Influence of external factors on growth and development of sugar-beet (*Beta vulgaris* L.).

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Abstract

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Several trials on the quantitative influence of photophase, chilling (vernalization) and high temperature (devernalization) on bolting of sugar-beet were analysed on the basis of a simple physiological model, in which bolting is considered as the final event of dynamic, momentary and quantitative processes in the plant. Trials in the field and in growth chambers examined factors in chilling and in light response. The inhibitory effect on bolting of high temperatures and the role of photophase in this process was investigated for several periods after vernalization.

Growth and bolting seem to be correlated, as plants with just visible bolting were usually heavier.
A possible relation between bolting resistance and vigour was investigated. Also the influence of photophase and cold treatment on growth was measured in a trial.

Some ways are shown of using a climatic factor like temperature to predict bolting in the field. Finally some recommendations for sugar-beet breeders are drawn up.

Free descriptors: vernalization, photoperiod, bolting, daylength, temperature, chilling, model, flowering, generative, devernalization, regression, optimization, breeding.

Contents

Abbreviations and symbols

*	$P<0.1$	
**	$P<0.05$	
***	$P<0.01$	
****	$P<0.001$	
ANOVA	Analyses of variance	
d.05	Studentized range, Tukey, at the 5 % level	
F	Hypothetical flower hormone	
LA	Leaf area per plant	(cm^2)
LAR	Leaf area ratio	($cm^2\ g^{-1}$)
NAR	Net assimilation rate	($g\ cm^{-2}\ d^{-1}$)
r	Coefficient of correlation	
r^2	Coefficient of determination	
RER	Relative expansion rate	(d^{-1})
RGR	Relative growth rate	(d^{-1})
SLA	Specific leaf area	($cm^2\ g^{-1}$)
W	Dry weight per plant	(g)
V	Hypothetical substance thought to accumulate during chilling	

1 Introduction

1.1 BOLTING IN SUGAR-BEET CROPS

Usually sugar beet (*Beta vulgaris L.*) behaves as a biennial. In the first year of growth, the plant produces a rosette of leaves and a root. After overwintering, in the second year of growth, dry matter distribution becomes totally different. In May or June, the stem appears and then the plant flowers. In most years all overwintered plants may flower, although in certain years some plants do not. Also in some years, some plants run to seed in their first season, the beet production year, and do not behave as a biennial. These plants are called bolters. Bolters appear from the beginning of July until the harvest of the crop in October. In general, plants whose stem is visible in the Netherlands before 1 August are arbitrarily called early bolters and after that date late bolters.

For several reasons bolters are not wanted in a root crop. Especially early bolters, which do not have time to develop a normal root, reduce yield. According to Longden et al. (1975), yield is reduced by about 1 % for every 4 % of plants showing signs of bolting. There is also an effect on sugar content of roots; over a 0-50 % range in the frequency of bolters, a 1 % increase causes a 0.05 % decline in sugar content (Longden et al., 1975). The values vary according to whether bolters are removed in the field and whether the remaining plants can compensate for the gaps. Other disadvantages are the woody texture of the root, especially of early bolters, so that farmers have difficulty in topping their crop properly and sugar factories have to sharpen their knives more often. Finally bolters give rise to self-sown beet in following crops, especially if early bolters are not removed and are allowed to shed viable seeds (Van Steyvoort & Van Stallen, 1973). Whether bolters appear in the sugar-beet crop depend on the following factors:

- agricultural practice
- choice of the cultivar
- environmental conditions (temperature, daylength)

1.2 INFLUENCE OF AGRICULTURAL PRACTICE

The farmer can alter the risk of bolting by shifting the sowing date. However, extension of the growing season by aiming at an early closing of the canopy has proved more advantageous for yield of sugar than avoidance

of bolting. So in recent years, there has been a shift towards earlier sowing dates, encouraged also by the improved quality of seed from more southerly areas.

Some other influences on bolting have been reported. Nitrogen fertilizer and lower plant density seem to promote bolting. It seems as though all factors that enhance growth promote bolting. As there are several indications to a relationship between bolting and rapid growth, growers are unlikely to curb bolting by changing cultivation methods: the measures against bolting could reduce yield. So to prevent excessive bolting in sugar-beet, cultivars resistant to bolting must be developed.

1.3 BREEDING OF CULTIVARS RESISTANT TO BOLTING

Although the environment plays a major role in the bolting behaviour of sugar-beet, there are large differences between cultivars. The inheritance of the bolting resistance is not fully understood. Dominant, recessive and polygenic control have been reported in literature.
Although great progress has been made in the past 20 years in developing cultivars resistant to bolting, bolting has not disappeared. In certain years, it can still reduce yield.

Breeders, however, have to solve several problems before a bolting-resistant cultivar can be obtained. Because sugar-beet cross-pollinates, the plants are rather varied in characteristics, including bolting. A self-pollinating crop would show an all or nothing response, which makes selection easier.

Selection for bolting resistance is nowadays by sowing early in the season (February, March). When bolters are discarded, the remaining plants will, in general, have less tendency to bolt. Sowing early about 500 km north of the beet-growing area turned out to be even more successful.

The seed crop is at present grown in more southerly countries like France and Italy, where climate during ripening is better for yield and quality of seed. An other advantage of that area for seed production is that ripening seed on the plant does not vernalize, whereas in N.W. Europe, bolting behaviour of the next generation is affected by conditions during seed ripening (O'Connor, 1970; Bosemark, 1970; Bornscheuer, 1972; Lexander, 1969). When sugar-beet is grown for seed, breeders need to be certain of flowering in all plants, otherwise there will be a shift towards more bolting in the progeny. Here lies also one of the limits for the breeders in creating a cultivar resistant to bolting. A cultivar extremely resistant to bolting would bring problems in how to obtain seed. Moving seed production to southern countries only aggravates this problem: duration of vernalizing temperature will be shorter and also daylength will be shorter than in northern Europe.

Over a number of years, another complication has emerged of seed production in the south of Europe. The seed stock can be contaminated by cross-

pollination with annual wild beets like *Beta maritima* and *B. patellaris*, because the tetraploid pollinators used nowadays in modern breeding produce less pollen and reach their daily pollen peak later than diploid wild beet plants (Scott & Longden, 1970). However, breeders can test for contamination with annual beets by growing seed stocks in long days above 20 °C.

This defect of hygiene during seed multiplication is outside the scope of this report.

1.4 SCOPE OF THE PRESENT STUDY

Several research workers have studied the relationship of environmental factors to bolting, including the extensive research by Chroboczek (1934) on beetroot (red garden beet) and studies by Curth (1955; 1960; 1962; 1963). Both workers, however, worked without growing rooms, which are essential to quantitative measurements of influences of temperature and lightphase (day-length).

Chapter 2 describes, based on data from literature, the influence of light and temperature on bolting and Chapter 5 presents trials on this matter with four genotypes of different susceptibility to bolting. Such information should help breeders in developing more specific selection methods to reduce susceptibility to bolting, and to improve adaptation to local conditions. Practical implications will probably also be found in this chapter, as breeders are interested in making their crosses in greenhouses to shorten the growth cycle.

Chapter 6 analyses a relation between growth and appearance of bolters. Chapter 7 describes techniques to relate a climatic factor like temperature to proportion of bolters.

In recent years, bolters have not been such a severe problem in the Netherlands. In surrounding countries, where beet can be sown very early in the year on sandy soils, crops have bolted severely, forcing farmers to abandon some fields for beet growing. If autumn sowing of sugar-beet should ever become possible, extremely resistant cultivars will have to be bred, utilizing quantitative information about the bolting process.

2 Factors influencing bolting in sugar-beet

2.1 INTRODUCTION

Many extensive reviews of flowering physiology are available (Chouard, 1960; Lang, 1965; Napp Zinn, 1961, 1973; Naylor, 1961; Purvis, 1961; Zeevaart, 1976). So only data will be reviewed here that is comparable with the situation in beet.

Sugar-beet requires cold for flowering: a long interval of low temperature for vernalization. Vernalization has two meanings: first, the physiological process in the plant; secondly, a treatment (in a cold room or in the field) to hasten development. Vernalization induces to flower. The term 'ripeness to flower' is used (Napp Zinn, 1973) because no external modification can be seen at the end of the chilling period and the plant needs another stimulus (daylength) before the apex starts to differentiate. Sugar-beet, being a long-day plant, therefore needs chilling first, then a time with a relatively long photoperiod at somewhat higher temperature (in the following the words light phase or photophase will be used for daylength and photoperiod).

Not only low temperature but also moderate or high temperature can influence bolting. A relatively short time at high temperature can nullify or moderate the effect of previous vernalizing temperatures. This phenomenon is called devernalization.

In the normal 2-year cycle of sugar-beet, all prerequisites for flowering are fulfilled in the second year. By overwintering, the plants have undergone a long period of vernalizing temperatures and the plants all start bolting when daylength gradually increases in May or June.

In the first season of growth, beets are fairly well protected against undesired bolting. With a normal sowing date, long vernalization does not usually occur. Moreover some authors report a juvenile phase, in which sugar-beet in the germination or early-leaf stages is not very sensitive to low temperature (Chroboczek, 1934).

The influence of environmental conditions will be considered in more detail in the following sections.

2.2 TEMPERATURE

Temperature influences many processes in the plant. Among the least understood is vernalization which induces the plant to flower. Although widely

used since Lyssenko introduced vernalization for germinating wheat seeds, the mechanism of the paradoxal forcing effect of low temperature is unknown.

Chroboczek (1934) demonstrated that only part of the plant needs to be exposed to low temperature. Winding a rubber tubing around the crown of beetroots just below the petioles and running cold water through the coils, made all plants flower, whereas control plants did not flower at all. Cooling the lower part of the root caused only 10 % of the plants to flower. Also for other rosette plants, chilling of only the apex was sufficient to obtain flowering (Curtis & Chang, 1930). However, the influence of vernalizing temperatures is not restricted to apices alone, since Wellensiek (1964a) demonstrated the possibility of vernalizing leaf cuttings of *Lunaria biennis*. The earlier concept that dividing cells were a prerequisite for vernalization was almost abandoned because, in some plants, vernalization took place under conditions that practically excluded cell division. Instead it was thought possible that also cells preparing themselves for division could perceive the vernalizing action of low temperature. Likewise treatment of root segments of *Cichorium intybus*, which are known for rapid regeneration, resulted in flowering, although it was less effective than seed or plant vernalization (Wellensiek, 1964b).

As mitotic cells seem to play an important role in the vernalization process, some authors prefer to speak of a vernalized condition of the plant, rather than to assume a diffusible substance accumulating during cold treatment. Grafting trials to transfer the vernalized condition of the donor to the receptor almost always failed. If they were successful, photoperiod played an important role, so it can be argued that not the immediate product of vernalization is transmitted, but the flower-inducing end-product. Barendse (1964) concluded from his trials with *Cheiranthus allionii* that the direct vernalization effect was immobile and was translocated by cell division only.

One way of studying vernalization is to measure organic substances in vernalized and unvernalized plants. However one cannot be sure whether a change in content of a substance is due to vernalization (the flower-inducing process), to 'cold metabolism' or to other differences between vernalized and unvernalized plants. Adequate tests must include also forms not requiring cold. Carbohydrates, especially sucrose, were supposed to play an important role in vernalization, since Gregory & Purvis (1938a,b) demonstrated that vernalization of caryopses of Petkus winter rye failed in the absence of oxygen and sufficient sugars.

Nowadays many authors try to relate the vernalization process with DNA and RNA synthesis. According to Besnard-Wibaut (1977) induction at low temperature specifically acts on the axial cells of the shoot apex of *Arabidopsis thaliana*, where the DNA synthetic capacity was increased. Also Shiomi & Hori (1973) observed an increase in DNA synthesis in vernalized barley seedlings. In wheat embryos, Tateyama et al. (1978) found an increased content

of RNA and DNA during cold treatment compared to germination at a normal temperature.

Numerous reports mention an increased or decreased content of several growth substances in vernalized plants, especially the gibberellins are often linked with the vernalization process. In sugar-beet, Margara (1967) showed that application of GA_3 to unvernalized plants could lead to stem elongation. In fully vernalized plants, GA advanced the date of flowering and increased the number of flower buds. After vernalization periods too short to obtain total flowering, GA application was effective to complete the vernalization process (Margara, 1960; Gaskill, 1957). Although gibberellin could be involved in the vernalization process, Margara (1960) did not detect differences in gibberellin content between vernalized and unvernalized plants by several biological tests. Suge (1970) measured a doubled content of gibberellins in response to vernalization of radish seeds or seedlings. This may, however, have been an indirect effect of vernalization. Accumulation of gibberellins may also occur as a consequence of vernalization, when precursors of the gibberellins are produced during the vernalization treatment, or it might even be a direct consequence of the flower initiation process itself. Moreover, gibberellins cannot be considered as real flowering hormones, their main effect being stem elongation, even in unvernalized plants under non-inductive conditions. In *Hyoscyamus niger*, gibberellin participates in the mechanism of flowering only by its indirect effect on stem elongation and does not act directly on flower formation itself, according to Mugnier (1977). In sugar-beet, content of gibberellin in apices increased sharply just before or after visible bolting (Lenton et al., 1975), suggesting that gibberellins are somehow involved in the expression of the vernalization stimulus.

A different approach to vernalization is to reveal more of the kinetics of the process by studying different conditions before, during and after cold treatment. The effectiveness of a certain environmental condition to induce 'ripeness to flower' can be measured as the number of leaves produced before flowering, as the proportion of plants flowering or bolting or as the time from chilling to first visible symptoms of flowering. In sugar-beet, the proportion of plants flowering is often used but can depend also on temperature and light phase (photoperiod) after vernalization. If there is an interaction between effectiveness of cold treatment and climatic conditions after vernalization, the proportion of plants flowering will not be a good measure of the effectiveness of the applied cold treatment.

The effect of cold treatment can depend on the presence of a juvenile stage, temperature, duration of cold treatment, and on temperature after vernalization.

A juvenile stage, in which sugar-beet is less responsive to low temperature was reported by Chroboczek (1934), who found that the younger the plants

at the beginning of cold treatment, the lower the proportion of seed stalks. He also suggested that low temperature might have no less effect at that stage but that subsequent devernalizing temperatures were more effective in young plants. Margara (1960) found no flowering when plants were vernalized at the cotyledonary stage, not even under subsequent continuous illumination. However in his trial temperatures after vernalization were rather high (18-22 °C), so devernalization may have played a role. Gaskill (1963) and Curth (1955), the latter with steckling beet, observed that age of plants at the time of cold treatment was positively correlated with the proportion of bolters.

In beetroot, Junges (1959), also observed that older plants could be vernalized more readily. In his trial, there could, however, have been some induction of older plants before vernalization, as temperature during raising was rather low, 10-15 °C. The same holds for Voss (1936) for fodder beet. Wood & Scott (1975) sowed sugar-beet in autumn but encountered excessive bolting in the following spring, except for plots sown late in autumn, perhaps because of a juvenile stage.

By contrast, Heide (1973) showed that beetroots were responsive to low temperature at any age, though the sensitivity to chilling increased somewhat with age. Kloen (1952) and Wiebosch (1965) indeed found that even seed of sugar-beet could be vernalized. Even in immature seed on the mother plant, vernalization seems possible (Lexander, 1969; O'Connor, 1970; Scott & Jaggard, 1978; Bosemark, 1970).

The literature does not agree on optimum temperature of vernalization, of which several have been reported. Curth (1960) states 3 °C for steckling beets, Fife & Price (1953) 6 °C, Bachmann et al. (1963) 8 °C, Stout (1946) 6-9 °C, Curth (1962) normally 4 °C and with a 'photothermic' treatment (simultaneous low temperature and long light phase) 8 °C and Lasa & Silvan (1976) with 'photothermal' treatment also 8 °C.

The duration of vernalization influences proportion of plants flowering. Plants longer exposed to low temperature, more bolt and the first bolters appear sooner after cold treatment (Curth, 1955; Heide, 1973; Wellensiek & Verkerk, 1950). The minimum duration of the cold period depends on cultivar, since unsusceptible cultivars require far stronger induction than susceptible ones.

Chroboczek (1934) was probably the first research worker who did systematic research with beetroot on the influence of temperature *after* vernalization. High temperature (21-27 °C) tended to reverse the effects of previous cold treatment. But this reversal could be counteracted by extending cold treatment and also by extending the light phase up to continuous illumination. Heide (1973) also mentions interactions between daylength and deverna-

lizing temperatures in beetroot: shorter light phase allowed reversal at lower temperature (18 °C or more) than did a longer light phase (24 °C or more). Apart from the influence of high temperature as such, duration of the warm period is decisive: Curth (1960) found complete reversal with 25 days at 30 °C. Curth also found that the region of the growing point is the receptor of temperature, as for vernalization.

In crops like wheat or rye, devernalization occurs especially when plants are exposed to high temperature immediately after vernalization (Purvis & Gregory, 1952). After a long cold treatment or when vernalization is followed by a rather short period of intermediate temperature (12-15 °C), the process of vernalization was assumed to be fixed and high temperature could not exert a devernalizing influence anymore. This fixation is often called stabilization. In *Arabidopsis thaliana*, however, devernalization can occur irrespective of the length of the vernalization period (Napp Zinn, 1957). But Napp Zinn showed that also in *A. thaliana* the devernalizing action of high temperature strongly depends on the time elapsed after the end of the cold treatment, immediately after vernalization being the most effective.

For beet, there is little evidence whether a stage or condition is ever reached where vernalization is fixed or 'stabilized': in certain other plants, certain treatments result in fixation and reversal is no longer possible.

2.3 LIGHT PHASE

Photoperiodism is a response to the phase and period of light and darkness. Incident radiant energy above a certain threshold is of secondary importance. When sugar-beet is vernalized, it behaves as a long-day plant: stem elongation and subsequent flowering is advanced and accelerated in proportion to light phase. Response depends largely on duration of previous cold treatment. Usually there is no response without cold treatment.

According to Schneider (1960), the apex of sugar-beet starts to differentiate only after cold treatment. During cold treatment lasting 84 days, he observed no changes in the structure of the growing point. Obviously, after chilling, a certain time with suitable light phase and good growing conditions were required for actual stem and flower formation.

Curth (1960) measured the influence of daylength. In sugar-beet chilled for a given time, continuous illumination was slightly superior to a light phase of 21 h. With shorter light phase, response decreased sharply until with 8 h stems did not elongate and no plants flowered. Also after the stem started elongating, a long photophase was still required. Margara (1960) reported a standstill of stem elongation and a delay in flowering after transfer from long to short photophase. Usually the plants start to form a rosette of leaves again and even the root starts to swell again at the top. Margara observed that transfer back to long photophase after protracted shor

photophase no longer provoked flowering. This phenomenon is often called 'SD devernalization' but I restrict the words vernalization and reversal (devernalization) to temperature-dependent processes.

Research workers usually study light phase with incandescent tungsten lamps to extend natural daylight. For sugar-beet Curth (1960) found low luminous flux densities (areic luminous flux) to be sufficient. Above 100-200 lx, an increase in flux from tungsten lamps did not advance bolting. During that extension of daylight, such a flux allows negligible photosynthesis and avoids differences in growth rate.

Curth (1960) also studied the influence of spectral distribution of several light sources used for daylength extension. Most effective for photoperiodical responses were sources with a peak of luminous flux density in the blue or orange-red regions. Sources with a peak in the green region were less effective.

Lane et al. (1965) compared red light (wavelength 600-700 nm) with far-red light (700-770 nm) for daylength extension. In an annual sugar-beet strain, far-red extension was more effective to obtain flowering plants than red light. In other plants, however, like henbane and petunia, a mixture of red and far-red was superior. Of the luminous flux from incandescent lamps in the waveband 600-770 nm, half is red and half far-red.

In floral initiation, plants perceive photoperiod by the leaves (Withrow et al., 1943; Naylor, 1961; Lang, 1952). The pigment phytochrome is likely to be involved as the receptor of the daylength stimulus, considering the red and far-red action spectra.

For phytochrome, there are two types of reactions: induction-reversion reactions and 'high-irradiance' reactions. Involvement of phytochrome in a ligth mediated response requires that an induction effect by a pulse of red light can be fully reversed by a subsequent pulse of far-red light. With an exposure time of less than 5 min, the action spectrum for the induction of a light-mediated response shows a peak at wavelength 660 nm and reflects the absorption spectrum of the red-absorbing form of phytochrome (P_r).

The high-irradiance reaction occurs with prolonged irradiation (e.g. daylength extension). Its action spectrum is completely different from the induction spectrum: always a peak in the far-red region of the spectrum (wavelength 700-730 nm) and several peaks in the blue region of the spectrum (Schäfer, 1976). Curth (1960) and Lane et al. (1965) reported peaks of the action spectra in the blue and red to far-red regions for beet, suggestive of the high-irradiance reaction.

2.4 INTERACTIONS BETWEEN LIGHT PHASE AND TEMPERATURE

The most common interaction between the effects of daylength and temperature is that unvernalized plants fail to respond to light phase. The 'cri-

tical photophase' seems to shift with the degree of vernalization. For unvernalized plants, the threshold is apparently so high that the stem does not elongate with long photophases. In other words, the vernalized condition seems to lower the critical photoperiod: sufficiently vernalized plants do not need an extremely long photophase to bolt. Fife & Price (1953) showed complete substitution of vernalization and light phase for sugar-beet. When steckling beets were vernalized at 4 °C for extremely long (100-300 d), plants bolted and flowered at 21 °C, even in complete darkness.

For beetroot, Heide (1973) reported bolting and flowering in continuous light, without any cold treatment at temperatures usually considered as 'vernalizing', but temperature in his trial was not above 18 °C.

2.5 GROWTH AND BOLTING

Several research workers have pointed out a connexion between growth and bolting in sugar-beet. Usually a vigorous early growth leads to more bolters; inhibiting circumstances on the other hand reduce bolting. According to Röstel (1968) there is a positive correlation between the proportion of bolters and soil fertility. Röstel also reported a positive influence of irrigation on bolting. He stated that bolting resistance was more needed when growth-stimulating cultural practices were successful. Many reports deal with the influence of nutrient supply, especially nitrogen, on the proportion of bolters (Mann, 1951; Gorodnii & Sereda, 1975; Hoekstra, 1960; Lüdecke, 1938; Schneider, 1960; Lysgaard, 1978).

Also an influence has been reported of plant density, which affects individual plant growth, (Jorritsma, 1978), lower plant densities causing more plants to run to seed. Warne (1949) observed in an experiment that border row plants had a greater tendency to bolt. Dowker & Jackson (1975) observed the same phenomena in carrots.

Good growing conditions clearly promote the tendency to run to seed for these species.

3 Models for bolting

Several models have been elaborated for the flower-promoting action of low temperature and of subsequent light phase.

3.1 MODELS IN LITERATURE BASED ON (HYPOTHETICAL) PLANT HORMONES

Lang & Melchers (1947) and Purvis & Gregory (1952) conceived of two reactions within the plant: synthesis of a flower-promoting substance B and destruction of this substance (Figure 1).

Reaction 1 was thought to proceed even at low temperatures, but the rate of Reaction 2 is low at low temperatures and increases with temperature much more rapidly than for Reaction 1. At low temperature, substance B would accumulate and at higher temperature destroyed (converted to C, or perhaps back to A). At moderate temperature, B was thought to be converted by Reaction 3 to D, which was not destroyed by high temperature. So after a few days at these moderate temperature reversal was no longer possible. Reaction 3 might be governed by light phase, whereas Reaction 1 and 2 might be independent of daylength. Substance B might be identical with the hypothetical substance vernalin, considered to build up during vernalization (Melchers, 1939), and D might be the final flowering substance florigen. In this model 'vernalin' is a precursor of florigen.

Another model to explain experimental results was that of Napp Zinn (1957). He assumed the more complicated system of several 'labile' and 'stable' stages in vernalization, some of which, however, could be bypassed.

As Chouard (1960) states: 'these formulations are handy to memorize and they stimulate further investigations on hypothetical substances, but they provide no more clarification than the authors' description of their own results. Further they may require adjustment for each new discovery and they also change for each plant type that behaves in a particular way and does not fit the particular representation'.

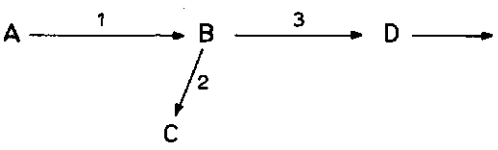

Fig. 1. Reactions in induction of flowering (Lang & Melchers, 1947). Reaction 1, synthesis of a flower-promoting substance B; Reaction 2, destruction of the substance.

A major assumption of the models is build-up of a substance, usually called vernalin, during vernalization. This substance could be a precursor of florigen or could, together with light phase, regulate florigen formation. However there is no experimental evidence for 'vernalin', in contrast to the final flowering hormone(s).

3.2 A MATHEMATICAL MODEL FOR BOLTING IN SUGAR-BEET

To account for the experimental results for sugar-beet of Chapter 5, a different approach was necessary: as it is likely that the flowering process as a whole is a dynamic, continuous and quantitative process, a relational diagram was drawn with the conventions of Forrester (1961), like models used in simulation studies (Figure 2).

The rectangles in the model represent a quantity of specific substances. Such a quantity is subject to change, the rates of which are indicated with valve symbols. Factors influencing the rate of these changes are drawn with dotted arrows. Flow of material (substance) is drawn with solid arrows. The model assumes two substances:

V: A substance resulting from vernalization.
F: A final flowering substance. The term flowering substance needs explanation as this publication reports only 'bolting'. In the trials, bolting was always followed by flowering. According to Heide (1973) the period between visible bolting and flowering is almost constant. Stem elongation and flowering may, however, differ in physiological mechanism. An illustration was given by Curtis (1964), who showed in a grafting experiment that flowering could occur without previous stem elongation. Under normal circumstances, however, it may be assumed that conditions favouring the synthesis of substances involved in stem elongation also favour the synthesis of the flowering substance.

The model distinguishes the following processes.

Process I (vernalization) is the synthesis of a substance V at a rate that is positively temperature-dependent but still proceeds at low temperature.

Process II (devernalization) is the breakdown of V at a rate that is also temperature-dependent. At low temperature, Process II is slower than Process I. So after a long time in the cold, a considerable amount of substance V is available, because Process II is practically still.

Process III is the synthesis of a final flowering substance F. After vernalization, synthesis of F starts, if temperature is raised and light phase (photoperiod) is long. The rate of synthesis of F is (in this quantitative model) determined by three conditions.

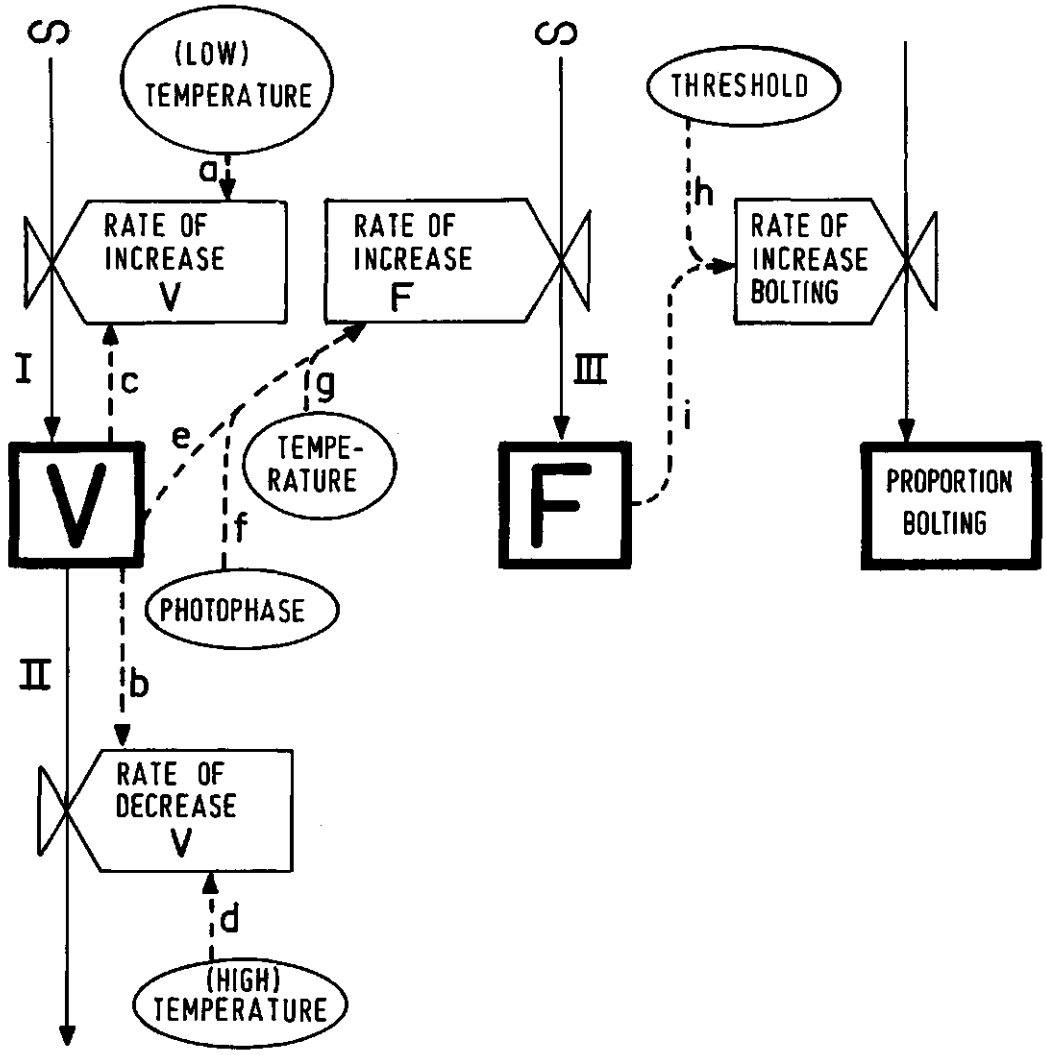

Fig. 2. Relational diagram of a simple model for the bolting process in sugar-beet. Substance V is hypothetical and is involved in vernalization. Substance F is thought to be associated with one or more flowering hormones. Solid lines mean flow of substance. Dotted lines are transfer of information: e.g. dotted line e means that the amount of V, together with photophase (f) and temperature (g), regulates the rate of synthesis of F.

- The light phase influences the rate of synthesis of F, longer lightphase speeding up this rate (relation f in the model).
- After longer cold treatments, bolters appear sooner and in larger number. So there must be a relation between the amount of V and the rate of synthesis of F (Figure 2, relation e): the larger the amount of V, the higher the rate of synthesis of F.
- Temperature must also influence synthesis of F, since most biochemical reactions are temperature-dependent, higher temperatures accelerating syn-

thesis and since synthesis also might depend on the growth rate of the plants, which is itself a temperature-dependent process. Rapid expansion of leaf area or leaf number accelerates reception of the light phase. Favourable conditions of growth have increased the number of bolting plants in some cases (relation g in the model).

The following mathematical equation fulfills all three conditions and gives the momentary rate of the synthesis of F at any time:

$$dF/dt \tilde{\sim} k_\theta \ V \ k_p \qquad (1)$$

in which

F, V is substance content of F and V
t is time
k_θ is a temperature coefficient
k_p a photophase coefficient

The temperature coefficient increases with temperature and the photophase coefficient with light phase.

The model simulates the following observations of other workers. Unvernalized plants do not bolt, although enough leaf area is produced to perceive a suitable light phase.

Vernalized plants (with enough V) do not bolt with a short photophase, which does not allow synthesis of F.

Under certain conditions higher temperatures advance bolting, for instance after long cold treatment. Then plants start to bolt earlier under better growth conditions.

Rates introduced depend on state variables. For instance, rate of synthesis of F depends at any moment on the amount of V. As the amount of V is continuously changing, not only during vernalization but also after vernalization, the direct relation of content of V with rate of synthesis of F implies that the rate of increase in F is also continuously changing. So a plot of the content of F against time can have different shapes according to temperatures after vernalization, duration of chilling and photophase.

When conditions are favourable for bolting and in the right sequence, enough F is produced and bolting begins when a certain threshold content of F is reached. This threshold is probably subject to variation between plants, according to genotype. When F is produced at a low rate, with short photophases or after less cold treatment, the interval from appearance of the first bolter and the final one will be long. When F is produced very rapidly, however, the threshold will be passed quickly for all plants, so that they

will bolt in a few days. For example, when sugar-beet is grown for seed, plants vernalized during the winter have produced a considerable amount of V; in May or June when the light phase is adequate and the raised temperature has allowed formation of several leaves, synthesis of F is unlimited.

Although the model is probably much too simple, it accounts for most of the observations reported in the literature. Its framework can be extended and modified to results reported in Chapter 5. The model can serve as an aid in quantitative interpretation of these results. Although the proposed model is based on the experimental results, it is presented already at this point of the manuscript to make it easy for the reader to instantly compare the results of the experiments with the model.

4 Materials and methods

4.1 PLANT MATERIAL

The cultivars used in growing rooms, greenhouses and in the field differed widely in susceptibility to bolting (Table 1). Because sugar-beet is cross-pollinated, each cultivar, even a single cross, would include different genotypes. Most trials were with four single crosses (G1-G4) supplied by the breeding firm D.J. van der Have B.V. (Kapelle). Commercial cultivars were sometimes included. For most indoor trials, each treatment consisted of 20 plants in two replicates of 10 placed randomly. Trials were of factorial design.

4.2 FIELD TRIALS

Triald fields were at three sites near Wageningen in the years 1975-1978:
- Wageningen-Hoog on a coarse-sand soil with a low content of organic matter.
- Wageningen (Haarweg, on the western outskirt of Wageningen) on a heavy-clay soil.
- Achterberg (near Rhenen to the west of Wageningen) on a black peat soil.

The experimental design was randomized blocks or split plots. In 1975 and 1976, daylength was extended in the field with incandescent light from bulbs of 100 W at a height of 1.5 m above the ground and with 1 bulb for every 5 m^2. At plant height this resulted in an areic luminous flux of 50-110 lx, according to distance from the bulb. For technical reasons, the assigned subblocks could not all be lit at the same time. So, some were lit from sun-

Table 1. Cultivars used in the project.

Code	Cultivar	Type	Bolting resistance	Breeder
G1	P2272	single cross	strong	D.J. van der Have
G2	35848-74	single cross	rather strong	D.J. van der Have
G3	35872-74	single cross	low	D.J. van der Have
G4	P6672	single cross	very low	D.J. van der Have
G5	Donor	comm. cultivar	strong	Hilleshög Fro
G6	Kawepoly	comm. cultivar	rather low	Kleinwanzleben
G7	Monohil	comm. cultivar	rather strong	Hilleshög Fro
G8	Monokuhn	comm. cultivar	rather low	Kuhn & Co
G9	Polykuhn	comm. cultivar	rather low	Kuhn & Co
G10	Zwaanpoly	comm. cultivar	rather strong	Zwaanesse
G11	MK711	test cultivar	low	Kuhn & Co

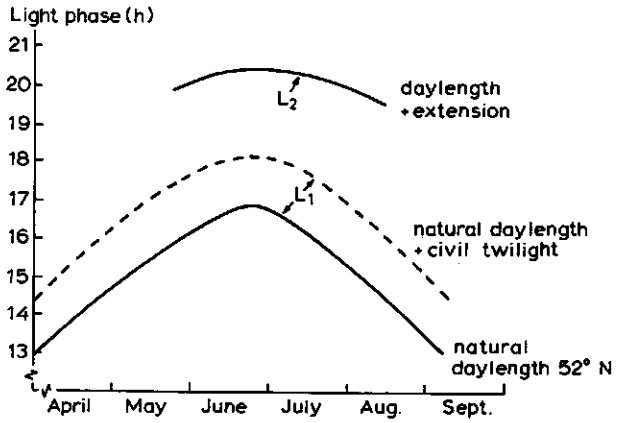

Fig. 3. Daylength in the Netherlands in the period April-September (L1) and light phase in field trials at Wageningen extended with incandescent bulbs (L2).

set till 00h30, the rest from 01h00 till sunrise. At Wageningen, astronomical midnight is 00h45. The sequence was reversed every week. The control sub-blocks were protected from illumination by their distance from the light source (at least 3 m for the nearest plots) and by plastic shades. The day-length (light phase) so obtained is shown in Figure 3. For the unlit plots, the graph of daylength + civil twilight probably reflects the effective day-length.

4.3 INDOOR TRIALS

The Department of Field Crops & Grassland Science of the Agricultural University at Wageningen provided growing rooms for raising plants, cold treatment and subsequent treatments as follows.

4.3.1 Growing rooms

Six growing rooms 4.5 m x 3.2 m x 2.2 m, regulated in temperature, humidity and partial pressure of carbondioxide, were used. Areic radiant power in the waveband 400-10.000 nm was 125 W/m^2 and in the waveband 400-700 nm 80 W/m^2 from 187 'TL' MF 140 W/33 + 17 'TL'M 140 W/33 fluorescent tubes, together with 18 incandescent bulbs of electrical power 150 W and 18 incandescent bulbs of 100 W. That areic radiant power in the visible spectrum corresponded to an areic luminous flux of about 20 klx, according to data of Gaastra (1959). In the middle of these cells temperature could be kept constant to within about 0.4 °C. Near the cell walls, however, temperature could deviate about 1 °C from the desired value. By moving the plants, placed on carts, at least twice a week around the cells, differences due to position were reduced. These cells were used mainly for raising plants and for treatments after chilling.

4.3.2 Rooms for cold treatment

Four rooms were used for cold treatment, equipped like those used for raising plants, lit by 8 HPLR bulbs and 4 incandescent lamps of electrical power of 100 W, giving an areic radiant power in the waveband 400-10000 nm of 45 W/m^2 and in the waveband 400-700 nm 23 W/m^2. The areic radiant power in the visible spectrum corresponded to an areic luminous flux of about 6.6 klx. Unless otherwise stated, cold treatment was at 3 °C.

4.3.3 Greenhouses

Three greenhouses with a floor area of 40 m^2 each, were used. Temperature was controlled to within 0.3-0.5 °C at night and 2 °C with low radiation during the day and 4-5 °C or even more in summer with high solar radiation. In winter, natural radiation was supplemented by 32 HPLR bulbs, giving an additional radiant power in the waveband 400-10.000 nm of 45 W/m^2 and in the waveband 400-700 nm of 23 W/m^2.

4.3.4 Small greenhouses

Three smaller greenhouses with a floor area of 30 m^2 each were used, in which 20 HPLR bulbs could be installed if supplementary lighting was needed.

4.4 LIGHT CONDITIONS

The photosynthetic phase of the daily cycle was extended with normal incandescent bulbs of electrical power 100 W, giving an areic radiant power in the waveband 400-10.000 nm of 5.3 W/m^2 and in the waveband 400-700 nm of 0.88 W/m^2 and an areic luminous flux of about 210 lx. The extension was divided equally before and after the phase of high-intensity lighting. The light phase will be annotated below as 2 numeric values, the first the phase of high intensity and the second the phases of extension. For instance, 14 h + 4 h indicates a total photophase (TP) of 18 h, 14 h of high intensity and 4 h of extension. The greenhouses could be covered with blinds during the dark phase to exclude natural light or artificial light from adjacent greenhouses.

4.5 RAISING AND CHILLING OF PLANTS

Plants were grown in a mixture of equal volumes of sandy soil (pH of KCl extract 5.6; mass fraction of organic matter 3.8 %) and peat soil (pH of KCl extract 4.6; organic matter 62 %). The KCl extract of the mixture had a pH of 5.2. Sugar-beet was sown either directly in plastic square 2L pots (0.13 metre cube) or in plastic boxes 0.46 m x 0.31 m x 0.16 m and trans-

planted into the square pots after chilling. When seed was sown in boxes, heat-sterilized soil was used to prevent infection by soil pathogens. Ten rows were sown in each box and after emergence thinned in the row to a distance of 2 cm between seedlings. Usually the plants were grown in the six raising rooms (Section 4.3.1) with a light phase 14 h + 0 h and temperature 15 °C until the first true leaves were about 2-3 cm. Boxes or pots were then transferred to the cold rooms. When chilling or light phase was not under test, a temperature of 3 °C and a light phase of 14 h + 0 h was used.

When the duration of chilling or plant age before chilling was a factor in the experiment, sowing and chilling were started on such dates that the cold treatments could be finished on the same date, so that all plants were under identical conditions from then on. When the plants were sown in boxes, they were usually kept a week at 10 °C after the cold treatment before transplanting into the square 2L pots and transferred to either growing rooms or greenhouses, where light phase or temperature were varied, 15 °C and a phase of high-intensity of 14 h being the basic conditions. Immediately after the transfer, however, the plants were usually kept yet another week at 10 °C to acclimatize after transplanting. The plants received 100 ml of a nutrition solution at least once a week according to growth stage (Table 2). Although the plants were grown in small pots to save floor space and would lose some nutrients by percolation, they grew vigorously in all trials. Times of changes in conditions during growth are expressed as far as possible with respect to the date of sowing until cold treatment and with respect to ending of cold treatment thereafter.

Table 2. Composition of the nutrient solution for sugar-beet grown indoors.

8	ml $Ca(NO_3)_2$ (1 mol/l)
8	ml KNO_3 (2 mol/l)
5	ml KH_2PO_4 (1 mol/l)
3	ml of a solution containing:
	2 g $MnCl_2.4H_2O$/l
	3 g H_3BO_3/l
	0.5 g $ZnSO_4.7H_2O$/l
	0.1 g $CuSO_4.5H_2O$/l
	0.1 g MoO_3/l
2	ml of a solution containing 35 g FeEDTA/l
5	ml $MgSO_4$ (1 mol/l)
69	ml H_2O
---	---
100	ml

4.6 OBSERVATIONS

In the field and indoors number of beet bolting was recorded regularly, sometimes twice a week. A plant was considered a bolter when the stem had elongated by at least 5 cm. When batches were harvested periodically, plants were separated in leaf blades, petioles and beet root. Leaf area of young plants was measured with an electronic device and of older plants by matching the leaves against photocopies of leaves of known area. Yield of dry matter was estimated by drying at 105 °C for at least 18 h.

4.7 STATISTICS

Data were processed on the DEC-10 computer of the Agricultural University, either with Fortran programs or with SPSS (Statistical Package of the Social Sciences). Data on the proportion of bolters were transformated (arc-sine-method) for statistical analyses to obtain a more normal distribution (Bliss, 1937).

5 Results

5.1 THE JUVENILE STAGE AND VERNALIZATION

The possibility of a juvenile stage in sugar-beet is of considerable relevance to undesired bolting. Figure 4 shows the average course of temperature during spring in the Netherlands. If temperatures below about 11 °C vernalize, the process can continue for the whole day until about 10 April. By then, plants in the Netherlands are usually germinating or in the cotyledonary stage. The following trials were designed to test for vernalization in these early stages.

5.1.1 Effect of daylength and covering with plastic

5.1.1.1 Introduction

In 1976 at Wageningen-Hoog, emergence was accelerated by covering the seed-bed with a perforated polyethene sheeting. If a juvenile stage exists, such a treatment should increase proportion of bolters because it would shorten this stage. The light phase was also extended with artificial light (Section 4.2).

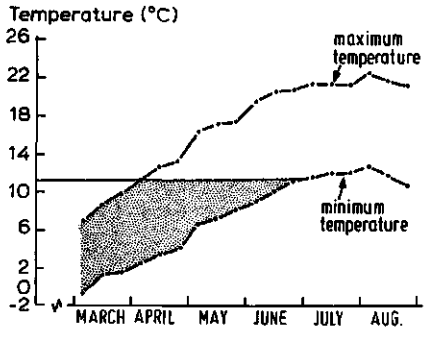

Fig. 4. Average course of temperature (minimum and maximum) from March to September in the Netherlands (data for De Bilt near Utrecht).

5.1.1.2 Materials and methods

The experimental scheme (split plot) included the following factors:

- Covering of the seed-bed:
  M1: control
  M2: covered
- Light phase (main factor):
  L1: natural daylight
  L2: light phase extended to about 20 h (Figure 3)
- Two genotypes: G1 and G4 (Table 1)
- Sowing dates:
  T1 = 2 March
  T2 = 17 March
  T3 = 1 April
  T4 = 15 April
- Two replicates.

The trial consisted of 64 plots, each of area 30 m^2 (net area 20 m^2) in the lit parts and 42 m^2 (net 30 m^2) in the unlit parts. Directly after sowing, the assigned plots were covered with the sheeting. As soon as seedlings emerged the sheeting was removed, in order to protect the seedlings from too high temperatures, which can easily occur under plastic. After emergence, the plants were thinned to a stand density of 9-10 m^{-2} (rows 50 cm apart). Fertilizers were applied on a normal basis.

5.1.1.3 Results

Measurements with a temperature recorder showed that on sunny days the plastic raised maximum temperatures at sowing depth to about 4 °C above those of controls. Germination took much less time (Table 3). On 17 May, covered plants had grown to almost twice the plant mass and leaf area of the control plots (Table 4). Sowing date and covering both had a significant effect on mass and area ($P < 0.001$).

Although it could be expected that these bigger plants would have been

Table 3. Effect on date of emergence of covering the seedbed with perforated transparant plastic sheeting during germination.

Sowing date (month-day)	Date of emergence (month-day)		Time of germination (d)	
	control	mulched	control	mulched
03-02	04-09	03-27	38	25
03-17	04-15	04-04	29	18
04-01	04-19	04-12	18	11
04-15	04-29	04-23	14	8

Table 4. Leaf area and mass of dry matter per plant for covered and open plots on 17 May 1976. Average for the genotypes G1 and G4.

Sowing date (month-day)	Leaf area (cm^2)		Mass of dry matter (g)	
	open	covered	open	covered
03-02	27.7	52.1	0.28	0.52
03-17	20.8	36.2	0.22	0.39
04-01	15.0	22.6	0.15	0.24
04-15	3.1	7.2	0.04	0.08

vernalized more effectively, the opposite was true (Table 5): the covered plots bolted much less ($P < 0.05$). Perhaps covered plants were devernalized in the few days after emergence and before removal of the plastic mulch, especially for the third sowing for which emergence started on a Friday but the cover was not removed until Monday. With the sunny weather of that week-end air around the plants would have been very hot. Such an explanation would assume either that vernalization proceeded during germination or that the seeds were already more or less vernalized when sown.

Alternatively vernalization could not proceed to such an extent as in the controls during germination because of the higher soil temperature. That explanation requires also that vernalization was already proceeding during germination.

Extension of the light phase to about 20 h induced more bolting ($P < 0.025$, Table 5) and may thus be a useful tool for breeders to test bolting behaviour of their plant material or to be used in more specific selection methods. In 1976, the effect of sowing date was marked ($P < 0.001$), especially in the cultivar unsusceptible to bolting, presumably because of the very high (devernalizing) temperatures during spring and summer. Cultivar G1 and G4 showed large difference in tendency to bolt ($P < 0.001$).

Table 5. Influence of covering, light phase and sowing date on proportion of bolters on 23 August 1976 for 2 cultivars.

Genotype	Cover	Natural photophase				Light phase extended to 20 h			
		sowing date (month-day)				sowing date (month-day)			
		03-02	03-17	04-01	04-15	03-02	03-17	04-01	04-15
G1	-	6.8	0.3	0.0	0.0	12.9	3.2	0.0	0.0
G1	+	6.6	0.0	0.0	0.0	8.8	0.3	0.3	0.0
G4	-	88.9	77.0	43.8	23.0	94.6	88.6	71.3	50.9
G4	+	89.0	66.0	21.8	17.7	100.0	86.1	58.4	41.5

5.1.2 Effect of germination temperature

5.1.2.1 Introduction

In 1977, temperature of soil at germination depth was kept *down* by covering field plots with plates of polystyrene insulating material 2 cm thick. This was very effective in keeping the soil temperature low at sowing depth.

5.1.2.2 Materials and methods

The experimental field at Wageningen-Hoog was laid out in a factorial block design with plots 4 m x 2.5 m including the following factors:

- Three sowing dates: 3 and 15 March and 5 April; only cultivar G1 (unsusceptible to bolting) was sown.
- Some plots were covered with 3 polystyrene plates (1.22 m x 2.44 m x 0.02 m) as soon as possible after sowing. Immediately after emergence of the first plants in a plot, the plates were removed.
- Sowing of ready germinated seed. Seed was soaked for 2 h in running water at 25 °C, and allowed to germinate for 20 h between two layers of filter paper at a temperature of 20/30 °C (day/night cycle) in an incubator. At sowing, almost all seeds were visibly germinated.
- Four replicates.

All combinations were included, so that the trial consisted of 48 plots. Temperature was recorded with a Flat-Bat 24-points recorder in covered and

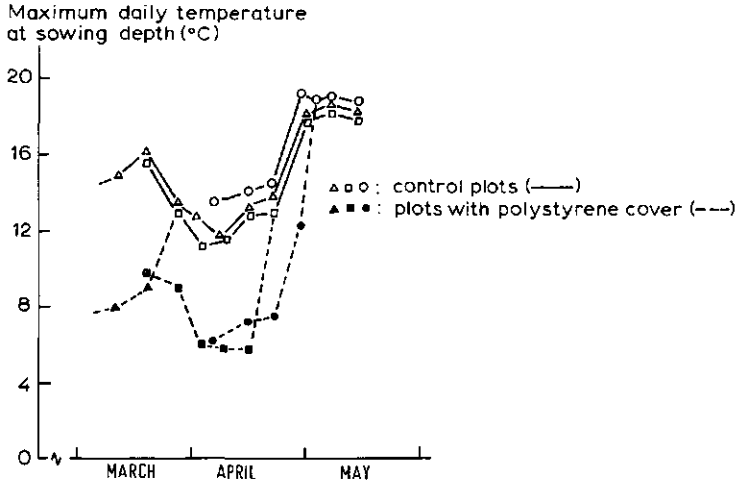

Fig. 5. Maximum soil temperatures at sowing depth as influenced by covering (filled symbols) with polystyrene plates in 1977.
△▲, sown 3 March; □■, sown 15 March, ○●, sown 5 April.

Table 6. Influence of covering the seedbed with polystyrene plates during germination on the date of emergence, areic number of plants (stand density) and proportion of bolters on 4 October 1977 for ready germinated and control seed.

Date of (month-day)			Ready germinated	Date of emergence (month-day)	Areic number (m^{-2})	Proportion of bolters (%)
sowing	covering	removal				
03-03	control		-	03-23	9.3	6.5
03-03	control		+	03-20	8.1	4.6
Mean					(8.7)	(5.5)
03-03	03-07	03-22	-	03-27	3.9	13.8
03-03	03-07	03-22	+	03-25	4.1	13.7
Mean					(4.0)	(13.8)
03-15	control		-	04-10	10.3	1.7
03-15	control		+	04-04	10.0	1.7
Mean					(10.1)	(1.7)
03-15	03-15	04-18	-	04-30	5.0	8.2
03-15	03-15	04-06	+	04-20	3.7	5.6
Mean					(4.3)	(6.9)
04-05	control		-	04-30	11.1	0.3
04-05	control		+	04-30	10.3	0.0
Mean					(10.7)	(0.1)
04-05	04-06	05-02	-	05-04	5.9	0.9
04-05	04-06	05-02	+	05-04	5.2	2.0
Mean					(5.6)	(1.4)
Significance of the effects of:						
Sowing date					($P < 0.1\%$)	($P < 0.1\%$)
Imbibition etc.					($P < 3.9\%$)	(n.s.)
Polystyrene plates					($P < 0.1\%$)	($P < 0.1\%$)

open plots at sowing depth. After complete emergence, plants were thinned to obtain, if possible, a stand density of 10 m^{-2}.

5.1.2.3 Results

With polystyrene plates, the seed-bed was 4-6 °C cooler, somewhat different for each sowing date (Figure 5). However, the plates also hindered germination, possibly because of saturation of the seed-bed with condensation water from the plates in combination with the lower temperature during germination. Only an irregular stand with a density of about 4 m^{-2} was achieved in the covered plots.

Covering delayed emergence and considerably increased bolting (Table 6). The effect on bolting could be due either to germination temperature or to stand density, since Jorritsma (1978) reported 2.4, 3.1 and 3.5 % bolters for respective stand densities of 12.6, 6.9 and 3.4 m^{-2}. The effects I observed were, however, more pronounced than those reported by Jorritsma. So

very probably, bolting is enhanced mainly by the lower germination temperature in the covered plots. Covered plots of the second sowing gave even more bolters than the control plots of the first sowing, as might be expected from the different temperatures (Figure 5). Sowing of ready germinated seed had no significant influence on bolting. Despite the disturbing effect of different plant densities, this experiment provided another indication that, already during germination, temperature may play an important role in achieving a vernalized condition.

5.1.3 Effect of plant size on vernalization in the field

5.1.3.1 Materials and methods

In 1976, single cross G4 was transplanted into the field at different stages. The trial (block design with 4 replicates and with plots of area 20 m^2) consisted of the following treatments.

A. Sown normally outdoors on 18 March and emerging on 21 April.

B. Sown in paper pots (Nippon Tensai Seito Kabushiki Kaisha) of diameter 1.9 cm and a height 13 cm on 12 April in a greenhouse with respective day and night temperatures of 18 and 15 °C. On 21 April, plants were as large as those sown in the field on 18 March (Treatment A) and were transplanted to the field.

C. Sown in 16 March and transplanted outdoors on 18 March, being for two days in the greenhouse at day and night temperatures of 18 and 15 °C.

D. Sown in the greenhouse in paper pots on 25 February and transplanted to the field on 18 March when two true leaves had developed. In the week after transplanting, severe frost damage occurred and transplanting was repeated (with spare plants) at 25 March.

E. Sown in the greenhouse in paper pots on 12 February and transplanted outdoors on 18 March in the 4-leaf stage. Also in this treatment, because of the frost damage, a new batch was transplanted on 25 March.

The plants were transplanted or, for direct sowing, were thinned to a stand density of 12 m^{-2}.

5.1.3.2 Results

Although on 21 April, outdoor-sown (Treatment A) and later indoor-sown plants (Treatment B) were at the same stage of growth, the outdoor-sown beet were heavier on 1 June, probably because of an initial inhibition by the paper pot. At the end of the season, plants of Treatment B, without a 'cold' germination period, had bolted significantly less than the outdoor sown plants of Treatment A (Table 7). Temperature during the spring of 1976 is shown in Figure 6. The timing of the different treatments is presented at the bottom of this graph.

Table 7. Effect of transplanting at several stages of growth on the mass of dry matter per plant on 1 June 1976 and bolting on 22 September.

Treatment	Site	Sowing date	Stage on 21 April	Mass of dry matter (g)	Proportion of bolters (%)
A	Outdoors	03-18	cotyledonary	4.2	47.3
B	Indoors	04-12	cotyledonary	1.3	3.0
C	Indoors	03-16	2-4 leaf	2.1	57.0
D	Indoors	02-25	4-leaf	10.3	26.4
E	Indoors	02-12	6-leaf	14.7	69.5
Significance of treatment effect				($P < 0.01$)	($P < 0.01$)

Again results indicate that vernalization proceeds very early in growth, considering the difference in bolting between Treatment A and B. Beet sown indoors in March and transplanted outdoors two days later bolted more than beet sown directly outdoors (Treatment C and A). Beet sown early February and planted out late in March (Treatment E) bolted severely, indicating easy vernalization of that older plants, but those sown later in February and planted out at the same time (Treatment D) bolted less than treatment C. Proper comparisons with Treatment A, B and C cannot, however, be made because of the delayed planting of treatments D and E.

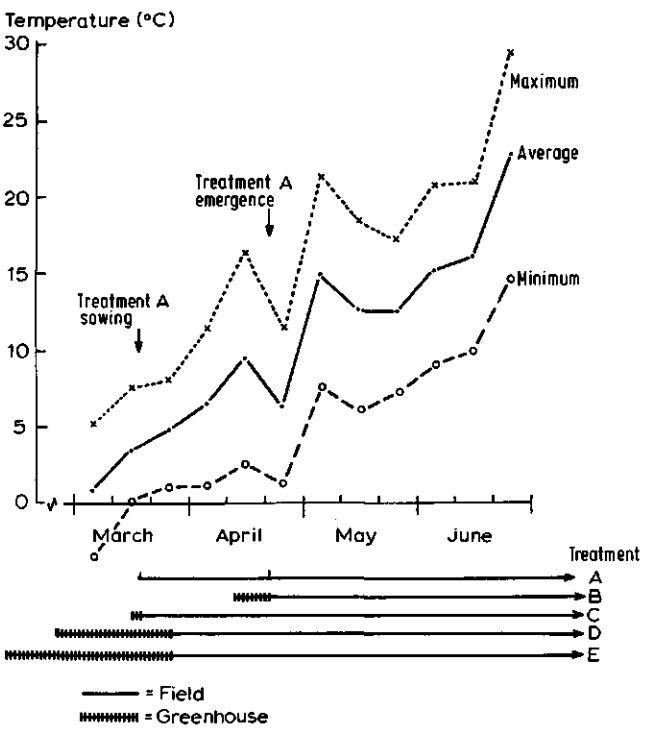

Fig. 6. Course of maximum, minimum and average temperature in spring 1976. Timing of the treatments A, B, C, D and E is indicated below the graph. Treatment A is field sown. Treatment B, C and D: plants were raised in the greenhouse at 18/15 °C for various durations and afterwards transplanted to the field.

5.1.4 Effect of plant size on cold treatment

5.1.4.1 Materials and methods

In the raising rooms, plants of cultivars G1, G2 and G3 (Table 1) were raised to different ages before cold treatment. They were sown in 2L pots and raised at 20 °C with a light phase of 14 h during raising and cold treatment. Sowing dates were so organized that plants were of 5 ages at the beginning of cold treatment and that both cold treatments of 49 and 28 days at 4 °C were completed simultaneously:

S1 = Immediately chilled. Sown and immediately transferred to the cold rooms.
S2 = Chilled after 2 days. Sown 2 days before transfer to the cold treatment.
S3 = Chilled after 14 days.
S4 = Chilled after 28 days.
S5 = Chilled after 42 days.

After cold treatment, the plants were transferred to a greenhouse where the temperature was kept at 10 °C for 1 week and then raised to 15 °C, which could be rigidly maintained. Only towards the end of the trial (90 days after cold treatment) did the sun cause temperature to rise to a maximum of 18 °C during some days. In the greenhouse, the total light phase was 14 h + 10 h = 24 h.

5.1.4.2 Results

At the beginning and end of cold treatment, number of leaves per plant was counted for each treatment (Table 8). Despite the low temperature, treatments S1 and S2 emerged during the cold treatment. Germination may have been stimulated by use of sprinkling water of 10-15 °C. At the end of cold treatment for 49 days, S2 seedlings already had stretched cotyledons, whereas those of S1 were just visible.

Although plants were genetically as uniform as possible, Figure 7 indi-

Table 8. Number of true leaves per plant (mean for 3 cultivars) for up to 42 days at 20 °C before cold treatment for 28 or 49 days at 4 °C.

Stage of treatment	Number of leaves for plants									
	chilled for 28 d					chilled for 49 d				
	time of raising at 20 °C					time of raising at 20 °C				
	0	2	14	28	42	0	2	14	28	42
Start of chilling	-	-	2	9	12	-	-	2	6	11
End of chilling	*	*	2	10	14	*	*	3	10	15

\- no emergence visible
* cotyledons were visible

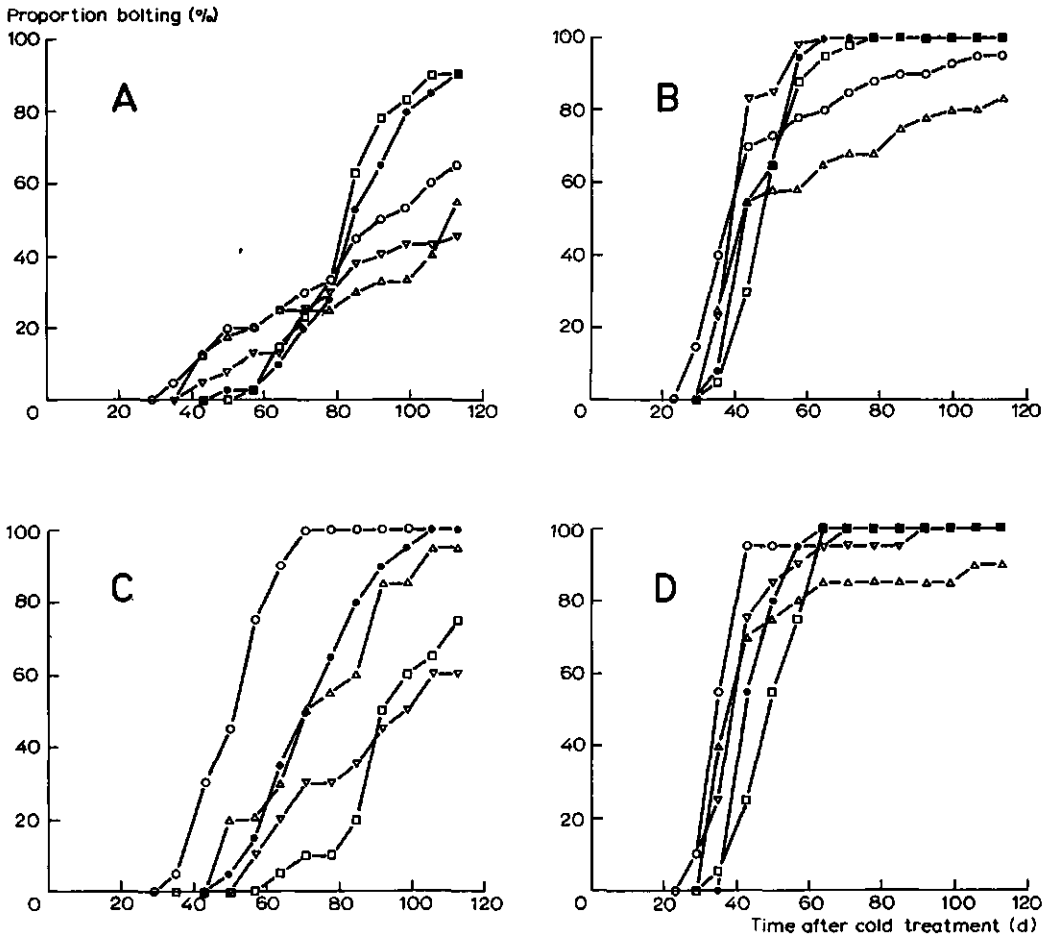

Fig. 7. Bolting as influenced by plant age at begin of cold treatment.
A. Cultivars G1 and G2 with cold treatment of 28 days.
B. Cultivars G1 and G2 with cold treatment of 49 days.
C. Cultivar G3 with cold treatment of 28 days.
D. Cultivar G3 with cold treatment of 49 days.
Age at start of cold treatment 0, 0 days; Δ, 2 days; ∇, 14 days; □, 28 days; ●, 42 days.

cates the great variability in bolting date. Longer cold treatment and (in other trials) longer photophases seemed to reduce that variability, as all plants then bolted within a shorter time interval. The results do not indicate how much of the variability was genotypic or phenotypic. In some treatments, bolting and non-bolting plants can be distinguished, but the difference could be caused by rather small genotypic or phenotypic differences. Perhaps the non-bolting plants were near the threshold for bolting.

In Figure 7A and 7B, the mean is taken for the two cultivars, as no differences in reaction were observed. Against expectation, the plants chilled at a younger stage showed the earliest bolters, 30 days after cold treatment (Figure 7A). At the final count however, those treatments resulted in signi-

ficantly fewer bolters. In plants chilled for 7 weeks, the differences are less pronounced (Figure 7B), although even there the plants chilled at the youngest stages started bolting first.

The more susceptible cultivar G3 reacted differently (Figures 7C and 7D). The quicker onset of bolting with direct chilling was similar to all cultivars but all plants of cv. G3 had bolted with that treatment within 60 days, before the other treatments. With plants chilled at increasing stages, a depression in sensitivity to cold seems to be followed by an increase. Also against expectation, plants chilled after germination for 2 days bolted less than those directly chilled.

Longer cold treatment leads to more bolters and a faster onset of bolting, especially in the more resistant genotypes.

5.1.5 Conclusions

Whether younger plants can be vernalized is pertinent to commercial beet growing and to breeders wanting to vernalize plants in a controlled room. If young plants can be vernalized, space, labour and time can be saved.

The results (Section 5.1) indicate that a true juvenile stage does not exist in sugar-beet, although chilling during germination induces somewhat less bolting than during later stages of growth.

5.2 TEMPERATURE OF COLD TREATMENT

5.2.1 Materials and methods

Several values for the optimum temperature of vernalization have been reported in literature (Section 2.2). Four cultivars were therefore chilled to four temperatures in an indoor trial. To separate effects of light phase from those of low temperature, a rather short photophase of 14 h was maintained during raising and chilling. All four single crosses G1, G2, G3 and G4 were directly sown in 2L pots and kept in a room at 20 °C until two true leaves were formed after 14 days. The plants were then transferred to 4 cold rooms with temperatures of 3, 7, 11 and 15 °C respectively for 55 days.

After chilling, all plants were transferred to a greenhouse where temperature was kept at 12 °C for the first 8 days. The temperature was then maintained at 15 °C and the light phase was altered to 14 h + 10 h = 24 h. Temperature was reasonably well regulated, rising to 18 °C on only 7 days in the interval 15-40 days after chilling.

Unchilled plants of cultivars G3 and G4, sown 3 weeks before the end of the cold treatment, raised at 20 °C and a total light phase of 14 h, were transferred on the same day to the greenhouse as the chilled plants.

The experiment can be summarized as follows.

Factors 1) Genotype: G1, G2, G3, G4
2) Cold treatment: V1; unvernalized (only G3 and G4)
V2; 55 days
3) Temperature of cold treatment:
T1 = 3 °C
T2 = 7 °C
T3 = 11 °C
T4 = 15 °C

5.2.2 Results

As a consequence of the different temperatures of chilling, the number of leaves per plant differed at the end of the vernalization treatment (Table 9).

The unsusceptible cultivars G1 and G2 have reacted identically to temperatures of chilling (Figures 8A en 8B). The lower the temperature, the sooner plants started to bolt. Even the unsusceptible cultivars chilled at 15 °C started bolting about 100 days later. If the temperature had been higher after chilling (in this trial also 15 °C), there might have been few bolters, if any.
The susceptible cultivar G4 responded in the same way to temperature of chilling: the lower temperature was more effective (Figure 8D). Only in the cultivar G3 did the cold treatment at 7 °C result in slightly more bolting than that at 3 °C (Figure 8C). Unchilled plants of cultivar G3 differed considerably from those chilled at 15 °C but the difference was very small in cultivar G4.

5.2.3 Conclusions

A tentative conclusion in the terms of the model in Section 3.2 is that either Process 1 proceeds faster at lower temperature or the difference be-

Table 9. Number of leaves per plant at the end of the cold period (mean of the four cultivars) in relation to temperature and duration of cold treatment.
Unchilled plants had 3 leaves.

Temperature (°C)	Duration of the cold period (d)	
	31	55
3	4	3
7	4	6
11	9	12
15	10	14

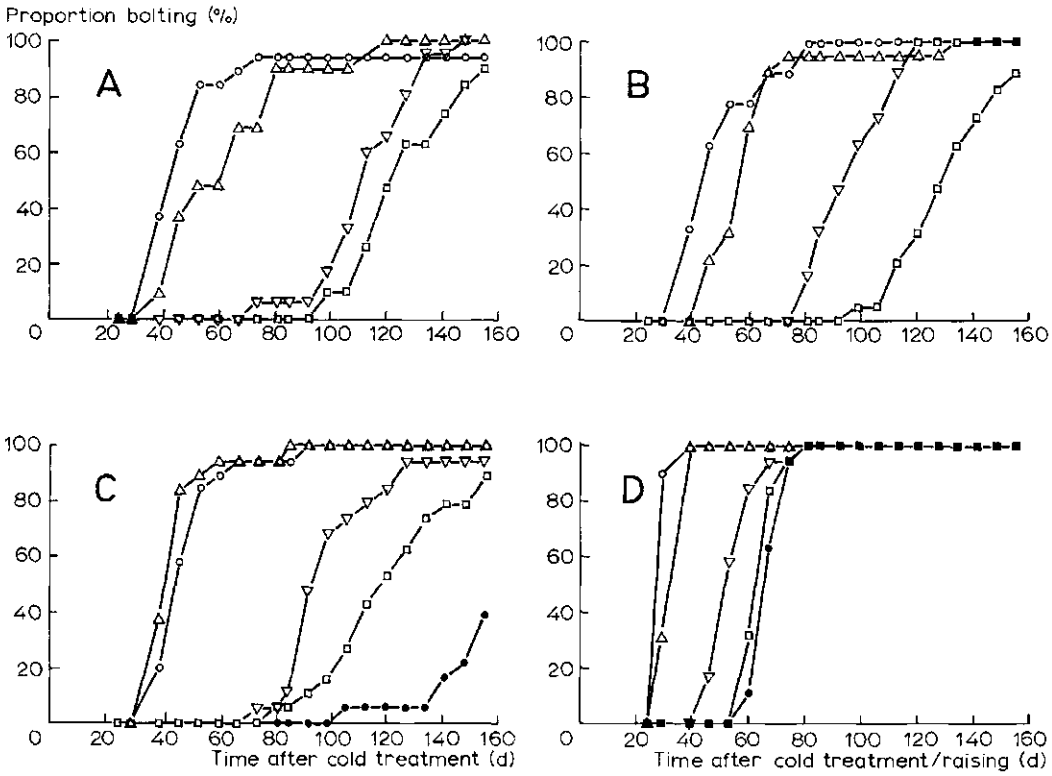

Fig. 8. Bolting as influenced by temperature of cold treatment 0, 3 °C; Δ, 7 °C; ∇, 11 °C; □, 15 °C; ●, unchilled.
A. Cultivar G1; b. Cultivar G2; C. Cultivar G3; D. Cultivar G4.

tween the rates of Processes 1 and 2 is greatest at temperatures as low as 3 °C. But even at 15 °C V accumulates continuously, though very slowly. Although the trial suggests chilling at the lowest temperature to obtain earliest flowering, this might be wrong (especially for susceptible cultivars), since there is a second temperature-dependent step in the model, the synthesis of F. Synthesis of F during chilling was probably slow, because of the short photophase and, in some treatments, the low temperature. Had the light phase during chilling been 24 h, the outcome would have been completely different. For example, in unchilled plants of cultivar G4, bolting started in continuous light 55 days after raising, whereas plants chilled at 3 °C with a light phase of 14 h for 55 days required already 55 + 24 = 80 days (Figure 8D). So, for several cultivars vernalization could be most effective at intermediate temperatures like 8-10 °C with long photophases, because V could accumulate and also synthesis of F could start. The optimum temperature for vernalization was found indeed higher if the light phase during chilling was longer (Section 2.2). In a trial reported below (Section 5.4), however, light phase during chilling at 3 °C had no significant effect on subsequent bolting, whereas in another (unpublished) trial at 8 °C, light

phase had a slight influence. Thus the action of light phase seems to be limited by lower temperatures, as has been assumed in the model.

5.3 LIGHT PHASE AND VERNALIZATION

5.3.1 Influence of light phase after vernalization

5.3.1.1 Materials and methods

The response of bolting to light phase after different duration of cold treatment was estimated in the four single crosses (G1-G4). Plants were raised in plastic boxes (Section 4.5) in a greenhouse at 15 °C, the first true leaves appearing after 14-15 days. They were then transferred to a cold room at 3 °C with a light phase of 14 h for 0, 14, 28 and 42 days. Afterwards they were transferred to a greenhouse at 15 °C, still with light phase 14 h, and after 4 days transplanted into 2L pots. The plants were then divided between 3 greenhouses at the same temperature but after another 3 days the light phase was either unchanged or supplemented for 4 or 10 h. Temperature in the greenhouses could not be regulated as accurately as in previous trials. On sunny days, the temperature rose 4-5 °C above the intended 15 °C, though to almost the same degree in the three greenhouses. However, in the greenhouse with a photophase of 14 h, a technical failure caused a rise to 28 °C for a few hours 23 days after cold treatment. The consequent devernalization was probably not too disastrous but could not be assessed quantitatively.

A comprehension of the experimental treatments:

- Genotypes: G1, G2, G3 and G4
- Duration of the cold period:
  V1, 0 weeks
  V2, 2 weeks
  V3, 4 weeks
  V4, 6 weeks
- Photophase: P1, 14 + 0 = 14 h
  P2, 14 + 4 = 18 h
  P3, 14 + 10 = 24 h

5.3.1.2 Results

To some degree, induction by cold and subsequent light phase were interchangeable in a susceptible cultivar (Figure 9B). With continuous light, shorter chilling retarded appearance of the first bolters and new bolters appeared slower, but even in unchilled plants 80 % of the plants finally bolted. With a subsequent light phase of 18 h the effect of chilling was more pronounced but even of unchilled plants, 5 % bolted. With the shortest

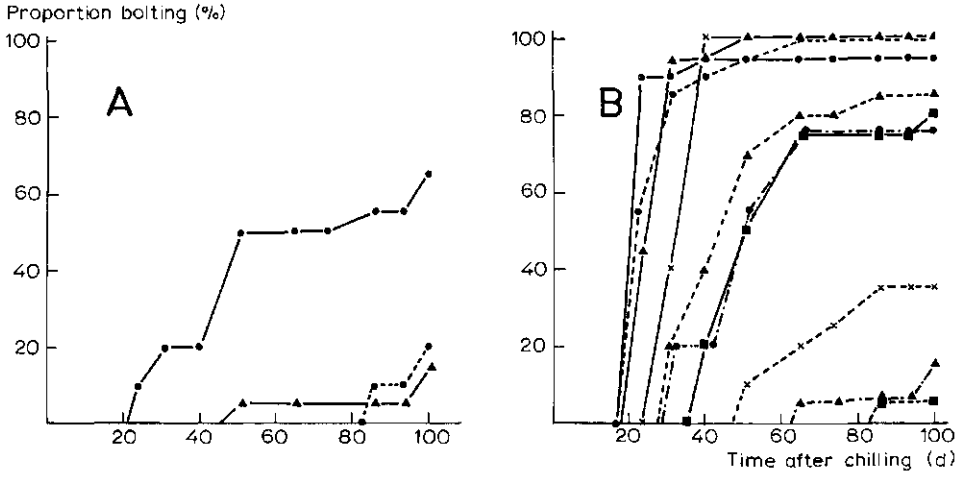

Fig. 9. Bolting without chilling (■) or after chilling for 14 (X), 28 (▲) or 42 days (●) and with subsequent light phase of 14 (-.-.-), 18 (----) or 24 h (——). A. Cultivar G2; B. Cultivar G4.

light phase of 14 h, bolters appeared only after chilling for 42 or 28 days.

However, for the more resistant cultivar (Figure 9A), no beet bolted with a light phase of 14 h and even under continuous light the plants only bolted if they had been chilled for 28 to 42 days. A certain proportion of bolters, for instance 50 %, after a certain time after cold treatment can be obtained in different ways: longer chilling and shorter light phase or shorter chilling and longer light phase (Figure 10A and 10B). Low temperature and subsequent long light phase are complementary.

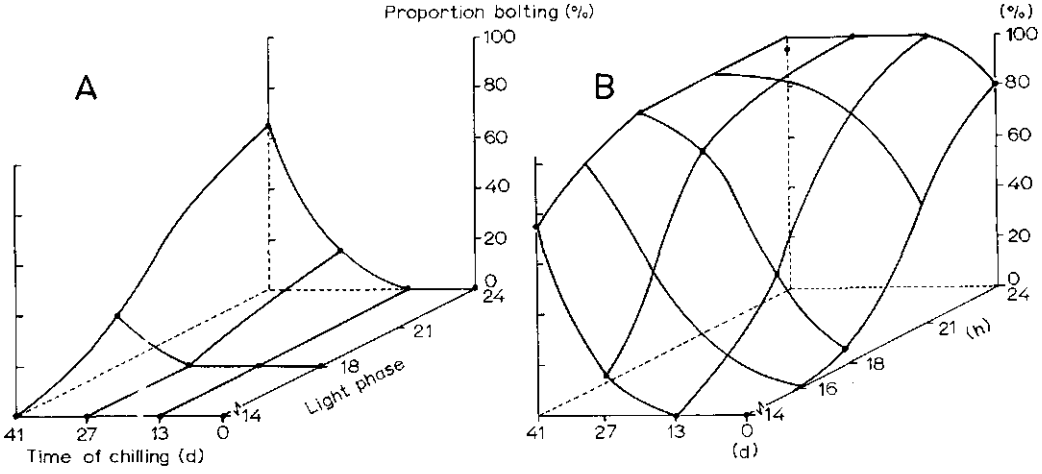

Fig. 10. Proportion of bolters after 100 days as a function of duration of chilling and of subsequent light phase.
A. Cultivar G2; B. Cultivar G4.

The other cultivars G3 and G1 reacted similarly to cultivars G4 and G2.

Interpretation in terms of the model suggests that during vernalization, only Process 1 proceeds. The light phase during vernalization (14 h) was not appropriate for Process 3; also because of the low temperature (3 °C) probably no F would be synthesized. After vernalization, different amounts of V would be present in the plants according to the duration of chilling. With temperature raised to 15 °C under different light phases, differences in synthesis of F depend on the amount of V and on light phase in this trial. In Figure 9B, the combination with 6 weeks chilled and then 14 h light phase obviously results in the same pattern of synthesis of F as unchilled and 24 h photophase, as is possible when assuming a relation between synthesis of F and temperature, light phase and amount of V as in Section 3.2

5.3.2 Effect of cultivar

5.3.2.1 Introduction

In a similar trial to the previous one and in a field trial, all 10 cultivars of Table 1 were tested to find out whether the four single crosses were in a similar range of susceptibility to commercial cultivars of sugarbeet used in the Netherlands.

5.3.2.2 Materials and methods

At Achterberg, the ten cultivars were sown on 2 and 29 March and 12 April 1978 in plots of 15 m^2 in 4 replicates. After emergence, the plants were thinned to a stand density of 10 m^{-2}. Indoors, the cultivars were raised for 6 days at 20 °C, then 5 days at 15 °C with a light phase of 14 h and 3 days at 10 °C. Afterwards they were chilled for either 31 or 49 days at 3 °C, switched to 10 °C for 3 days, transplanted and transferred to greenhouses with the following light phases:

- P1; 14 h + 0 h for 56 days, afterwards 14 h + 4 h = 18 h.
- P2; 14 h + 4 = 18 h until the end of the trial.
- P3; 14 h + 10 h = 24 h until the end of the trial.

After chilling, temperature was kept at 13 °C for the first 4 days and subsequently at 15 °C.

5.3.2.3 Results

The results (Table 10) were comparable with those in the previous trial. The proportion of bolters for the first sowing date in the field trial are shown at the bottom of the table for 2 dates, 3 August (early bolters) and

Table 10. Proportion of bolters for the cultivars G1-G10 (defined in Table 1) in a greenhouse at 5 intervals (30, 44, 74, 102, 125 days) after chilling and in a field trial (on 2 dates). Treatment V1, chilled for 31 days; V2, chilled for 49 days; P1, light phase of 14 h for 56 days and later 14 h + 4 h; P2, 14 h + 4 h; P3, continuous light (14 h + 10 h).

Time after chilling (d)	Treatment	Proportion of bolters (%)										Spearman's r with proportion bolting in the field trial on:	
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	08-03	09-21
30	V1P1	0	0	0	0	0	0	0	0	0	0	-	-
	V2P1	0	0	0	0	0	0	0	0	0	0	-	-
	V1P2	0	0	0	55	0	0	0	0	0	0	0.52*[1]	0.52*
	V2P2	10	0	25	84	0	20	5	10	20	5	0.39	0.52*
	V1P3	0	0	0	100	0	18	5	10	20	0	0.26	0.46*
	V2P3	60	55	90	95	30	60	50	75	70	75	0.48*	0.48*
44	V1P1	0	0	0	5	0	0	0	0	0	0	0.52*	0.52*
	V2P1	0	0	0	50	0	0	0	0	0	0	0.52*	0.52*
	V1P2	0	0	0	90	0	0	0	10	0	6	0.44	0.44
	V2P2	55	25	75	100	5	35	5	31	45	25	0.31	0.27
	V1P3	20	5	45	100	5	59	25	44	30	5	0.58**	0.64**
	V2P3	65	90	100	100	65	80	60	80	95	85	0.52*	0.35*
74	V1P1	0	0	0	45	0	0	0	0	5	0	0.16	0.39
	V2P1	0	10	5	85	0	5	0	5	0	5	0.77***	0.25
	V1P2	5	0	10	95	0	0	0	10	10	11	0.22	0.39
	V2P2	70	55	100	100	20	55	10	48	60	30	0.32	0.17
	V1P3	45	20	70	100	10	59	45	50	58	35	0.50*	0.59**
	V2P3	70	100	100	100	75	85	75	85	100	90	0.51*	0.30
102	V1P1	0	0	10	75	0	5	0	5	5	0	0.66**	0.72**
	V2P1	10	20	60	90	5	40	45	30	20	20	0.73***	0.72**
	V1P2	5	5	30	100	5	10	10	25	25	11	0.54*	0.76***
	V2P2	85	80	100	100	55	85	35	58	70	50	0.47*	0.19
	V1P3	50	30	85	100	25	69	60	78	79	40	0.49*	0.68**
	V2P3	70	100	100	100	90	85	90	90	100	100	0.35	0.30
125	V1P1	10	0	25	90	0	10	0	15	5	5	0.53*	0.51*
	V2P1	20	35	70	90	20	50	55	65	45	35	0.77***	0.84****
	V1P2	15	20	35	100	20	20	25	40	45	23	0.47*	0.78***
	V2P2	95	90	100	100	80	90	65	73	80	75	0.36	0.08
	V1P3	55	40	90	100	35	74	65	84	84	60	0.55**	0.73***
	V2P3	95	100	100	100	90	90	95	90	100	100	0.10	0.02

	Proportion of bolting (%) in field trial									
Date	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
08-03	1.4	4.5	29.1	85.5	4.1	4.3	4.1	4.8	3.1	4.0
09-21	2.1	9.8	42.1	89.5	13.5	10.8	15.4	15.7	11.2	10.5

1. *, $P < 0.10$; **, $P < 0.05$; ***, $P < 0.01$; ****, $P < 0.001$

21 September (total bolters).

Single crosses G1 and G2 were less susceptible and G3 and G4 were more and far more susceptible, respectively, than commercial cultivars.

For breeders using regulated growing conditions, these conditions should be such that genotypic differences in bolting behaviour, as observed in the

field, could be reproduced. If the differences are large enough, there is no problem. However, small but significant differences in bolting are difficult to distinguish indoors. Although it was not the purpose of these trials and the number of plants was small, the problem might be solved as indicated in the last two columns of Table 10. To compare ranking orders of the genotypes in the field and greenhouse, non-parametric Spearman correlation coefficients were calculated for the proportion of bolters in the field at 2 dates with those in each treatment at the 5 intervals after cold treatment.

Chilling for 49 days and continuous light did not reveal differences in bolting found in the field. Chilling for 49 days and a light phase of 14 h and later 18 h, which is closer to natural circumstances, gave better correlation. Curth & Fürste (1960) state, however, that continuous illumination improves the accordance between field and greenhouse bolting trials.

Cultivars could differ in rate of devernalization (Process 2), sensitivity to light phase (Process 3), cold requirement (Process 1) and the value of the threshold for bolting (Process 4). Because of the interdependence of these processes, it would be difficult to distinguish these characteristics for the cultivars. If genotypes mainly differed in the rate of devernalization, the method could be improved by a high temperature for a short time after chilling instead of a constant temperature of 15 °C. If, however, the difference were in sensitivity to light phase, temperature after chilling should not be too high. Instead the light phase should be that which gives best indication of sensitivity towards light phase. It is unlikely that differences in bolting are due to a single characteristic. So to test for bolting resistance the conditions should imitate those of early spring (with the same sequence of low temperature and high temperature, and a rather short light phase. If, however, the breeder would like to select for a particular characteristic, the conditions can be chosen accordingly.

Probably every climatic region where beet is grown has specific conditions for bolting. Dutch experience is that plant material from elsewhere, for instance Poland and North America, tends to bolt more than the Dutch commercial cultivars. Temperature conditions in spring in those countries are quite different and may allow more devernalization, or less vernalization, or both.

5.4 DEVERNALIZATION

5.4.1 Materials and methods

To find the effect of high temperature after vernalization, the four single crosses (Table 1) were vernalized and then kept at two constant temperatures 15 and 25 °C. They were raised at 15 °C in plastic boxes in a greenhouse for 14 days with light phase 14 h and then kept cold at 3 °C for 0, 14, 28 and 42 days. Besides chilling, the effect of light phase during

cold treatment was tested with 14 + 0 = 14 or 14 + 10 = 24 h. After cold treatment, the temperature was raised to 10 °C, and the plants were transplanted into 2L pots 2 days later. The plants were divided between 2 greenhouses with a light phase of 14 h + 10 h = 24 h and, in contrast to other trials, immediately kept at 15 °C. One week after transplanting, the temperature in one greenhouse was raised to 25 °C and the other maintained at 15 °C. Temperature could not be regulated very rigidly in the cooler greenhouse. On several sunny days, temperature rose to 18-20 °C. The warmer house became infested with aphids and mites, despite precautions, and that part of the trial had to be terminated earlier than planned. The experimental treatments can be summarized as follows:

1. Single crosses: G1, G2, G3 and G4
2. Duration of cold treatment:
   V1; 0 weeks
   V2; 2 weeks
   V3; 4 weeks
   V4; 6 weeks
3. Photophase during cold treatment:
   P1; 14 h + 0 h = 14 h
   P2; 14 h + 10 h = 24 h
4. Temperature after cold treatment:
   T1; 15 °C and photophase 14 + 10 h = 24 h
   T2; 25 °C and photophase 14 + 10 h = 24 h

5.4.2 Results

Light phase during vernalization had no significant influence on bolting, possibly because of the low temperature during vernalization. With a temperature of 15 °C after vernalization, the 4 cultivars bolted in the same way as in the trials on light phase (section 5.3). At 25 °C, however, only the most susceptible cultivar bolted. So apparently devernalization in the less susceptible cultivars was sufficient to prevent all the plants from bolting. Bolting of cultivar G4 was reduced by high temperature after all times of cold treatment, although the difference between 15 °C and 25 °C was more pronounced after shorter chilling or no chilling (Figure 11). Unchilled plants did not bolt at 25 °C, but had almost all bolted within 90 days after raising at 15 °C.

According to the time of chilling, different amounts of V would be present in the plants. Plants chilled for 42 days would bolt first: after vernalization, F would be rapidly synthesized at 15 °C, also because the amount of V was not destroyed at that temperature and positively influences this synthesis. Shorter chilling would result in less V and reduce, but still allow, synthesis of F. Plants would reach the threshold for bolting later.

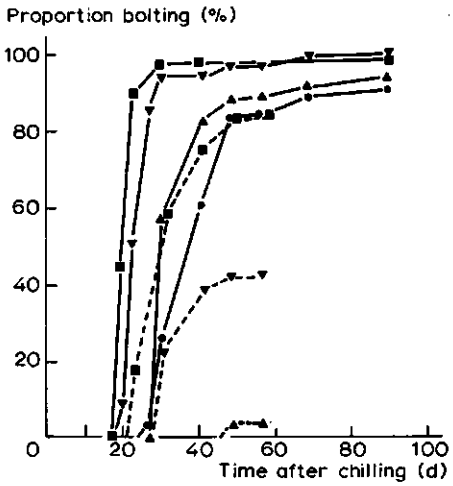

Fig. 11. Influence of temperature (——, 15 °C; -----, 25 °C) after cold treatment for different times (●, 0 d; ▲, 14 d; ▼, 28 d; ■, 42 d) on bolting of cultivar G4. Average data for light phases of 14 and 24 h during chilling. Unchilled plants did not bolt at all at 25 °C.

At 25 °C, V would break down. If so, the smaller amount of V would directly influence the rate of synthesis of F in proportion to that amount. *During* breakdown of V, F would still be synthesized, though at progressively lower rates until V was depleted. Obviously, the amount of F produced until that stage allowed bolting in only 40 and 80 % of the plants chilled for 28 and 42 days respectively.

After shorter chilling, less V will be available, which means that synthesis of F will stop earlier, the amount of F may then not be sufficient to allow any of the plants to bolt. In bolting-resistant genotypes, depletion of V might take place in such a short time that insufficient amounts of F can be synthesized, even at that long daylength.

5.5 TEMPERATURE AND LIGHT PHASE IMMEDIATELY AFTER VERNALIZATION

In the next two trials, high temperature was applied for only a short time, to avoid treatments that induced no bolting.

5.5.1 Trial 1

5.5.1.1 Materials and methods

Seeds of cultivars G1-G4 were sown in plastic boxes, temperature being kept at 25 °C until emergence was complete and then lowered to 15 °C. After 13 days, cold treatment started. After 52 days at 3 °C, the plants were transplanted into 2L pots and transferred to 4 growing rooms at 15 °C. After

Table 11. Temperature and light phase from the time of ending of cold treatment in Trial 1.

Treatment	Time interval (d)		
	17-28 (Period I)	29-41 (Period II)	41-end (Period III)
1 Temp. (°C)	15	15	15
Phase (h)	18	18	18
2 Temp. (°C)	15	15	15
Phase (h)	24	24	24
3 Temp. (°C)	25	15	15
Phase (h)	18	18	18
4 Temp. (°C)	25	15	15
Phase (h)	24	24	24
5 Temp. (°C)	15	25	15
Phase (h)	24	24	24
6 Temp. (°C)	15	25	15
Phase (h)	18	24	24

8 days, the light phase was increased from 14 + 0 = 14 h to 14 + 4 = 18 h and, after 17 days, the plants were subjected to 6 combinations of temperature and lighting (Table 11).

Besides the chilled plants, unchilled plants of cultivars G3 and G4 were included in the trial. They were sown 16 days before the end of chilling in the same way as the chilled plants and allowed to germinate at 25 °C. Three days before chilling of the other plants ended, they were cooled to 11 °C. They were transplanted at the same time as chilled plants and further treated identically.

5.5.1.2 Results

In G1 and G3, high temperature in the early interval (Period I) reduced bolting considerably (Treatment 3), even with continuous light (Treatment 4, Figure 12A and 12B). In unchilled plants of the most susceptible cv. G4 (Figure 12C) with continuous light bolting was delayed rather than reduced. At a photophase of 18 h, no bolting took place. In the vernalized plants of G4, devernalization seemed to have less influence, probably the applied cold treatment of 52 days had induced this susceptible genotype so much that other factors did not have much influence any more (Figure 12D).

With continuous light in the unsusceptible cultivar G1, warmth was more effective in preventing bolting if it was earlier (Treatment 4) than if it was later (Treatment 5, Figure 13A). However, a later interval of warmth also reduced bolting, if the light phase in the earlier interval was shorter

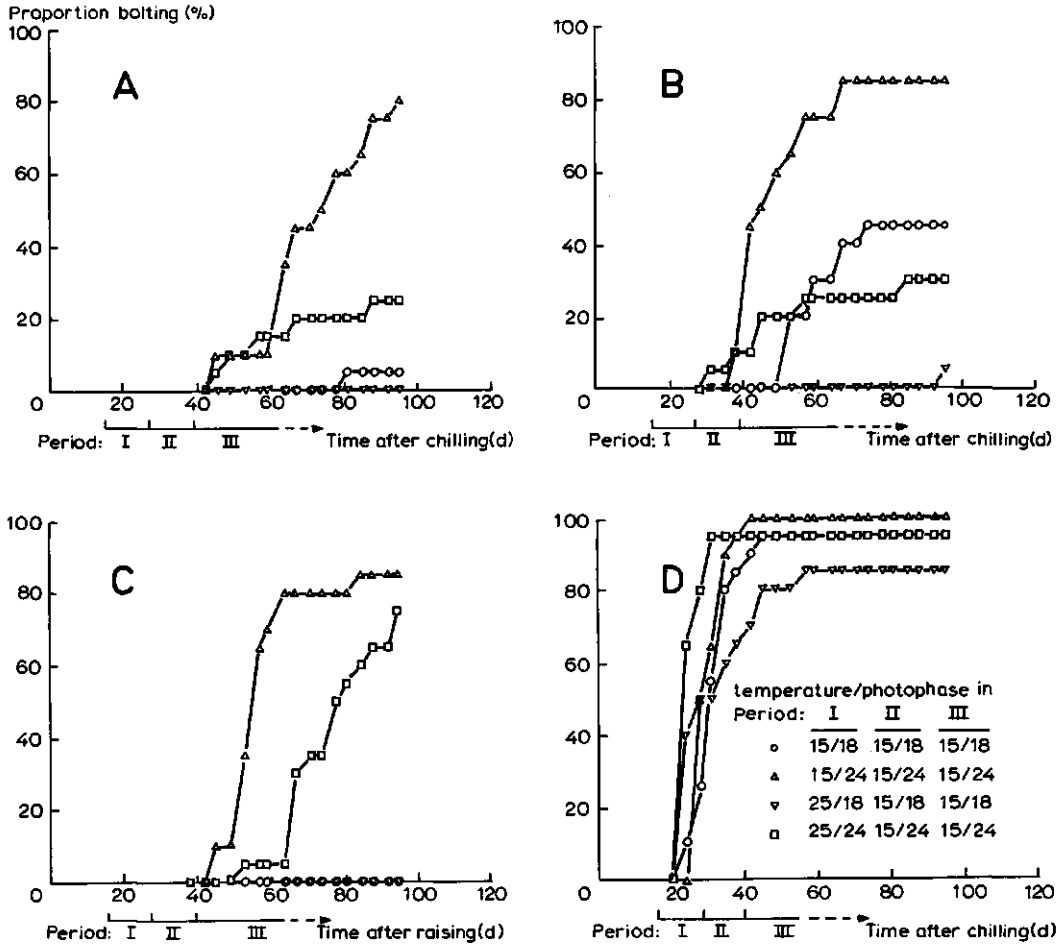

Fig. 12. Bolting in Treatment 1 (0), 2 (Δ), 3 (∇) and 4 (□) of Trial 1 (Table 11). A. Cultivar G1; B. Cultivar G3; C. Cultivar G4, unchilled; D. Cultivar G4, chilled.

(Treatment 5 and 6).

In the more susceptible genotype G3 (Figure 13B), later warmth did not reduce bolting at all (Treatment 5), except if the early light phase was short (Treatment 6).

Unchilled plants of the most susceptible cultivar (Figure 13C) reacted towards warmth like chilled plants of the moderately susceptible cultivar (Figure 13B). However, devernalization shifted the bolting to a later date and did not prevent ultimate bolting of all plants. Probably this effect was associated with a better 'vernalizability' than G3 under the cool conditions of Period III. After some time under the conditions of that period (15 °C, 24 h total light phase) the plants can be considered as vernalized. Bolting is then rapidly complete. For this cultivar intervals of high temperature retard this date of being vernalized.

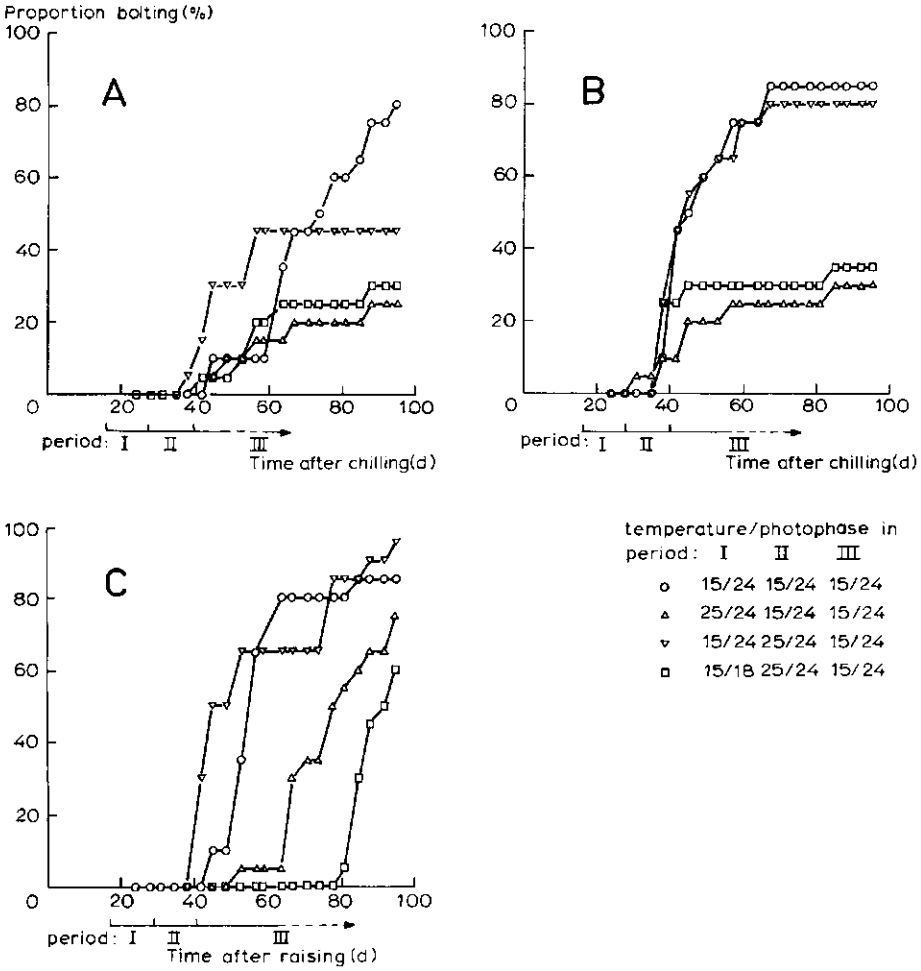

Fig. 13. Bolting in Treatments 2 (0), 4 (Δ), 5 (∇) and 6 (□) of Trial 1 (Table 11). A. Cultivar G1; B. Cultivar G3; C. Cultivar G4, unchilled.

An attempt will be made now to examine the results of this experiment in a more quantitative way, with respect to the presumed processes occurring in the plants. The question arises how the different effects of high temperature in Period I or II can be explained. (Treatments 2 or 4 and 5, all three treatments having a constant photophase of 24 h throughout the trial.

If in Period I temperature is raised to 25 °C, this has consequences for the destruction rate of V, but also for the rate of synthesis of F. Both processes will be enhanced by high temperature. However, since the rate of synthesis of F is also directly dependent on the amount of V, high temperature has a positive influence on synthesis of F in a direct way (via temperature dependence of the true synthesis of F) and an indirect (negative) way (via the amount of V in the plants). When at the end of Period I V is

depleted totally and not enough V can be produced any more in following periods, no further synthesis of F takes place in succeeding periods. This explains the curves for Treatment 4: not enough F has been produced at the end of Period I to allow complete bolting in cultivars G1 and G3.

When the devernalizing temperatures act upon the plants in the second period, the amount of F produced in the previous period governs how far bolting is reduced. For genotype G3 this amount was clearly sufficient to induce bolting. Probably also in this genotype, V is destroyed in the second period, but this will have less effect, because enough F has already been synthesized in the previous period to surpass the bolting threshold for 80 % of the plants. In such a way, one can also explain why high temperatures some time after vernalization can still reduce bolting, as long as shorter photophases prevail in the previous period.

Comparing Treatment 5 with 6 and remembering that Process 3 proceeds at a much slower rate in Treatment 6, one can see the consequences for the amount of F at the end of Period I. In the plants of Treatment 6, the destruction by high temperature in Period II does have an effect, because the level of already synthesized F is still too low for many of the plants. In this treatment, synthesis of additional F will also be reduced because of the lower amount of V. The final amount of F is clearly then too low to allow bolting to a level comparable to Treatments 5 or 2.

So stabilization of the vernalized condition in sugar-beet, if any, depends not on time, but especially on photophase.

5.5.2 Trial 2

5.5.2.1 Materials and methods

Plants of cv. G1 and G2 were raised as in Trial 1 but at 20 °C initially. Six days after sowing the temperature was lowered to 15 °C for 8 days, then plants were chilled to 10 °C for 4 days, 6 °C for 2 days and 3 °C for 54 days. The temperature was then raised to 10 °C; after 2 days, the plants were transplanted and transferred to growing rooms; and after another 8 days the temperature was raised to 15 °C and chilling ended. The cold induction was thus probably stronger than in the previous trial. After 12 days (photophase 14 h + 0 h = 14 h), treatments for time intervals of 8 days began (in contrast to 12 days in the previous trial) (Table 12). As before, unchilled plants of G1 and G2 were included in Treatments 2, 4 and 6, but none of the plants bolted during the experimental period. Unchilled plants of G4 were present in all treatments. After the first interval, all plants except of Treatments 7 and 8 were subject to the same temperature of 15 °C and to continuous light. Treatments 7 and 8 measured the effect of a later interval of warmth after different light phases in the first interval.

Table 12. Temperature and light phase from the time of ending of cold treatment in Trial 2.

Treatment	Time interval (d)		
	12-19 (Period I)	20-27 (Period II)	28-end (Period III)
1 Temp. (°C)	10	15	15
Phase (h)	14	24	24
2 Temp. (°C)	10	15	15
Phase (h)	24	24	24
3 Temp. (°C)	15	15	15
Phase (h)	14	24	24
4 Temp. (°C)	15	15	15
Phase (h)	24	24	24
5 Temp. (°C)	25	15	15
Phase (h)	14	24	24
6 Temp. (°C)	25	15	15
Phase (h)	24	24	24
7 Temp. (°C)	15	25	15
Phase (h)	14	24	24
8 Temp. (°C)	15	25	15
Phase (h)	24	24	24

5.5.2.2 Results

As expected, the lower the temperature in the first interval, the more and the sooner bolting appeared (Figure 14A, Treatment 1, 3 and 5). High temperatures later (Treatment 7) had far less effect than earlier (Treatment 5). By contrast, with continuous light during the first interval, the higher the temperature in Period I, the sooner bolters appeared (Figure 14B). With continuous light in Period I, high temperature (Figure 14B, Treatment 6) did not reduce early bolting although later a lower final value was reached than with the lower temperature treatments. That picture was common to the two unsusceptible cultivars (Figures 14A, B, C, D).

The unchilled plants of G4 in the sequence of light phases 14 h, 24 h, 24 h (Figure 14E) behaved like chilled plants of G1 and G2. In continuous light (Figure 14F) the pattern was somewhat different, in that Treatment 4 and not one of the high temperature treatments gave (as in Figures 14B, 14D) the earliest bolters.

An interpretation in terms of the model would assume that when plants were subjected to a photophase sequence of 14 h, 24 h, 24 h (Figures 14A, C), synthesis of F in Period I would be low because of the rather short day-

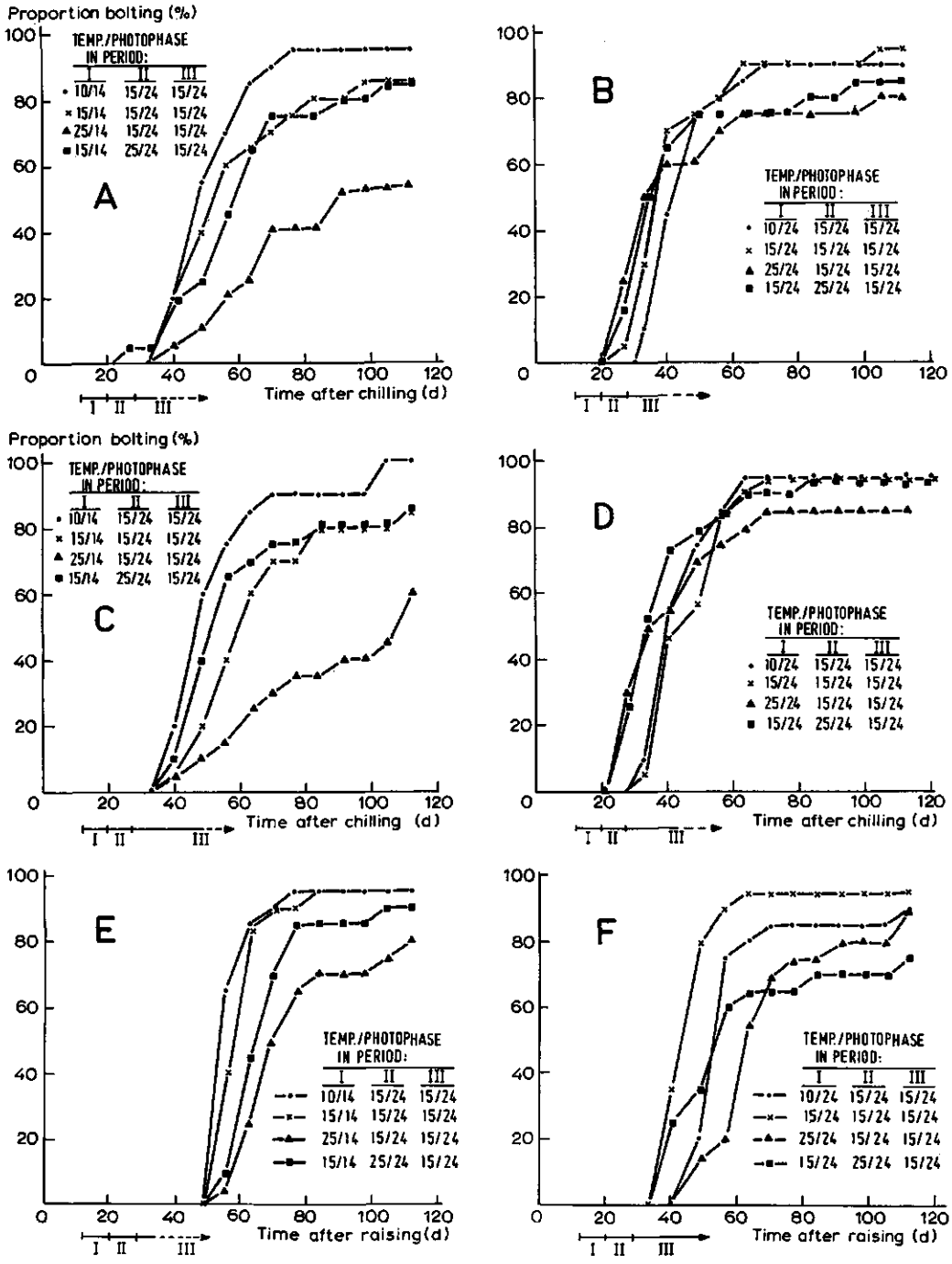

Fig. 14. Bolting in the cultivars G1, G2 and G4 as influenced by temperature and light phase after chilling (G1 and G2) and after raising (G4).
A. Cultivar G1, Treatments 1 (●), 3 (X), 5 (▲) and 7 (■)
B. Cultivar G1, Treatments 2 (●), 4 (X), 6 (▲) and 8 (■)
C. Cultivar G2, Treatments 1 (●), 3 (X), 5 (▲) and 7 (■)
D. Cultivar G2, Treatments 2 (●), 4 (X), 6 (▲) and 8 (■)
E. Cultivar G4, unchilled; Treatments 1 (●), 3 (X), 5 (▲) and 7 (■)
F. Cultivar G4, unchilled; Treatments 2 (●), 4 (X), 6 (▲) and 8 (■)
Treatments are defined in Table 12.

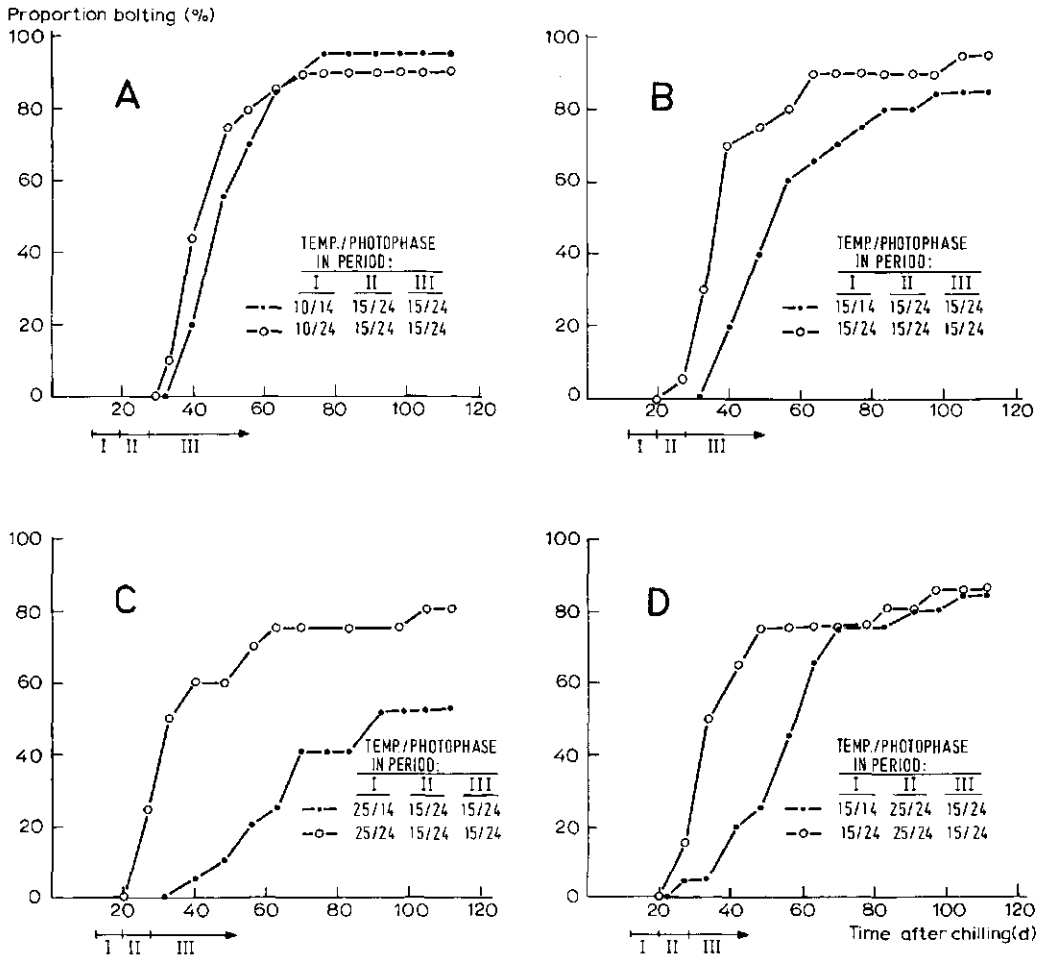

Fig. 15. The influence of photophase in Period I (14h (0) and 24h (0) on the proportion of bolters of genotype G1 at the various temperatures in the same Period 10 °C (A), 15 °C (B), 25 °C (C) and 15 °C (+25 °C in Period II) (D).

length in that period. The amount of V, at the beginning of Period II; which is influenced by temperature in Period I, will then greatly influence final yield of F. When, however, photophase in Period I is 24 h (Figures 14B, D), synthesis of F and destruction of V will take place concomittantly in this period. Considering Treatment 6 (25, 15, 15 °C), the only conclusion can be that at a high temperature V is in fact broken down at a certain rate, but in the mean time, before V is depleted completely, rapid synthesis of F can take place (in contrast to Treatment 5) because both (high) temperature and photophase favour this synthesis. Obviously the result is that Treatment 6 is the first all treatments to reach the threshold level. In Figures 15 and 16 the main features are given of the response of synthesis of F to temperature and V-level. In these graphs the 2 photoperiodic treatments in Period I

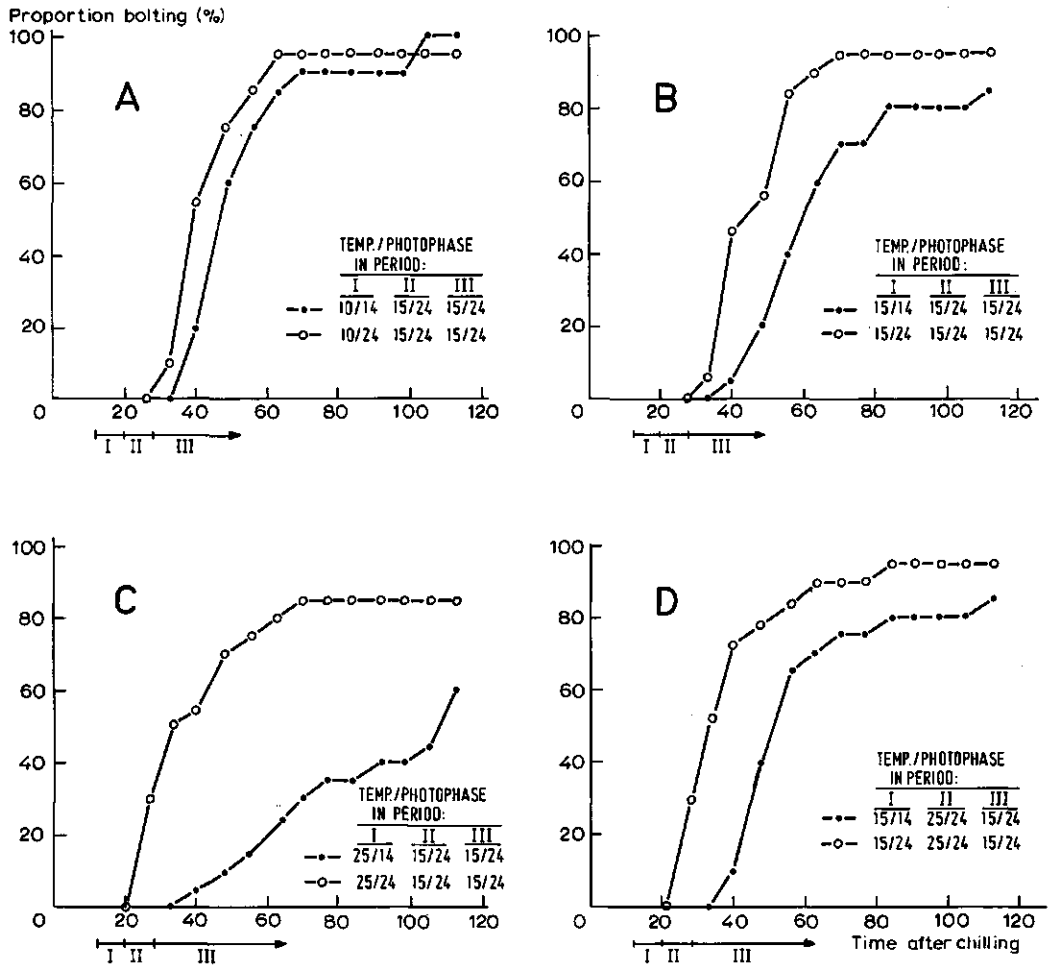

Fig. 16. Like Fig. 15, now for genotype G2.

are drawn for each temperature sequence. When in Period I temperature is low (10 °C) (Figures 15A, 16A) little difference in reaction between 14 and 24 h is observed. Obviously in both treatments synthesis of F is almost at the same level, despite the difference in photophase: the low temperature reduces synthesis of F in this period. When temperature in Period I is higher (Figure 15B, 16B) the effect of the difference in photophase in Period I becomes more pronounced, which is in agreement with the assumed temperature dependence of synthesis of F. At still higher temperatures in Period I (Figure 15C, 15D, 16C, 16D) not only temperature dependence of synthesis of F is involved, also the dependence on the amount of V accounts for the difference in the ultimate amounts of F synthesized and the corresponding number of bolting plants.

In unchilled plants of G4 a somewhat different situation will occur. One

may assume that at day 0 no V is present. Then high temperature in Period I will only *delay* V-synthesis, because in this genotype it may be assumed that afterwards at 15 °C V-synthesis will still take place. As can be seen in Figure 14F, 15 °C is optimal for rapid bolting, possibly because both synthesis of F and V proceed at this temperature, while at 10 °C though synthesis of V may be faster, synthesis of F is slower. Higher temperatures cause these plants, in contrast to chilled plants of G1 and G2, to bolt later in continuous light.

The difference may be explained by assuming that the amount of V is still rather low at the beginning of the high temperature interval, whereas in G1 and G2, the high amount of V at the start of 25 °C already allows synthesis of F right from the beginning and so induces bolting more rapidly.

5.6 DISCUSSION

5.6.1 Introduction

This section summarizes the main effects before, during and after vernalization, and discusses their physiology in the light of the model.

5.6.2 Seedling stage

Is there a minimal size sugar-beet plants must reach before vernalization can take place? The trials suggested no true juvenile stage, though vernalization seemed less effective in very young plants. This lowered effectivenes is less pronounced than stated by Margara (1960; 1968) who did not succeed in bringing sugar-beet plants to flower, when vernalized in the cotyledonary stage. Also Junges (1959) and Chroboczek (1934) detected such a juvenile stage in red garden beet. Both authors, however, could not avoid high temperatures after vernalization. Especially the early vernalized plants can also perhaps be devernalized more easily. Heide (1973) on the other hand showed results in beetroot that were similar to the effects described in Section 5.1. Despite a somewhat lower vernalizability in the germination stage, a great part of the vernalization process in practical sugar-beet growing should take place before emergence, as judged by the temperature course in most years.

The depressed vernalizability of germinating seeds may be due to shortage of carbohydrates. These are, according to Purvis (1944), essential for vernalization. Figure 7A, B, C, D showed that pre-germinated seeds kept for 2 days at 20 °C were slightly inferior in bolting than unpre-germinated seeds. Especially this pre-germination at a rather high temperature may have resulted in a low carbohydrate content, with consequences for the final proportion of bolters in this treatment. Also Wellensiek (1964b), who vernalized roots of *Cichorium intybus*, found negative effects of previous exposure to

20 °C, and thought it might have diminished the substratum for the vernalization process. Highkin (1956) reports a retarding effect of a pre-treatment at 20 °C or 26 °C for up to 5 days before the optimum cold treatment for peas. This treatment resulted in a progressive loss of the ability to be vernalized.

Besides causing a shortage of carbohydrate, high temperatures before vernalization could also have an effect similar to devernalization, assuming that plants have an 'initial degree of vernalization'. Raising at rather warm temperatures determines then:

- loss of the initial degree of vernalization
- increase in vernalizability (because of the larger plants)

In that way it is more understandable that the first appearing bolters were observed in treatments vernalized at the youngest stage (Section 5.1.4) (the plants kept their initial "degree of vernalization") but the highest final proportion of bolters were found when vernalized as larger plant sizes (because they could be vernalized easier).

5.6.3 Vernalization

Vernalization (accumulation of V in the model) was faster, the lower the temperature (Figure 8), even to 3 °C. There was no interaction between cultivars and vernalization temperature.

In this trial a long-day influence *during* vernalization was precluded. A longer photophase during vernalization would have favoured especially the treatments at 7 °C and 11 °C, because according to the model such temperatures allow both vernalization and synthesis of F. This explains the shift towards higher optimum temperatures of vernalization, as found by Curth (1962) when a photothermal treatment was applied (simultaneous action of low temperature and long photophase). Gaskill (1952) mentions an optimum temperature of vernalization of 9 °C, but in fact he too applied a 24 h photophase during chilling. Recently Lasa & Silvan (1976) reported 8 °C as optimum, also with a photophase of 24 h. It explains further also why Heide (1973) found that a long photophase during vernalization prevented subsequent devernalization to some extent.

The question arises whether a constant temperature during vernalization, as is usual maintained in trials, is the most effective procedure. In bolting-resistant cultivars, it might be useful first to create a high amount of V at 3 °C (the duration of this period could be adjusted according to the susceptibility of the cultivar) and to 'convert' it cautiously in the second part of the cold treatment, at somewhat higher temperatures and with long days, to the more stable substance F (without destroying V by a too high temperature). Even daily fluctuations in temperature might be more ef-

ficient. Wellensiek (1979) reported that vernalization of *Silene armeria* L. during only the dark period was even more effective than vernalization during the whole day.

Without cold-treatment and at temperatures as high as 15 °C but under continuous light, bolting is bound to start after a long period, also in the most bolting-resistant single-cross. Obviously some vernalization proceeds at these high temperatures, but at such a low rate that bolting only starts at extremely long photophases and not before 6 months after sowing. Vernalization does not seem to be a process restricted to the low temperature, as is usually thought, but proceeds slowly, even at 15 °C. It might be a common process also in plants without a quantitative or qualitative cold requirement. Heide (1973) also mentions that in beetroot, though generally more liable to bolt than sugar-beet, a constant temperature of even 18 °C under continuous illumination triggered flowering.

5.6.4 Post-vernalization

According to the proposed model, the synthesis of the final flowering substance in the post-vernalization period is dependent on several factors, which also interact. This makes it almost impossible to discuss the different processes like devernalization, stabilization and photoperiodical influences separately. When only length of cold treatment and light phase after vernalization were varied, these two factors were more or less interchangeable (Figures 9 and 10). According to Fife & Price (1953), these factors can even be interchanged completely, as extremely long chilling periods of 100-300 days could provoke flowering in complete darkness. The reverse seems to be possible also (unchilled plants flowering under continuous light, e.g. Figures 8 and 9). According to the results shown in Section 5.5, temperature conditions immediately after vernalization were crucial for final proportion of bolting plants. High temperatures immediately after cold treatment strongly inhibit flowering. Yet there is an important interaction with photophase as in Trial 1 (Section 5.5.1). Also after a period of neutral (stabilizing in the literature) temperatures, devernalization remains possible, provided the plants received a shorter light phase in the neutral period. For practical sugar-beet growing, this would mean that especially in early spring with a daylength of only 14-15 h as in the Netherlands, days with a high maximum temperature would thus be highly effective in preventing bolting.

If thermostable end-products of the vernalization process do exist, as proposed by Napp Zinn (1957) for *Arabidopsis* and Devay et al. (1976) for winter wheat, their rate of synthesis should depend not only on temperature but also on photophase. For sugar-beet, within the quantitative and momentary approach of the model, there seems to be no necessity to assume other intermediate substances than the final flowering substance.

One of the properties of the presented model is the positive temperature

dependence of photoperiodic action, which means that photophase is of less or no influence at very low temperatures. In practice, this would mean that photophase during vernalization was of minor importance because of the usually low temperatures in this process. When vernalized at 3 °C the plants showed no significant difference between light phases of 14 and 24 h during cold treatment (Section 5.4). Voss (1940) also reported that photophase during chilling had no influence on bolting. Heide (1973) on the other hand, mentions that in beetroot, already at 5 °C a 24 h photophase prevented devernalization slightly in the post-vernalization period. Hence photophase plays a major role at moderate temperatures after vernalization (Figure 9B). In the described trials the difference between 18 and 24 h seems to be more pronounced than indicated by Curth (1960).

5.6.5 Physiological background of the model

It is worthwhile to recall some recent physiological results, that support the validity of the model. It must, however, be borne in mind that many remarks or assumptions have not been tested experimentally directly and are only suggestions.

Why does the model assume 2 substances to be active? Could not low temperature and a long photophase influence the synthesis of only one substance, because photophase and temperature are more or less complementary? Arguments against such a concept are the different sites of perception (apex and leaves) and the existence of a major interaction between vernalization and photophase. Vernalization plus photophase are much more effective than vernalization or a long photophase alone. The fact that long light phases after vernalization have far more effect than before vernalization suggests that cold treatment influences subsequent reaction towards photophase. Although it is suggested in the proposed model that V is a specific substance that accumulates during vernalization, there is hardly any evidence for such a substance, as many grafting experiments have failed. Schneider (1960) found that partial plant vernalization (bud vernalization) in sugar-beet did not lead to flowering in unvernalized buds, which shows the untransferability of vernalization.

Therefore it has been thought that vernalization, rather than producing a substance, brings forth a certain condition, which is transmissable by cell division only (Barendse, 1964). If the plant is in a different condition after vernalization, what would be the effect? One possibility is to assume that the apex region has obtained an increased sensitivity towards flowering hormone(s). In this view the growing point would react (by differentiation into stem and flower buds) only after vernalization to substances exported by leaves in long-day conditions. This idea is, however, unlikely since the grafting experiments of Curtis & Hornsey (1964) and Margara (1960) showed that unvernalized growing points could be brought into flower,

provided they were grafted on a flowering plant, which indicates that also unvernalized apices can be brought into differentiation. Instead it is more likely that vernalization is a process necessary for subsequent synthesis of the final flowering hormone in leaves grown out of such a vernalized growing point. The rate of this synthesis, moreover, seems to depend on the length of the photophase. The existence of transmissable hormones exported by leaves is, in contrast to the vernalization substance, beyond doubt (Lang, 1965).

So the amount of V in the model could quantitatively represent a certain condition of the plants, rather than a substance, as has been suggested until now, mainly for simplicity. What could be the nature of such a plant condition? Wellensiek (1977) discussed the principles of flower formation in general and distinguished between the following groups of genes.

- Flower-forming genes: a collective name for all genes influencing flower formation in some way.
- Flower-hormone-forming genes: those genes that are mainly active in the leaves by production of floral hormone.
- Floral genes: genes which act in the apex and determine, for instance, shape and colour of the flowers and shape of the inflorescence.

Wellensiek discussed the possibility that certain groups of genes are blocked, repressed or inhibited: 'this blocking is immobile, in the sence of not-translocated, and it may occur in different quantities, in different intensities, at different levels'. Vegetative plants presumably produce leaves in which the genes that produce flower hormone are inactive by a blocking. This blocking can be prevented, however, by a period of low temperature. Leaves growing out of a vernalized growing point, carry the genes forming flower hormone in a more or less deblocked state. The longer the cold treatment lasts the more the flower-hormone-forming genes of the subsequently appearing leaves are deblocked. The deblocked state is maintained by cell division, unless reblocking takes place at high temperature, which corresponds to the devernalizing action of high temperatures. In agreement with this is the conclusion of Curth (1960) that the site of perception of devernalizing temperatures also lies in the apex.

Assuming that blocking of such specific genes can occur at different intensities, it would not lead to an elementary change in the model if substance V in the model were replaced by a variable V indicating the intensity of deblocking (of flower-hormone-forming genes). Should this intensity refer to the cellular or the plant level? Looking at a cellular level, the flower-hormone-forming genes themselves might then be blocked in different intensities. If it would turn out that only two states of blocking exist, blocked and unblocked, the intensity of deblocking might be considered at plant level, where it might represent the ratio between the number of blocked and un-

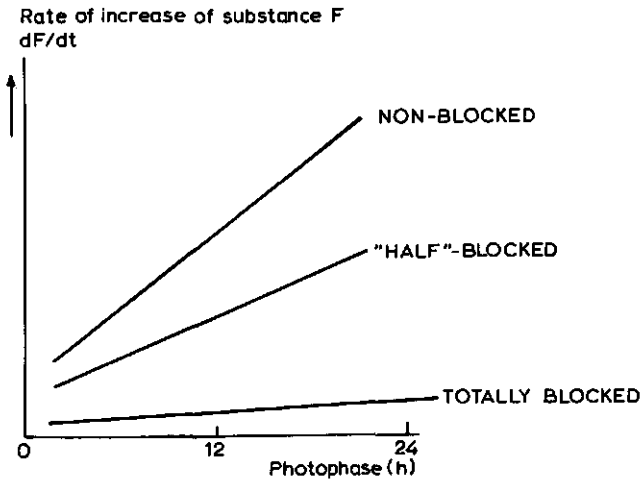

Fig. 17. The hypothetical relation between the intensity of blocking of flower-hormone-forming genes, photophase and the rate of synthesis of the hypothetical flower hormone(s).

blocked cells. One may assume that the ratio of unblocked to blocked cells would shift if vernalization were longer. Wellensiek (1964a) also mentioned competition between the number of vernalized and unvernalized cells.

Without the occurrence of devernalizing temperatures, leaves grown from a vernalized growing point can then export final flowering substances to the apex, where they play a role in the differentiation into stem or flower buds. Whether these deblocked leaves would export these final flower promoting substances or not is, however highly dependent on photophase. The following scheme could elucidate the relation between blocking and photophase (Figure 17). Such a relation could account for the complementary action of low temperature and photophase. As can be seen, photophase determines the rate of synthesis of F in dependence on the intensity of blocking of the flower-hormone-forming genes.

The model also assumes that true synthesis of F is dependent on temperature. Salisbury (1963, p. 163) described the 'photoperiodical' process as being temperature sensitive. Not only the synthesis of the flower hormone, but also sensitivity to the hormone might be temperature-dependent. Stoddart et al. (1978) for example found in lettuce hypocotyls a sharp decrease in the response to gibberellic acid at temperatures lower than 13 °C. This positive temperature-dependence is usually masked in plants with a cold requirement. In these plants, high temperatures have a twofold effect after vernalization: more rapid initial synthesis of F and enhancement of the reblocking of the flower-hormone-forming genes (devernalization).

The model further assumes that the flower hormone F accumulates until a certain level is reached, after which differentiation of the apex starts. The validity of this assumption is supported by Zeevaart (1976) who inter-

preted grafting experiments in *Perilla* by King & Zeevaart (1973) to mean that the stimulus has to accumulate to a threshold in the apex before flower formation can take place. The flower hormone will act in the apex where a possible deblocking of the floral genes will take place, resulting in differentiation into stem and flower buds.

It remains to be seen whether only one substance is involved. Bernier (1976) offers a plausible picture, showing the existence of at least two components in the floral hormone in *Sinapis alba*, one of them being cytokinin. In sugar-beet, it could be imagined that one of the components is a gibberellin-like substance mainly active in stem elongation.

More attention should be given to the consideration that flowering is the final result of a strictly quantitative process. This might connect various response types (e.g SD plants, LD plants, with or without a cold requirement) to the same basic principles.

6 Relation between growth and bolting of sugar-beet

6.1 INTRODUCTION

External factors that encouraged growth also enhanced bolting, for instance low plant density (Jorritsma, 1978; Warne, 1949), heavy nitrogen dressing (Gorodnii & Sereda, 1975; Mann, 1951; Hoekstra, 1960; Ludecke, 1938; Lysgaard, 1978) and irrigation (Röstel, 1968).

Generalizing, one could assume that almost any external factor, that stimulates growth after vernalization could lead to more bolters. This would also imply that plants in a field crop with locally better growing conditions (e.g. more nitrogen, more space or early emergence) run a greater risk of bolting. Such a relation could well account for the greater plant weight of bolting plants, as found by Lysgaard & Holm (1962) who showed that late bolters had considerably higher root and top weights than vegetative plants. The smaller root weight at the final harvest, observed for early bolters no doubt has been caused by the changed dry-matter distribution after onset of stem elongation. One could imagine that early bolters also show a higher plant weight, when measured immediately after visible stem formation. In the following some data will be given on this subject.

More rapid growth of bolting plants before visible stem elongation need not result only from better external growing conditions but also from quite different factors. In the literature, some evidence can be found that plants that have advanced more towards flowering show an increased growth. Behaeghe (1975) observed growth differences between vernalized and unvernalized *Lolium perenne* and *Dactylis glomerata*. Vernalized plants showed an increased top growth, their specific leaf area was considerably greater and net photosynthesis per unit leaf area was higher. Davies (1971) reported similar results in perennial ryegrass. Relative growth rates in swards of vernalized plants were 50 % greater than in swards of comparable unvernalized material. Davies attributed this effect to a changed distribution of the products of photosynthesis and to differences in the rate of losses of dead matter. Jones et al. (1975) reported that vernalization of a winter wheat cultivar increased the photosynthetic rate by 16 %, in contrast to a spring wheat where no such increase was measured.

Such observations could indicate that the advanced state of flower induction itself or other circumstances leading to flowering also promote growth of the plants. Such a relation would *also* lead to higher weights of bolting plants, assuming that especially those plants more sensitive to low

temperature and photophase and therefore further advanced to flowering would show a more vigorous growth. So one may suspect that selection for bolting resistance by breeders could have a negative effect on vigour. Selecting for vigorous growth would, inversely, increase bolting susceptibility. If such a relation should exist, breeders of sugar-beet are caught in a vicious circle.

For carrots, with a similar relation between bolting and growth, Dowker & Jackson (1975) suggested that a check was necessary whether a selection for reduced bolting does not lead to an undesirable reduction in growth rate in the selected lines. A very clear example was given by Parlevliet (1967) in spinach cultivars. Parlevliet determined the growth rate of five groups of spinach cultivars, differing in earliness. Earliness in spinach can be taken to be similar to bolting susceptibility in sugar-beet. He found that after equal growing periods, the later the cultivar (more bolting-resistant), the lower its yield. Parlevliet also reported that selection for late bolting in a population of mainly fast-growing plants almost inevitably leads to slower growth. If, however, selection for fast-growing plants was carried out in a population of mainly late-bolting plants, the population will become earlier.

However, the literature provides no clear evidence that such a relation also holds for sugar-beet. This chapter reports some trials on the influence of external growth stimulating factors, such as additional nutrients and irrigation.

6.2 INFLUENCE OF CONDITIONS OF GROWTH ON BOLTING

6.2.1 Introduction

Considering the positive effect of extra mineral nitrogen on bolting, a first question was whether this effect was associated with the sluggish response to the vernalizing action of low temperature during the early stages of growth. Nitrogen could play a role by accelerating progress towards the stage that is more sensitive to cold. However, differences in growth caused by nitrogen usually appear after the periods of vernalizing temperatures. Therefore the influence of nitrogen on bolting might be mainly active after vernalization.

6.2.2 Materials and methods

In 1976 and 1977 trials were done to study the relation between growth and bolting. In 1976 a trial of factorial design (split plot) was set out at Wageningen (Haarweg) in three replicates, being sown on 25 February with cultivar G1 with a Stanhay precision drill, with rows 50 cm apart and seeds 5 cm apart in the row. After emergence on 8 April the plants were thinned

to a stand density of 10 m^{-2}. In the trial the following factors were investigated:

1 Irrigation (main factor):
R0 = control
R1 = irrigated
The crop was regularly irrigated from 5 May to 1 July. In total, the irrigated plots received 145 mm of water. After 1 July, all plots were irrigated as necessary.

2 Rate of N:
N1 = 25 kg/ha
N2 = 100 kg/ha
N3 = 175 kg/ha
N4 = 250 kg/ha
N1 + T = 25 + 75 = 100 kg/ha (T = second time of application)
N2 + T = 100 + 75 = 175 kg/ha.

On 15 March the whole field was dressed with 250 kg of 43 % superphosphate and 500 kg of K-40. On 31 March, before emergence, nitrogen was applied at 25 (N1) and 100 kg/ha (N2, N3 and N4). Some plots given 100 kg/ha were given a further 75 or 150 kg/ha after emergence on 29 April (N3 and N4). For the top-dressing treatments, N1 + T and N2 + T, the first dressing of 25 and 100 kg/ha, respectively, was given on 31 March, together with the N1-N4 treatments, the remainder of 75 kg/ha was given on 15 June.

3 N source. The nitrogen was supplied as either
S1 = $Ca(NO_3)_2$ or
S2 = $(NH_4)_2SO_4$
To avoid any differences in pH, an additional dressing of 0.61 kg of acid-binding material was given for each kilogram of ammonium sulphate.

The trial consisted of 72 field plots, each of 6 m x 7 m. Each plot was divided into two parts, one half used for periodical harvests of vegetative plants on the following dates: 19 May, 24 June, 21 July, 25 August and 14 September. On these dates samples of 10, 20, 20, 20 and 40 plants, respectively, were taken from each plot.

In the other half of each plot with about 150 plants, bolting plants were harvested almost weekly on the following dates: 14 June, 24 June, 1 July, 9 July, 15 July, 22 July, 29 July, 5 August, 12 August, 25 August and 14 September. On each side of the plot, 2 rows were left as border rows, to avoid interferences between plots. For the same reason plants were left unharvested between successive gaps resulting from harvesting.

Also in the following year (1977) dry weights of bolting plants were compared with those of vegetative plants. For this purpose the two single-crosses G1 and G2 were sown on 16 March at Achterberg. For each cultivar four plots of 9 m x 14 m were sown, which were thinned on 25 and 26 May to a stand of 10 m^{-2}. During the growing season 25 vegetative plants per plot were harvested at regular intervals together with bolting plants, which had started bolting since the previous harvest. From both groups of plants, dry weights were calculated.

6.2.3 Results

Effects on plant growth were as follows. Due to the low temperature in the month of March 1976, field emergence did not take place before 8 April. During this period, vernalization of the germinating seeds was probably strong. After emergence, however, an unusually dry and warm summer ensued which no doubt reduced the potential number of bolting plants by devernalization. Especially in the first week of May, when the plants had reached the two-leaf stage, there were several days with high maximum temperatures.

Irrigation until July encouraged growth early in the season (Table 13). Afterwards the irrigated plots did not maintain their lead and ultimately there was no significant influence of irrigation. Especially with little N, growth was even retarded, perhaps because of poor rooting in the irrigated plots, which would have had influence in the following dry and warm summer.

Table 13. Mass of dry matter per plant at successive harvest dates as influenced by irrigation, form and rate of nitrogen dressing.

Treatment	Mass (g) on: (month-day)				
	5-19	6-24	7-21	8-25	9-14
Irrigation					
Unirrigated	0.47	38.4	105.5	193.8	206.8
Irrigated	0.58	39.2	108.6	183.9	198.9
effect	*[1)]	-	-	*	-
Form of N					
$Ca(NO_3)_2$	0.57	42.3	109.8	190.5	202.2
$(NH_4)_2SO_4$	0.48	35.4	104.3	187.2	203.5
effect	****	****	**	-	-
Rate of N (kg/ha)					
25	0.44	32.3	100.2	185.1	192.9
100	0.55	38.3	103.3	180.2	209.9
175	0.60	44.1	112.5	191.1	209.6
250	0.57	47.5	115.7	204.3	215.1
25 + 75	0.45	30.3	103.6	181.0	198.7
100 + 75	0.54	40.5	107.2	191.4	199.7
effect	****	****	***	*	**

1) *, $P < 0.10$; **, $P < 0.05$; ***, $P < 0.01$; ****, $P < 0.001$

Table 14. Mass of dried leaf per plant (g) at successive harvest dates, as influenced by irrigation and rate of nitrogen.

Rate of N (kg/ha)	Mass (g/pl.) on				
Unirrigated	5-19[1]	6-24	7-21	8-25	9-14
25	0.45	22.5	45.6	60.6	51.5
100	0.46	23.2	40.2	64.1	61.2
175	0.54	24.7	47.9	75.0	65.2
250	0.48	26.4	55.0	81.0	70.8
25 + 75	0.41	22.6	50.3	66.2	58.5
100 + 75	0.47	26.2	49.1	67.7	66.8
Irrigated					
25	0.44	16.7	38.5	58.7	48.0
100	0.64	24.7	50.1	61.6	59.1
175	0.66	32.5	57.2	67.8	65.7
250	0.66	37.4	55.1	77.8	67.5
25 + 75	0.49	16.6	44.6	63.6	53.9
100 + 75	0.61	27.2	50.7	69.6	62.9
Significance of interaction	***	****	****	-	-

1. For the harvest on 19 May total plants rather than leaves were taken.

Ammonium sulphate initially retarded growth, as would be expected. After 21 July, however, total plant weight did not differ significantly, whereas leaf weight remained different throughout the season. Especially in the first part of the season, nitrogen dressing and irrigation interacted on mass of leaves (Table 14). More nitrogen had more effect in the irrigated than unirrigated plots, as would be expected in the dry spring of 1976. Later in the season, growth increased with top-dressing but not to the extent (for mass of leaf) of early dressing at 100 or 175 kg/ha, the same total amount. In summary, irrigation up to 1 July especially had a positive influence in the first part of the season, except for the N1 treatments, where growth was reduced.

Bolting was influenced in this trial by irrigation although the difference did not reach statistical significance until 14 September (Figure 18). The form of nitrogen had no significant effect (Figure 19). Rate of nitrogen had a significant positive effect on bolting, except on 14 June, 9 July and 15 August. It had little effect in unirrigated plots (Figure 20A) but had more pronounced effect on irrigated plots (Figure 20B). Top dressings enhanced bolting, so that improved growth after vernalization is responsible for bolting rather than early stimulation of growth and consequent increased responsiveness to low temperature. The same was suggested by a greenhouse trial on the effect of nitrogen. In this trial more N after vernalization increased the proportion of plants bolting.

Final proportion of bolters was thus correlated to (leaf) growth, especially growth in June and July seemed crucial for bolting. For leaf mass on 21 July, correlation to proportion of bolters was 0.59.

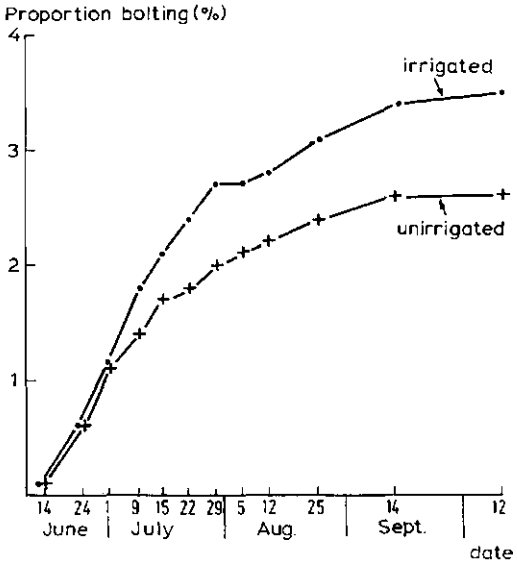

Fig. 18. Bolting as influenced by regular irrigation.

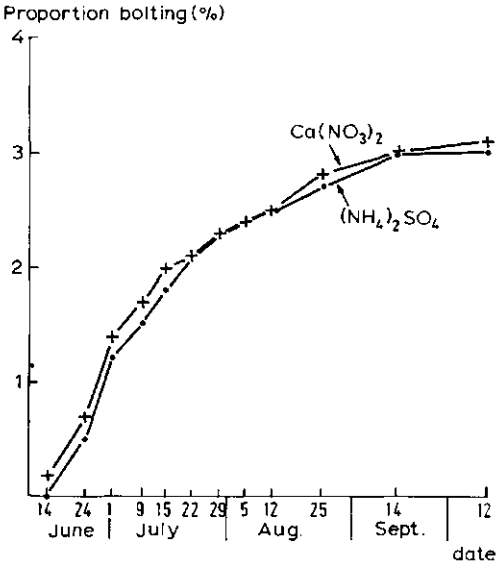

Fig. 19. Influence of two nitrogen sources $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ on bolting.

6.2.4 Plant weights of bolters and non-bolters

The mass ratio of incipiantly bolting plants to vegetative plants on the same date for each treatment (Figure 21) was usually more than 1. The weight of vegetative plants at a date between two harvests was estimated with a

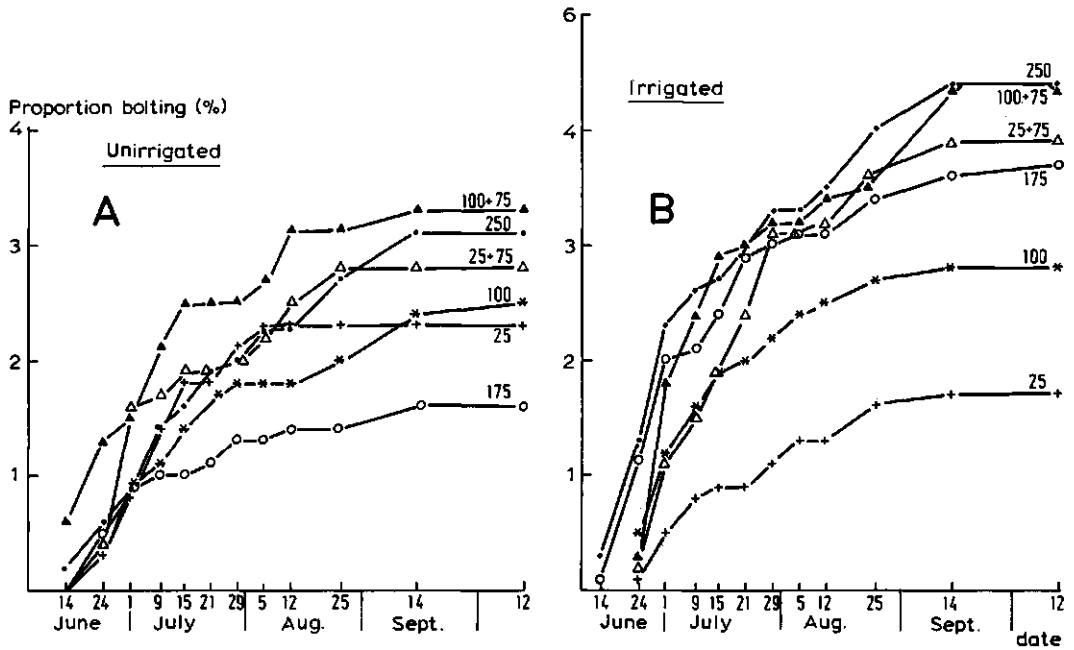

Fig. 20. Influence of rate (areic mass) of nitrogen dressing bolting.
A. Unirrigated plots. B. Irrigated plots. Labels of lines are rates of nitrogen in kg/ha either as base dressing up to 250 kg/ha or with top-dressing of 75 kg/ha (indicated by +).

regression equation (weight against time) for each of the 24 treatments in this trial. Coefficient of determination (r^2) was between 0.940 and 0.996, with an average of 0.977. Calculating the ratios for each treatment separately avoided bias from treatments, in which bolting as well as growth was increased, which would lead to an overestimate of the differences in plant weights between bolting and vegetative plants. In general, bolters were heavier than vegetative plants in the corresponding treatments. That relation held for early or late bolters. The difference was due to leaf more than to root.

The number of harvested bolting plants for each genotype in the 1977 trial can be found in Table 15, which also shows the significance of the differences in plant weight. Bolting plants turned out to have a distinct lead in plant weight throughout the season, as in the 1976 trial (Figure 22A, B).

6.2.5 Conclusions

The results presented in this section show that plants that run to seed have a larger plant weight throughout the growing season, if harvested when their dry matter distribution is still comparable with vegetative plants. The irrigation and fertilization experiment showed, however, that stimula-

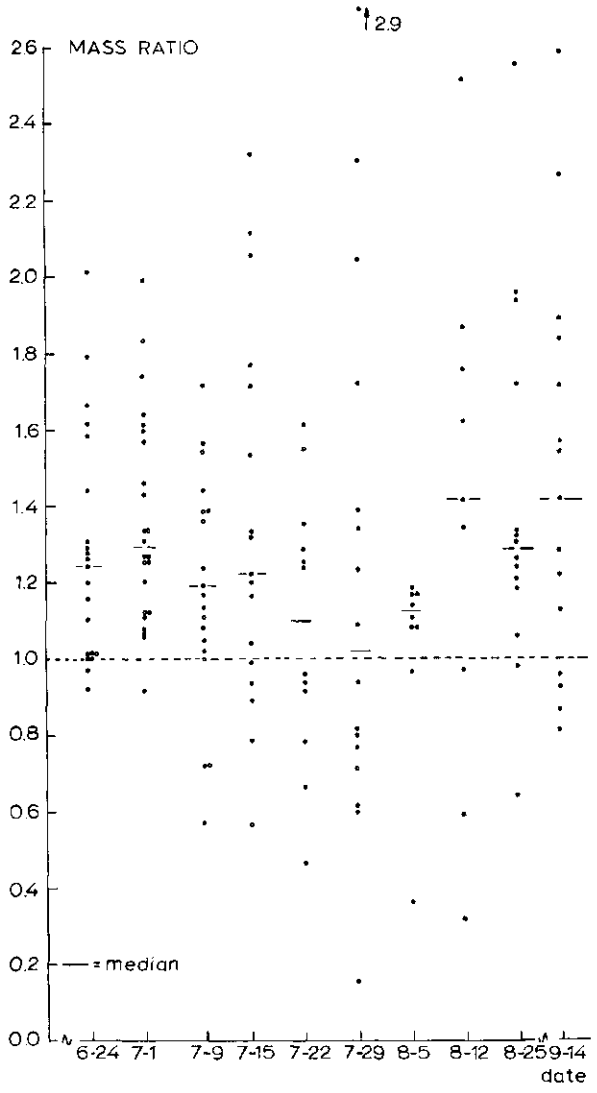

Fig. 21. Mass ratio of dry matter per beet plant with just visible bolting and vegetative plants at the same date. The median on a date is indicated by ———.

tion of growth by external factors enhanced bolting, so this larger plant weight might be largely explained by better local circumstances of growth (e.g. plant space) which would favour growth and enhance undesirable bolting. The mechanism of how growth stimulation influences bolting remains uncertain. The explanation might be that in the faster growing plants the devernalizing temperatures have a smaller influence because the threshold for bolting is reached earlier in the season. The period in which devernalizing temperatures can have their effect is then shortened.

Table 15. Number of bolters and non-bolters harvested on different dates in the 1977 trial

Cultivar	Type	Harvest date (month-year)							
		06-16	06-27	07-06	07-13	07-26	08-09	08-22	09-14
G1	bolters	5	6	26	8	22	16	16	14
G1	non-bolters	101	100	100	100	100	101	102	100
G2	bolters	2	4	39	37	67	53	58	42
G2	non-bolters	100	100	100	100	100	100	101	100
Significance of difference in plant weight between bolters and non-bolters		-	***	**	-	***	***	**	**

6.3 INFLUENCE OF COLD TREATMENT AND PHOTOPHASE ON GROWTH

6.3.1 Introduction

The observed larger plant weights of bolting plants could also be explained by assuming that especially those plants more responsive to low temperature or light phase reacted with an improved growth. To study the influence of vernalization and photophase on growth, sugar-beet plants were subjected to various conditions in growth chambers, with extreme differences in time of chilling and in photophase.

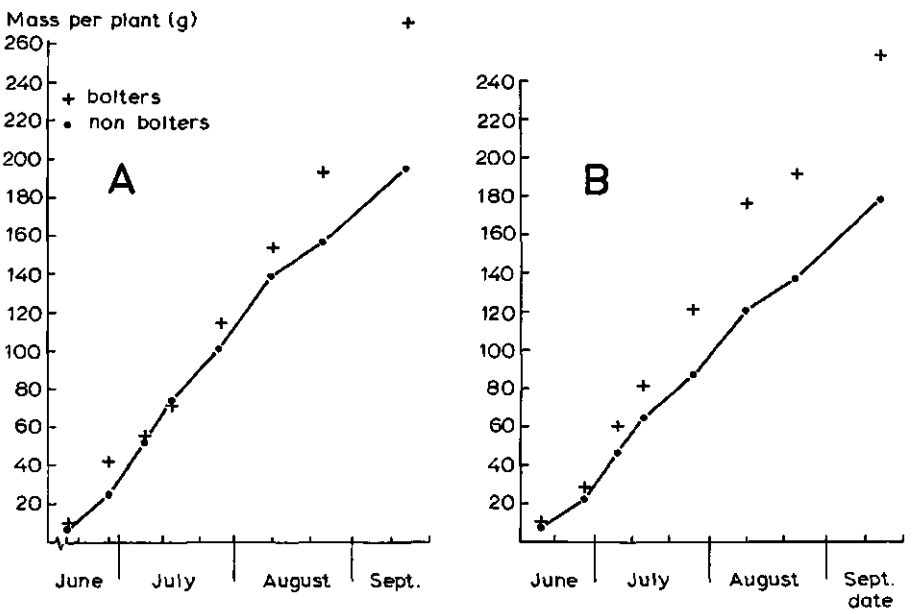

Fig. 22. Mass of dry matter per plant of just visibly bolting beet plants and (at that moment) vegetative plants.
A. Cultivar G1; B. Cultivar G2.

6.3.2 Materials and methods

The influence was investigated of two factors on growth, each in three levels (3^2 factorial).

1 Time of chilling:
  V1, unchilled
  V2, 27 days at 3 °C
  V3, 55 days at 3 °C

2 Photophase after vernalization:
  P1, 9 h + 0 h = 9 h
  P2, 9 h + 6 h = 15 h
  P3, 9 h + 15 h = 24 h

All possible 9 combinations were included in the trial.

- Raising the plants. Seeds of cultivar G1 were sown in paper pots and were placed in a glasshouse at 25 °C for 8 days, after which emergence was complete. The temperature was then lowered to 15 °C until two leaves developed three weeks after sowing.
- Vernalization. Plants were chilled in growth chambers (Section 4.3.1) at a temperature of 3 °C, total photophase 15 h + 0 h, rel. humidity 0.7. Vernalization took 0, 27 and 55 days for the three experimental groups. Sowing dates were so organized that the cold treatments ended on the same day.
- Post-vernalization. After vernalization the plants were transplanted into white plastic 6L pots, and kept at a temperature of 10 °C for three days. Before transplanting 2 x 10 plants per cold treatment were sampled and mass of dry matter and leaf area per plant were measured. Next, the plants for each vernalization group were split up into three subgroups, each henceforth receiving one of the following photophases: 9 h + 0 h = 9 h, 9 h + 6 h = 15 h, and 9 h + 15 h = 24 h at a temperature of 15 °C and a rel. humidity of about 0.80. The photophase was extended in the way described in Section 4.4. The 9 treatments consisted of 18 plants.

From these 18 plants, 16 plants were harvested on three successive harvest dates: three weeks after vernalization (4 plants), 5 weeks after vernalization (4 plants) and finally 8 weeks after vernalization (8 plants). The plants were taken at random from each group. To exclude variation within the growth chambers, the plants were placed on carts and were switched about twice per week. Differences between the growth rooms were prevented as far as possible by exchanging the plants at regular intervals between the chambers, thereby, of course, also changing the assigned photophase. This resistant genotype bolted in the V3P3 treatment after the 3rd harvest (1 plant, which was not harvested). At the 4th harvest, 3 plants showed visible bolt-

Table 16A. Mass of dry matter per plant and leaf area per plant at t = 0 for the three cold treatments.

	Treatment			
	V1	V2	V3	d.05[1]
Dry matter (mg)	13.7	31.4	92.3	20.0
Leaf area (cm^2)	1.52	2.42	6.12	1.98

1. d.05 = studentized range, (Tukey) at the 5 % - level.
V1, unchilled ; V2, 27 days at 3 °C; V3, 55 days at 3 °C

ing. As these plants were then still comparable with vegetative plants, they were included to obtain an orthogonal scheme, which facilitated the statistical procedures. At each harvest date, the following measures of dry matter were calculated: beet-root per plant, petioles per plant and leafblades per plant. Also leaf area per plant was recorded with either an electronic device at the first 2 harvests or later by scaling to photocopies of leafblades of known area.

6.3.3 Results

At the end of cold treatment (t = 0) and before submitting the plants to different photophases, dry matter and leaf area were first measured. Table 16A shows that longer chilling resulted in more dry matter and larger leaf area, because plant growth did not stop entirely at 3 °C. The differences in dry matter at t = 0 between the chilling treatments may make a later comparison spurious. For example, even when assuming exactly the same (exponential) growth for the three chilling treatments, differences in dry matter would become even greater at following dates. To overcome this problem and still to investigate the possible positive effect of cold treatment on subsequent growth, a procedure was devised for comparisons within each vernalization level, i.e. between the three light phases. Such comparisons can be made at each moment after vernalization without objection, as the (average) start position at t = 0 is the same for those treatments.

In Tables 16B, C and D, the first three lines present the main effect of chilling (averaged over the three light phases). The fourth line indicates the significance of the vernalization effect and also gives the Studentized Range (Tukey) at 5 %, for comparisons between two means. The next four lines indicate the main effect of light in the same way. The following lines of the tables show the mean for each of the nine combinations. The significance of an interaction can be read in the last line, together with the Studentized Range for comparisons between any two means.

Table 16B. Harvest 3 weeks ($\underline{t}$ = 3) after end of chilling.

Treatment	Leaf blade (g)	Petioles (g)	Beet root (g)	Total dry matter (g)	Leaf area (cm^2)	SLA ($cm^2 g^{-1}$)	LAR ($cm^2 g^{-1}$)	Petiole fraction of sprout (%)	Number of leaves
V1	0.21	0.07	16	0.30	73	349	243	25.1	7.3
V2	0.32	0.10	46	0.47	94	296	200	24.8	7.4
V3	0.57	0.21	86	0.86	164	299	193	27.2	9.2
d.05	0.09	0.04	24	0.14	26	30	17	-	1.1
	****	****	****	****	****	****	****	-	***
P1	0.41	0.09	49	0.55	106	265	200	17.7	7.8
P2	0.40	0.14	58	0.60	123	323	216	26.4	8.3
P3	0.30	0.15	43	0.49	103	356	221	33.0	7.8
d.05	0.09	0.04	-	-	-	30	17	3.4	-
	***	***	-	-	-	****	**	****	-
V1P1	0.24	0.05	22	0.30	69	288	224	16.2	7.0
V1P2	0.19	0.07	14	0.28	68	351	244	26.4	7.8
V1P3	0.20	0.10	13	0.31	83	408	262	32.7	7.0
V2P1	0.31	0.07	38	0.41	79	256	191	18.1	7.0
V2P2	0.44	0.13	68	0.64	129	295	203	23.4	7.8
V2P3	0.22	0.11	33	0.37	76	337	206	32.8	7.5
V3P1	0.68	0.16	86	0.92	170	250	184	18.6	9.5
V3P2	0.56	0.23	91	0.88	174	322	201	29.5	9.3
V3P3	0.47	0.23	81	0.78	149	324	195	33.4	8.8
d.05	0.21	-	-	-	60	-	-	-	-
	**	-	-	-	*	-	-	-	-

V1, unchilled; V2, 27 days; V3, 55 days at 3 °C
P1 = total light phase 9 h; P2 = 15 h; P3 = 24 h
d.05 = Studentized range at the 5 % level for comparisons between means.
SLA = specific leaf area
LAR = leaf area ratio.

Influence of photophase Light phase had great influence, even though all plants received the same amount of photosynthetically active radiation. (The light was varied with incandescent bulbs of low radiant flux density, which had negligible effect on photosynthesis). Yet the different photophases had a pronounced influence on dry matter production. The crop at t = 3 (3 weeks after chilling, Table 16B) showed that sprout growth was especially influenced. Lengthening the light phase from 9 h (P1) to 15 h (P2) increased total leaf area, though not yet significantly, and Specific Leaf Area (SLA = cm^2 leaf area per g dry matter of leaf laminae).

There was also a marked influence of light on petiole lenght and on petiole dry matter. With longer light the mass fraction of dry matter of petiole to total sprout was significantly larger.

A still longer light phase (24 h) did not further increase dry matter production. On the contrary, a slightly inhibitory effect could be observed with respect to 9 h. The plants under continuous light developed narrower and yellower leaves. Petiole growth was however not inhibited to such an extent, so that the proportion of petiole dry matter increased even further.

Table 16C. Harvest 5 weeks ($\underline{t}$ = 5) after chilling.

Treatment	Leaf blade (g)	Petioles (g)	Beet root (g)	Total dry matter (g)	Leaf area (cm_2)	SLA $(cm^2 g^{-1})$	LAR $(cm^2 g^{-1})$	Petiole fraction of sprout (%)	Number of leaves
V1	1.42	0.59	0.39	2.40	378	265	158	28.9	10.2
V2	1.87	0.86	0.38	3.11	475	254	152	31.4	10.2
V3	2.58	1.34	0.49	4.41	621	242	141	34.3	11.2
d.05	0.61	0.38	-	1.02	158	19	18	3.5	1.3
	****	****	-	****	***	**	*	***	*
P1	2.03	0.71	0.51	3.25	434	220	141	25.4	10.8
P2	2.27	1.13	0.44	3.85	636	281	165	32.4	10.9
P3	1.58	0.95	0.30	2.82	404	259	145	36.9	9.8
d.05	0.61	0.38	0.20	1.02	158	19	18	3.5	1.3
	**	**	*	*	***	****	***	****	*
V1P1	1.31	0.41	0.20	1.92	311	244	166	24.1	10.0
V1P2	1.65	0.71	0.59	2.95	480	292	159	30.4	10.8
V1P3	1.32	0.64	0.37	2.33	342	259	148	32.3	9.8
V2P1	1.81	0.61	.55	2.96	376	208	127	25.4	10.5
V2P2	2.33	1.11	.33	3.77	673	290	180	31.6	10.8
V2P3	1.47	.87	.26	2.60	376	263	147	37.2	9.3
V3P1	2.97	1.11	.79	4.88	616	208	129	26.6	11.8
V3P2	2.84	1.57	.40	4.81	754	261	154	35.3	11.3
V3P3	1.94	1.34	.26	3.54	494	256	140	41.1	10.5
d.05	-	-	.47	-	-	-	42	-	-
	-	-	***	-	-	-	**	-	-

At the third harvest (Table 16C), a similar phenomenon was observed. Lengthening photophase from 9 to 15 h increased leaf area per plant from 434 to 636 cm^2. The increase was not due to an increase in number of leaves, but to a higher massic area of leaves (SLA). The photophase induced faster expansion of leaf area, resulted in an increase in dry matter of all plant parts at the final harvest for the P2 plants (Table 16D). At that time the P3 plants had practically caught up with the P2 group, so that a positive influence of this photophase on root dry matter was observed. The negative influence on laminae growth however remained, but together with the positive effect on petiole growth, P3 plants were significantly heavier than P1 plants. Of the three photophases, 15 h was optimal for dry matter production per plant. An increase of 42 % was observed: from 13.91 g to 19.76 g!

Influence of cold treatment Throughout the trial the chilled plants (V2 and V3) were heavier and had larger leaf area than unchilled plants. The tables do not indicate how much of the increase should be attributed to a positive effect of chilling on subsequent growth and how much to the natural consequence of the different start of the three treatments. To overcome this difficulty and to investigate the chilling effect in more detail, the following procedure was developed.

Table 16D. Harvest 8 weeks (t = 8) after chilling.

Treatment	Leaf blade (g)	Petioles (g)	Beet root (g)	Total dry matter (g)	Leaf area (cm^2)	SLA ($cm^2 g^{-1}$)	LAR ($cm^2 g^{-1}$)	Petiole fraction of sprout (%)	Number of leaves
V1	5.61	3.56	2.90	12.07	1156	209.3	97.2	39.2	13.6
V2	6.71	4.74	4.77	16.22	1315	198.6	82.8	41.1	15.5
V3	8.23	6.48	6.04	20.74	1569	192.4	77.2	43.6	16.3
d.05	1.26	1.12	0.93	2.64	233	15.6	7.4	4.2	1.6
	****	****	****	****	****	**	****	**	****
P1	7.05	4.02	2.84	13.91	1387	198.6	101.2	36.3	18.3
P2	7.90	5.89	5.98	19.76	1510	194.4	78.6	42.4	13.6
P3	5.60	4.87	4.89	15.36	1143	207.3	77.3	45.2	13.5
d.05	1.26	1.12	0.93	2.64	233	-	7.4	4.2	1.6
	****	***	****	****	***	-	****	****	****
V1P1	5.46	2.91	1.98	10.35	1147	210.4	111.5	34.8	16.6
V1P2	6.84	4.46	3.65	14.95	1403	211.1	94.0	40.7	12.9
V1P3	4.54	3.30	3.08	10.91	919	206.4	86.1	42.0	11.3
V2P1	7.23	4.07	2.86	14.15	1396	193.7	97.8	36.5	18.9
V2P2	7.74	6.00	6.80	20.54	1480	191.3	72.9	42.6	13.4
V2P3	5.16	4.15	4.66	13.97	1069	210.6	77.6	44.2	14.1
V3P1	8.47	5.08	3.68	17.22	1619	191.6	94.4	37.7	19.3
V3P2	9.11	7.21	7.48	23.81	1647	180.7	68.8	43.9	14.5
V3P3	7.11	7.14	6.95	21.20	1441	205.0	68.4	49.3	15.1
d.05	-	-	2.16	-	-	-	-	-	-
	-	-	**	-	-	-	-	-	-

Standard deviations of plant dry matter and leaf area increased with time. To render the variability more homogeneous with time, values to total dry matter per plant (W) and leaf areas per plant (LA) were transformed to natural logarithms (Hunt & Parsons, 1974).

For each of the 9 VP combinations, a 2nd-degree polynomial was then fitted to the transformed data, according to the following regression model:

$$\ln (x_{ijk}(t)) = \alpha_i + \beta_{ij}\, t + \gamma_{ij}\, t^2 + e_{ijk}(t) \qquad (2)$$

$$[e_{ijk}(t) = v(0,\sigma)]$$

in which $\ln (x_{ijk}(t))$ refers to the natural logarithm of either mass (W) or leaf area (LA) of plants submitted to the ith chilling treatment (i = 1, 2, 3) and afterwards growing under the jth light regime (j = 1, 2, 3). The subscript k refers to the ordinal number of the harvested plant (k = 1, 2, 3, 4 or k = 1, 2 ....8) within a ViPj combination at time t after vernalization (t = 0, 3, 5, 8 weeks). The model required that all observations with the same degree of vernalization were fitted with equations with the same constant α_i. Such a regression model was chosen because all plants with the same cold treatment Vi had had the same treatment until t = 0. Hunt & Parsons

Table 17. Analysis of variance for mass of dry matter and leaf area per plant.

Plant parameter	Source of variation		Sum of squares	Degrees of freedom	Mean square	F	P
		1	40.02	1			
Dry	c.f[1]	2	77.73	1			
matter		3	137.10	1			
(W)			254.85	(3)			
	linear		425.26	9	47.25	593.01	<0.001
	quadratic		8.14	9	0.91	11.35	<0.01
	error		10.28	129	0.08		
	total		698.53	150			
		1	1683.98	1			
Leaf area	c.f.	2	1803.47	1			
(LA)		3	2016.05	1			
			5503.50	(3)			
	linear		288.95	9	32.11	473.18	<0.001
	quadratic		30.90	9	3.43	50.60	<0.001
	error		8.75	129	0.07		
	total		5832.09	150			

1. c.f. = correction factor.

(1974) and Nicholls & Calder (1973) have warned against overfitting, meaning that quadratic or cubic terms should not be included in the regression model if insignificant. The ANOVA tables for W and LA (Table 17) show that also the quadratic term is significant for both plant parameters. So a 2nd-degree polynomial was fitted to the transformed data.

The estimated regression coefficients for W and LA are presented in Tables 18 and 19. They allow estimates to be made of W and LA throughout the trial without any awkward interpolation. Figures 23 and 24 show the resulting curves for dry mass and area for each of the combinations.

Relative growth rates (RGR) can easily be computed, as:

$$RGR(t) = 1/W \times dW/dt = d[\ln (W(t))]/dt \qquad (3)$$

in which $\ln W(t)$ represents the estimate of the logarithm of mass at time t weeks after vernalization.

Differentiation of Equation 2 allows the RGR to be expressed as a function of time:

$$RGR(t) = \beta + 2\gamma t \ (week^{-1}) \qquad (4)$$

Figures 25 A, B, C shows the calculated course of the relative growth

Table 18. Regression coefficients for mass total dry matter per plant as a function of time:

$$\ln (W_{ijk}(t)) = \alpha_i + \beta_{ij} t + \gamma_{ij} t^2$$

Treatment		Regression coefficients		
chilling (V_i)	photophase (P_j)	α_i	β_{ij}	γ_{ij}
V1	P1	-4.52	1.275	-0.0526
V1	P2	-4.52	1.309	-0.0505
V1	P3	-4.52	1.338	-0.0599
V2	P1	-3.68	1.116	-0.0409
V2	P2	-3.68	1.232	-0.0497
V2	P3	-3.68	1.029	-0.0299
V3	P1	-2.55	0.987	-0.0390
V3	P2	-2.55	0.920	-0.0256
V3	P3	-2.55	0.831	-0.0163

For explanation of treatment codes see Table 16B.

rate for each of the 9 combinations. For each of the three chilling treatments, P2 plants had a higher RGR than the corresponding P1 plants during the greater part of the trial. Continuous light (P3) reduced the RGR in unchilled plants.
Chilled P3 plants initially grew slower too but later grew faster than P1 and P2 plants.

Figure 25 also shows that the RGR decreased with advancing stage of development, hampering direct comparison of relative growth rates between different chilling treatments, because of the different plant weights at time $t = 0$. Rather than to compare the RGR at a given moment of time, a comparison at the same growth stage would be a better approach. Although rather ar-

Table 19. Regression coefficients for leaf area per plant as a function of time.

Treatment		Regression coefficient		
chilling (V_i)	photophase (P_j)	α_i	β_{ij}	γ_{ij}
V1	P1	0.44	1.471	-0.0814
V1	P2	0.44	1.544	-0.0868
V1	P3	0.44	1.585	-0.0993
V2	P1	0.85	1.386	-0.0743
V2	P2	0.85	1.653	-0.1062
V2	P3	0.85	1.392	-0.0784
V3	P1	1.86	1.303	-0.0769
V3	P2	1.86	1.347	-0.0822
V3	P3	1.86	1.228	-0.0692

For explanation of treatment codes see Table 16B.

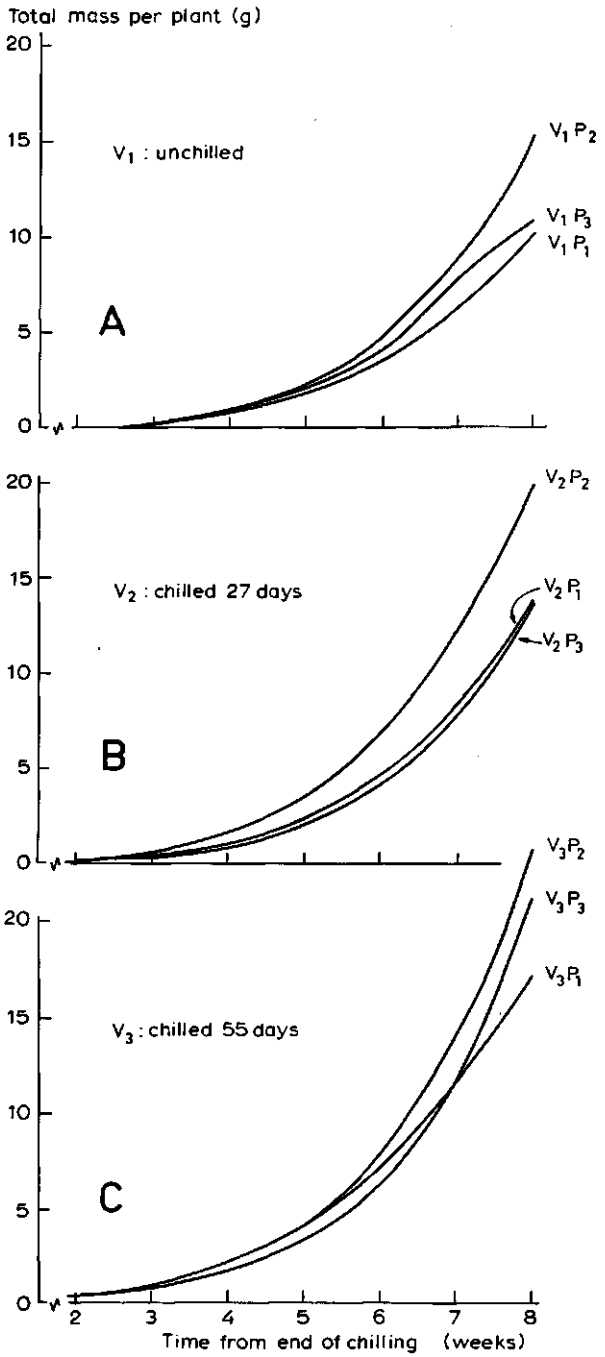

Fig. 23. Mass of total dry matter per plant as a function of time with different periods of chilling (V1, unchilled; V2, 27 days and V3, 55 days chilled) and different photophases after chilling (P1, 9 h; P2, 15 h and P3, 24 h).

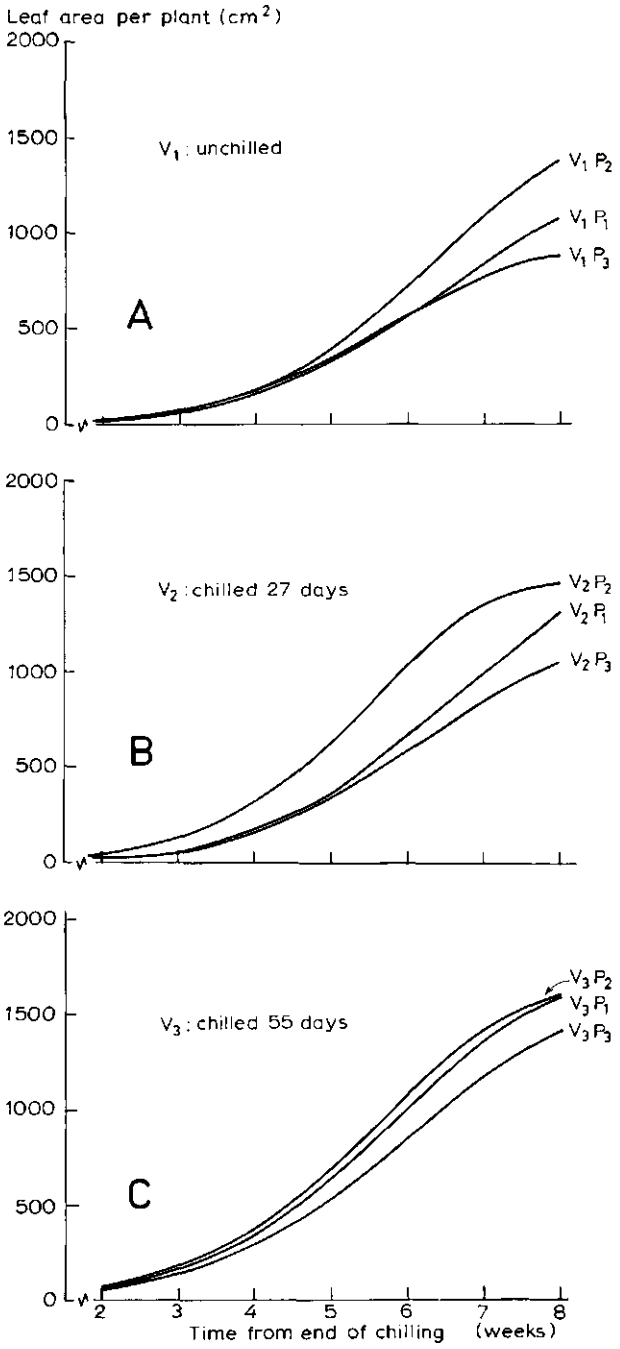

Fig. 24. Leaf area per plant as a function of time in the various combinations of chilling and photophase (as in Figure 23).

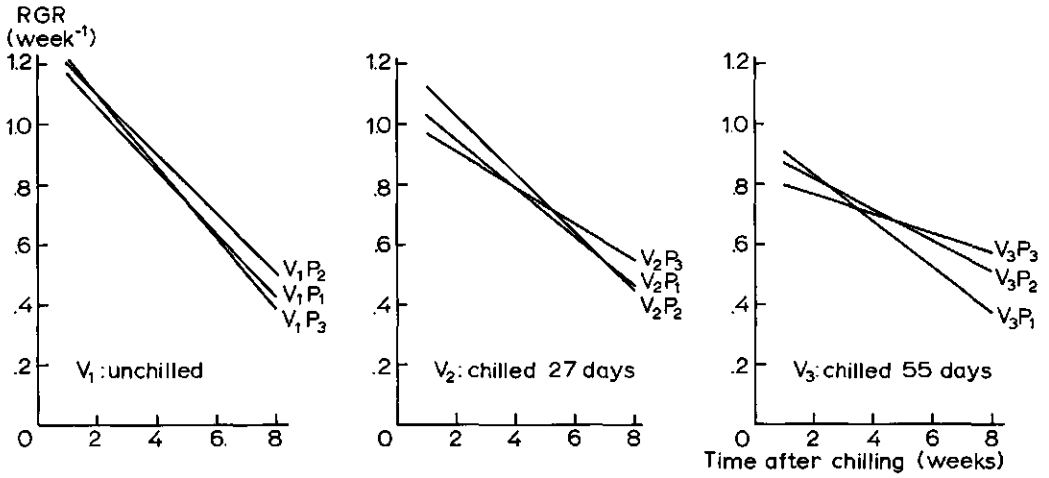

Fig. 25. Relative growth rates (RGR) after cold treatment as influenced by length of the chilling period and subsequent photophase.

bitrarily, in the following procedure, total dry weight per plant is chosen as a yardstick for development.

At time t after vernalization (Equation 2), mass of dry matter W will be:

$$\ln (W) = \alpha + \beta t + \gamma t^2 \qquad (5)$$

or

$$(\alpha - \ln (W)) + \beta t + \gamma t^2 = 0 \qquad (6)$$

A common type of equation,

$$t = \frac{-\beta + \sqrt{\beta^2 - 4(\alpha - \ln (W))\gamma}}{2\gamma} \qquad (7)$$

can be converted to

$$\beta + 2\gamma t = \sqrt{\beta^2 - 4(\alpha - \ln(W))\gamma} \qquad (8)$$

Comparison with Equation 4 shows that the left term represents relative growth rates which can be calculated from the right side when plant mass equals W. Relative growth rate is then a function of α, β, γ and W, rather than a function of β, γ and t as in Equation 4:

$$RGR(W) = \sqrt{\beta^2 - 4(\alpha - \ln (W))\gamma} \qquad (week^{-1}) \qquad (9)$$

This equation allows comparison between cold treatments at a given plant mass rather than at a certain moment, thereby preventing differences in growth parameters arising from differences in development.

In Figure 26, the RGR is plotted against plant mass. The natural decrease

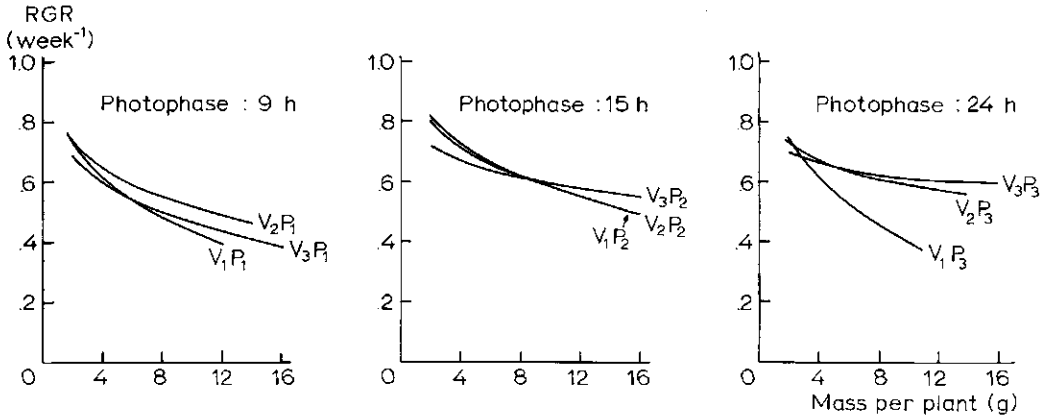

Fig. 26. Relative growth rates as function of mass of dry matter per plant for various combinations of chilling and photophase (as in Figure 23).

in relative growth rate with advancing stage was less marked in chilled plants.

At a plant mass of 16 g, V3 and/or V2 plants grew faster with each light phase. Immediately after chilling, the contrary seems to be true, perhaps because cold-treatment plants had more acclimatization problems in resumption of growth than unchilled plants.

In the same way as relative growth rate based on plant mass, a growth parameter can be based on leaf area, the relative expansion rate (RER). Normally the formule is:

$$RER\ (t) = 1/LA(t)\ \ dLA/dt \qquad (week^{-1}) \qquad (10)$$

To make RER a function of leaf area rather than time, the same procedure can be followed as for the RGR:

$$RER(LA) = \sqrt{\beta^2 - 4(\alpha - \ln\ (LA))\gamma} \qquad (11)$$

in which α, β and γ are the regression coefficients for the estimate of leaf area per plant. In Figure 27, RER is plotted against leaf area per plant. At each leaf area, P2 leaves expanded faster than P1. Those of P3, however, expanded slower in all chilling treatments. In Figure 28, the effect of a previous cold treatment on expansion of leaf area can be read for each light phase, again at a particular leaf area. The impression is obtained that cold treatment affects this rate too. Especially the plants with 9 and 24 h light phase had a gradually increasing advantage with increasing cold period.

Yet another growth parameter, net assimilation rate (NAR), can easily be computed with the calculated regression coefficients:

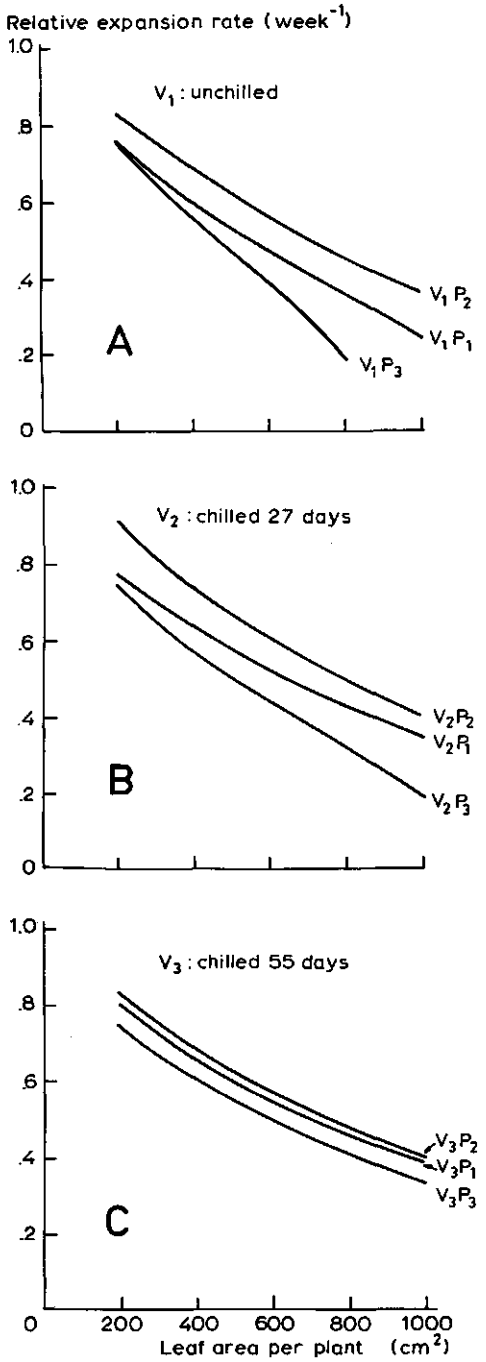

Fig. 27. Influence of photophase on relative rate of increase in leaf area (as a function of leaf area) after chilling for 0, 27 and 55 days.

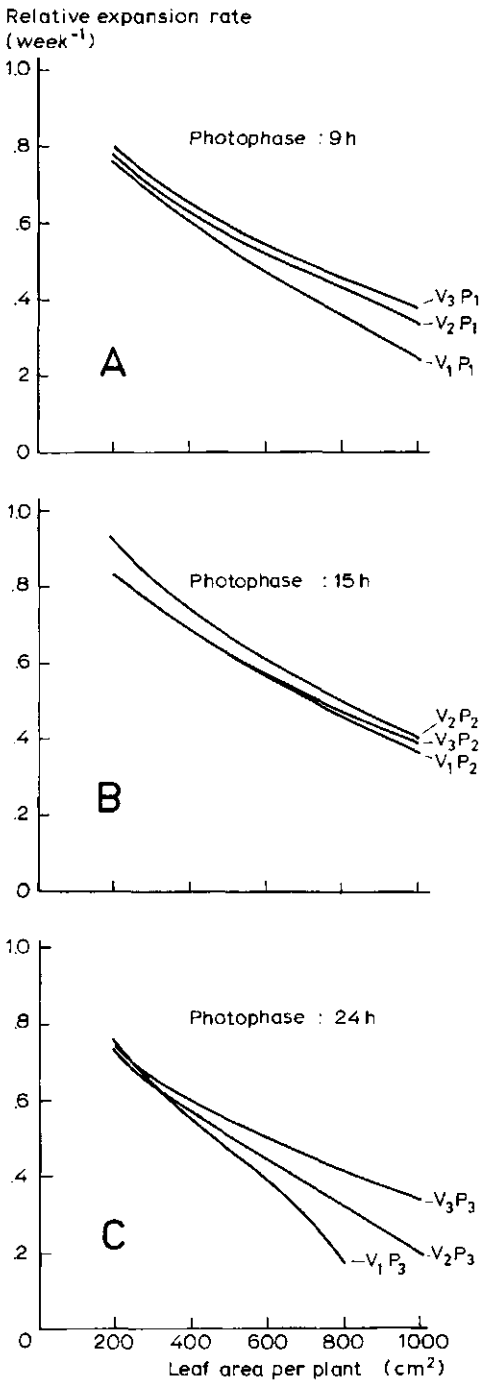

Fig. 28. Influence of time of chilling (V1, 0; V2, 27 and V3, 55 days) on the relative rate of increase in leaf area (as a function of leaf area) with photophases of 9 h (A), 15 h (B) and 24 h (C) after chilling.

$$NAR(t) = 1/LA(t)\ dW/dt \quad (12)$$

$$= 1/LA(t)\ W(t)\ 1/W(t)\ dW/dt \quad (13)$$

$$= W(t)/LA(t)\ RGR(t) \qquad (week^{-1}) \quad (14)$$

Figure 29 shows assimilation rate for the various treatments as a function of time. Until at least 5 weeks after the end of chilling, the higher growth of P2 treatments did not result from higher assimilation.

After that date, assimilation of P2 and P3 plants increased markedly. It might be simplistic to attribute this to daylenght as such. For example, it could be caused by a higher senescence rate of leaves. Table 16C and 16D indeed show that the number of leaves of the P2 and P3 treatments was much reduced after the third harvest. This might point towards a suddenly increased loss of older leaves by the long-day plants. Such a phenomenon could account for the higher NAR, as the remaining leaf area seems then to be more efficient.

Though often considered representative of photosynthetic capacity, one of the main drawbacks of using a growth parameter like NAR, is that it does not take into account the quality of the leaf area. The efficiency of the P2 and P3 plants after the harvest at the fifth week might therefore be overestimated.

6.3.4 Discussion

Both factors known to have a strong promoting influence on bolting also stimulated growth. Especially the influence of light was pronounced. The results were similar to those of Milford & Lenton (1976), who concluded that

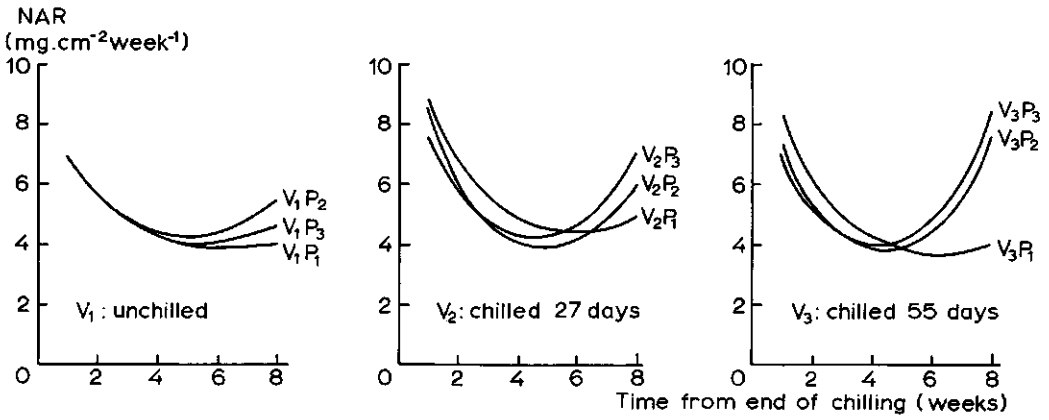

Fig. 29. Net assimilation rate after chilling for the various combinations of vernalization and photophase.

the increased growth at the longer photoperiod (light phases of 12 h and 12 h + 4 h) was effectuated by a change in leaf area ratio and specific leaf area and not by an increase in the photosynthetic activity of the leaf surface.

Until the 5th week, Figure 29 supports that view. As has already been mentioned, the rapid increase of the NAR after the 5th week for P2 and P3 plants may be unreliable.

The observed negative effect of continuous light was also observed in spinach by van Oorschot (1960), who found an optimum curve for dry matter production. An optimum for fresh and dry matter was with a photophase of 21 h.

The extension of the light phase with incandescent lamps brought about morphogenetic effects, which resemble the effects when gibberellin is applied to sugar-beet plants. Especially the marked effect on petiole length and leaf shape was very similar. The same effect of long photophase and gibberellin was observed in spinach by Zeevaart (1971), who suggested that long days promote a higher rate of gibberellin biosynthesis and increased sensitivity to gibberellin to cause the observed growth responses. Probably this is a phytochrome-mediated response, induced by red or far red light emitted by the incandescent lamps.

Milford & Lenton (1976) mentioned that also growth in the field may be influenced by the spectral radiant energy of natural daylight. According to Smith (1975, p. 151), a shift towards far red can occur within the canopy of the sugar-beet crop. The field observation that petiole length and weight increased at high plant densities might also be connected with the change in the spectrum in such a canopy.

This morphogenetic behaviour (faster expansion of the leaf area) might help to achieve a closed canopy earlier in the season. Therefore, possible genotypic differences in reaction towards photophase or differences in reaction to particular wavelengths should perhaps receive more attention by breeders (or growers of indoor vegetables to save energy).

Also previous cold treatment had a positive effect on growth of the plants. RGR decreased at a slower rate in chilled plants than in unchilled ones and leaf area expanded faster.

Are these effects, if caused by the cold treatment, related to vernalization (the flower-inducing process)? Or does the low temperature cause some changes independent of vernalization? Behaeghe (1978), for grasses, considered the positive effect of low temperature on growth completely independent of vernalization and so coined the name 'hibernation' (Figure 30).

Yet, for my trial and that of Behaeghe stimulation of growth should perhaps not be ascribed mainly to the differences in previous treatment but rather as a side effect of low temperature. For example, during cold treatment, root development might be relatively favoured, which could have a positive influence in the following period.

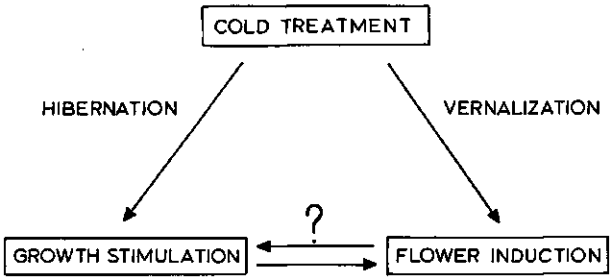

Fig. 30. Hypothetical relation between cold treatment, growth stimulation and flower induction (after Behaeghe, 1978).

Considering the positive effect on productivity resulting from photophase and from low temperature one must ask also whether there is a definite relation with the usual larger plant weight of just visibly bolting plants. Just as Behaeghe (1978) believed that the growth-stimulating effect of low temperature could be independent of the flower-inducing effect, the same could apply for photophase. Figure 31 represents such a relation.

Arguments for such an independent action of photophase on growth and on flower induction are:

- Only chilled plants respond to photophase in flowering, whereas both chilled and unchilled plants respond to the morphogenetic action of photophase.
- The photophase causing the most rapid flowering seems to be 24 h (Chapter 2; Curth, 1960), whereas for growth a shorter photophase was optimum.

Despite these discrepancies, there were similarities. Gibberellin may be one of the components of the flowering hormone or may play some role in the stem-formation process. The enhanced biosynthesis of gibberellin with longer

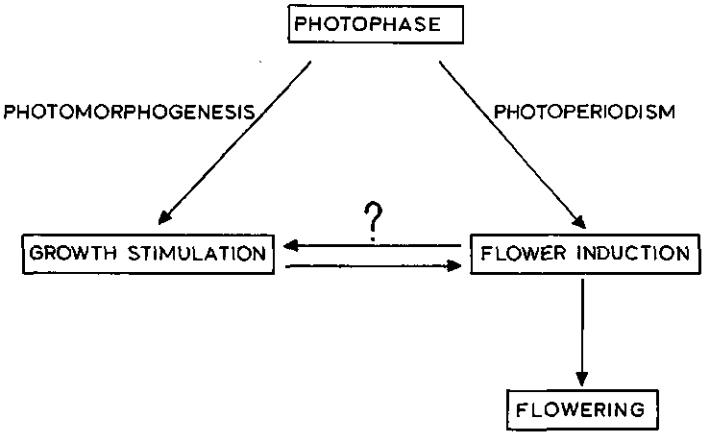

Fig. 31. Hypothetical relation between photophase, flower induction and growth stimulation.

photophases (Zeevaart, 1971) could have two effects, one on stem formation and one on increasing leaf expansion. These processes may have a different optimum concentration of gibberellin.

6.4 INFLUENCE OF SELECTION TOWARDS BOLTING RESISTANCE

6.4.1 Introduction

In the schemes describing the influence of photophase and cold treatment, an arrow was drawn with a question mark (Figures 30 and 31), since evidence was required whether plants induced to flower (or more liable to flower) had an increased growth rate. If so, selection by breeders to improve bolting resistance could mean a loss in growth rate. To answer this question, the plant breeders van der Have B.V. supplied several genotypes in which a possible negative influence on productivity of such a selection could be tested.

6.4.2 Materials and methods

Most modern cultivars are triploids (3*n*) obtained by crosses between a tetraploid (4*n*) and a diploid (2*n*) monogerm malesterile (*MoMs*) genotype. Selection towards bolting resistance in three tetraploids (T1, T2 and T3) was carried out by van der Have in 1975 by sowing early. From the non-bolting plants, seed was grown in the year 1976: TS1, TS2 and TS3. Crosses were made between the selected tetraploids and one diploid monogerm male-sterile genotype and also between the original populations and the same diploid genotype. The resulting 6 triploid single crosses were sown out, together with the 6 tetraploid genotypes (Table 20) at 5 sites in 1977: at Wageningen, Frederika Polder and Zimmerman Polder, being sown on 20, 27 and 21 April, respectively, and in Germany at Coverden and Sollingen on 21 March and 26 April, respectively. Except at Wageningen, the trials were supervised by van der Have, as part of their varietal trials.

6.4.3 Results

In the early-sown trial at Coverden, many beet bolted. Comparison of Selection 1 with 2, 3 with 4, and so on, showed that selection had been effective both for tetraploids and triploids (Table 20).

To investigate the influence of selection on productivity, periodical harvests were carried out at Wageningen. Table 21 shows the relevant figures of the first and second harvest. The data of the first harvest indeed suggest a slight reduction in dry matter and leaf area per plant. Analysis of variance showed, however, that this was not significant. ($P < 0.143$) and ($P < 0.151$), respectively. For the second harvest on 29 June especially root

Table 20. Influence of a selection for bolting resistance on the final proportion of bolters in a field trial at Coverden.

No.	Genotype	Seed production year	Ploidy	Selection for bolting resistance	Proportion bolting at Coverden, 11 October
1	T1	1971	4 n	-	10.8
2	TS1	1976	4 n	+	1.7
3	T1*MoMs	1975	3 n	-	5.1
4	TS1*MoMs	1976	3 n	+	0.3
5	T2	1974	4 n	-	36.5
6	TS2	1976	4 n	+	5.4
7	T2*MoMs	1975	3 n	-	13.0
8	TS2*MoMs	1976	3 n	+	6.0
9	T3	1973	4 n	-	15.1
10	TS3	1976	4 n	+	3.0
11	T3*MoMs	1976	3 n	-	6.3
12	TS3*MoMs	1976	3 n	+	0.8

weights tended to be less for the more bolting-resistant genotypes ($P < 0.079$). In succeeding harvests of the trial, the effect disappeared, however. At the final harvest, there was no significant difference in plant dry matter between selected and unselected genotypes.

For the other four trials, only the final harvest was available. Because bolting in the Coverden trial prevents proper comparison for productivity between selected and unselected genotypes, those data were excluded. The mean for the three other trials are shown in Table 22. The selection effect

Table 21. Influence of a selection for bolting resistance on mass of total dry matters (g) and leaf area (cm^2) per plant at two harvest dates.

Genotype	Harvest date				
	27 May		29 June		
	dry matter	leaf area	sprout dry matter	beet root dry matter	leaf area
T1	0.25	33.1	32.0	8.8	3556
TS1	0.27	33.6	32.4	8.5	3668
T1*MoMs	0.27	32.6	32.3	10.3	3587
TS1*MoMs	0.25	31.5	31.8	9.7	3553
T2	0.25	32.7	29.9	8.6	3240
TS2	0.25	31.6	30.3	8.4	3450
T2*MoMs	0.26	33.3	32.9	10.5	3590
TS2*MoMs	0.24	30.1	32.8	9.8	3546
T3	0.28	33.1	29.6	8.7	3402
TS3	0.24	29.8	29.2	8.8	3211
T3* MoMs	0.29	35.6	34.1	11.5	3440
TS3*MoMs	0.27	34.3	33.7	11.0	3744

Table 22. Influence of a selection for bolting resistance on final root yield and sugar content.

Genotype	Average of three trials (without Coverden):		
	root yield (t/ha)	*sugar content* (g/kg)	*white sugar* (g/kg)
T1	51.97	173.9	150.7
TS1	52.03	175.1	151.8
T1*MoMs	54.53	179.3	154.9
TS1*MoMs	55.27	178.8	154.9
T2	45.97	189.1	165.9
TS2	46.10	189.7	166.4
T2*MoMs	51.83	186.6	162.7
TS2*MoMs	49.80	189.1	166.4
T3	53.37	177.6	151.4
TS3	52.03	178.8	152.9
T3*MoMs	57.13	181.8	157.1
TS3*MoMs	54.83	182.4	157.6

was insignificant ($P < 0.19$). Surprising was that the selected genotypes showed a consistently higher content of sugar and of white sugar ($P < 0.017$) and ($P < 0.015$).

6.4.4 Discussion

The possible negative effect on productivity of previous selection against bolting could not be detected. Only the second harvest in the Wageningen trial showed less root but at a low significance. The influence of selection might, however, be confounded with differences in seed age (Table 20). Further the question arises whether these trials were sufficiently discriminative to detect possible small effects of selection. The question also arises whether there had been only a selection against bolting or that, unwittingly, also other selection criteria had played a role. Of the non-bolting plants in the selection year, only the most vigorous plants might perhaps have been chosen for production of the improved tetraploids. Such a selection would counteract possible negative effects of selection only against bolting.

For the positive effect on sugar content, no explanation is available. According to the breeder, selection was only against bolting and not for sugar content.

Lysgaard (1978) investigated the effect of selection against bolting on yield in fodder-sugar-beet. His trials, in which none of the plants bolted, showed that selection against bolting did not reduce dry matter productivity. In two of the cultivars even an increase was found. Also Yusubov (1977) stated that elimination of bolting biotypes from tetraploid sugar-beet populations did not reduce yield.

So there is no strong relation of bolting resistance with productivity, in contrast to spinach where selection inevitably leads to reduced growth (Parlevliet, 1967). In spinach, however, the difference between early and late cultivars might be larger than in sugar-beet cultivars, which already possess an acceptable resistance to bolting (biennial character). In genotypes with more extreme differences in bolting, a relation might come to light.

7 Temperature and bolting under field conditions

7.1 INTRODUCTION

In certain years, bolting in sugar-beet occurs to such an extent that yield is reduced. In the Netherlands the years 1972 and 1973 were known as such "bolter years". Often it is not known *why* such years deviate. It might be due to early sowing, which lengthens the subsequent period of low temperature. Further the temperature in corresponding periods between the years might be below average. Furthermore in some years, the absence of high (devernalizing) temperatures might increase the risk of bolting.

This chapter correlates the course of temperature after sowing to the observed final percentage of bolters in the field. Data were kindly supplied by the 'Instituut voor Rationele Suikerproduktie' (IRS) at Bergen op Zoom and also by the 'Rijksinstituut voor Rassenonderzoek' (RIVRO) at Wageningen. These institutes arrange annual trials on sowing date for several cultivars at sites throughout the country. Sowing and emergence dates, and final proportion of bolters were recorded.

Data were from trials with cvs. Monohil and Polykuhn. For the period 1966-1976, 53 sowing dates (further indicated with cases) were available for Monohil and 60 sowing dates for Polykuhn. The final percentage of bolters was transformed to arcsines: angle (%) = arcsine ($\sqrt{\%/100}$). In the trials, true 'annual' plants (caused by 'contamination' with annual beet types) were not included in the final proportion of bolters.

7.2 THE RELATION BETWEEN BOLTING AND SOWING DATE

A first impression of the data is given by Figure 32, which shows the transformed percentages in the different years for cv. Monohil and suggests rather good relation with the sowing date, as expected. A 2nd-degree polynomial against time from 1 March to the sowing date had a coefficient of determination (r^2) of 0.508. Figure 33 shows the same angles against date of emergence. The relation was poorer: r^2 = 0.282. In cv. Polykuhn, the respective coefficients were 0.620 and 0.412. The better relation with sowing date than date of emergence supports remarks made in Section 5.1 about the absence of a true juvenile phase. If vernalization took place only after emergence, the relation with date of emergence would be at least as good.

Figure 32 already allows satisfactory prediction of the proportion of bolters (r = 0.7!). However, the course of temperature after sowing or after

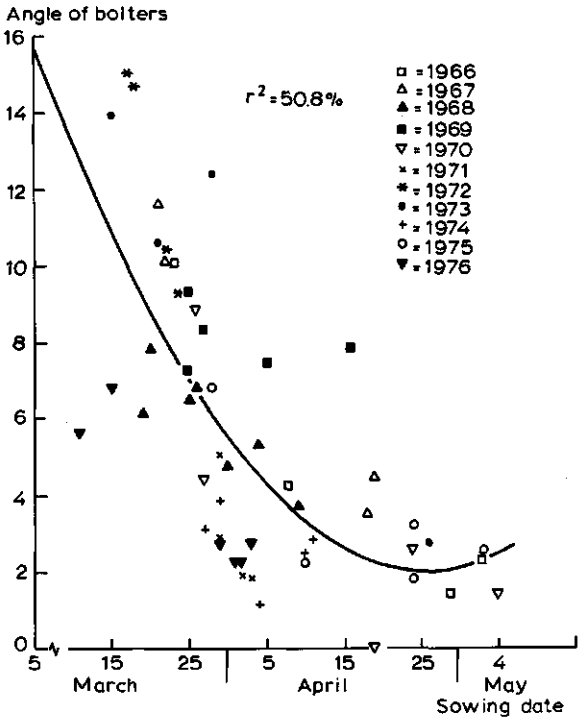

Fig. 32. Relation between sowing date and the proportion of bolters (transformed into angles) under field conditions for cultivar Monohil.

emergence for each case may give a still better relation, which would also be more causal than the indirect relation with the sowing date. Certain years showed a systematic deviation from the proportion of bolters predicted (Figure 32). In 1969, 1972, and 1973, more bolters appeared, whereas in 1971, 1974 and 1976 fewer bolters developed than would be expected from the sowing date. An abnormal course of temperature was probably responsible for those deviations. Although 1976 had a cold spring, probably the proportion of bolters was reduced by the high temperatures in late spring and in summer.

7.3 A REGRESSION APPROACH

How can the relation temperature and proportion of bolters be analysed? Several choices have to be made.

- In what period should the temperature be considered: after emergence or after sowing, and how long should this period be?
- Should daily, weekly or monthly temperatures be used?
- Which temperatures should be used, maxima, minima or average temperatures?

First it was decided to use only the temperature data of the Meteorologi-

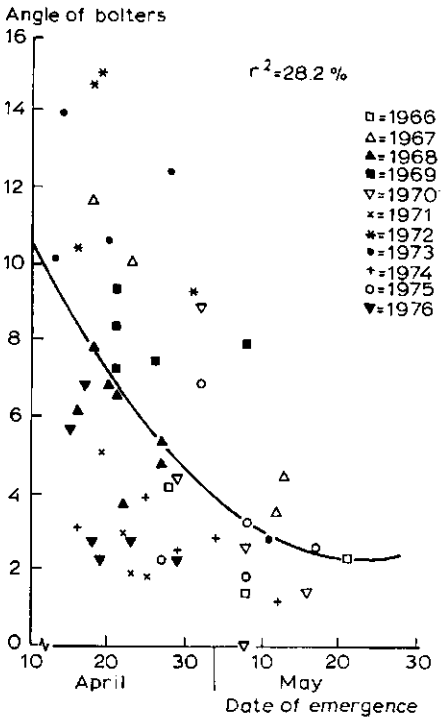

Fig. 33. Relation between date of emergence and the final proportion of bolters (angles) under field conditions for cultivar Monohil.

cal Station De Bilt near Utrecht. Of course the most accurate procedure would have been to use the temperature of the nearest station to each of the trials. Inspection of the bolting data, however, revealed no consistent regional deviation. There are no extreme temperature differences between the different parts of the Netherlands.

As the final proportion of bolters is related to the number of 'vernalizing' days after sowing or emergence, one must decide what should be considered as a 'vernalizing' day. Therefore, for each case (observation, sowing date) the days were counted with a temperature of up to 0, 2, 4, 6, 8, 10, 12, 14 and 16 °C, giving 9 numbers per case. These counts were made for the period between sowing date and 1 July, for minima, maxima and average daily temperatures. With each of the counts, a simple correlation coefficient was computed with the angle (arcsine) of the final proportion of bolters (Table 23). For both cultivars the closest correlation (0.76 and 0.83) was with number of days with a maximum temperature up to 12 °C. The same procedure was followed for number of days from emergence rather than sowing (Table 24). On average, the correlation coefficients were smaller, as one would expect by comparison between Figures 32 and 33. The relevant period should therefore begin at sowing.

Table 23. Simple correlation between the angle of bolters and the number of days from sowing date until 1 July with a (minimum, average or maximum) temperature ≤0, 2, 4 .... 16 °C.

Variety	Temperature	Temperature (°C)									
		≤0	≤2	≤4	≤6	≤8	≤10	≤12	≤14	≤16°C	
Monohil	minimum	0.52	0.50	0.54	0.64	0.67	0.67	0.69	0.68	0.68	n=53
	average	0.06	0.14	0.37	0.49	0.75	0.73	0.73	0.68	0.70	n=53
	maximum	-	-	0.02	0.09	0.43	0.72	0.76	0.76	0.74	n=53
Polykuhn	minimum	0.53	0.68	0.66	0.76	0.75	0.71	0.72	0.72	0.72	n=60
	average	0.02	0.03	0.23	0.63	0.78	0.79	0.78	0.76	0.73	n=60
	maximum	-	-	0.06	0.06	0.35	0.76	0.83	0.79	0.81	n=60

-: correlation coefficient cannot be computed.

Daily maxima gave the best correlation with bolting. Daily minima are probably less indicative of a really vernalizing day. In the climate of the Netherlands, days in May or June with a low minimum temperature can be abnormally warm during daylight. Further studies revealed that also for the relation with a devernalizing day the best relation was found when daily maxima were used. Daily maxima were therefore used.

The following question had to be answered now: in what period should temperature be considered? A choice had to be made between two options:

- a fixed period from sowing, e.g. 8 weeks after sowing
- a period from sowing to a fixed date, e.g. from sowing until 1 July

A fixed period has the advantage that time is equal for each case but the disadvantage that an early sowing date gives quite a different interval from a later sowing date and results in a different photophase.

An interval to a fixed date means that at least the last part of the interval is equal for each case. The beginning will differ for each sowing

Table 24. Simple correlation between the angle of bolters and the number of days from emergence until 1 July with a (minimum, average or maximum) temperature ≤0, 2, 4 .... 16 °C.

Variety	Temperature	Temperature (°C)									
		≤0	≤2	≤4	≤6	≤8	≤10	≤12	≤14	≤16 °C	
Monohil	minimum	0.25	0.24	0.32	0.50	0.58	0.57	0.58	0.55	0.55	n=53
	average	-	-	0.24	0.15	0.60	0.58	0.59	0.60	0.59	n=53
	maximum	-	-	-	-	0.39	0.50	0.50	0.62	0.56	n=53
Polykuhn	minimum	0.48	0.53	0.57	0.64	0.68	0.64	0.64	0.61	0.61	n=60
	average	-	-	0.23	0.54	0.63	0.63	0.63	0.70	0.64	n=60
	maximum	-	-	-	-	0.29	0.60	0.61	0.66	0.69	n=60

-: correlation coefficient cannot be computed.

date, but temperature will then be low and probably photophase is then less important (Section 5). A fixed date proved more succesful, according to total r^2 in the regression models.

Now the following regression model is proposed. For each observation, the following counts were made (and tested as variates) from sowing to six fixed dates: 15 May, 1 June, 15 June, 1 July, 15 July and 1 August:

n_1 = number of days with a maximum temperature $\leq$ 12 °C
n_2 = number of days with a maximum temperature > 12 and $\leq$ 16 °C
n_3 = number of days with a maximum temperature > 16 and $\leq$ 20 °C
n_4 = number of days with a maximum temperature > 20 and $\leq$ 24 °C
n_5 = number of days with a maximum temperature > 24 and $\leq$ 28 °C
n_6 = number of days with a maximum temperature > 28 °C

To find the relation with the process of vernalization, a quadratic relationship with n_1 was assumed, therefore the variable x_1 and x_2 were included in the regression model:

$$x_1 = n_1$$
$$x_2 = n_1^2$$

Further it is reasonable to assume that the effect of a devernalizing day will depend on the degree of vernalization in the preceding period. A devernalizing day will not reduce the number of bolters after a late sowing date to the same extent than after an early sowing, because the number of potential bolters is already lower with late sowing. Therefore an interaction is assumed with the number of 'vernalizing' days.
The following dependent variables were computed (for each case):

$$x_3 = n_2 . n_1$$
$$x_4 = n_3 . n_1$$
$$x_5 = n_4 . n_1$$
$$x_6 = n_5 . n_1$$
$$x_7 = n_6 . n_1$$

The following expression was chosen as the complete regression model:

$$p_{ij} = ax_1 + bx_2 + cx_3 + dx_4 + ex_5 + fx_6 + gx_7 + C + e_{ij} \qquad (15)$$

where p_{ij} was angle of bolters in the year j after a sowing date i.

The bolting resistance of the cultivars was assumed to remain the same from year to year, although this may not be true. Breeders are constantly

selecting in their families and lines. It could only be hoped that this would not be too disturbing a factor. Regression analysis was with the computer package SPSS (SPSS manual, 1975). Stepwise inclusion was combined with hierarchical inclusion. The variables x_1 and x_2 (vernalization) were entered together in the first step, the variables x_3 to x_7 were entered in a stepwise inclusion, provided they met the statistical criteria. The statistical criteria for these variables were the F value, and a parameter called tolerance. The tolerance of an independent variable being considered for inclusion is the proportion of its variance not explained by the independent variables already in the regression equation. The tolerance (a variable between 0 and 1) was set at 0.3, which means that 30 % of the variance of a potential independent variable is unexplained by predictors already entered. The model as a whole has the advantage of a limited number of regression variables (at most 7). Especially in a small population a large number of regression variables leads to spurious results. Hanus & Aimiller (1978) have discussed this problem predicting cereal yields in Germany from weather data. A further advantage of the model is that it is based on physiological processes in the plants.

To assess in what period temperature exerts the largest influence on bolting, the regression was analysed for 6 intervals up to 1 August, at the most.

Table 25 shows the proportion of variance explained by each variable included in the regression equation. For all variables, total r^2 (coefficient of determination) is presented in the last column. The contribution to total r^2 can be read for each of the variables in the model, and it gives there-

Table 25. Attributed proportion of variance to each of the regression variables in data of the years 1966-1976.

Time from sowing date until	'Cold'			Interactions					Total r^2
	≤12 °C (X_1)	$(\leq 12\ °C)^2$ (X_2)	SUM	12-16 °C (X_3)	16-20 °C (X_4)	20-24 °C (X_5)	24-28 °C (X_6)	>28 °C (X_7)	
Monohil									
15 May	59.1	1.1	60.2	-	5.3	3.0	-	8.0	76.5%
1 June	61.3	1.6	62.9	-	-	4.3	1.6	6.3	75.1%
15 June	59.3	1.2	60.5	-	-	-	15.7	1.7	77.9%
1 July	58.3	1.3	59.6	-	-	-	12.6	5.9	78.1%
15 July	58.3	1.3	59.6	-	-	-	17.3	4.0	80.8%
1 August	58.3	1.3	59.6	-	-	-	12.5	2.6	74.7%
Polykuhn									
15 May	74.5	9.7	84.2	-	3.6	4.0	1.2	1.6	94.5%
1 June	69.6	11.4	81.0	-	-	9.6	-	1.0	91.6%
15 June	69.3	11.5	80.8	-	-	7.2	-	1.0	89.0%
1 July	68.4	11.7	80.1	-	-	-	5.1	-	85.2%
15 July	68.4	11.7	80.1	-	-	-	4.5	0.4	85.1%
1 August	68.4	11.7	80.1	-	-	-	2.1	0.6	82.8%

-: regression variable is not included in the equation.

Table 26. Regression coefficients included in the equation on the basis of statistical parameters. Years 1966-1976.

Time	Regression coefficients for variables							
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	C
Monohil								
15 May	0.399E-1*	0.779E-2	.	0.203E-1	-0.142E-1	.	-0.730E-1	0.18
1 June	0.391E+0	0.279E-2	.		-0.189E-1	-0.197E-1	-0.426E-1	0.94
15 June	0.224E+0	0.489E-2	.	.	.	-0.277E-1	-0.155E-1	1.41
1 July	0.140E+0	0.878E-2	.	.	.	-0.203E-1	-0.122E-1	2.16
15 July	0.226E+0	0.806E-2	.	.	.	-0.209E-1	-0.612E-2	1.95
1 Aug.	0.250E+0	0.683E-2	.	.	.	-0.148E-1	-0.483E-2	1.58
Polykuhn								
15 May	-0.190E+0	0.208E-1	.	0.432E-1	-0.429E-1	0.294E-1	-0.908E-1	0.98
1 June	0.443E+0	0.124E-1	.	.	-0.474E-1	.	-0.723E-1	0.43
15 June	0.453E+0	0.116E-1	.	.	-0.300E-1	.	-0.415E-1	0.38
1 July	0.696E-1	0.207E-1	.	.	.	-0.355E-1	.	1.31
15 July	0.798E-1	0.217E-1	.	.	.	-0.253E-1	-0.851E-2	1.26
1 Aug.	0.306E-1	0.224E-1	.	.	.	-0.148E-1	-0.967E-2	1.14

.: variable not included in the equation.
*: the values are presented in 'E-format'; the number following the E is the power of 10.
0.123E-2 = 0.00123
0.123E+2 = 12.3
0.123E+0 = 0.123

fore an impression of the influence on bolting of the temperature range corresponding with the variables. Table 25 shows that besides the variables x_1 and x_2 (which are always included and represent the low temperature effect), some of the higher temperature variables were included in the equations. These variables represent devernalization, because they reduce the number of bolters. For Monohil, increasing the interval from sowing only slightly increased total r^2. For Polykuhn, the largest value (the best explanation) was obtained when only the temperatures in the short interval from sowing until 15 May were considered.

There were other differences between the cultivars. Total r^2 was larger for Polykuhn and the variables x_1 and x_2 contributed much to total r^2 (about 80 %). For Monohil, total r^2 was somewhat less, x_1 and x_2 contributed only 60 % and in this more resistant cultivar, high temperature variables seem largely to determine final proportion of bolters. Table 26 shows the corresponding regression coefficients for the variables included. The coefficient was positive for x_1 and x_2 and, of course, negative for high temperature variables.

The observation that total r^2 for Polykuhn decreased with a longer interval (until 1 August) indicates that later in the season temperature does not have much influence on bolting, as observed in Section 5.5. For the resistant cultivar Monohil, not only high temperatures seemed to have more influence but also the interval in which these temperatures were effective seems to be longer. Total r^2 increased if the interval were extended to 15 July.

Table 26 shows that upon extending the period in which temperature was considered from 15 May to 1 August variable x_7 shifted from -0.07 to -0.0048

Table 27. Attributed proportion of explained variance to each of the regression variables. Data from the years 1967-1975 (without 1966 and 1976).

Time from sowing date until	'Cold'			Interactions					Total r^2
	<12 °C (X_1)	(<12 °C)^2 (X_2)	SUM	12-16 °C (X_3)	16-20 °C (X_4)	20-24 °C (X_5)	24-<28 °C (X_6)	>28 °C (X_7)	
Monohil									
15 May	64.7	2.0	66.7	-	7.8	4.8	-	2.8	82.0%
1 June	65.4	2.1	67.5	-	-	7.3	1.4	3.2	79.5%
15 June	64.5	1.8	66.3	-	-	2.7	9.7	-	78.6%
1 July	63.1	2.0	65.1	-	-	-	13.1	-	78.2%
15 July	63.1	2.0	65.1	-	-	-	14.5	-	79.6%
1 August	63.1	2.0	65.1	-	-	-	8.3	-	73.4%
Polykuhn									
15 May	73.5	9.8	83.3	-	3.7	5.7	1.4	-	94.1%
1 June	69.4	11.1	80.5	-	-	11.3	0.4	-	92.2%
15 June	70.3	10.0	80.3	-	-	9.0	0.5	2.9	92.7%
1 July	69.1	10.5	79.6	-	-	-	9.0	1.5	90.1%
15 July	69.1	10.5	79.6	-	-	-	6.5	3.1	89.2%
1 August	69.1	10.5	79.6	-	-	-	2.5	3.0	85.2%

-: regression variable not included in the equation.

for Monohil and from -0.0908 to -0.00967 for Polykuhn, in line with an observation in Trial 1 and 2 (Section 5.5) that warm days later after vernalization had less influence on the final proportion of bolters.

To investigate the effect of discarding some years from the data collection, the regression procedures were repeated without the years 1966 and 1976. The results are presented in Table 27 and 28. Some differences can be observed between Tables 25 and 26. Variables x_7 was less often included in the equations. Discarding a year like 1976 altered the significance of this variable. This illustrates the importance of having a sufficiently large and representative population in order to prevent irrelevant variables from being included by coincidence or important variables from being left out. In Table 27 (in contrast to Table 25), the largest r^2 (Monohil) was for temperature from sowing until 15 May. However, this equation does not seem very reliable, considering the positive regression coefficients for x_4 and x_7. Days with a maximum temperature above 28 °C would hardly increase the proportion of bolting plants. So, it is still possible that high temperature has a stronger influence, which remains active for a longer period, in bolting-resistant cultivars.

The larger proportion of low temperatures in r^2 does not necessarily mean that Polykuhn would be more sensitive to low temperature. If devernalizing temperatures less influenced the proportion of bolters in this cultivar, bolting would mainly be determined by low temperatures and would be easier to predict (higher r^2) than if bolting were dependent on two processes.

Similar studies on the relation of temperature to bolting were made by

Table 28. Regression coefficients included in the equation on the basis of statistical parameters. Years 1967-1975.

Time	Regression coefficients for variables							
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	C
Monohil								
15 May	-0.528E+1	0.112E-1	.	0.213E-1	-0.220E-1	.	0.125E+0	2.26
1 July	0.465E+0	0.271E-2	.	.	-0.296E-1	-0.178E-1	0.113E+0	0.64
15 June	0.496E+0	0.119E-3	.	.	-0.104E-1	-0.252E-1	.	0.13
1 July	0.944E-1	0.103E-1	.	.	.	-0.213E-1	.	2.47
15 July	0.194E+0	0.895E-2	.	.	.	-0.221E-1	.	2.07
1 Aug.	0.219E+0	0.729E-2	.	.	.	-0.142E-1	.	1.55
Polykuhn								
15 May	-0.267E+0	0.229E-1	.	0.410E-1	-0.468E-1	0.291E-1	.	1.92
1 June	0.454E+0	0.136E-1	.	.	-0.535E-1	-0.141E-1	.	0.57
15 June	0.323E+0	0.153E-1	.	.	-0.251E-1	-0.139E-1	-0.112E+0	1.06
1 July	-0.380E+0	0.320E-1	.	.	.	-0.324E-1	-0.592E-1	4.89
15 July	-0.321E+0	0.327E-1	.	.	.	-0.229E-1	-0.424E-1	4.49
1 Aug.	-0.161E+0	0.312E-1	.	.	.	-0.181E-1	-0.464E-1	3.34

.: variable not included in the regression equation

Wood & Scott (1975). They, however, studied temperatures in the period 4-6 weeks after emergence, in contrast to the foregoing approach with temperature from sowing. Also Lasa (1977), in correlation studies in Spain found it was best to start the interval at emergence or even 30 days after emergence. Spanish conditions are, however, quite different from the Netherlands, as the crop is sown in dry soil and the seed does not germinate until the soil is wetted. According to Lasa, emergence is much delayed in some years, which explains why the best relation is found with temperatures from *emergence* rather than sowing. Lasa (1977) also stated that devernalizing temperatures did not have a great role in the field. However, with the approach of the regression model (inclusion of higher temperatures with an interaction term), high temperatures still seem to reduce the proportion of bolters in the field (at least for Dutch conditions). A further difference is that Lasa used average and minimum temperatures instead of maximum temperatures, which gave best correlation in my study.

7.4 AN OPTIMIZATION APPROACH

The regression method of Section 7.3 has shown that proportion of bolting can be predicted from a few weather data, especially low and high temperatures. Some objections can be raised to this method:

- In most of the resulting models, only days with a maximum temperature above 28 °C influence the final proportion of bolters. It is reasonable, however, to assume that days with a somewhat lower maximum temperature also have an effect, though less.

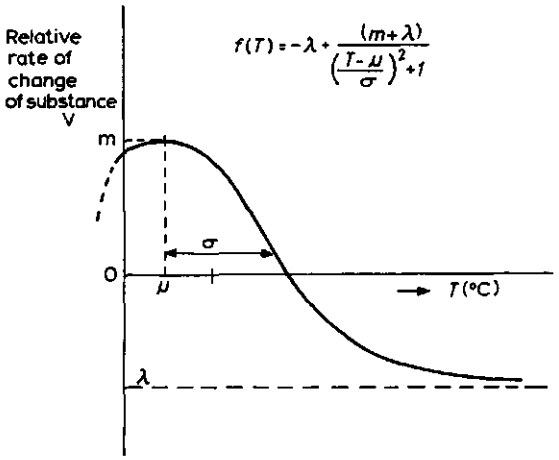

Fig. 34. Hypothetical relation between relative rate of change of substance V and temperature.

- Because of the small sample, some spurious correlations were probably picked up.

A different approach would be to regard the process of flowering more in terms of the model (Figure 2). The final amount of F synthesized in the plants will, of course, show a perfect relation with the final proportion of bolters. Presumably the amount of V at a specific date also shows a good relation with the proportion of bolting plants. Suppose the relative rate of increase and decrease of substance V as a function of daily temperature is known. Probably a function like the one in Figure 34 would result. At lower temperatures, V is synthesized and at higher temperatures V is depleted. The following function is used to describe the relative rate of increase and decrease in substance V:

$$f(T) = -\lambda + \frac{m + \lambda}{\left\{\frac{T - \mu}{\sigma}\right\}^2 + 1}. \qquad (16)$$

in which T = daily maximum temperature, λ = lower asymptotic value of the daily rate of change, m maximal daily rate of change at $T = \mu$ and σ deviation round $T = \mu$.

Use was made below of data for Monohil (p_j, j = 1, 2, ... 52, 53). It was assumed that at the sowing date the amount of V was 1 (in arbitrary units) and that the amount of V on 15 July would show a good (linear) relation with the final angle of bolters. For each case, p_j, the rate of change i days after sowing will be:

$$r_{ij} = -\lambda + \frac{m + \lambda}{\left\{\frac{T_{ij} - \mu}{\sigma}\right\}^2 + 1}. \qquad (17)$$

The amount of V on 15 July (n days after sowing) for a case p_j will then be:

$$V_j = 1(r_{1j} + 1.)\ (r_{2j} + 1.)....(r_{ij} + 1).... .(r_{nj} + 1) \qquad (18)$$

where V_j is final content of V on 15 July and r_{ij} rate of change on day i after sowing for case j.

To predict the final angle of bolters $\hat{p}_j$, the following relation with the content of V_j was assumed:

$$\hat{p}_j = aV_j \qquad (19)$$

With the least-square method, the following function F was defined:

$$F = \sum_{j=1}^{j=53} (\hat{p}_j - p_j)^2 \qquad (20)$$

It is a function of the temperature course after sowing in each case and of the parameters λ, m, σ, μ and a. It was minimized with the non-linear optimisation package OPTPAC3, (van Kilsdonk, Philips, Eindhoven, 1977) using the zero-order method of Hooke & Jeeves. For those values of the parameters that minimized function F, predictions of the proportion of bolters should be good.

To simplify the problem and to reduce the number of parameters, it was assumed that μ = 5 °C. Although the problem required a large core-memory and a long computer run, a minimum of F was found at the following values of the parameters:

λ = 0.052
m = 0.062
σ = 10.5 °C
a = 4.7

Figure 35 gives the corresponding function of the daily relative change in the content of V in the plants. With such a relation to predict p_j, 76.2% of the variance in angles of bolters could be accounted for. Figure 36 shows the prediction for the years 1976, 1977 and 1978 as a function of temperature after sowing. The year 1976 cannot be considered a true prediction, because the data of 1976 were part of the data the model was based on. The curves for 1977 and 1978 are on the other hand true predictions, because these observations are additional data, made available by the two research institutes at a more recent date. The number of additional observations was, however, too small to decide whether the approach was valid. The method was probably less dependent on occasional deviations in temperature, because

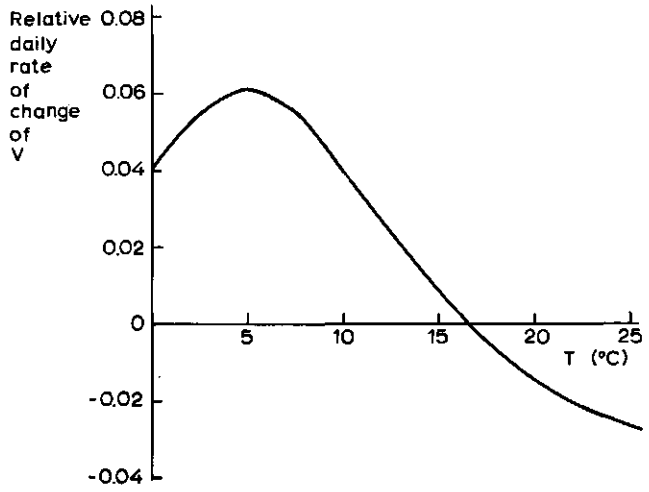

Fig. 35. Relation between rate of change of substance V and temperature with optimization.

every day between sowing and 15 July was included in the calculations. This is in contrast with the regression method where in some models only days with a maximum temperature ≤12 and >28 °C. were used to predict the final angle of bolters. Despite this more realistic approach, total r^2 was not higher than in the regression method. However, temperature of every day from sowing date was used. This is much more 'difficult' for prediction than in the regression method, which easily could give coefficients of determination over 90 %, for a larger number of x-variables and without restricted inclusions.

This optimization method will certainly require further research and perhaps a modified physiological basis. Its principles, however, seem to be useful and could also be applied for other temperature-dependent processes like growth, for example. An optimized temperature function instead of tem-

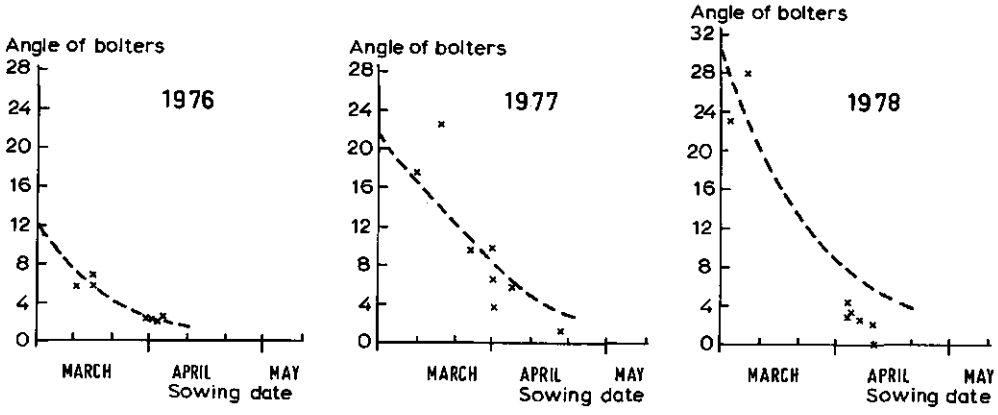

Fig. 36. Prediction of bolting angles for the years 1976, 1977 and 1978. The data for 1977 and 1978 were not part of the data on which the model was based.

perature sums, time-temperature product of growth (in °C.d) might be a better physiological basis for prediction. A disadvantage is that it is still not a dynamic approach. Equation 18 to calculate the final amount of V shows that in this method a warm day in June will have the same effect on the predicted proportion of bolting than a warm day in May. In the dynamic approach of the model (Figure 2), however, this certainly makes difference. Early destruction of V hinders F synthesis more than destruction later in the season (Trials 1 and 2).

A simulation approach is then the solution. The estimation of the relevant parameters is for the moment, however, impossible as only the final result (bolting) is known and not the intermediate steps in the process of bolting and flowering.

8 Final remarks

Let us first consider bolting in sugar-beet from the point of view of breeders. To reduce bolting in their plant material, breeders as a rule sow early and discard bolting plants. In certain years, however, such a selection is hardly possible, because one cannot sow early or because the weather is not cold after sowing. The degree of flower induction will therefore vary from year to year. Not only the total strength of the induction can be different between years, also the pattern of flower-inducing factors may be quite different in some years from others. In certain years, a cold spring is followed by a period of high temperatures; in others, a shorter period of vernalizing temperatures is followed by a period of 'neutral' temperatures. So the nature of the selection is dependent on the weather. To put it in physiological terms: some years, readily devernalizing plants will remain in the population; in other years, plants will be retained that have a high cold requirement. It would be interesting to find out whether such differences exist and whether more specific selection is possible.

Which components of the flowering process are relevant for breeders when selecting for a bolting resistant cultivar? It is possible that differences in bolting resistance of cultivars is due to differences in:

- juvenility
- cold requirement
- maximum temperature for vernalization or minimum temperature for devernalization
- sensitivity to long days
- sensitivity to devernalization.

If possible, which component should be chosen by breeders to achieve bolting resistance? My trials indicated that bolting resistance is due to several components, because of the observed interaction between the several factors involved.

However, it may still be possible and useful in a selection programm to emphasize a single component of the bolting process. If so, photophase should be a selecting tool. A smaller sensitivity to long photophase will have a direct effect, because the synthesis of flowering substances will slow down and the final proportion of bolters will be reduced. It will also have an indirect effect: a lowered synthesis of F (in terms of the model) would postpone reaching the threshold level of flowering to a later date in

the season. This delay, will give devernalizing temperatures longer the opportunity to reduce the potential number of bolters. Therefore, especially photophase should be a tool to reduce the tendency for bolting to acceptable levels in plant populations.

To detect specific genotypic differences, breeders will have to do their selections under conditioned circumstances, which will make them less dependent on the weather conditions. Moreover, the breeder will then be able to choose climatic conditions which result into optimum adaptation to the climate of a given beet-growing area.

To select for bolting resistance, long induction by low temperature gives opportunity to discard easily bolting plants. The breeder does not necessarily need to select for strictly non-bolting plants. When the low temperature induction is so strong that all plants bolt in due time, probably a very good selection will be made by selecting the late flowering plants. Selecting for the latest 10 % of flowering plants would probably faster lead to a bolting-resistant population than discarding of the earliest 10 % of flowering plants.

If one attempts to select for fast devernalization, periods of higher temperature should be applied to follow the low temperature period. In such a way also, plants insensitive to devernalizing temperatures (if any) can be removed.

Longden & Scott (1979) mentioned also that seed-testing institutes have reason to be interested in comparing cultivars under conditioned circumstances. But also with this technique of testing, the problem arises what kind of 'climate' should be applied. When specific differences in separate components of the bolting resistance exist, one must expect that a specific testing climate overestimates the resistance of one variety and underestimates that of another. Temperature should then be similar to that of the beet-growing area. Moreover the assessment of bolting resistance is valid only for that area.

Although a relation certainly exists between growth and bolting (Chapter 6), there is not enough convincing evidence to state that selection towards bolting resistance will have a negative influence on growth rate. Such differences in growth rate between selected and unselected genotypes may only be detectable when the plants have been submitted to a cold period. This could be one of the reasons why no such differences were found in the trial described in Section 6.4. Sowing early, however, introduces the problem that comparisons are obscured because of the differences in bolting. Measurements should be made then before the plants start bolting. If, however, further trials prove such a relation, breeders should be reluctant to discard bolting plants that otherwise grow vigorously. Bolting plants that show a smaller than average plant weight should always be removed from the plant population, but later bolting plants having a distinct lead could be of advantage in maintaining productivity.

Besides the use of bolting-resistant cultivars, bolting in early sowings also could be reduced if chemicals were available to inhibit bolting. Caution should be taken that such substances do not reduce growth. Because of the relation between growth and bolting, a screening of chemicals might show up some that inhibit bolting but by means of reduced growth. In the literature, bolting-inhibiting chemicals like maleic hydrazide also inhibited growth (Lasa & Silvan, 1976).

Summary

Bolting of sugar-beet (*Beta vulgaris* L.) was studied in a number of trials. In general, the beet plant is fairly well protected from premature bolting: at first, the plant has to be vernalized by means of a period with relative low temperature and further the plants have a long-day requirement (the longer the day after chilling, the sooner plants start bolting and the higher the proportion of bolting plants).

The purpose of the study was to clarify the quantitative effects of temperature and daylength on bolting of sugar-beet in its first growing season. A relative simple quantitative and dynamic model was developed based on trials in growth rooms. The model assumed a hypothetical substance V, which accumulated during vernalization and a final substance F, whose rate of synthesis was influenced by amount of V, temperature and daylength. The final amount of F determines when and how many of the plants would become generative.

Some aspects of the bolting process in trials were as follows. In growth rooms and in the field, plants in the very early stages could be vernalized somewhat less efficiently, but no true juvenile stage was detected in contrast to published observations. Therefore, the course of temperature in the Netherlands after sowing in most years suggests that flower induction by low temperature occurs mainly before emergence.

In a trial with several temperatures during chilling, the lowest temperature in the trial (3 °C) was also most effective. The true vernalization process probably proceeds fast at that temperature, although the processes following vernalization will perhaps start earlier with a higher temperature. Vernalization proceeded also at temperatures as high as 15 °C; when daylength is then extremely long, all plants will finally bolt. There were no reasons to assume an interaction between degree of bolting resistance and optimum vernalization temperature.

The duration of cold and daylength after chilling strongly influenced final proportion of bolters. A certain exchange between cold period and daylength became apparent: longer cold period and shorter daylength could give the same proportion of bolting plants as shorter chilling and longer daylength afterwards.

High temperatures (25 °C) after chilling reduced bolting considerably (devernalization), especially immediately after chilling. Yet an important interaction with daylength became visible because high temperature could

still devernalize later after chilling, provided that in the period between chilling and high temperature a shorter daylength were applied. So there seems to be no fixation of the vernalized condition under moderate temperatures in contrast to reports for other plant species.

A reasonable interpretation of the model is that the hypothetical substance V, accumulating during chilling, represents a certain condition of the plant: the intensity of deblocking of flower-hormone-forming genes in leaves, originating from a vernalized growing point. This intensity of deblocking together with the daylength determine whether and how much of the final flowering hormone is translocated to the growing point, where differentiation in stem and flower buds can follow.

Another aspect investigated was possible physiological and genetic coupling between growth and bolting. For other plant species (e.g. spinach), fast-growing cultivars tend to bolt earlier than slow-growing cultivars. If such a relation applies also for sugar-beet, breeders have a serious difficulty in creating higher-yielding cultivars: selecting for bolting resistance would mean loss of productivity and selecting for productivity would mean an increase in susceptibility to bolting.

Incipiently bolting plants indeed had a larger plant weight than (still) vegetative ones. Further, growth-stimulating factors (like nitrogen fertilizer and irrigation) increased bolting. So the larger weight of bolting plants could be caused by locally better conditions of growth e.g. more space and N of some plants in the population of a field. With such an explanation, it is not necessary to assume genetic coupling between fast growth and bolting. Although chilling and long days as such also stimulated growth in a trial in a conditioned room, the conclusion was drawn that these effects were almost independent of the flower(-inducing) process and were photomorphogenetic effects. Also a decreased productivity of a number of genotypes after selection for bolting resistance did not show up in three field trials.

From the point of view of breeders, the suggestion was given to let daylength play a role in the selection procedures for bolting resistance. If cultivars are possible that react slowly to daylength, a twofold aim can be reached. A slower daylength reaction will directly prevent some plants from reaching the threshold for bolting but will also extend the period in which devernalizing temperatures can be active, so strongly reducing the proportion of bolting plants.

A last aspect of the study was to account for the variation in proportion of bolting plants within and between years (1966-1979), based on the course of temperature after sowing. A regression approach showed that temperature immediately after sowing already influences bolting, in agreement with the trials in which no true juvenile phase was found. Maximum daily temperature was a particularly good predictive variable. For the cultivars used (Monohil

and Polykuhn), induction by cold could be fairly well estimated by counting the number of days with a maximum temperature up to 12 °C. With this number, 60 % (Monohil) to 80 % (Polykuhn) of the variation in bolting could be accounted for. When temperature above 20 °C was also taken into consideration from sowing to 15 July (Monohil) and from sowing to 15 May (Polykuhn), this percentage was raised to 80 % (Monohil) and 95 % (Polykuhn). In a second method, based on an optimization procedure, about 76 % of the variation in bolting for Monohil could be accounted for. With this method, the complete course of temperature from sowing to mid June was included in the calculations.

Samenvatting

Hoewel de suikerbiet (*Beta vulgaris* L.) tot de tweejarige planten gerekend wordt, kunnen in bepaalde jaren bij de teelt veel schieters optreden, planten die reeds in het eerste groeiseizoen generatief worden. De plant is tamelijk goed beschermd tegen voortijdig schieten. Voorwaarde voor schieten is een gevernalizeerde toestand, die tot stand komt in een periode met betrekkelijk lage temperatuur, vervolgens moet nog aan de zogenaamde langedagbehoefte worden voldaan. Hoe groter de daglengte is na de koudeperiode, hoe sneller en hoe meer planten er schieten. Normaal gesproken zal alleen na overwintering van de planten aan beide voorwaarden voldaan worden. Toch kan ook na vroege zaai een aanzienlijk aantal planten in bloei komen. Dit kan tot moeilijkheden bij de oogst en volgteelten leiden.

Deze studie beoogt vooral de kwantitatieve effecten van temperatuur en daglengte op het schieten van de planten (speciaal in het eerste groei-jaar) duidelijker te maken.

Nadat het merendeel van de experimenten was uitgevoerd, werd een kwantitatief relatiemodel ontwikkeld wat de gevonden resultaten kon verklaren. In het model werd voorlopig aangenomen, dat er een hypothetische substantie V onder invloed van lage temperatuur gevormd wordt tijdens het vernalizatieproces. In het model werd verder aangenomen dat de synthesesnelheid van het uiteindelijke bloeihormoon F positief beïnvloed wordt door de aanwezige hoeveelheid V, de temperatuur en de daglengte. De hoeveelheid F die gevormd wordt, bepaalt of, en hoeveel, planten uiteindelijk generatief worden.

In klimaatkamers en in het veld bleek, dat planten iets minder effectief in jonge stadia gevernalizeerd konden worden. Uit deze proeven bleek dus niet dat de bietenplanten in de jonge stadia ongevoelig zijn voor de vernalizerende werking van lagere temperaturen (juveniele fase), zoals dat in de literatuur wordt vermeld. Verwacht mag worden dat onder Nederlandse omstandigheden, gezien ook het temperatuurverloop in de meeste jaren, de bloeiinductie door deze lage temperaturen vooral in de tijd vòòr opkomst plaats zal vinden.

In een proef met verschillende temperaturen tijdens de koudebehandeling, bleek de laagste temperatuur (3 °C) het meest effectief. De indruk werd verkregen dat weliswaar het vernalizatieproces bij deze lage temperatuur optimaal plaats vindt, maar dat bij vernalizatie bij iets hogere temperaturen, processen die gewoonlijk op de vernalizatie volgen (daglengtereactie) reeds op gang kunnen komen, wat uiteindelijk positief kan werken. Het vernalizatieproces bleek, indien tegelijkertijd een extreem lange daglengte heerste,

zich zelfs af te kunnen spelen bij temperaturen van 15 °C. Er werden geen aanwijzingen verkregen, dat rassen die verschillen in schietergevoeligheid, interacties vertonen voor wat betreft de optimale vernalizatietemperatuur.

De lengte van de koudebehandeling en de daglengte na de koudebehandeling, bleken beide het percentage schieters sterk te beïnvloeden. Een zekere uitwisselbaarheid tussen koudeperiode en daglengte kwam naar voren: een langere koudeperiode en kortere daglengte kon een zelfde percentage schieters geven als een kortere koudeperiode en een langere daglengte.

Hogere temperaturen (25 °C) na de koudeperiode beperkten het percentage schieters aanzienlijk (de zgn. devernalizatie), vooral vlak na de koudeperiode. Toch bleek een belangrijke interactie met de daglengte, omdat ook geruime tijd na de koude periode de hoge temperaturen nog effectief konden zijn, mits in de periode tussen lage en hoge temperatuur een korte daglengte werd toegepast. Bij bieten lijkt er dus geen sprake te zijn van een fixatie van de gevernalizeerde toestand onder gematigde temperaturen, zoals van andere gewassen gemeld is.

Bij herinterpretatie van het model werd het aannemelijker geacht, dat de hypothetische stof V, die zich op zou hopen tijdens het vernalizatieproces, meer een bepaalde toestand representeert van de plant; een toestand die door middel van celdeling kan blijven bestaan. Omdat de lage temperatuur specifiek op het groeipunt werkt en het verder aannemelijk is, dat bladeren die uit een gevernalizeerd groeipunt komen verantwoordelijk zijn voor de synthese van het bloeihormoon, werd verondersteld dat deze toestand de mate van deblokkering van de genen die het bloeihormoon vormen, representeert. Of, en in welke hoeveelheid, bladeren uit een gevernalizeerd groeipunt inderdaad bloeihormonen exporteren, hangt dan af van de daglengte. Indien het bloeihormoon in het groeipunt aankomt, volgt differentiatie in stengel en bloemknoppen.

Een ander onderzocht aspect was de mogelijke genetische fysiologische koppeling van groei en schieten. Bij andere gewassen (bv. spinazie) hebben snelgroeiende rassen een sterkere neiging tot schieten dan traag groeiende rassen. Indien dit verband ook bij bieten bestaat, zou dit voor kwekers een ernstige handicap betekenen om tot produktievere rassen te komen: selectie op produktie zou leiden tot schietergevoeligheid en selectie op schieterresistentie tot verminderde produktiviteit.

Uit veldwaarnemingen bleek inderdaad dat juist schietende planten veelal aanzienlijk zwaarder zijn dan (nog) vegetatieve planten. Verder bleek echter dat ook groeibevorderende maatregelen zoals stikstof (over)bemesting en beregening tot meer schieters leidde. Het overgewicht van schietende planten zou dus veroorzaakt kunnen zijn door toevallig betere groei-omstandigheden van deze planten (bv. meer ruimte, stikstof, enz.). Zowel de groei als het schieten wordt dan bevorderd.

In deze verklaring is het niet nodig een genetische koppeling van snelle groei en schietneiging aan te nemen. Wel bleek, dat een koudeperiode en lange dagen op zich groeibevorderend kunnen werken, maar op grond van de proeven werd geconcludeerd, dat dit effect min of meer los staat van het proces van (bloei) inductie en meer te maken heeft met fotomorfogenetische effecten. Ook een verminderde produktiviteit van een aantal genotypen geselecteerd op schieterresistentie, kon in een drietal proeven niet significant aangetoond worden.

Vanuit het gezichtspunt van de plantenveredelaar, dient overwogen te worden of de daglengte niet een rol kan spelen in de selectie op schieterresistentie. Indien een genotype mogelijk is dat traag op lange dagen reageert, kan een tweeledig doel bereikt worden. Door deze trage daglengtereactie zullen minder planten de drempelwaarde voor schieten overschrijden, anderzijds zal de periode voor het bereiken van deze drempelwaarde langer worden, zodat devernalizerende temperaturen een grotere kans krijgen om het potentiële aantal schieters terug te dringen.

Het laatste aspect in deze studie was om de variatie in schieterpercentages tussen en binnen de jaren 1966 tot en met 1979 te verklaren met behulp van het temperatuurverloop na zaai. Met behulp van regressieberekeningen bleek, dat de temperatuur onmiddelijk na zaai al invloed heeft, hetgeen in overeenstemming is met de in proeven gevonden afwezigheid van de juveniele fase. Speciaal de dagelijkse maximumtemperatuur kon goed als verklarende variabele gebruikt worden. Voor de twee gebruikte rassen (Monohil en Polykuhn) kon het effect van koude-inductie goed worden benaderd door na zaai het aantal dagen te tellen met een maximumtemperatuur kleiner dan 12 °C. Hiermee kon 60 % (Monohil) tot 80 % (Polykuhn) van de variatie in schieterpercentages verklaard worden.

Als het aantal dagen met een maximumtemperatuur van meer dan 20 °C in de periode van zaai tot bv. 15 juli (Monohil) of tot 15 mei (Polykuhn) in de berekeningen werd opgenomen, kon het percentage verhoogd worden tot 80 % (Monohil) en 95 % (Polykuhn).

Een andere methode, m.b.v. een optimaliseringsprocedure leverde voor Monohil een percentage van ca. 76 % op. Hierbij werden dagelijkse temperatuurwaarnemingen gebruikt over de gehele periode van zaai tot half juli.

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