is not affected by the preservation as no percentage of flowering is considerably lower than the control, whereas the mean numbers of days to budding also do not differ significantly from the control.

4.3. DISCUSSION

Accumulation of the vernalization effect has been clearly demonstrated in *Cheiranthus allionii*. Partial vernalization was achieved by interruptions of PV with non-vernalizing conditions in such a way that the PV period was

divided into two periods, each being in itself insufficient for vernalization. Similar results have been obtained for winter rye by GREGORY and PURVIS (41).

High temperature of a rather long duration before PV causes anti-vernalization.

Short interruptions of PV by 35°C were less disadvantageous than corresponding interruptions by 20°C. This effect was ascribed to an advantageous effect on subsequent revernalization besides the devernalizing effect on the preceding vernalization. Equal results have been reported earlier (3), whereas similar results have been obtained with wheat by EFEIKIN (cited after GREGORY and PURVIS, 46), with winter rye by GREGORY and PURVIS (46) and with *Arabidopsis thaliana* by NAPP-ZINN (104).

After SV nearly complete devernalization remains possible, irrespective of the duration of SV. However, devernalization becomes harder with increasing duration of SV. Similar results have been reported for winter rye by PURVIS and GREGORY (116) and for *Arabidopsis thaliana* by NAPP-ZINN (105, 107). In *Cheiranthus allionii* moreover the effect of high temperature increases, when applied at later moments after the SV.

After PV partial devernalization is possible. Devernalization becomes harder with increasing duration of PV. After optimal PV, followed by a few days at a neutral temperature, devernalization is not possible any more. Similar results are reported for biennial *Hyoscyamus niger* by LANG and MELCHERS (79, 98) and for *Arabidopsis thaliana* by NAPP-ZINN (104, 107).

Successful devernalization by high temperature after SV or PV has been reported for winter wheat (25, 26), for *Beta vulgaris* (19), for *Dianthus barbatus* (134) and for celery (130), whereas negative results have been reported for *Brassica campestris*, *Cicer arietinum*, *Linum esculentum*, *Lens esculenta* (13) and for *Sinapis alba* (49).

Since revernalization after devernalization by high temperature is possible in *Cheiranthus allionii* as well as in the cases mentioned in the literature, the true reversion of the vernalization is demonstrated. The result that devernalization after SV remains possible, irrespective of its duration, suggests that the achieved vernalisation is never completely stable. It is assumed that as soon as flower initiation starts, no reversion will be possible any more. The so-called stabilization of the vernalization effect after PV by a neutral temperature may therefore probably be ascribed to the fact that flower initiation starts very soon after PV.

Short days of rather long duration before PV cause anti-vernalization. SD or CD during interruptions of PV cause a devernalization of the preceding vernalization and anti-vernalization with respect to the subsequent revernalization. A similar effect of SD has been described by CHOUARD (90, p. 75) for *Oenothera biennis*.

SD or CD after SV as well as after PV may cause devernalization in *Cheiranthus allionii*. However, the results after SV demonstrated that SD and CD were most disadvantageous just before flower bud differentiation takes place. It could be shown that darkness is the determining factor. It is obvious that this

devernalization by darkness is indirect. SD or CD probably cause a low level of carbohydrates and decrease the mitotic activity in *Cheiranthus allionii*, both necessary for rapid flower initiation. Hence the processes leading to flower initiation are delayed and this enables the relatively high temperature of e.g. 20°C to exert its devernalizing effect. The above view is supported by the fact that GA_3 may partially overcome the disadvantageous effect of SD after SV since GA enhances cell division, but does not increase the carbohydrate supply.

SCHWABE (125) reported that devernalization of *Chrysanthemum* by low light intensity was largely dependent on the temperature. In literature (32, 104, 105, 107) devernalization by high temperature was most effective in darkness, as has been explicitly shown by FRIEND and PURVIS (32), who demonstrated that the devernalization by high temperature could be diminished by weak light during this treatment. It becomes obvious from the above results that the temperature is the predominating factor in devernalization, while its effect may be enhanced by darkness.

The disadvantageous effect of SD after vernalization is generally recognized and its devernalizing character has been pointed out for the first time by CHOUARD (16) for *Oenothera biennis*. On the other hand, LANG and MELCHERS (79) demonstrated that the vernalized condition of *Hyoscyamus niger* was maintained in SD for a long time. This effect is probably due to the food reserves available in the roots of *Hyoscyamus niger*.

Desiccation of vernalized seeds of *Cheiranthus allionii* does not affect the vernalized condition, irrespective of temperature or duration of preservation. Similar results are reported for *Brassica campestris*, *Cicer arietinum*, *Linum usitatissimum* and *Lens esculenta* (13, 128). Furthermore, FRIEND and PURVIS (32) reported that devernalization of winter rye could be diminished by restricting the water supply of the seeds during the high temperature treatment. These results suggest that the achieved vernalization is more or less stabilized in the desiccated seeds and that devernalization is possible in growing tissues only. On the contrary, devernalization by desiccation is reported for wheat (83) and for winter rye (43) after preservation for more than 6 weeks.

CHAPTER 5

LOCALIZATION AND TRANSLOCATION OF THE VERNALIZATION EFFECT

5.1. THE ROLE OF GROWING-POINTS IN VERNALIZATION

When plants are not decapitated, laterals are formed only incidentally and if so, they normally develop from basal nodes only. It was observed that when such laterals developed early after SV, they may come into flower, whereas when they developed late after SV, they remained vegetative. Decapitation induced the development of laterals of which those from the upper nodes developed most rapidly and heavily. These observations initiated the following experiment.

After 6 weeks of SV the following treatments were applied in LD: removal of roottips 3 days after SV (-rt.); removal of cotyledons 7 days after SV (-cot.); decapitation of the plants after 2, 3,..., 11 or 12 weeks from the base upwards above the different nodes indicated as 1 (above the cotyledons), 2, 3, 4, etc. The number of plants varied from 40 in the first to 24 in the last treatments. After 7 weeks or later after SV only flowering plants were decapitated. Normally only the laterals from the node just below the decapitation were taken into consideration for the results presented in table 25. Ten weeks after SV or later an extra decapitation was applied just below the terminal inflorescence, the results of which are given within brackets. The mean numbers of days to budding were counted from the date of decapitation.

Removal of the roottips causes a decreased percentage of flowering and an increased mean number of days to budding, which may be ascribed to the delay in the growth owing to this treatment. Removal of the cotyledons has no effect on the percentage of flowering and on the mean days to budding, thus demonstrating that they are not of importance after SV, at least from the 8th day.

The percentages of flowering as well as the mean numbers of days to budding are rather irregular, but nevertheless show some definite trends.

When the decapitation takes place later, the percentages of flowering per node tend to decrease and to become zero: when is decapitated above the 2nd node after 7 weeks or later, above the 4th node after 8 weeks or later, above the 7th node after 10 weeks or later, and above the 9th node after 11 weeks or later. These results demonstrate that initially all the lateral buds formed after the SV are in the vernalized condition, but that they gradually lose this condition with time, which is probably due to devernalization.

The mean numbers of days to budding of the laterals generally decrease as these laterals are formed on higher nodes. Moreover, flowering laterals from basal nodes were much longer and possessed more and larger leaves than those from higher nodes (see photo 5). In extreme cases flowering laterals from the highest nodes did not possess leaves at all and consisted of an inflorescence only. These results demonstrate that laterals from basal nodes have to reach a certain

TABLE 25. The effect of removing roottips (–rt.) or cotyledons (–cot.) and of decapitation above different nodes and at different moments, after 6 weeks of SV. Details in text

Treatments	% flowering	Days to budding
Control	95	42
–rt.	64	51
–cot.	94	44

Decapitated after (weeks)	% flowering from node															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	59	43															
3	9	7	33														
4	–	17	25	22													
5	–	25	11	19	40												
6	–	9	6	16	38												
7	–	0	–	25	–	88	100										
8	–	0	–	0	–	–	94	–	86								
9	–	0	–	0	–	–	33	–	57								
10	–	0	–	0	–	–	0	–	7	(20	25	43	43	60	100)		
11	–	–	–	0	–	–	0	–	0	(0	7	33	46	55	73	100)	
12	–	–	–	–	–	–	0	–	0	(0	14	35	57	71	73	70	100)

	Days to budding																
2	46	34															
3	49	33	21														
4	–	33	28	22													
5	–	32	31	34	15												
6	–	27	37	19	18												
7	–	∾	–	23	–	17	16										
8	–	∾	–	∾	–	–	17	–	14								
9	–	∾	–	∾	–	–	13	–	15								
10	–	∾	–	∾	–	–	∾	–	13	(12	12	12	11	12	11)		
11	–	–	–	∾	–	–	∾	–	∾	(∾	12	12	12	11	11	11)	
12	–	–	–	–	–	–	∾	–	∾	(∾	12	12	12	11	11	11	11)

size in order to make flower formation possible i.e. possess a juvenile phase for flowering. These results furthermore suggest that the juvenile phase for flowering is confined to the basal parts of the plants, while the juvenile character disappears gradually with the height of the plant.

In a following experiment grown-up plants were vernalized for 6 weeks and decapitated at different moments after the PV. The decapitations were somewhat arbitrary as the plants consisted of rather perched rosettes. Decapitation consisted on the one hand of removal of the apex together with some primordial leaves and on the other hand of removal of that part of the apex which ensured removal of all parts formed after the PV. If any of the laterals obtained in this way came into flower, the plant was considered as flowering. The mean numbers of days to budding were counted from the date of decapitation.

Decapitation of that part of the apex which was formed after the PV never

resulted in flowering of the laterals. The remaining results are presented in table 26.

TABLE 26. The effect of decapitation at different moments after 6 weeks of PV

Decapitated after (days)	% flowering	Days to budding	Decapitated after (days)	% flowering	Days to budding
control	94	15	5	73	23
0	0	∞	8	65	20
2	13	18	14	81	20

When the decapitation takes place immediately after the PV, no flowering occurs. Furthermore, the percentages of flowering increase with decapitation at a later time. The mean days to budding after decapitation are higher than in the control. The results of this experiment demonstrate that the vernalized condition is achieved in the apex only, while only laterals formed after the PV attain a vernalized condition.

In conclusion, it is apparent that the results with SV as well as with PV are principally the same and that they demonstrate that only growing-points formed after the vernalization attain a vernalized condition.

These results are in accordance with those reported for wheat by ISHIHIRA (60) and for *Chrysanthemum* by SCHWABE (125).

5.2. THE ROLE OF LEAVES IN VERNALIZATION

An experiment has been carried out in which 7 week old plants were defoliated during or after 6 weeks of PV, followed by LD or SD. With defoliation some of the primordial leaves were kept in order to prevent the apex from dying. According to expectation, in SD the percentage of flowering of non-defoliated plants was considerably lower than in LD. However, none of the defoliated plants came into flower in LD neither in SD.

In a following experiment plants of about 4 months of age were defoliated during and/or after 8 weeks of PV, followed by LD. Each treatment consisted of 10 plants. All control plants flowered after about 11 days. None of the plants defoliated during PV or during *and* after PV came into flower. All plants defoliated only once, immediately after the PV, flowered after about 17 days. Of the plants defoliated twice, immediately and 1 week after PV, 3 out of 10 plants flowered after 20 days. These results demonstrate that in *Cheiranthus allionii* leaves are necessary for an effective PV, probably for the carbohydrate supply during PV. Defoliation after PV delays flowering and repeated defoliation even reduces the number of flowering plants. These results are probably due to a carbohydrate deficiency.

The above results with *Cheiranthus allionii* seem to be in contradiction to those mentioned in the literature for biennial *Hyoscyamus niger* (78), for endive (55), and for *Lunaria biennis* found by WELLENSIEK (oral communication), which demonstrated that leaves were not essential for vernalization. However, this

may be explained by the fact that *Hyoscyamus niger* possesses considerable food reserves in the roots, whereas endive requires a relatively short vernalization in comparison with plants of *Cheiranthus allionii*. Several reports on vernalization of excised embryos (39, 43, 47) or of dissected growing tips (59, 60, 68, 69) demonstrated that carbohydrate supply is necessary to obtain an effective vernalization. TASHIMA (129) demonstrated that the vernalizability of *Raphanus sativus* decreased as one or two cotyledons were removed, whereas KIMURA (64) showed that this plant could be vernalized without cotyledons, if sugars were applied during the vernalization. All these results demonstrate that leaves are not essential for the vernalization, but that they merely serve as a carbohydrate source, whereas their relative importance depends on other sources in the plant.

5.3. THE ROLE OF DIVIDING CELLS IN VERNALIZATION

As has been mentioned earlier (p. 38), the majority of the seeds of *Cheiranthus allionii* do not germinate during the vernalization, but remains in a swollen condition. With cytological methods no cell divisions have been observed during the course of the vernalization. The first mitoses were observed in the roottips 36 to 48 hours after the end of the vernalization, whereas cell divisions in the apex occurred at least another 24 hours later. With unvernalized seeds the earliest cell divisions were observed 72 hours after the beginning of the imbibition. Similar results have been reported by GRIF (48).

WELLENSIEK (139, 141) arrived at the concept that dividing cells are the prerequisite for vernalization. The above results seem to oppose this concept, as do other observations, which have shown that vernalization can take place at temperatures below freezing point, as is reported by HÄNSEL (50), JUNGES (61), GRIF (48), and SCHMALZ (123), while no cell divisions or no detectable growth were observed. However, as MAZIA (94, p. 98) states: 'Some of the most important events of mitosis actually take place during the so-called interphase'. With cytological methods only the actual division of a cell is observed and therefore they cannot be decisive for establishing whether cells are *not* in the process of mitosis.

5.4. TRANSLOCATION OF THE VERNALIZATION EFFECT BY MEANS OF CELL DIVISION

The observation that only growing-points derived from vernalized tissue attain a vernalized condition suggest that the direct vernalization effect in itself in *Cheiranthus allionii* is immobile and is translocated by cell divisions only. The translocation of the vernalization effect is thus entirely restricted to the meristematic cells derived from actually vernalized ones, whereas the formation of a transmissible flower stimulus owing to the vernalization is unlikely as far as *Cheiranthus allionii* is concerned. Equal ideas have been put forward by MARGADANT (88), WYCHERLEY (148) and CHOUARD (17).

SCHWABE (126) pointed out that the vernalization requirement in winter rye seems to arise very early in the formation of the seed i.e. possibly at meiosis or

fertilization and that therefore probably no subsequent transmission of the vernalization effect from the parent plant occurs. This implies that the vernalized condition of the parent plant disappears at least at meiosis, but probably earlier owing to devernalization, which has been demonstrated by the loss of the vernalized condition in laterals formed after SV in *Cheiranthus allionii*.

5.5. Translocation of the vernalization effect by means of grafting

Cleft-grafts were made between unvernalized and vernalized cold requiring plants or between unvernalized cold requiring and non-cold requiring plants, each of which were used as stock or scion respectively. Graft-combinations and their controls were grown under LD. Although these grafting-experiments were carried out repeatedly, no translocation of the vernalization effect could be established in *Cheiranthus allionii*.

Successful translocation of the vernalization effect has been reported in literature, of which the graft experiments with biennial *Hyoscyamus niger* by Melchers (96, 97) are best known. Two examples are mentioned by Wellensiek (140), whereas others are compiled by Lang (72) and Mathon and Stroun (90). Negative results with respect to the translocation have been reported as well (90, 124, 125). These results demonstrate that the final vernalization effect may be translocated in some cases, but, as Hillman (57, p. 82–83) pointed out, there has never been any clear demonstration of the translocation, by grafting or otherwise, of a stimulus resulting from vernalization alone rather than vernalization followed by LD; such a demonstration would be necessary to establish the existence of the so-called vernalin.

CHAPTER 6

GENETICAL BASIS OF THE VERNALIZATION REQUIREMENT

6.1. VARIABILITY OF THE VERNALIZATION REQUIREMENT

The variability of the vernalization requirement in *Cheiranthus allionii* is apparent from the observation that with increasing duration of the vernalization, the percentage of flowering increases, thus demonstrating that different plants reach their vernalized condition consecutively. Even an almost non-cold requiring strain (143) has been selected from the original commercial seed. However, this strain proved to be still heterogeneous as a part of the plants come into flower in LD as well as in SD, another part in LD only, whereas a small part of the plants do not flower without vernalization. A few plants of the non-cold requiring strain flower simultaneously in LD as well as in SD, while the flowering of the remaining plants is spread over a rather long time, which may be shortened by vernalization. These results together demonstrate that the cold requirement within *Cheiranthus allionii* varies from none and a quantitative cold requirement to a qualitative cold requirement. There is no doubt that different genetical constitutions are responsible for this variability. *Cheiranthus allionii* furthermore is hexaploid (23) and this may increase the great variability of the cold requirement within one species.

Examples of a more or less great variability in the cold requirement are found in literature, as compiled by NAPP-ZINN (109) for *Triticum*, *Hordeum*, *Secale*, *Arabidopsis*, *Lolium* and others, whereas at least annual strains are known for usually biennial plants such as *Hyoscyamus niger*, Brussels sprouts and *Beta*.

6.2. INHERITANCE OF THE VERNALIZATION REQUIREMENT

The hexaploid character of *Cheiranthus allionii* made it practically impossible to obtain decisive results on the mode of inheritance of the cold requirement. Another limitation was that no homogeneous cold requiring or non-cold requiring material was available. Nevertheless, a crossing was made between the earliest flowering plant of the non-cold requiring strain and a cold requiring plant of a clone. The F_1 grown in LD, consisted of 208 plants of which initially only 7 plants came into flower without vernalization. The remaining 201 plants, which remained vegetative for more than 3 months, were then treated with 100 ppm. GA_3 every other day for two weeks. Unexpectedly, this treatment resulted in a rapid flowering of another 175 plants. The still remaining plants had to be vernalized before flowering.

The earliest flowering plant of the F_1 as well as an early, middle and late flowering plant of GA_3-treated plants were crossed with cold requiring plants from the same clone as used for the original crossing. Three out of 100 plants, from the first crossing only, flowered, whereas no more plants could be brought to flower with GA_3.

A crossing was also made between an early flowering plant of the F_1 with a GA_3-treated plant and between two plants of the GA_3-treated ones. Cold requiring plants only were obtained. These results suggest that the cold requiring character in *Cheiranthus allionii* tends to be dominant over the non-cold requiring character.

CHAPTER 7

GENERAL DISCUSSION

The results presented in the preceding chapters will now be discussed in as far as they are concerned with the mechanism of vernalization in general.

7.1. The vernalized condition

Evidence has been presented that the effect of vernalization has a quantitative character and that its effect is accumulated until a maximum is reached. Decapitation treatments after the vernalization have clearly demonstrated that the vernalization product is immobile. We therefore speak of a 'vernalized condition', being the immediate result of vernalization. The vernalized condition was found to be never completely stable. A reversion of this condition remains possible throughout. A so-called stabilization by an increasing duration of PV or by some days at a neutral temperature after PV can be carried back to the fact that realization of flowering has started. A reversion i.e. devernalization can be obtained directly by high temperature or indirectly be means of SD or CD, darkness being the effective agent. Its effect can be carried back to a temperature effect as well (see p. 40).

Desiccation of vernalized seeds, even at high temperatures, did not cause devernalization which made it obvious that devernalization is possible in growing tissues only.

Decapitation experiments have shown that only growing-points subjected to vernalization and those derived from vernalized tissue attain a vernalized condition. This would suggest that the vernalization effect is achieved by growing-points only. However, as the vernalization effect can only be expressed by the growing-points and as its product is immobile in *Cheiranthus allionii*, the results cannot be conclusive on this point. Wellensiek (138, 139) demonstrated in *Lunaria biennis* that leaves and roots could be vernalized, since shoots regenerated after the cold treatment flowered.

Defoliation experiments demonstrated that the achievement of the vernalized condition in *Cheiranthus allionii* is highly dependent on the leaves. These results emphasized the necessity of a sufficient carbohydrate supply during the vernalization (p. 44).

In conclusion, it is apparent that the immediate product of vernalization in *Cheiranthus allionii* is rather a 'condition' than a 'substance', though it discloses the possibility that substances are involved on cellular level.

Gott, Gregory and Purvis (37, 115, 116), Lang and Melchers (78), van de Sande Bakhuijsen (121) and Napp-Zinn (104, 105) suppose that the immediate product of vernalization is a hormonal substance. Margadant (88), Wycherley (148) and Chouard (17) conclude that it is an immobile product.

7.2. Translocation of the vernalization effect

From the decapitation experiments it was concluded that a translocation of the vernalization effect occurs only by means of cell divisions and that only cells derived from actually vernalized ones attain a vernalized condition. With grafting-experiments no translocation of the vernalization effect could be established. Thus the existence of a hormonal flowering stimulus as an after-effect of the vernalization appears to be unlikely in *Cheiranthus allionii*.

As the vernalized condition is carried on by means of cell divisions and hence remains located at its places of action i.e. the growing-points, it becomes obvious that in fact there is no need for a hormonal vernalization product.

However, the successful graft experiments known in the literature point at least to a hormonal substance formed after the vernalization. Grafting-experiments so far only demonstrated the translocation of the effect of vernalization in combination with favourable day length, but not of the vernalization effect alone (p. 46). Thus the prevailing experimental evidence in literature rather supports than contradicts the existence of an immobile vernalized condition.

7.3. Vernalization in relation to photoperiodism

The results with *Cheiranthus allionii* have clearly demonstrated that the effectiveness of vernalization itself as well as the final expression of the vernalization effect i.e. flowering are largely determined by photoperiodical conditions. It has been shown that SD or CD before or during PV decreases its effectiveness, whereas SD or CD after SV as well as after PV may lead to a reversion of the vernalized condition. The same holds true for unfavourable light conditions.

However, no real photoperiodical behaviour with respect to flower induction could be established. The effect of different day lengths on the effectiveness of vernalization could be carried back to the disadvantageous effect of darkness. The influence of different light qualities could be more or less traced back to the effect of light intensity, low light intensity acting in the same way as darkness.

It has been pointed out (p. 40) that the effect of darkness presumably consists of a disadvantageous effect on the carbohydrate supply, being necessary for optimal vernalization. The so-called anti-vernalization caused by SD or CD may then be ascribed to a decreased vernalizability on account of an insufficient carbohydrate supply. Devernalization by means of SD or CD is probably largely due to the decreased mitotic activity, which may include longer durations of the mitotic cycles and thereby enables the relatively high temperature, usually prevailing after vernalization, to exert its devernalizing effect. Thus eventually the effect of darkness in turn can be carried back to the effect of temperature.

In conclusion, it becomes apparent that devernalization is always caused by unfavourable temperature conditions and that unfavourable photoperiodical conditions may only enhance their effect. These results emphasize the indirect and secondary action of photoperiodism with respect to vernalization in *Cheiranthus allionii*. If this is generally true, it may be the reason that among the various species of plants all possible combinations of different vernalization and

photoperiodic requirements occur, as appears from the different reaction types compiled by CHOUARD (17) and by SALISBURY (120).

LANG (71) pointed out that biennial *Hyoscyamus niger* after cold induction and after defoliation flowered in SD as well as in CD, while non-defoliated ones flowered in LD only. He concluded that light has probably no significance in this plant, that flower formation is primarily independent from photoperiodical conditions and that day-lengths effects rest on an inhibition of a secondary nature, which is bound to darkness and located in the leaves. His view is thus completely in accordance with that established for *Cheiranthus allionii*.

7.4. HYPOTHESES CONCERNING THE MECHANISM OF VERNALIZATION

LYSENKO (84, 85) based his well known theory of the stadial development of plants on his studies on vernalization. This concept appeared to be untenable at least with respect to vernalization, as it denied a possible reversion of the different 'phases', including the so-called 'vernalization phase'.

GOTT, GREGORY and PURVIS (37, 115, 116). LANG and MELCHERS (78) VAN DE SANDE BAKHUIJSEN (121) and NAPP-ZINN (104, 105) developed descriptive formulations of the facts observed with respect to vernalization studied in individual plants, namely Petkus winter rye, biennial *Hyoscyamus niger*, wheat and *Arabidopsis thaliana* respectively. All their concepts are based on the assumption that the immediate effect of vernalization consists of a substance which is transformed into the final flowering hormone by several labile or stable steps.

MARGADANT (88) concludes for *Lolium perenne* that the direct effect of vernalization is immobile and he assumes that as cell divisions proceed the vernalization product will be distributed throughout the protoplasm of the new tissues and will eventually be so dispersed that insufficient product will be available to be effective. He furthermore points out that as inflorescences are determinate structures, the immobile vernalization product is largely lost with them.

WYCHERLEY (148) concludes that the vernalization product is immobile in three perennial grass species, but he seems to assume that a transmissible flower stimulus is formed after the vernalization.

CHOUARD (17) emphasized that in general an autocatalytic transmission of the vernalization effect is entirely restricted to those meristematic cells derived from actually vernalized ones, but he discloses the possibility of a substance diffusing from cell to cell.

WELLENSIEK (141) states that the direct effect of low temperature in *Lunaria biennis* would be a 'vernalized condition' which only arises in dividing cells and which is only transmissible from cell to cell by mitosis, while cells in the vernalized condition can produce a floral stimulus which can be translocated to cells which themselves need not be vernalized.

The author confines himself to the concept that the immediate product of the vernalization is immobile and is only transmitted by cell division, basing himself mainly on the results obtained with *Cheiranthus allionii*. As stated earlier

(p. 50) the author sees no necessity for the formation of a transmissible flower stimulus formed after the vernalization, although he admits the possibility. If such a transmissible flower stimulus has to be assumed on the grounds of successful graft experiments, it still remains possible that this stimulus is of a secondary nature and its effect may possibly be compared with e.g. that of GA. It is known for example, that GA induces flowering in biennial *Hyoscyamus niger* (75, 80) without vernalization. Furthermore, it is known that vernalization increases the level of native gibberellins in plants in LD (9), whereas gibberellin-like substances have already been detected in annual *Hyoscyamus niger* (77). Thus the grafts between unvernalized and vernalized *Hyoscyamus niger* can possibly be explained as an induction of flowering in the unvernalized shoot by gibberellins produced in the vernalized shoot. It is known that the translocation of gibberellins offers no difficulties. Such an explanation is of course speculative, but it points out that the necessity of a specific flower hormone formed after vernalization is not proven yet.

Most of the above theories are hardly concerned with the actual location of the vernalization effect. They all more or less imply the necessity of meristematic cells present in the growing-points for the achievement of the vernalization effect.

WELLENSIEK (54, 131) arrived at the concept that dividing cells are a prerequisite for vernalization, no matter where they occur in the plant. Lately WELLENSIEK (oral communication) enlarged the actual locus for vernalization even further to cells which are preparing themselves for cell division. In connection with this concept it should be emphasized that cell divisions *after* vernalization prove to be of great importance as well and probably are even more important, as we have seen that the vernalized condition is restricted to vernalized cells themselves and to those derived from them by cell division.

Moreover, WELLENSIEK (141) presented evidence for a competition between vernalized and unvernalized cells. Thus the ultimate effect of vernalization will depend on the ratio of vernalized and unvernalized cells.

RESENDE (118) presented evidence that in plants, which require low temperature i.e. vernalization, the *open* state of heterochromatin is carried into the metaphase and anaphase during the low temperature period, whereas this persistence of genetic *openness* of heterochromatin induced by low temperature would be a consequence of the quantitative action of native chemical substances which are temperature controlled. Some of these substances would function as activators of the genes, which trigger the change from the vegetative to the generative state. RESENDE envisages a parallelism with the action of ecdyson (hormone of metamorphosis), which induces 'puffs' in *Chironomus*.

This curtails the localization of the vernalization effect to an indirect action of low temperature on the activation of genes. Although the above concept is not completely proven yet and will probably undergo modifications, it is attractive and in combination with the concept put forward by WELLENSIEK gives a good hold for further investigations.

SUMMARY[1])

1. The effect of vernalization in *Cheiranthus allionii* was studied in connection with external conditions, particularly temperature and day length.
2. *Cheiranthus allionii* is a qualitative cold requiring winter annual. However, an almost non-cold requiring strain could be selected.
3. *Factors interacting with the effect of vernalization*

3.1. SV as well as PV may lead to flower formation. However, the vernalizability varies in consecutive stages of the plant.

3.2. The temperature influences flower formation after vernalization, high temperatures being disadvantageous.

3.3. Fluorescent light during PV increases its effectiveness in comparison with incandescent light. This effect could be partly carried back to the effect of light intensity, whereas it interacted with the effect of day length. The influence of light after SV could be carried back to the effect of light intensity as well.

3.4. Increasing day lengths consisting of fluorescent light during suboptimal PV may decrease its effectiveness. Increasing day lengths consisting of incandescent light during PV increase its effectiveness. After SV flower formation takes place in LD only and not in SD.

3.5. A close relation exists between age, duration of vernalization and day length after vernalization. In LD after vernalization the sensitivity to vernalization varies with age, seeds and older plants being the most sensitive to vernalization, while plants of 12 to 16 days old possess a consistent minimum for vernalizability. An increasing duration of the vernalization causes both an increase of the percentage of flowering and a decrease of the number of days to budding, irrespective of the age on which the plants are vernalized. With grown-up plants the number of days to budding still further decreases with increasing duration of vernalization until the plants may even flower during the vernalization treatment itself. SD after SV and after PV of young plants has a disadvantageous effect on flower formation which can be partly overcome by increasing duration of the vernalization. Grown-up plants are day neutral after PV.

3.6. GA_3 may substitute for the vernalization requirement only partially. No direct influence of 5-FU on vernalization could be established.

4. *Factors involved in fixation and reversion of the vernalization*

4.1. The vernalization effect is achieved gradually and quantitatively, whereas its effect may be reversed.

4.2.1. High temperature (35°C) before PV may cause anti-vernalization. Short interruptions of PV with LD at 35°C were less disadvantageous than corresponding interruptions at 20°C, which has been ascribed to an advantageous effect of 35°C on subsequent revernalization besides the devernalizing effect on the preceding vernalization.

[1]) The figures in this summary refer to corresponding chapters and sections. Compare list of abbreviations on p. 5.

After SV devernalization by high temperature remains possible, irrespective of the duration of previous SV.

After PV usually only partial devernalization is possible, whereas the extent to which devernalization takes place depends on the duration of the previous PV, and on the duration and time of application of the high temperature treatment after the PV.

4.2.2. SD before PV may lead to anti-vernalization.

SD or CD during interruptions of PV result in devernalization of the preceding vernalization and anti-vernalization of the subsequent revernalization.

The disadvantageous effect of SD after SV, also found for CD, is particularly strong just before flower initiation takes place.

The disadvantageous effect of SD after PV increases with the duration of the SD, but decreases with age and with increasing duration of the PV.

The disadvantageous effect of SD or CD had to be ascribed to the effect of darkness, which in turn could be carried back to the effect of temperature.

4.2.3. Desiccation of vernalized seeds does not affect the vernalized condition, irrespective of temperature or duration of preservation.

5. *Localization and translocation of the vernalization effect*

5.1. Only growing-points subjected to vernalization and those derived from vernalized tissue attain a vernalized condition.

5.2. Leaves are necessary for vernalization in as far as they serve as a carbohydrate source.

5.3. The experiments with respect to the necessity of dividing cells for vernalization do not give conclusive results.

5.4. Translocation of the vernalization effect could not be established by means of grafting.

5.5. The immediate vernalization effect, the vernalized condition, is translocated by means of cell division.

6. *Genetical basis of the vernalization requirement*

6.1. *Cheiranthus allionii* possesses a great variability in the cold requirements of different plants, which has been largely ascribed to its hexaploid character.

6.2. The cold requiring character tends to be dominant over the non-cold requiring character.

7. The mechanism of vernalization has been discussed with respect to the results obtained with *Cheiranthus allionii* and the different theories prevailing in literature. It is concluded that the immediate product of vernalization is immobile and that the formation of a specific flower hormone after vernalization does not necessarily have to be accepted.

ACKNOWLEDGEMENT

This study was carried out at the Horticultural Laboratory of the Agricultural University, Wageningen, The Netherlands. The author is much indebted to Prof. Dr. Ir. S. J. WELLENSIEK for involving him in this fascinating subject and for his continuous guidance and interest during the investigations.

SAMENVATTING

VERNALISATIE IN *Cheiranthus Allionii* HORT.

1. De samenhang tussen het effect van vernalisatie en uitwendige omstandigheden, vooral temperatuur en daglengte, werden in *Cheiranthus allionii* bestudeerd.
2. *Cheiranthus allionii* is een kwalitatief koubehoeftige winterannuel. Er kon evenwel een bijna niet-koubehoeftige lijn worden geselecteerd.
3. *Factoren die het effect van vernalisatie beïnvloeden.*

3.1. Zowel zaad- als plantvernalisatie is mogelijk. De vernaliseerbaarheid is echter afhankelijk van het stadium van de plant.

3.2. De temperatuur beïnvloedt de bloemknopvorming na de vernalisatie, waarbij hoge temperaturen een nadelige invloed uitoefenen.

3.3. TL-licht gedurende plantvernalisatie verhoogt het effect van de vernalisatie in vergelijking met gloeilicht. Dit effect kon gedeeltelijk teruggevoerd worden tot het effect van lichtintensiteit, terwijl het nauw samenhangt met de invloed van de daglengte. De invloed van licht na zaadvernalisatie kon eveneens worden teruggevoerd tot het effect van lichtintensiteit.

3.4. Toenemende daglengten, bestaande uit TL-licht gedurende suboptimale plantvernalisatie, kunnen het effect van de vernalisatie verminderen. Toenemende daglengten, bestaande uit gloeilicht gedurende plantvernalisatie, verhogen het effect van de vernalisatie. Na zaadvernalisatie treedt alleen bloei op in lange dag.

3.5. Er bestaat een nauw verband tussen leeftijd, vernalisatieduur en daglengte na de vernalisatie. In lange dag na de vernalisatie varieert de gevoeligheid voor vernalisatie met de leeftijd. Zaden en oudere planten zijn het gevoeligst voor vernalisatie, terwijl planten van 12 tot 16 dagen oud een duidelijk minimum voor vernaliseerbaarheid bezitten. Een toenemende vernalisatieduur veroorzaakt een toename van het bloeipercentage en een gelijktijdige afname van het aantal dagen tot bloemknopvorming, ongeacht de leeftijd waarop wordt gevernaliseerd. Bij volwassen planten neemt het aantal dagen tot bloemknopvorming verder af onder een nog steeds toenemende vernalisatieduur, totdat de planten zelfs gedurende de koudebehandeling zelf in bloei geraken.

Korte dag na zaadvernalisatie en na plantvernalisatie van jonge planten heeft een nadelige invloed op de bloei, welke gedeeltelijk door een langere vernalisatieduur teniet gedaan kan worden. Volwassen planten zijn dagneutraal na de vernalisatie.

3.6. GA_3 kan de koubehoefte slechts gedeeltelijk vervangen. 5-FU heeft geen directe invloed op de vernalisatie.

4. *Factoren met betrekking tot fixatie en devernalisatie van de vernalisatie.*

4.1. Het vernalisatie-effect wordt geleidelijk en kwantitatief verkregen, terwijl dit effect teniet gedaan kan worden.

4.2.1. Hoge temperatuur (35°C) vóór plantvernalisatie kan anti-vernalisatie veroorzaken.

Korte onderbrekingen van plantvernalisatie met lange dag bij 35°C waren

minder nadelig dan overeenkomstige onderbrekingen bij 20°C, hetgeen toegeschreven is aan een gunstige invloed van 35°C op de revernalisatie naast een devernaliserende invloed op voorafgegane vernalisatie. Na zaadvernalisatie blijft devernalisatie door middel van hoge temperatuur steeds mogelijk, ongeacht de vernalisatieduur.

Na plantvernalisatie is gewoonlijk slechts gedeeltelijke devernalisatie mogelijk. De mate van devernalisatie hangt af van de vernalisatieduur, en van duur en tijdstip van toediening van de hoge temperatuurbehandeling na de vernalisatie.

4.2.2. Korte dag vóór plantvernalisatie kan tot anti-vernalisatie leiden.

Korte dag of continu duisternis gedurende onderbrekingen van plantvernalisatie leidt tot devernalisatie van voorafgegane vernalisatie en tot antivernalisatie met betrekking tot revernalisatie.

Korte dag of continu duisternis na zaadvernalisatie is het meest nadelig juist voordat de bloemknopvorming plaats vindt.

De nadelige invloed van korte dag na plantvernalisatie neemt toe met de duur van de korte dag, doch neemt af met een toenemende leeftijd van de planten en een toenemende vernalisatieduur.

De nadelige invloed van zowel korte dag als continu duisternis moest worden toegeschreven aan de nadelige invloed van duisternis, terwijl de invloed van duisternis op zijn beurt teruggebracht kon worden tot de invloed van temperatuur.

4.2.3. Drogen van gevernaliseerde zaden heeft geen invloed op de vernalisatietoestand, ongeacht de temperatuur of de duur van bewaring.

5. *Localisatie en translocatie van het vernalisatie-effect.*

5.1. Alleen gevernaliseerde groeipunten en die, welke afkomstig zijn van gevernaliseerd weefsel, bezitten een vernalisatietoestand.

5.2. Bladeren zijn noodzakelijk voor de vernalisatie in zover zij dienst doen als een bron voor koolhydraten.

5.3. De experimenten met betrekking tot de noodzakelijkheid van delende cellen voor vernalisatie geven geen beslissende resultaten.

5.4. Transport van het vernalisatie-effect kon door middel van enten niet worden aangetoond.

5.5. Het onmiddellijke effect van de vernalisatie, de vernalisatietoestand, wordt getransporteerd door middel van celdelingen.

6. *Genetische basis van de koubehoefte.*

6.1. *Cheiranthus allionii* bezit een grote variatie in de koubehoefte van de verschillende planten, welke grotendeels toegeschreven moet worden aan het hexaploïde karakter.

6.2. Het koubehoeftige karakter vertoont de neiging om te domineren over het niet-koubehoeftige karakter.

7. Het mechanisme van de vernalisatie is besproken met betrekking tot de resultaten verkregen met *Cheiranthus allionii* en de in de literatuur geponeerde theorieën. Geconcludeerd werd dat het onmiddellijke product van vernalisatie immobiel is en dat de aanname van de vorming van een specifiek bloeihormoon door vernalisatie niet noodzakelijk is.

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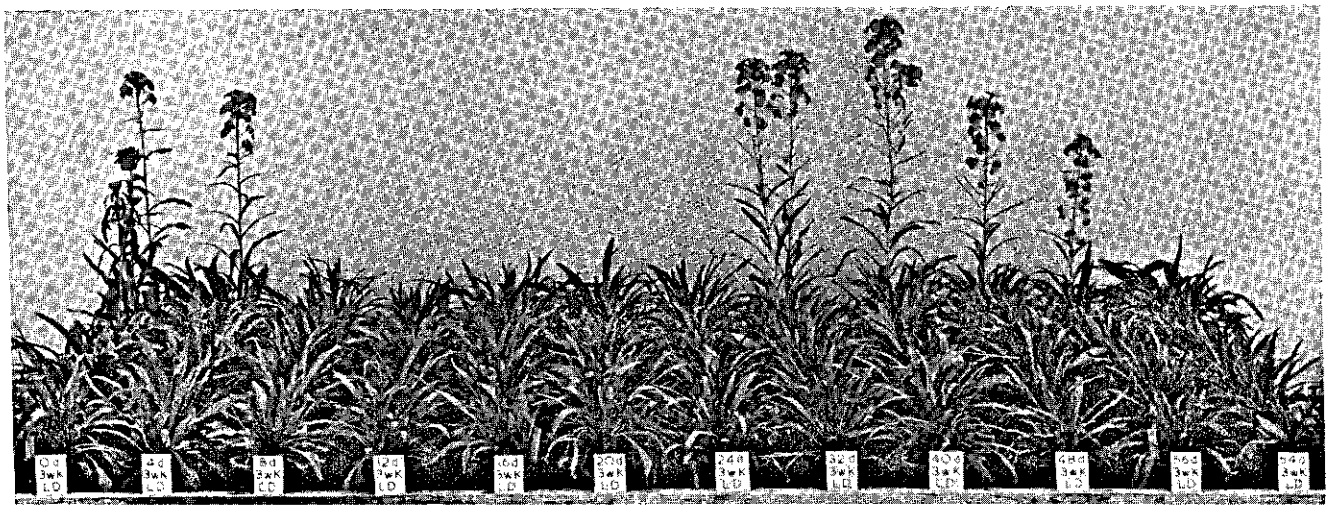

PHOTO 1. The relation between age and duration of vernalization, in LD after vernalization. From left to right plants of 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56 or 64 days, vernalized for 3 weeks. Photo taken 65 days after the vernalization.

PHOTO 2. The relation between age and duration of vernalization, in LD after vernalization. From left to right plants of 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56 or 64 days, vernalized for 4 weeks. Photo taken 65 days after the vernalization.

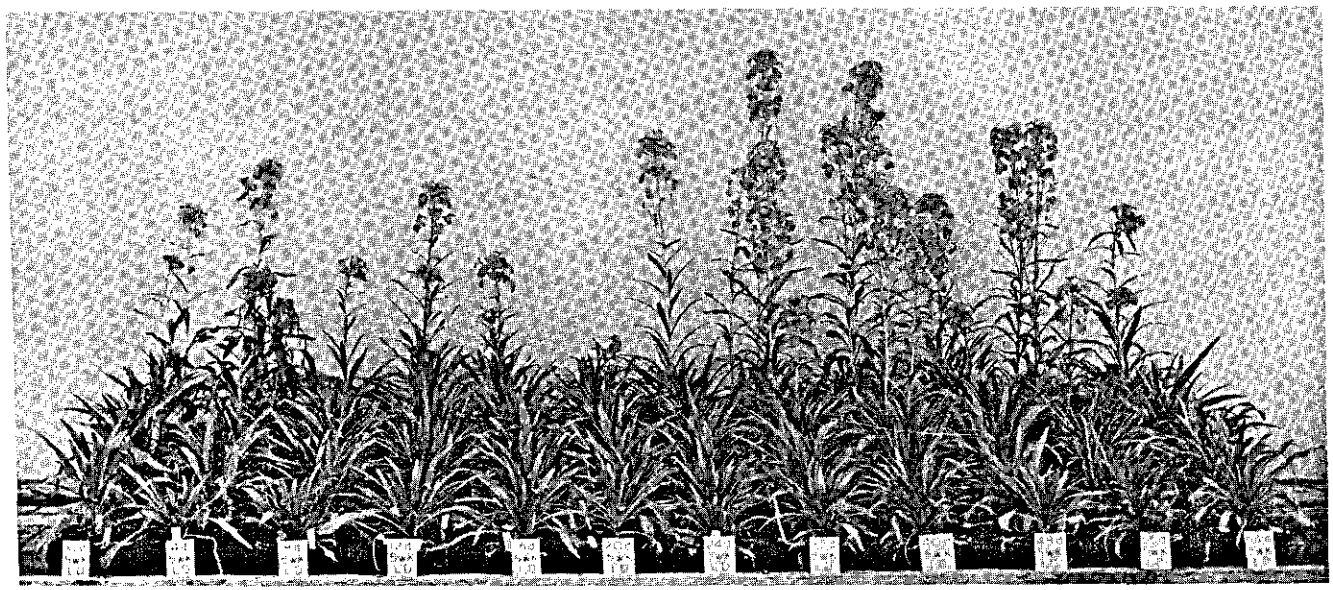

PHOTO 3. The relation between age and duration of vernalization, in LD after vernalization. From left to right plants of 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 56 or 64 days, vernalized for 5 weeks. Photo taken 65 days after the vernalization.

PHOTO 4. The relation between age and duration of vernalization, in LD after vernalization. From left to right plants of 0, 4, 8, 12, 16, 20, 24 or 28 days, vernalized for 10 weeks. Photo taken 70 days after the vernalization.

PHOTO 5. From left to right: control, plants decapitated 2, 6 and 10 weeks after the end of SV for 6 weeks, followed by LD. Photo taken 87 days after the vernalization.