THE INFLUENCE OF EXTERNAL FACTORS
ON THE GROWTH AND
DEVELOPMENT OF SPINACH CULTIVARS
(SPINACIA OLERACEA L.)

. PARLEVLIET

THE INFLUENCE OF EXTERNAL FACTORS ON THE GROWTH AND DEVELOPMENT OF SPINACH CULTIVARS *(SPINACIA OLERACEA* L.)

DE INVLOED VAN UITWENDIGE FACTOREN OP DE GROEI EN ONTWIKKE-
LING VAN SPINAZIE CULTIVARS *(SPINACIA OLERACEA* L.)

PROEFSCHRIFT

J. E. PARLEVLIET

STELLINGEN

I

De conclusie van FRIEND and NELSON, dat één lange dag bloei induceert in *Brassica campestris* L., is onvoldoende gefundeerd.

D. J. C. FRIEND and V. A. NELSON, Science **153**, 1966: 1115.

II

Het geslachtbepalend mechanisme van spinazie bevindt zich in een vroeg stadium van evolutie.

J. R. ELLIS and J. JANICK, Amer. J. Bot. **47**, 1960: 210–214.
S. BOSE and J. JANICK, Amer. J. Bot. **48**, 1961: 238–241.

III

Betreffende de overerving van éénhuizigheid bij spinazie is de theorie van JANICK and STEVENSON de meest waarschijnlijke.

J. JANICK and E. C. STEVENSON, Genetics **40**, 1955: 429–437.

IV

Het optreden van een niet te verwaarlozen percentage zuiver mannelijke spinazieplanten in éénhuizige handelsrassen vindt zijn oorzaak veeleer in het niet volledig zuiver zijn van het uitgangsmateriaal dan in een ongewenste bestuiving door tweehuizige spinazierassen bij de zaadvermeerdering.

V

Bij de veredeling van de champignon verdient de toepassing van mutagene agentia meer aandacht.

VI

Het feit, dat alle soorten binnen het geslacht *Citrus* te kruisen zijn en dit ook vaak zonder tussenkomst van de mens doen, heeft onvoldoende expressie gevonden bij de classificatie binnen het geslacht *Citrus*.

W. T. SWINGLE, In: The Citrus Industry I. University of California Press., Berkeley and Los Angelos 1946: 129–474.

VII

Het verbod tot incest, bij vele volkeren voorkomend, vindt zijn oorzaak niet in het voorkomen van inteeltdegeneratie.

CLAUDE LEVI-STRAUS, In: Man, Culture and Society. Oxford University Press., New York, 1960: 169–200.

Proefschrift J. E. PARLEVLIET
Wageningen, 21 april 1967.

VIII

Het nastreven van een groot gezin in overbevolkte gebieden is strijdig met de belangen van de gemeenschap.

IX

Binnen de Westeuropese landen tenderen de kinderen groter te zijn, naarmate de ouders afkomstig zijn uit verder van elkaar verwijderde plaatsen.

Welternährungskongress 1966, Hamburg.

X

De verkiezing van Mexico als plaats voor de Olympische Spelen is een stimulans voor de verdere ondermijning van de amateurbepalingen.

J.BLANKERS, Richting 20, 1966: 222–223.

VOORWOORD

Gaarne wil ik op deze plaats dank zeggen aan allen die tot mijn vorming en tot het gereed komen van dit proefschrift hebben bijgedragen.

Vader, jij hebt mij deelgenoot gemaakt van je grote belangstelling voor de levende natuur; je ging helaas veel te vroeg heen. Moeder, ik ben je zeer dankbaar voor het feit, dat je mij, ondanks vele moeilijkheden, de wens van vader, de studie aan de Landbouwhogeschool, hebt laten volbrengen.

U, Hoogleraren en Lectoren van de Landbouwhogeschool dank ik voor alles wat ik op Uw colleges en practica, maar ook in persoonlijk contact, heb mogen leren.

Dr. CAMERON, I am still very grateful for the excellent way you supervised my first steps in research.

Hooggeleerde PRAKKEN, U en Uw medewerkers op het Laboratorium voor Erfelijkheidsleer ben ik zeer dankbaar voor de inzichten en kennis die U mij hebt meegegeven.

Hooggeleerde WELLENSIEK, hooggeleerde promotor, het was een genoegen op Uw laboratorium te mogen werken en dit onderzoek uit te voeren. Uw enorme werkkracht en Uw uitstekende werkverdeling hebben grote indruk op mij gemaakt. Voor de grote mate van vrijheid en de vruchtbare kritiek bij het onderzoek ben ik U zeer erkentelijk.

Mijn collega's ben ik dank verschuldigd voor de vruchtbare discussies.

Zeergeleerde JENSMA, ik ben dankbaar voor het initiatief, dat leidde tot dit onderzoek. UNILEVER N.V. ben ik erkentelijk voor de toestemming de vele onderzoekresultaten te mogen gebruiken voor een proefschrift.

Vele leden van het personeel van het Laboratorium voor Tuinbouwplantenteelt en van het Unilever Research Laboratorium Duiven, hebben bijgedragen tot de totstandkoming van dit proefschrift. In het bijzonder dank ik ANNEKE MANGER voor haar toegewijde en enthousiaste assistentie bij de meeste proeven. Ook PIET V. D. BERG en HENK V. BAARSEL dank ik voor hun medewerking. Ook ben ik dank verschuldigd aan het technisch- en tuinpersoneel van beide laboratoria voor hun toewijding, MIMI VAN VOORTHUYSEN voor het typen van het manuscript, Mej. WOLDA voor de correctie van de drukproeven, REYER JANSEN en HENK VAN LENT voor het foto- en tekenwerk. Een speciaal woord van dank geldt de heer SELWAY voor zijn hulp en adviezen bij de correctie van de Engelse tekst.

CONTENTS

1. INTRODUCTION

1.1. ORIGIN AND HISTORY OF SPINACH

Spinach (*Spinacia oleracea* L.) is a summer annual. Sown in spring it will flower in early summer. It is generally described as a dioecious species, although monoecious plants quite often occur.

The genus *Spinacia* belongs to the *Chenopodiaceae*. Within the genus *Spinacia* several species have been described by a number of authors. SNEEP (67), summarizing their data, concluded that it was questionable whether all these described species are, in fact, separate and independent. As most authors give Persia and surrounding countries as the place of origin of their *Spinacia* species, it is highly probable that this is also the centre of origin of the cultivated spinach. Although the precise relationship between spinach and the recorded *Spinacia* species is not known, it is a fact that samples of wild or run-wild *Spinacia*, collected in Persia and Manchuria, could be crossed to cultivated spinach without difficulty.

The oldest known records mentioning cultivated spinach come from Arab and Chinese writings, indicating that spinach was consumed in the 10th century or even earlier in these countries (67). The first records concerning the growing of spinach in Europe (Spain) date from the 12th century (67). From this time onward spinach gradually spread over Europe, becoming a regularly used vegetable in many countries. Spinach is now grown and used as a vegetable in most countries of the world, with the exception of the tropical regions.

1.2. SPINACH IN THE NETHERLANDS

1.2.1. *Economic importance*

Spinach, a leaf vegetable with a high nutritive value, is of considerable economic importance. Nowadays it is used regularly as a fresh vegetable, and it is the best sold frozen vegetable in Western Europe with the exception of England where spinach is not particularly popular.

TABLE 1. Production and use of spinach in the Netherlands

Period	Production in 1000 tons per year		Processed in 1000 tons per year		
	Total	To auction	Total	Canned	Frozen
1951/1954	29.1	28.6	7.5	–	–
1955/1958	36.2	35.5	12.5	9.6	2.6
1959	30.3	29.8	11.0	6.1	4.4
1960	38.1	32.4	17.3	10.5	6.0
1961	39.1	32.7	19.4	10.1	8.4
1962	43.5	33.8	20.0	9.6	9.3
1963	43.4	27.7	24.9	11.0	12.8
1964	48.4	28.8	27.9	10.5	16.9

Table 1 gives the production and processing data over the period 1951–1964 (73). The total spinach production has increased strongly over this period, this increase being largely due to the increase in frozen spinach. The amount of canned and fresh spinach has not changed much.

The production of spinach seeds is also of some importance. In the period of 1955–1964 the average yearly acreage was 1375 ha with a seed yield of about 2000 tons (73).

The total commercial value of spinach, as a vegetable and a seed crop, amounted to approximately D.Fl. 11,000,000 in 1964 (73).

1.2.2. *The growing of spinach*

Before 1960 most spinach was grown by market-gardeners. Spinach is grown by them the whole year round, although the main harvest period is the spring, approximately 85% of spinach being produced in the months March–June.

The processing industry, however, started to contract farmers as they could mechanize the growing of spinach considerably. How quickly the farmers have adopted this contract growing of spinach can be seen in table 1. The difference between 'Total' and 'To auction' is approximately equal to the tonnage produced by the farmers. This was almost negligible in 1959 (500 tons or 1.5%), but had increased to 19,600 tons or 40% in 1964.

A small proportion of the spinach produced by the market-gardeners is grown in non-heated glasshouses. The spinach is then either sown in early autumn to be harvested in November, or during the winter to give an early spring crop (March–April). The field-grown spinach is sown from February, or sometimes even earlier, until the end of September, with a heavy peak in the early spring. The late winter and early spring sowings are often on sheltered fields. The earliest field-grown spinach is harvested in April, this spinach coming from overwintered spinach sown in September and from winter sowings on sheltered sites. The latest spinach in autumn, harvested in October, is sown in August.

The spinach grown by the farmer on contract shows clearly 2 sowing periods. The spring spinach, sown in March and April, is harvested from the middle of May until the middle of June. The autumn spinach is sown from the end of July until the third week of August to give a harvest period from the beginning of September until the second week of October.

The sowing date is a main factor in the choice of the cultivar. In general, the closer the sowing date to December, the earlier the chosen cultivar and the closer to June, the later the cultivar should be. This is valid both for the field-grown spinach and for the glasshouse spinach.

The spinach grown by the market-gardener is generally sown broadcast at a high seed rate, which may vary from 200–500 kg/ha and sometimes even higher. Especially for the early field sowings, very high seed rates are used. The farmer uses much lower seed rates varying between 40 and 80 kg/ha, while the spinach is sown in rows 11–22 cm wide.

The time of harvest is nearly always determined by the bolting behaviour of

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the spinach. As the spinach should not be too stalky and should contain practically no visible flower buds, it is generally harvested when the flower stalk has just started to elongate. The spinach grown by the market-gardener is generally harvested at an earlier stage than the spinach grown for processing by the farmer. The latter crop is often harvested when the first flower buds become visible.

1.3. SCOPE OF THE PRESENT INVESTIGATIONS

The growing of spinach appears to be closely related to the bolting behaviour of this crop. As previously mentioned, the farmer harvests the spinach when the first flower buds become visible. The market-gardener generally cuts the spinach somewhat earlier. The cultivar choice, too, is related to the bolting behaviour.

The present investigations were started in order to obtain more information about the relation between growth and development in spinach. This implied the study of those factors which control or influence the bolting and flowering of spinach. At the same time, investigations were made into the relation between the rate of growth and the rate of development. The collected data enable us to explain most of the characteristic features of the cultivation of spinach. The implications for breeding will also be discussed.

2. DEVELOPMENTAL FACTORS INFLUENCING STEM AND FLOWER FORMATION IN SPINACH

2.1. INTRODUCTION

The flowering process is strongly influenced by temperature (33, 75) and daylength (32, 51, 75, 76, 82). These factors, together with the genotypic variation in response to temperature and daylength, formed the main object of this investigation.

In this chapter, a number of concepts are used. To avoid misunderstanding, the meaning accorded here to these concepts is briefly stated below:

Vernalization is a low temperature treatment of the germinating seeds or the plants resulting in a changed flowering response in the period following this treatment. Vernalization is therefore an inductive process.

Induction is said to have taken place if the response to the environment is changed after the inducing treatment has been completed.

Stem initiation is the activation of the sub-apical meristem, resulting in the formation of the flower-bearing central stem.

Stem formation is the process of the formation of stem tissue starting after stem initiation and resulting in a visible stem (see stem elongation).

Progress to stem elongation is any stage between stem initiation and stem elongation and results for the greater part from cell division and only for a small part from cell elongation.

Stem elongation is the stage at which the stem becomes macroscopically visible.

Flower initiation is the formation of flower primordia.

Flower formation is the process of the formation of flower tissue starting after flower initiation and resulting in open flowers (see flowering).

Progress to flowering is any stage between flower initiation and flowering proper.

Flowering is the stage at which flowering proper starts, i.e. when the first flowers have opened (anthesis).

Development comprises the gradual progress of the combined processes of stem and flower formation.

Earliness is determined by the time lapse between sowing and readiness for harvest. This agrees reasonably well with the time needed for bolting. A spinach crop is therefore said to be late when it bolts late.

Quantitative long day (LD) plant or cultivar is one in which flower formation occurs at any daylength, but faster in LD than in short day (SD).

Qualitative LD plant or cultivar is one which makes no progress to flowering in SD.

2.2. LITERATURE

Spinach is an LD plant (32, 51, 76, 82). In SD it forms a rosette of leaves; in LD a central stem carrying the flowers is produced (Photos 1 and 2).

MOSKOV (51) determined the relation between days to flowering and the daylength. At daylengths of 13h or shorter he did not observe any flowering. From 14 until 17h the plants flowered earlier, the longer the daylength. At 17h a maximum was reached. The leaves, and not the growing point, react to daylength (32, 82). Leaves kept in SD have an inhibiting effect, those kept in LD a promoting effect on flower initiation and formation (6, 82). WAGENAAR (76) demonstrated that an optimal LD-effect also could be obtained by a light break in the middle of an otherwise flower-inhibiting long night. For a LD effect some LD plants need red light, while others need far-red as daylight extension. Some LD plants can use both (48, 69). Spinach belongs to the group needing red light for day extension (69).

GENTCHEFF and GUSTAFSSON (17) mentioned that spinach flowered in complete darkness. They did not, however, state how long it took before flower buds were formed. WILMAR (81) also observed that spinach of the early cultivar 'Indian Thorny' formed flower buds in the dark. The rate of flower formation was considerably higher in the dark than in SD and almost as high as in LD at comparable temperatures.

According to EGUCHI (15) spinach is day-neutral as far as flower initiation is concerned. For the flower formation LD is required. EGUCHI, however, did not state what precisely he meant by flower initiation and how he had determined it. WARD (77) observed that spinach sown in late summer and early autumn remained in the rosette stage during the winter, although the flower primordia were present, suggesting that flower initiation can precede visible stem formation.

Low temperatures (3–5 °C) during germination result in earlier flowering in the subsequent LD (28, 68, 74, 75). JONES (25) also observed an effect of plant vernalization. According to VLITOS and MEUDT (75) unvernalized spinach plants of the cultivar 'Nobel' do not flower at photoperiods shorter than 14h. When vernalized for 2–4 weeks flower buds were even formed at photoperiods of 8 and 10h. Other authors (28, 53, 74), however, did not observe flower formation at daylengths of 8h after seed vernalization. KAGAWA (27) observed that the effect of seed vernalization was not reversed by drying the seeds after vernalization; germination, however, was badly affected.

Although KNOTT (33) worked under only partially controlled conditions, he observed a tendency for spinach to bolt at shorter daylengths, the lower the temperature within the range of 10–25 °C. JONES (25) observed that spinach plants flowered at longer stems, the earlier the sowing date in the spring. He supposed that this was due to the lower temperatures to which the earlier sowings were exposed.

MAGRUDER and ALLARD (49) compared 8 cultivars at daylengths of 8, 10, 12 and 14 h. The shortest daylength at which bolting occurred varied with the cultivar from 10–14h. 'Virginia Savoy' and 'Hollandia' rather early in the

field, showed some stem formation at 10 h, while 'King of Denmark', the latest of the 8 cultivars did so only at 14 h.

SNEEP and WIEBOSCH (68) vernalized two cultivars and KAGAWA (29) 13 cultivars, differing in earliness. All cultivars flowered earlier in the subsequent LD treatment when vernalized. The order of flowering was not affected.

WITTWER and BUKOVAC (83, 84) obtained flowering spinach plants in SD (9–11 h) by repeated application of gibberellic acid (GA_3). Depending on the concentration, flowering occurred 94–108 days after sowing on stems 80–120 cm long. KAGAWA (30) found that GA_3 applied to seedlings in the late summer caused stem formation but no flower initiation. His concentrations (1–40 ppm), however, were considerably lower than those of WITTWER and BUKOVAC (100–1000 ppm) which may explain the conflicting results. VERKERK and VOLOSKY YADLIN (74) concluded that gibberellin promotes stem growth the stronger, the less favourable the other factors are for bolting.

RADLEY (59) observed a temporary increase in gibberellin-like materials within 24 h, after transferring spinach plants from SD to continuous light.

2.3. MATERIAL AND METHODS

2.3.1. *Plant material and growth conditions*

In the present investigation a large number of cultivars has been used. Seeds of these cultivars were obtained from commercial seed firms with the exception of 'Tardive', obtained from the Institute of Horticultural Plant Breeding (I.V.T.) Wageningen, and a sample of wild spinach seed, collected in a valley north of Meshed in N.E. Iran by an assistant of Mr J. PETIET, vegetable seed expert of the F.A.O. The wild spinach is said to grow there abundantly. Table 2 gives the cultivars studied. As spinach is a cross fertilizing dioecious species, most cultivars are genotypically rather heterogeneous.

In most trials the seeds were sown in black plastic pots, 11 cm in diameter,

TABLE 2. List of cultivars and their source, mentioned in the following investigations

Cultivar	Source	
	Seed firm	Country
'Indian Thorny'	Umaje & Co	India (Bombay)
'Jiromaru'	Takii & Co	Japan
'Nobel'	A. R. Zwaan	Netherlands
'Noordland'	A. R. Zwaan	Netherlands
'Persian spinach' (wild spinach)	Meshed (N. E. Iran)*	Iran
'Spinoza'	A. R. Zwaan	Netherlands
'Supergreen'	Daehnfeldt	Denmark
'Tardive'	I.V.T. Wageningen	Netherlands
'Virtuosa'	Rijk Zwaan	Netherlands
'Vital R'	Sluis & Groot	Netherlands
'Vroeg Reuzenblad'	Sluis & Groot	Netherlands

*Location

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and grown under SD conditions until the experimental treatments started. The temperature in the SD was generally 18–22 °C, in summer quite frequently higher. The plants were never transplanted. In some trials flats or wooden containers measuring 50 × 40 × 15 cm or 70 × 35 × 15 cm were used; this is stated where appropriate.

The SD conditions were obtained by covering a bench in the glasshouse with lightproof curtains from 4.30 p.m. until 8.30 a.m. In this SD of 8 h, the qualitative LD cultivars remained vegetative. When the plants were ready for the experiment, they were given the required treatment, after which they were transferred to an LD after-treatment, which was in most trials 19 h. This long day was obtained by extending the natural daylight period with light from 160 Watt mercury tungsten lamps, mounted at a height of 80 cm above the bench with plants and spaced 80 cm from each other. During the winter months these lamps were also kept burning during the day for the full 19 h. The LD bench was in the same glasshouse as the SD bench.

Due to the variation in light intensity over the year, the rate of flower formation varied considerably from experiment to experiment.

2.3.2. General methods

Vernalization treatments consisted of seed or plant vernalization. The former was done by giving germinating seeds a low temperature treatment for 1–3 weeks. The seeds had already been sown in pots 0–3 days before the low temperature treatment started. The temperatures given were fairly constant over the whole period. The plant vernalization was done in cold rooms of 5 °C. The light in these cold rooms was supplied by fluorescent tubes (Philips TL No. 55, 40 W) of a light intensity of 6000–8000 erg. cm^{-2}. sec^{-1}.

The sowing dates of the different seed vernalization treatments and their controls were chosen in such a way that emergence occurred on approximately the same day, very shortly after the end of the low temperature treatment. The emergence occurred in SD at temperatures as close to 15 °C as possible in order to prevent devernalization. This SD treatment, to allow the plants to emerge, lasted 3–7 days and was given in the spinatron (see below), phytotron or the 15 °C glasshouse during the winter with its natural SD. After this emergence period the next treatment was given.

Photoperiodic, temperature and light intensity treatments were given in the phytotron, described by DOORENBOS (14) and in the so-called spinatron. This spinatron (Photos 3 and 4) was built up of 7 cabinets, each measuring 70 × 70 × 120 cm, the light being supplied by 8 fluorescent tubes (Philips TL No. 33, 40 W) mounted on 2 opposite sides, giving a light intensity of 27,000–33,000 erg. cm^{-2}. sec^{-1}. The variations in light intensity were due to variations in temperature and age of the tubes. In each cabinet, 2 incandescent 25 Watt bulbs, hanging in the centre of the cabinet, were kept burning together with the fluorescent tubes to increase the amount of red light. The incandescent bulbs in each cabinet could be regulated independent of those in the other cabinets, making it possible to give 7 photoperiods at the same time. The temperature, which

was the same in all 7 cabinets, could be controlled between 7 and 30 °C.

In the phytotron experiments have been carried out at daylengths of 8, 12 and 16 h at a light intensity of ca 46,000 erg. cm^{-2}. sec^{-1}. The light was obtained from fluorescent tubes (Philips TL No. 55, 40 W). A series of experiments was done with different light intensities. The normal light intensity, mentioned above, is denoted in these experiments as 100%. The light intensities at 75%, 50% and 25% were respectively 34,000, 23,500 and 12,000 erg. cm^{-2}. sec^{-1}. The light intensities were measured over a prolonged period (about 100 light hours) with the integrating light meter 01 constructed by the Wageningen technical-physical Foundation.

In all experiments the plants were moved regularly as especially in the glasshouse light intensity and temperature could vary within a small area.

The gibberellic acid used in the experiments was the GA$_3$ from I.C.I., which also contains about 10% of GA$_4$ and GA$_7$.

2.3.3. *General observations*

Unless mentioned otherwise 12 plants were used per treatment.

The date at which stem elongation and flowers became visible were noted for each plant, while the stem length was measured at the day of flowering. Plants were said to have flowered when the first flower parts (anthers or styles) had stretched.

The above-mentioned observations were generally made in the LD aftertreatment during which the treated plants were compared with the originally vegetative controls. The extent to which the treated plants are earlier than the controls in the LD after-treatment is a measure for the progress to stem elongation or flowering made during the treatment period. This progress is expressed as a percentage. An example may illustrate this. Suppose plants are given a photoperiod of 15 h for 20 days, and are then transferred to LD after-treatment, together with vegetative controls. The treated plants flower after 15 days whereas the controls need 25 days. This means that the treated plants have made a progress to flowering in the 20 days at 15 h equivalent to 10 LD, or otherwise expressed, the progress to flowering at the start of the LD after-treatment was $10/25 \times 100\% = 40\%$. This also means that flowering at the photoperiod of 15 h would have occurred after $100/40 \times 20$ days $= 50$ days. The progress to flowering after a certain period is therefore reversibly related to the number of days to flowering. This reasoning can be applied only if the rate of stem and flower formation in the LD after-treatment is not influenced by the treatment given to the plants before transferring to LD. Only in a few cases this was found to have occurred, namely, when the treatment had an inductive effect, as after seed or plant vernalization. In these cases the normal vegetative controls are not adequate. These problems, however, are discussed under the relevant experiments.

2.3.4. *Abbreviations*

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

Cv(s): – Cultivar(s)	'Sp' : – 'Spinoza'
GA_3: – Gibberellic acid	'Su' : – 'Supergreen'
'IT' : – 'Indian Thorny'	SV : – Seed vernalization
'Ji' : – 'Jiromaru'	'Va' : – 'Virtuosa'
LD : – Long day(s)	'VR': – 'Vroeg Reuzenblad'
'N' : – 'Nobel'	–V : – Not vernalized
'Nr' : – 'Noordland'	+V : – Vernalized
PV : – Plant vernalization	'WS': – Wild spinach
SD : – Short day(s)	

2.4. GENOTYPIC (CULTIVAR) RESPONSE TO PHOTOPERIOD

As far as studied, all cvs of spinach flower earlier in LD than in SD. From more than 100 cvs sown in the early spring of 1964, four cvs representing most of the earliness range, were chosen for a more detailed investigation. 'IT' is the earliest cv met so far. 'Va' is an early cv bred for Dutch glasshouse winter sowings. 'VR' is a cv used for the earliest spring sowings in the field, while 'N' is used for the late spring sowings. The latest cv 'Tardive' ('Ta') was less suitable for these investigations as its rate of flower formation is far too low. To get it to flower within a reasonable period, GA_3 has to be applied in the glasshouse at LD and rather high temperatures.

The above-mentioned 4 cvs were sown in SD. A week after emergence the young plants were transferred to 7 photoperiods and a temperature of 15–17 °C. The photoperiods ranged from $9 + 1\,^1/_4$ to $9 + 8\,^3/_4$ h. Each cv remained in the spinatron until the plants started to flower in the longest photoperiod. 'IT', 'Va', 'VR' and 'N' were transferred to the LD after-treatment after 33, 37, 37 and 42 days respectively. Of 'IT' and 'Va', controls were used, which were only one week old from emergence in SD. As these 2 cvs also flower ultimately in SD, the controls should have the same developmental stage as the treated plants at the start of the photoperiodic treatment. Of 'VR' and 'N', controls of the same age, but kept in SD, could be used as these do not show any progress to flowering under SD conditions.

The progress to stem elongation and to flowering made at the different photoperiods are given in figs. 1 and 2. Within each cv the response curves for stem and flower formation follow a similar course. On comparing the cvs with each other, it appears that stem and flower formation do not behave identically. 'Va' and 'VR' have, relative to their flower formation response, an early stem elongation. 'Va' appears always to flower later than 'IT', although it elongates earlier.

The course of the response curves for both stem and flower formation show interactions with each other. Especially 'VR' shows this, being late at shorter photoperiods and relatively early at longer photoperiods.
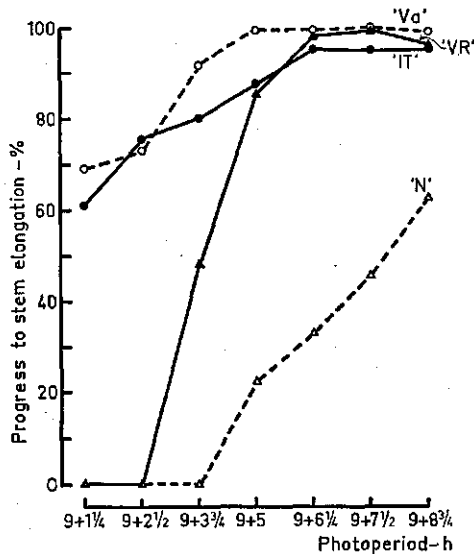
FIG. 1. Progress to stem elongation of 4 cultivars after 12 days at a range of photoperiods and a temperature of 15–17°C
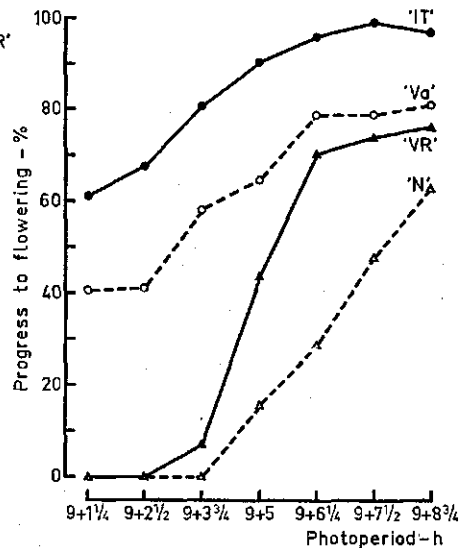
FIG. 2. Progress to flowering of 4 cultivars after 25 days at a range of photoperiods and a temperature of 15–17°C

'IT' and 'Va' are both quantitative LD cvs for stem and for flower formation, while 'VR' and 'N' show a qualitative LD response for both processes. Later it will be shown that this qualitative LD response of 'VR' and 'N' occurs only at relatively high light intensities and temperatures.

Data of the stem length at flowering of 6 cvs are given in table 3. As 'Va' and 'VR' start their stem elongation relatively early, it is to be expected that their stems at flowering will be longer than those of 'IT' and 'N'. This is, in fact, so, although 'Va' is only slightly longer than 'IT' and 'N'. In this connection it should be mentioned that 'Va' consists predominantly of female or nearly-female plants which, in spinach, flower at considerably shorter stems than do male plants. This is probably the reason why 'Va' has a shorter stem length at flowering than 'VR' which contains about 50% male plants.

TABLE 3. Stem length in cm at flowering in LD of 6 cultivars, obtained from 7 different trials

Cultivar	Stem length
'IT'	14.9
'Va'	17.3
'Ji'	18.5
'VR'	22.3
'N'	16.6
'Nr'	16.6

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It has been shown that in several daylength-sensitive crops the daylength response of regional cvs is adapted to the latitude at which they are grown (13, 58). In order to see whether this is also valid for spinach, other cvs were compared with 1 or 2 of the 4 cvs represented in fig. 2 at several daylengths. The data of 3 cvs have been interpolated in the data of fig. 2 resulting in fig. 3.

FIG. 3. Progress to flowering of 7 cultivars after 25 days at a range of photoperiods. For each cultivar the daylength of its growing-season has been indicated
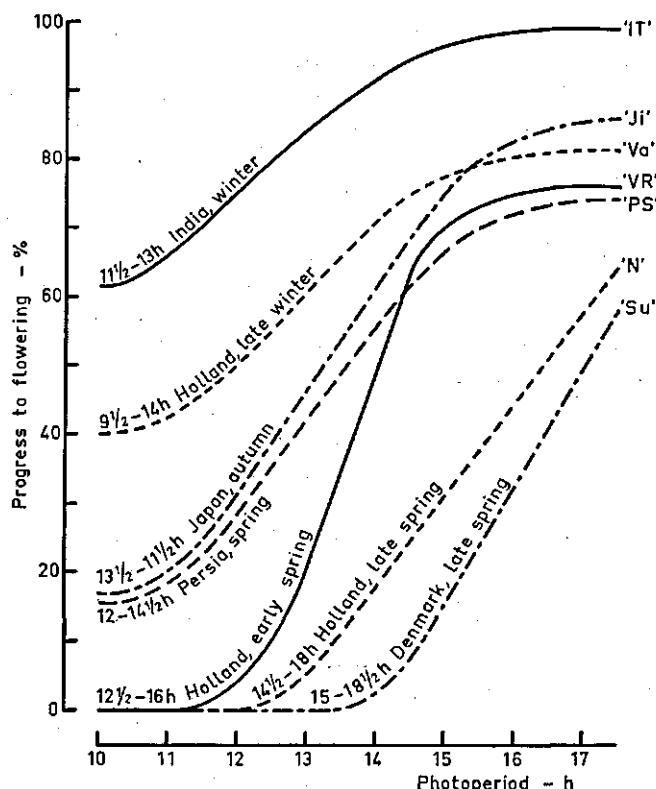
The 7 cvs of fig. 3 represent the complete range of cvs used in practice as far as earliness is concerned. For each response curve the growing region and season, together with the daylengths met during the growing period, are given. The cv response curve is strongly related to the daylengths met during the growing period; the longer the daylengths during the growing period, the more the response curve is shifted to longer photoperiods.

2.5. THE INFLUENCE OF TEMPERATURE

2.5.1. *Cultivar response to seed vernalization*

Like many LD plants, spinach shows an earlier flowering in LD after low temperature treatment of the germinating seeds (38, 54). VLITOS and MEUDT (75) observed that seed vernalization of 'Nobel' spinach resulted in a decrease in the critical daylength. To investigate whether all spinach cvs react in the same

way to seed vernalization, a number of cvs representing the complete range of earliness in spinach has been studied.

Seven cvs, of which 'IT' was the earliest and 'Ta' the latest, have been studied in 2 experiments. In Experiment I, 6 cvs were vernalized for 14 days at 5°C after 3 days at 10°C. The controls were germinated at 20°C for 4 days. The germination was done in petri dishes on moist filter-paper. After the vernalization treatment, the germinated seeds were planted in wooden boxes. In each box 12 –V and 12 +V seeds were planted. Of each cv 3 boxes were prepared in this way. The boxes were kept in SD for a week to allow all seeds to emerge, after which they were transferred to LD after-treatment. The +V plants emerged 1–2 days earlier than the –V plants.

The 3 cvs of Experiment II were treated in the same way. The germinated seeds, however, were not planted in boxes but in plastic pots, one plant in each.

Table 4 mentions the vernalization data and shows that all cvs responded to seed vernalization in a similar way, viz. earlier stem elongation, earlier flowering and flowering on a shorter stem. Only 'Nr' flowered on a longer stem in Experiment II. Other experiments verified the fact that flowering occurred on a slightly shorter stem if vernalized. This indicates that flowering is slightly more accelerated than stem elongation by seed vernalization. On studying the data more carefully, certain questions arise. The differences in stem length at flowering between –V and +V treatments vary with cvs. Part of these variations are not due to experimental error but are significant. 'Vr' has, in both experiments, the greatest decrease in stem length after vernalization. This agrees with the data of paragraph 2.4, where it was shown that 'Vr' had a relatively early and possibly a high rate of stem formation even without vernalization. It is therefore not surprising that in this cv the stem formation is accelerated less than the flower formation, compared with other cvs. For 'Nr' the reverse might be true, as in a number of other experiments too the stem length at

TABLE 4. Influence of vernalization and cultivar on days to stem elongation, flowering and on stem length at flowering

Cultivar	Stem elongation		Flowering		Stem length in cm	
	–V	+V	–V	+V	–V	+V
Experiment I						
'Va'	15.0	12.6	44	40	17.6	15.5
'VR'	16.0	13.3	50	45	23.3	19.1
'Vital R'	17.1	12.3	53	48	19.7	18.2
'N'	21.3	16.2	76	56	20.1	19.6
'Nr'	23.6	19.5	88	71	23.0	22.7
'Ta'	28.3	19.2	–	125	–	ca. 30
Experiment II						
'IT'	11.8	11.1	19.8	18.9	11.5	10.6
'VR'	12.2	10.2	25.2	23.5	19.6	16.6
'Nr'	13.5	12.5	32.2	28.6	11.8	14.4

flowering tended to become slightly longer after seed vernalization. The results, however, were not conclusive.

'Ta' is such a slowly developing cv that without vernalization flowering would have taken too long. There was, however, some progress to flowering as stem formation occurred. This cv should therefore not be seen as a cold-requiring one. It is just a very slowly developing cv.

To study the effect of vernalization at different photoperiods 3 cvs were vernalized as described in Experiment I. After emergence in SD of the −V and +V plants, they were transferred to 3 photoperiods in the spinatron at a temperature of 15–17°C. After 42, 28 and 20 days respectively at these 3 photoperiods the plants were transferred to LD after-treatment together with −V and +V controls, just emerged in SD. The number of days needed for stem elongation (which in most treatments occurred during the photoperiodic treatment) and flowering at those photoperiods was estimated from the progress to stem elongation and flowering made at those photoperiods.

From the data, given in table 5, it can be seen that all 3 cvs showed a changed photoperiodic response similar to the one described for 'Nobel' (75). The shorter the photoperiod, the greater the difference between −V and +V. 'VR' and 'Nr' became quantitative LD cvs after seed vernalization. Stem and flower

TABLE 5. Influence of vernalization on days to stem elongation and flowering at 3 photoperiods of 3 cultivars

	Photo-period in h	'IT'		'VR'		'Nr'	
		−V	+V	−V	+V	−V	+V
Stem elongation	8 + 0	21.5	−	∞	14.7*	∞	84
	8 + 4	17.0	15.1	20.1	15.0	∞	44
	8 + 10	13.1	12.3	13.7	11.5	17.9	16.5
Flowering	8 + 0	45	−	∞	111	∞	220
	8 + 4	40	31.3	140	71	∞	125
	8 + 10	26.0	24.9	33.3	31.0	48	40

*Probably unreliable

formation showed a parallel response, indicating, as in Experiment I, that both processes, stem and flower formation, are affected to approximately the same extent by seed vernalization. Since the vernalization effect becomes greater, the shorter the daylength, it may be expected that later cvs will show a greater vernalization effect than early cvs at one and the same daylength, as these later cvs have a higher daylength requirement. This, in fact, appears to be so, as was shown in table 4.

It may therefore be concluded that spinach is a LD annual, without cold-requirements, but reacting to seed vernalization, which induces a more quantitative LD requirement.

2.5.2. *Seed and plant vernalization*

JONES (25) has observed that young spinach plants can also be vernalized. It should be noted, however, that there is a substantial difference between seed and plant vernalization. Seeds are vernalized in the dark, whereas with plants light is needed to survive a prolonged period of low temperatures. It is therefore necessary to realize that during this period of low temperatures some progress to flowering may also occur.

To ascertain whether flower formation takes place at vernalization temperatures, vegetative 'N' plants, 2 weeks old from emergence in SD were placed at 5°C at 2 daylengths, viz. 8 and 16 h of fluorescent light. At each daylength 2 flats, each with 25 plants, were used. Table 6 shows clearly that the formation of visible flowerbuds occurred at both daylengths, but considerably faster at 16 h than at 8 h. At 8 h no flowering occurred within the period of the experiment (450 days), at 16 h flowering was observed after 180 days. Apparently flower formation takes place at such low temperatures. The daylength response of 'N', however, differs from the one at higher (15–25°C) temperatures. At these low temperatures the daylength response is a quantitative LD one, at the higher temperatures 'N' is a qualitative LD-cv.

TABLE 6. Number of days to visible flower buds and to flowering at a temperature of 5°C and 2 daylengths

Daylength in h	Days to	
	Flower buds	Flowering
8	380–440	–
16	140	180

The rate of flower formation at 8 h at low temperatures is extremely low, as has been shown. Using this daylength for plant vernalization for a few weeks, the effect of flower formation in this period is almost negligible and within the range of experimental errors.

In a second experiment seed and plant vernalization have been compared. Germinating 'N' seeds, 2 days old from sowing, and plants, 9 days old from emergence in SD, were vernalized at 5°C at a daylength of 8 h. After 14 days of low temperature the plants were transferred to SD at 15°C for 3 days to allow the SV plants to emerge. The SV and PV plants were treated together with –V plants, which were 7 days old from emergence in SD, at 3 photoperiods in the spinatron for 16 days at a temperature of 15°C, later rising to 20°C. After this photoperiodic treatment all plants were transferred to LD aftertreatment together with –V, SV and PV controls, which had not received a photoperiodic treatment.

The data, presented in table 7, show a surprising difference between SV and PV. The plants which were not exposed to the photoperiodic treatment but were transferred to LD directly after the pretreatment ended, show that PV was more effective than SV, as they flowered considerably earlier. This was also the case

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after 16 days at $10 + 7$ h, which acts as LD too. At the shorter photoperiods, however, the situation was reversed. At $10 + 1$ h the SV made considerably more progress to flowering than the PV plants. The reason of this reversion is not quite clear but it could be that PV plants are more liable to devernalization than SV plants. As will be seen later, devernalization occurs more readily at SD, when progress to flowering is slow, than at LD.

TABLE 7. Effect of no vernalization at all (–V), seed (SV) and plant vernalization (PV) at 3 photoperiods on the number of days to flowering in LD after-treatment and the progress to flowering made in the photoperiodic treatment

Pre-treatment	Photoperiod during 16 days	Days to flowering	Progress to flowering in %
–V		28.4	
SV	–	24.8	–
PV		22.1	
–V		28.5	0
SV	$10 + 1$	21.7	13
PV		21.9	1
–V		26.7	6
SV	$10 + 4$	18.0	27
PV		17.5	21
–V		16.8	41
SV	$10 + 7$	13.6	45
PV		10.6	52

From the above-mentioned experiments it can therefore be concluded that germinating spinach seeds, as well as young plants, can be vernalized by low temperatures. Flower formation can occur at these temperatures too.

2.5.3. *Vernalization in relation to stem and flower formation*

It has been shown that stem and flower formation react in a similar way. In paragraph 2.4 the photoperiodic response curves of the 2 processes were found to follow the same course within each cv. Vernalization also resulted in an earlier stem elongation and an earlier flowering. In the present paragraph, however, it will be shown that stem formation sometimes reacts independently of flower formation.

An experiment was carried out in which 'Nobel' plants were sown at 6 different temperatures in the phytotron. The daylength was 8 h. After 66 days from sowing, some of the plants were transferred to LD after-treatment, together with control plants kept in the SD of the glasshouse.

Table 8 shows that stem elongation and flowering occurred earlier in the LD after-treatment, the lower the temperatures were during the pretreatment with an irregularity at 15 °C. At 21 and 24 °C the plants flowered at the same time as the vegetative controls. The earlier stem elongation and flowering at the lower temperatures may be due to one or other of 2 causes. It may be a vernalization effect, inducing an accelerated rate of stem and flower formation in the sub-

TABLE 8. Influence of the temperature during a SD period of 66 days on the number of days in LD after-treatment to stem elongation and flowering

Short day (8 h) treatment		Number of days to	
Temperature	Days from sowing	Stem elongation	Flowering
9	66	10.3	21.0
12	66	10.0	22.7
15	66	16.3	29.1
18	66	14.0	27.3
21	66	16.8	31.4
24	66	18.0	31.4
20–25*	66	17.8	31.4
20–25*	9	17.8	31.0

*SD in the glasshouse

sequent LD after-treatment. Or the effect is only direct and not inductive in nature, the plants having made some progress to stem elongation and flowering at the lower temperatures of the SD pretreatment period itself. To distinguish between these 2 possibilities the remaining plants were distributed over 7 photoperiods in the spinatron at a temperature of 13–15 °C. At each photoperiod, 6 plants of each temperature pretreatment were brought together. After 28 days of photoperiodic treatment, the plants were transferred to LD after-treatment to measure the progress to stem elongation and flowering made at these photoperiods. A fairly low temperature was used during this photoperiodic treatment to avoid possible devernalization. If vernalization were the cause of the earlier stem elongation and flowering, it would be expected that the difference in days to stem elongation and flowering between the higher temperatures (−V) and the lower ones (+V) is larger at the shorter photoperiods than at the longest ones, as vernalization induces a changed photoperiodic response. Tables 6 and 7 have shown, namely, that the difference between −V and +V becomes greater at the shorter photoperiods. Since, of each temperature pretreatment, only 6 plants were used per photoperiod, the data of the 2 lowest as well as of the 2 highest temperatures have been taken together.

Fig. 4 gives the number of days to stem elongation and flowering in the LD treatment following the photoperiodic treatment. As, in some cases, stem elongation occurred during the photoperiodic treatment, these data have been corrected to the same stem formation rate as occurred in LD after-treatment, to obtain comparable data. The difference in days to stem elongation was significantly greater at the shorter photoperiods than at the longer ones, exactly as expected, when vernalization had taken place. The difference in days to flowering, however, was completely independent of the photoperiod given, indicating that no vernalization had taken place as far as the flower formation process was concerned. The earlier flowering in LD after-treatment after 66 SD at lower temperatures is therefore due to flower formation during these 66 SD, while no flower formation occurred in SD at the higher temperatures (21

FIG. 4. Days in LD after-treatment to stem elongation and flowering after 66 SD at temperatures of 9–12°C and 21–24°C, followed by 4 weeks at 7 photoperiods
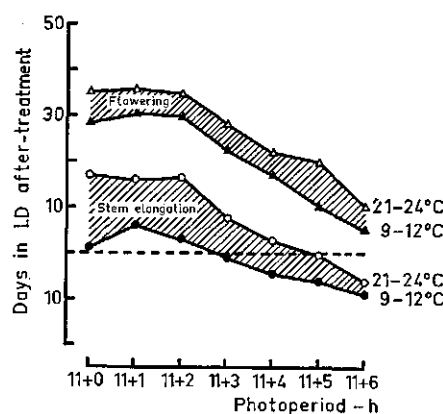
and 24°C). If temperatures of 9–15°C have a vernalizing effect on the stem formation process, but not on the flower formation process, it may be expected that the plants will flower on longer stems at these intermediate temperatures, as the rate of stem formation is accelerated relative to the rate of flower formation. This, in fact, appears to be so.

In table 9 stem length data have been collected from 4 cvs grown at a photoperiod of 18 h at 3 different temperatures. The lower the temperature, the longer the stem at which flowering occurred at least in the temperature range of 10–25°C. All 4 cvs, varying widely in earliness, showed the same effect, indicating that the temperature response of stem and flower formation is similar, irrespective of the earliness of the cv.

TABLE 9. Stem length in cm at flowering in a photoperiod of 18 h of 4 cultivars at 3 temperatures

Cultivar	Temperature		
	10–12°C	15–17°C	21–24°C
'IT'	19.5	16.0	12.5
'Va'	21.8	19.9	14.1
'VR'	26.8	21.0	16.5
'N'	22.1	13.9	11.1

In another experiment 4 cvs were given 7 photoperiods at 2 temperatures, 11 and 16°C. The former temperature should give a clear vernalization effect on stem formation; the latter, being almost the 'neutral' temperature, only a very slight effect, if any. For flower formation, both temperatures are without any vernalization effect. The experiment has already been described in paragraph 2.4 for the higher temperature. As the rate of development also depends on temperature, the progress to flowering is calculated over a period of 34 days at the lower temperature and of 25 days at the higher temperature. These have been chosen in order to get about the same progress to flowering at the longest

photoperiod as can be seen in fig. 5. If the response curves had changed due to temperature, it would be expected that the curves would deviate from each other at the other photoperiods. Fig. 5 shows that the photoperiodic response curves exhibit only slight, insignificant differences within cvs for 'IT' and 'Va'. The other 2, qualitative LD cvs, however, show significant differences, but only at the shorter photoperiods. At 11 °C some flower formation occurs; at 16 °C no progress to flowering occurs at the shortest photoperiods. This agrees with the results discussed in paragraphs 2.5.3. and 2.5.4.

FIG. 5. Progress to flowering of 4 cultivars after 34 days at 11°C or 25 days at 16°C. At each temperature 7 photoperiods were given

The progress to stem elongation has been calculated for periods equal to 35 % of the periods used for calculating the progress to flowering. If stem formation reacts in a similar way to temperature, the photoperiodic response curves should follow a course similar to those for flower formation. This, as can be seen in fig. 6, is not the case. All 4 cvs show considerably more progress to stem elongation at 11 °C than at 16 °C, while this was not so for flower formation, with the exception of 'VR' and 'N' at the short photoperiods. This indicates an increase in the stem formation rate at the lower temperature, relative to the one at the higher temperature. This increase is apparently highest at the longer photoperiods, as the difference between the 2 curves within each cv is greatest at the longest photoperiods.

The only case in which the flower formation response to temperature followed the stem formation response was at the short photoperiods for the cvs 'VR' and 'N'. In the following chapters (2.6, 2.7 and 2.8) it will again be shown that for these qualitative LD cvs, flower formation will occur at SD only if stem formation also occurs.

The conclusion is that vernalization in spinach results in an induced change of the photoperiodic requirement. The range of temperatures which result in
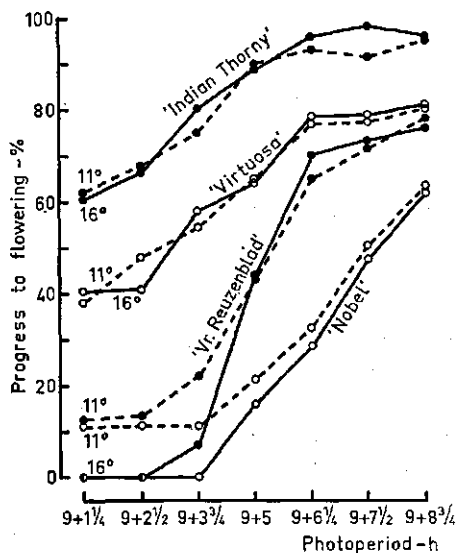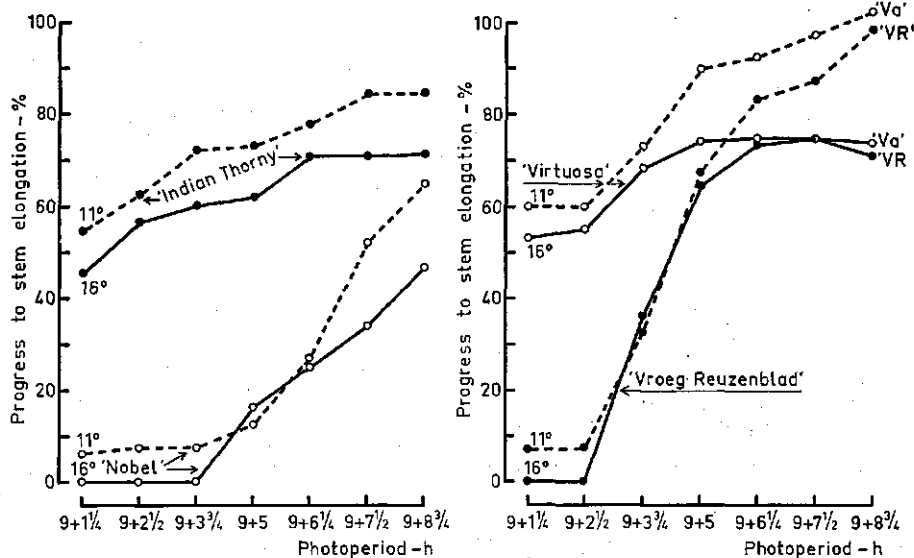
FIG. 6. Progress to stem elongation of 4 cultivars after 12 days at 11 °C or 9 days at 16°C. At each temperature 7 photoperiods were given

a vernalization effect is much broader for stem formation than for flower formation.

2.5.4. *Influence of temperature on the photoperiodic response of flower formation*

To investigate the photoperiodic response at different temperatures, 2 experiments were carried out.

Experiment I: 'Nobel' seeds were germinated at 20 °C for 4 days (–V) and at 5 °C for 21 days (+V), after which they were allowed to emerge in SD at 15 °C. After 7 days in SD the plants were transferred to 3 photoperiods in the spinatron at 10 °C. After 21 days at 10 °C, half of the 24 plants of each treatment were placed in LD after-treatment, together with –V and +V controls. The other 12 plants were kept at the 3 photoperiods for another 13 days, but now at 15 °C. At the end of this second period these plants were also transferred to LD after-treatment, together with –V and +V controls. From the data, the progress to stem elongation and flowering of each treatment during the photoperiodic treatment could be estimated and is given in fig. 7. At the shortest photoperiod some progress to stem elongation and flowering occurred during the 21 days at 10 °C. After raising the temperature to 15 °C the stem and flower formation of the –V plants showed a regression. For the +V plants neither progress nor regression was observed. At the photoperiod of 10 + 4 h the progress to stem elongation and flowering after 21 days at 10 °C was greater than at 10 + 1 h. At 15 °C the stem and flower formation of the –V plants did not proceed further, resembling the reaction of the +V plants at the shortest photoperiod. At the longest photoperiod stem formation (not given) and flower
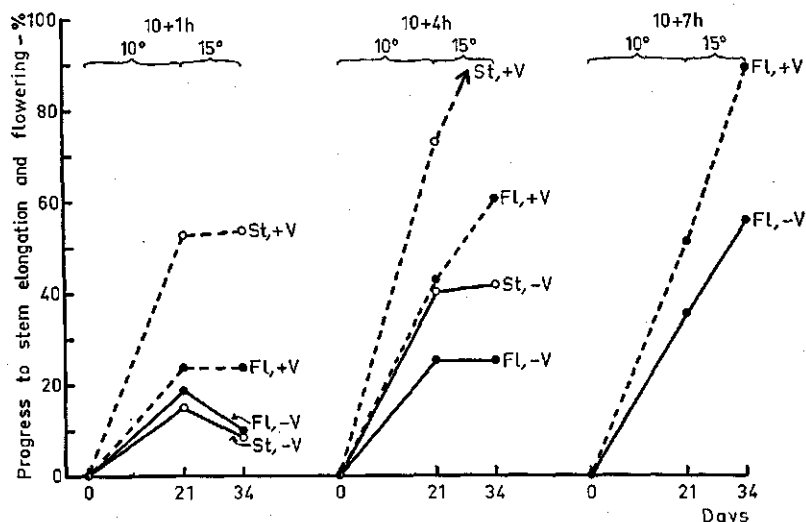
FIG. 7. Progress to stem elongation (St) and flowering (Fl) of unvernalized (–V) and vernalized (+V) plants after 21 days at 10°C, followed by 13 days at 15°C at 3 photoperiods

formation proceeded very rapidly, the +V plants showing a higher rate of progress than the –V plants. Stem elongation and flowering reacted similarly to temperature at all photoperiods.

At 10°C 'Nobel' behaved as a quantitative LD plant as, even at the shortest photoperiod, a progress to flowering was observed. At 15°C, however, it behaved like a qualitative LD plant with a critical photoperiod of $10+1$ h for the +V and $10+4$ h for the –V plants. Both the –V and the +V plants reacted to a change in temperature in the same way, except that the +V plants had a lower daylength requirement than the –V ones. The +V plants, namely, reacted to $10+1$ h in the same way as the –V plants did to $10+4$ h.

Experiment II: 'Nobel' seed was germinated in 15 cm clay pots at 20°C for 4 days (–V) and at 5°C for 18 days (+V), after which they were placed in flat lorries and given SD. This SD was obtained by transferring the lorries daily from the open to a light-tight shed and vice versa. The daylight period was from 8.00 a.m. to 4.00 p.m. This SD period started on 29th March and ended on 6th April, when all plants had emerged, and a photoperiodic treatment started. Each of the 4 lorries was given one photoperiod. The extension of the daylight period of 8 h was obtained by means of weak incandescent light (two 25 Watt bulbs/m², 0.60 m above the plants), given in the light-tight sheds. The temperatures, which only occasionally dropped below 9°C in the first weeks with a minimum of 7°C, increased steadily with time to 15–20°C, or sometimes even higher in the last weeks. After 0, 14, 23, 34 and 45 days from the start of the photoperiodic treatments 15 plants per treatment were transferred to LD after-treatment, together with vegetative unvernalized controls, kept in SD. As only –V controls were used, no progress to flowering for the +V treatments could be

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determined as the latter may have had a higher rate of flower formation in LD after-treatment. In this experiment, therefore, records were kept of the number of days the treated plants flowered earlier than the –V control plants.

Fig. 8 presents the results of experiment II. The results agree very well with those given in fig. 7 although here no progress to flowering, but earlier flowering than the controls, is recorded, this earlier flowering having almost the same meaning as progress to flowering. The –V plants initially showed some flower formation even at the shortest photoperiod, apparently because the temperatures were sufficiently low. Later, probably because of the rising temperatures, the –V plants exhibited regression of flower formation at all photoperiods, except the longest. The +V treatments regressed only at 8 + 2 and 8+4 h. Here, too, the daylength requirement is changed by seed vernalization. One other important fact becomes obvious at the shortest photoperiod. After 34 and 45 days the –V and +V plants flowered almost simultaneously, which means that the +V plants were practically devernalized. The results of other experiments had already led to the assumption that gradual devernalization occurs when flower formation does not proceed fast enough.
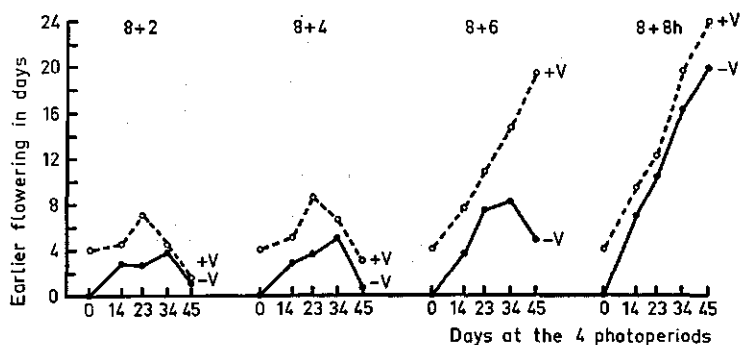
FIG. 8. Earlier flowering in LD after-treatment in days compared with control plants of unvernalized (–V) and vernalized (+V) plants after 0, 14, 23, 34 and 45 days at 4 photoperiods. During the period of 45 days the temperatures increased steadily

The conclusion can be drawn that the photoperiodic requirements for stem and flower formation depend on temperature. The lower the temperature the lower the daylength requirement. At low temperatures (vernalizing ones) this change in the photoperiodic requirement is induced, at non-vernalizing, higher temperatures the effect is not inductive, but direct.

2.6. LIGHT INTENSITY

It is a generally observed fact that LD plants flower later in LD at lower light intensities. This is primarily an energy effect.

Six cvs, representing the complete range of earliness, were sown at a daylength of 16 h at 2 light intensities in the phytotron. The temperature was 22–

24 °C. The emergence occurred at the same time in both light intensities. Table 10 shows that the flower formation was retarded at the lower light intensity. This retardation was greater, the later the cv, 'IT' forming the only exception. It is, however, questionable whether this deviation is reliable, as other similar experiments including 'IT' did not show such a deviation.

TABLE 10. Days to flowering at and the difference in percentage between 2 light intensities of 6 cultivars

Cultivar	Days at light intensity		Difference in %
	100 %	50%	
'IT'	19.1	27.6	44.5
'Va'	25.6	32.5	27.0
'Ji'	25.4	32.6	28.8
'VR'	30.7	40.1	31.3
'N'	36.9	51.2	38.7
'Nr'	49.0	70.0	43.0

This same light intensity effect was also observed in a different way. The experiment described in paragraphs 2.4 and 2.5.3 with 4 cvs and 2 temperatures was extended with a third temperature (21–24 °C) for the longest photoperiod $(9 + 8 \,^3/_4 \text{ h})$. This gave flower data for 3 temperatures of these 4 cvs at a long photoperiod. The light intensity during the 9 h of mainlight remained approximately constant. However, at higher temperatures the plants need more light; if the light intensity is kept constant, the plants react to a temperature increase as if the light intensity had been reduced. Fig. 9 gives the number of days to flowering. From 11 to 16 °C all cvs show approximately the same acceleration in flowering (decrease in days to flowering), apparently because the amount of light was sufficient. From 16 to 22 °C, however, the acceleration of flowering
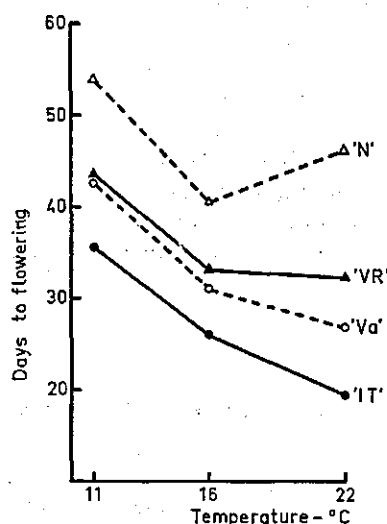

FIG. 9. Days to flowering of 4 cultivars at 3 temperatures in LD (photoperiod 9 + 8³/₄ h) at approximately the same amount of light

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was smaller, the later the cv, while 'Nobel' even exhibited a retardation of flowering.

These experiments show that the light requirements for flower formation of a cv increase, the later the cv is.

In the former experiments the impression was gained that the retardations in growth and flowering were related to each other. 'Nobel' plants, 4 days old from emergence in SD, were placed in the phytotron at 4 light intensities at a daylength of 16 h and a temperature of 21–23 °C. Table 11 shows that the rate of flower formation followed the same course as that of leaf formation, the latter being even more retarded by lower light intensities than the former. The size of the largest leaves together with the rate of leaf formation clearly indicates that the growth rate is strongly affected by light intensity. The data of this experiment suggest that the rate of flower formation decreased together with the growth rate, indicating that these two processes are not altogether independent of each other.

TABLE 11. Number of days to flowering, number of days to form one leaf and size of largest leaves (length × width) after 28 days at 4 light intensities in LD

Light intensity %	Days to flowering	Days needed to form one leaf	Size in cm²
25	71	5.2	7.2
50	50	2.9	22.8
75	42	2.3	42.4
100	39	1.9	67.6

To study the effect of light intensity on the daylength response, another experiment was designed. 'Nobel' plants, 5 days old from emergence in SD, were transferred to 4 light intensities at a daylength of 8 h in the phytotron. The temperature was 21–23 °C. After 4 weeks the plants were placed in LD after-treatment, together with vegetative controls, to measure the progress to stem elongation and flowering at these different light intensities. The data in table 12 are the results of 2 consecutive trials carried out in the same way. As the data of the 2 trials were almost identical, the results have been taken together.

At 100 % light the plants showed no progress to stem elongation or to flowering. At the lower light intensities, however, a considerable progress to stem elongation and a much smaller one to flowering were measured. At 50 % light, the progress was largest for both stem and flower formation.

TABLE 12. Progress to stem elongation and flowering in % after 4 weeks in SD (8 h) at 4 light intensities

Progress to	Light intensity (in %)			
	100	75	50	25
Stem elongation	0.0	47.3	79.9	55.4
Flowering	0.0	9.4	12.0	4.2

A 5th experiment was carried out with 3 cvs at 2 light intensities and 3 photoperiods. The plants, one week old from emergence in SD, were placed in the spinatron at a temperature of 20 °C. The lower light intensity was obtained by removing 4 of the 8 fluorescent tubes per cabinet, resulting in a light intensity of about 50 % compared with normal (100 %).

The results are shown in table 13. The progress to flowering was lower at 50 % light than at 100 % light for most treatments. At some treatments ('VR' at 10 + 1 h, 'N' at 10 + 1 and 10 + 5^{1}/$_{2}$ h), however, the progress to flowering was highest at 50 % light. As the progress to flowering is a measure for the rate of flower formation, it can be concluded that lowering the light intensity retards flower formation at high rates (progress to flowering at 100 % light > 40 %), but accelerates it at lower ones. As flower formation is retarded most at the longest photoperiods and least so, or even accelerated at the shorter ones, the actual effect of lowering the light intensity is a decreased photoperiodic requirement for flower formation, this effect being greater, the later the cv. This suggests, as also did the data given in fig. 9 and table 11, that the rate of flower formation is related to the general growth rate, but only when flower formation proceeds fairly rapidly.

TABLE 13. Progress to flowering in % of 3 cultivars after 21 days at 2 light intensities and 3 photoperiods

Cultivar	Light intensity in %	Photoperiod in h		
		10 + 1	10 + 5½	10 + 10
'Va'	50	46	66	59
	100	52	76	84
'VR'	50	20	57	58
	100	18	62	82
'N'	50	8	30	39
	100	0	27	72

If the rate of flower formation is low, it will be accelerated by decreasing the light intensity, as was shown in tables 12 and 13. At higher rates of flower formation, as occur in LD, a retardation is observed, which seems to be caused by the much stronger retardation of the growth (Table 11). This suggests that the flower formation process, although accelerated by the lower light intensities, cannot express this acceleration as the growth rate has become too low. Since the light intensity is known to have a strong formative effect on elongation (Table 12), it is possible that the acceleration of flower formation at lower light intensities is brought about by the strong promotion of stem formation. This, then, would parallel the action of gibberellic acid, to be discussed in the following paragraph.

2.7. GIBBERELLIC ACID

In many cold-requiring and LD plants, gibberellic acid causes or hastens flower formation under non-inductive conditions. LANG and REINHARD (41)

concluded, that gibberellins are stem-growth hormones, and that those plants in which flower formation depends directly or indirectly on stem growth will tend to respond to gibberellins. WITTWER and BUKOVAC (83, 84) obtained flowering spinach plants at photoperiods of 9–11 h by repeated application of GA_3. The plants flowered after 94–108 days on stems 80–120 cm long. Under LD conditions without GA_3, spinach plants normally flower within 20–50 days on stems 10–30 cm long.

'Nobel' plants, 16 days old from emergence in SD, were transferred to the spinatron at a temperature of 18–20°C. Two photoperiods were used, $11 + 0$ and $11 + 6$ h, acting as SD and LD respectively for this cv. At each photoperiod half of the plants were treated with GA_3, the others with distilled water. The GA_3 plants received an initial spray of 500 ppm at the start of the photoperiodic treatment, followed by a second spray 21 days later. Moreover, GA_3 was applied as drops of 100 ppm in the heart of the plants 4, 7, 11, 14, 25, 28, 32 and 35 days after the first spray. The SD-treated plants were transferred to LD after-treatment, together with vegetative controls, after 42 days in the spinatron. From the data the number of days to flowering in SD in the spinatron could be estimated.

Table 14 shows, that the stem formation was much more affected by GA_3 than the flower formation, in SD as well as in LD. The stem elongation occurred even earlier in SD, $+GA_3$ than in LD, $-GA_3$, although flowering was much later. Some flower formation took place in SD, when stem formation was stimulated.

TABLE 14. Number of days to stem elongation and flowering, and stem length in cm at flowering in SD or LD, with ($+GA_3$) or without ($-GA_3$) gibberellic acid

Treatment	Days to		Stem length in cm at flowering
	Stem elongation	Flowering	
SD, $-GA_3$	∞	∞	–
SD, $+GA_3$	13.2	80.0	–
LD, $-GA_3$	16.4	31.2	19.1
LD, $+GA_3$	8.6	28.0	35.6

A second experiment was carried out at 2 daylengths (8 and 12 h) in the phytotron, at a temperature of 21–23°C. 'Nobel' plants, 14 days old from emergence in SD, were kept for 8 weeks at these 2 daylengths. Five concentrations of GA_3 were given as leaf-sprays every 14 days starting at the beginning of the daylength treatment. After these 8 weeks the plants were transferred to LD after-treatment together with vegetative controls. Table 15 gives the results. During the 8 weeks at 8 and 12 h of daylength the $-GA_3$ plants (0 ppm) did not elongate or flower earlier than the vegetative controls, indicating that no progress to elongation or flowering had occurred. The $+GA_3$ plants (50–400 ppm) elongated 44.7–61.7 days earlier than the $-GA_3$ plants, while flowering was accelerated only 3.5–9.5 days. This agrees with the data of table 14, where stem formation was much more affected by GA_3 than flower formation. The differ-

ence between 0 and 50 ppm GA_3 was greater, especially for stem elongation, than between 50 and 400 ppm GA_3. The GA_3 effect on flowering at the day-length of 8 h was smaller than that at 12 h, suggesting that the daylength response of the flower formation process proper can express itself, if the inhibition of stem formation is removed by GA_3.

TABLE 15. Number of days in LD after-treatment to stem elongation and flowering after 8 weeks at 2 daylengths and 5 concentrations of GA_3 in ppm

Daylength in h	GA_3 in ppm	Days to	
		Stem elongation	Flowering
Control (SD)	0	14.1	22.6
8	0	14.4	23.0
8	50	− 40.3 (15.7)*	19.5
8	100	− 42.4 (13.6)	17.7
8	200	− 41.9 (14.1)	17.2
8	400	− 43.5 (12.5)	16.4
12	0	17.7	23.6
12	50	− 39.5 (16.5)*	18.7
12	100	− 42.4 (13.6)	15.9
12	200	− 44.0 (12.0)	14.7
12	400	− 43.7 (12.3)	14.1

* Number of days from the start of the GA_3 application in SD (8, 12 h).

The earlier flowering in LD after-treatment of the plants treated with GA_3 in SD is due to flower formation in this SD period. However, it cannot be ruled out that the GA_3 induces a higher rate of flower formation in the subsequent LD, resulting in the observed flower promotion. If this was the case, no difference between GA_3 treatment in 8 and 12 h daylengths is expected, as both act as SD. Since a difference was observed, the above mentioned possibility does not seem to be likely. Moreover, the flower promotion should be independent of the duration of the GA_3 treatment in SD.

To test this 'Nobel' plants were sprayed with 100 ppm GA_3 every 14 days for 4 or 8 weeks at daylengths of 8 and 12 h. The data of table 16, 5th and 6th column, indicate that the flower promotion, expressed as 'earlier flowering in days', was twice as great after 8 weeks as after 4 weeks of GA_3 treatment. This is shown best (6th column of table 16) when the average number of days to flowering of the 4 −GA_3 treatments is used as a control (23.6 days), this being permissible since both 8 and 12 h act as SD. These results therefore indicate that the flower promotion in the LD after-treatment is, at least for the greater part, due to flower formation in the preceding SD period.

Since low light intensity and GA_3 have approximately the same effect on stem and flower formation in SD, both factors have been studied in combination. 'Nobel' plants, 5 days old from emergence in SD, were transferred to 4 light intensities in the phytotron. The daylength was 8 h, the temperature 21–23 °C. Eight and 15 days from the start of the treatment in the phytotron, half of

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TABLE 16. Number of days to flowering in LD after-treatment of plants, treated with (+) or without GA₃ (–) during 4 or 8 weeks at 2 daylengths, and the earlier flowering in days of the +GA₃ plants compared with their own controls (– GA₃ plants) or with the average control (average of the 4 – GA₃ treatments)

Treatment			Days to flowering	Earlier flowering compared with	
Daylength in h	Duration in weeks	GA₃ (100 ppm)		Own controls	Average control
8	4	–	23.7		
8	4	+	20.6	3.1	3.0
8	8	–	23.0		
8	8	+	17.7	5.3	5.9
12	4	–	24.0		
12	4	+	19.9	4.1	3.7
12	8	–	23.6		
12	8	+	15.9	7.7	7.7

the plants were sprayed with 100 ppm GA₃. After 5 weeks in the phytotron the plants were placed in LD after-treatment.

The results, given in table 17, show that stem and flower formation did not proceed at 100% light and –GA₃. At lower light intensities, however, both processes showed progress, stem formation considerably more than flower formation. The application of GA₃ resulted in a strong increase in the progress to stem elongation at all 4 light intensities, compared with the –GA₃ treatments. For flower formation quite different results were obtained. Only at 100% light was flower formation stimulated considerably by GA₃; at the other 3 light intensities, with flower formation already proceeding, GA₃ had no important effect on flower formation. This shows quite clearly that the effect of GA₃ on flower formation is indirect, through its effect on stem formation.

TABLE 17. Progress to stem elongation and flowering of – GA₃ and +GA₃ treated plants after 5 weeks in SD (8 h) and 4 light intensities

Progress to	GA₃ 100 ppm	Light intensity			
		100%	75%	50%	25%
Stem elongation	–	0	58	72	43
	+	184	192	154	86
Flowering	–	0	14	18	6
	+	14	18	16	4

These results also reinforce the earlier mentioned suggestion that if stem formation is no longer inhibited, flower formation will proceed at a low rate in SD. This would mean that a qualitative LD cv such as 'Nobel' reacts qualitatively only with respect to the stem formation process. These results also agree with those of WELLENSIEK (79) who studied the effect of GA₃ in SD on flower formation in the subsequent LD. In some plants stem formation is a limiting factor, in others it is not.

2.8. DAYLENGTH

2.8.1. *The effect of short day on stem and flower formation*

In paragraph 2.4 it has been shown that some cvs have a quantitative, others a qualitative LD requirement. And, indeed, the following experiment shows that a qualitative LD cv such as 'Nobel' does not make any progress to flowering in SD. 'Nobel' plants, sown and grown for various lengths of time in SD (8 h) were transferred to LD after-treatment simultaneously. Table 18 clearly indicates, that no progress to stem elongation or flowering occurred in SD. The number of days to stem elongation has a tendency to increase for the older plants. This, however, is an observational error as it becomes gradually more difficult to determine the day of first-visible stem elongation with older plants, because of the increasing number and size of the petioles at the base of the plant rosette.

TABLE 18. Number of days to stem elongation and flowering in LD after-treatment of 'Nobel' plants 0–50 days old from emergence in SD

Age	Days to	
	Stem elongation	Flowering
0	15.4	29.4
4	15.2	28.1
8	15.2	28.4
12	15.0	27.0
16	16.1	28.9
22	15.1	26.6
30	16.0	28.8
40	17.1	29.9
50	18.0	30.7

Two more experiments were done with 2 quantitative and 2 qualitative LD cvs respectively. The first 2 cvs were sown and kept in SD or LD until flowering. In this connection it should be realized that the amount of light received per day was considerably higher in LD than in SD, the difference being about 50%.

Stem elongation, as well as flowering (table 19), occurred much earlier in LD than in SD. 'IT' flowered at a much longer stem in SD than in LD, while the difference was almost negligible for 'Va'. This could mean that stem formation is less retarded by SD than flower formation in 'IT', but not so in 'Va'.

TABLE 19. Number of days from emergence to stem elongation and flowering, and stem length in cm at flowering of 2 cultivars at 2 daylengths

Cultivar	Daylength in h	Days to		Stem length
		Stem elongation	Flowering	
'IT'	8	24.6	50.9	33.1
	19	10.5	18.4	14.4
'Va'	8	21.6	58.5	15.0
	19	9.8	21.1	13.6

The 2 cvs of the second experiment were kept for 3 or 45 days in SD, after which they were transferred to LD after-treatment. No consistent differences were found for the two age-groups (Table 20). Hence, like 'Nobel', these 2 cvs did not show any progress to stem elongation or flowering in SD.

TABLE 20. Number of days to stem elongation and flowering in LD after-treatment of 2 qualitative LD cultivars, 3 or 45 days old from emergence in SD

Cultivar	Age	Days to	
		Stem elongation	Flowering
'VR'	3	12.2	25.2
	45	13.5	25.0
'Nr'	3	13.5	32.2
	45	15.1	32.1

2.8.2. *The effect of daylength on the differentiation of the apex*

In paragraph 2.7 it was suggested that qualitative LD cvs were only so with respect to stem formation. This means that flower formation might occur in SD if it is not blocked by the inhibition of the stem formation process. EGUCHI (15) claimed that the flower initiation process in spinach is a day-neutral one, while the further development requires LD. To test this, an experiment with 2 qualitative LD cvs was carried out. Seed was sown in wooden boxes and kept in SD (8 h) or LD (19 h). Plants at both daylengths were collected periodically over a period of 85 and 12 days, respectively. At each sampling the growing tips of 8 plants were fixed in 96 % alcohol and stored in 70 % alcohol. The developmental stages of the growing-points were evaluated under a dissecting microscope according to the following scale:

  I Vegetative. Shoot apex relatively flat and small.
  II Slight swelling of the shoot apex.
 III Apex approximately convex.
 IV Apex slightly higher than broad.
  V Axillary bud primordia just visible.
 VI Axillary bud primordia clearly visible; the apex, however, not yet on a short stem.
VII Apex on a short stem, not yet macroscopically visible.

The development of the apex in LD, as shown in table 21, was approximately the same for both cvs; within 12 days stem formation, although not yet macroscopically visible, had started. The differentiation, consisting of the formation of flower primordia in the axils of the leaf primordia, was already visible within 9 days. This suggests that the differentiation process starts earlier than, or at the same time as, the stem formation process. After 15 days from emergence (not given in table 21) the plants of both cvs were completely generative: male and female plants could be readily distinguished.

In SD the development of the apex was much slower, but for the rest exactly the same as in LD until stage VI. This agrees with the general observation that

TABLE 21. Development stage of the apex at a different number of days from emergence (age) in LD and SD of 2 cultivars

Age	Stage			
	LD		SD	
	'VR'	'N'	'VR'	'N'
3	I	I	–	–
6	II	III	–	–
9	V	V	–	–
12	VII	VII	I	I
21	–	–	II	I
35	–	–	III	I
49	–	–	IV	III
63	–	–	V	V
74	–	–	VI	VI
85	–	–	VI	VI

the transition to the flowering phase is accompanied by a stimulation of the formation or outgrowth of axillary buds (2). The plants did not develop further than stage VI, the last stage before stem formation becomes visible in LD (stage VII). The rate of differentiation seemed to be slightly higher for 'VR' than for 'N'. At stage VI it was possible, although with difficulty, to distinguish male and female plants. The male plants generally have more axillary bud primordia per leaf primordium than the female plants. The above-mentioned observations explain why in SD the rate of flower formation is low if stem formation is made possible by lower temperatures, lower light intensities or GA_3 application. Apparently there are 2 processes necessary for flower formation, namely differentiation and growth of caulescent tissue.

Although plants which have reached stage VI in SD, are further differentiated than just emerged plants, they flower at the same time after transferring to LD. This means that the rate of the stem formation process is the limiting factor in determining the date of flowering. This also offers an explanation of the slightly earlier flowering in LD after GA_3 application, as observed in table 14, because stem formation is then no longer the limiting factor.

2.8.3. *The effect of short day intercalated in a long day treatment*

In paragraph 2.5 it has been shown that in SD regression can occur after a progress to flowering has taken place. The following experiment shows that regression may occur at different stages of flower formation. 'Nobel' plants, sown in flats, were given LD periods followed by SD periods. The treatments were given in such a way that the LD/SD treatments ended on the same day, whereupon the plants were transferred to LD after-treatment. Each treatment consisted of one flat with 25–40 plants. Table 22 shows that the control plants (no intercalated SD) needed 26.2–26.9 LD for flowering. If SD was intercalated into LD, more long days were necessary for flowering, indicating that the effect of the preceding LD period was partly annulled. Even after 20 LD, when

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TABLE 22. Effect of an SD period intercalated in an LD treatment on the total number of LD cycles to flowering

LD cycles preceding SD	Intercalated SD cycle		
	0	10	20
10	26.2	32.7	32.7
20	26.9	33.5	37.5

all plants had elongated and the first ones even had visible flower buds, this regression in SD occurred.

When the plants are transferred from a LD period to an intercalated SD period regression occurs, as was shown in table 22, but before this regression can occur it is necessary for the rates of stem and flower formation to be brought to a standstill. Whether this will take some time, has been studied in the following experiment.

'Nobel' plants were given 14 LD followed by 0 or 28 SD. Per treatment, 36 plants were used. The SD period resulted in some regression (6.2 days) as flowering occurred after 33.4 LD compared with 27.2 LD needed for the controls (0 SD). Stem formation had started at the beginning of the SD period and went on until all plants had elongated, as table 23 shows. The percentage of plants with visible flower buds also increased in the first 3 weeks of the SD period, after which the process of flower formation was stopped. These data suggest, therefore, that the regression in the progress to flowering occurred mainly in the last 7 days, as in the first 21 days of the intercalated SD period progress to flowering still occurred.

TABLE 23. The percentage of plants elongated and with flower buds in an intercalated SD period of 28 days, preceded by 14 LD

Stage	Days from the start of the intercalated SD period				
	0	7	14	21	28
Elongated	23	90	100	100	100
Flower buds	0	17	20	27	27

Not only qualitative LD cvs, like 'N', react to such an intercalated SD period. An experiment was done with 'Va', which flowers in SD too. 'Va' plants, one day old from emergence in SD, were exposed to different numbers of LD, ranging from 0–15, after which they were returned to SD. Table 24 shows the results. In SD the plants needed 49.2, in LD 23.6 days to flower. The rate of flower formation in SD, given in relation to that in LD, which is put at 1.0 is calculated as follows :The number of LD cycles which should still be needed for flowering at the start of the SD period (23.6–0.0 or 3.0 or 6.0 days, etc.) is divided by the number of SD cycles needed for flowering (49.2, 44.5, 34.8 days etc.). The rate of flower formation in SD for the 3rd treatment is, for example (23.6–6.0):34.8 = 0.51. These rates of flower formation in SD were about the

same for the plants receiving 0, 3, 6 or 9 preceding LD, while they increased for the 12 and 15 LD treatments. This latter increase might well be caused by the fact, that it takes some time before the high rate of flower formation of the preceding LD, is reduced to the rate of the SD, as was shown in the foregoing experiment. As this reduction takes some time, the rate of flower formation in SD will be overestimated, especially for the treatments needing relatively few SD for flowering like the 12 and 15 LD treatments. This reasoning, however, does not exclude the possibility that 'Va' tends to become more day-neutral at advanced stages of flower formation.

TABLE 24. Days to flowering and the rate of flower formation of 'Va' in the SD period following 0–15 LD

Number of cycles		Total days to flowering	Rate of flower formation in SD
LD	SD		
0.0	49.2	49.2	0.48
3.0	44.5	47.5	0.46
6.0	34.8	40.8	0.51
9.0	29.0	38.0	0.50
12.0	20.2	32.3	0.57
15.0	12.2	27.2	0.70
∞	–	23.6	–

An experiment was carried out to study the effect of GA_3 in SD. 'Nobel' plants, 16 days old from emergence in SD, were transferred to the spinatron at 2 photoperiods, $11 + 0$ h (SD) and $11 + 6$ h (LD), and a temperature of 18 °C. After 21 days the photoperiods were changed, the SD becoming LD, the LD becoming SD, with the exception of one treatment, which remained in LD. After the 2nd period of 21 days all plants received LD until flowering. Half of the plants in SD were treated with GA_3, which was given as a 500 ppm spray at the start of the SD period, followed by the application of one drop of 100 ppm GA_3 in the heart of the plant, 4, 7, 11 and 14 days after the initial spray.

Table 25 shows that stem elongation and flowering occurred after about 16 and 31 LD respectively in treatments 1 and 2, indicating that the 21 SD preceding LD did not influence flower formation in the subsequent LD. In treatment 3 stem elongation occurred in the first LD period, flowering, however, in the 2nd LD period, after 21 intercalated SD. The total number of LD needed for flowering was about 14 more than needed in the former 2 treatments. This points to regression in the SD period of the progress to flowering, made in the 21 LD preceding the SD. This regression was observed to occur in a rather advanced stage of flower formation. The axillary flower primordia were well developed, but not yet macroscopically visible. These primordia, however, did not grow out in the LD of period III, as the flowers were formed after a considerably greater number of leaves than on the plants given LD continuously. This indicates either that the most developed flower primordia become arrested in SD and remain so even in the succeeding LD, or that they revert back to a more

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vegetative stage, resulting in a side shoot, which ultimately will also flower in LD. The application of GA_3 in SD (treatment 4) caused a considerable progress to flowering in SD, as the flowering in the subsequent LD occurred 8.3 days earlier than treatment 2. In the intercalated SD, too, a progress to flowering instead of a regression was measured after application of GA_3 (treatment 5), the progress being about the same as in the SD of treatment 4.

TABLE 25. Total number of LD to stem elongation and flowering after 21 SD with ($+GA_3$) or without ($-GA_3$) gibberellic acid, the SD period preceded by or intercalated in LD

Treatment No.	Treatment during period				Long days to	
	I (21 days)		II (21 days)		Stem elongation	Flowering
	Daylength	GA_3	Daylength	GA_3		
1	LD	–	LD	–	16.4	31.3
2	SD	–	LD	–	15.9	31.0
3	LD	–	SD	–	15.1	45.3
4	SD	+	LD	–	–7.8*	22.7
5	LD	–	SD	+	15.2	21.8

* Elongation occurred in SD, 13.2 days after the first application of GA_3.

From the experiments with 'N' and 'Va' the conclusion can be drawn that both stem and flower formation react directly to daylength in approximately the same way, whether these processes have been started or not. This means that neither process is inductive in nature.

2.8.4. *The effect of photoperiods shorter than 8 hours*

DE LINT (43) and LONA (47) observed a decreased inhibition of flower formation in the LD plants *Hyoscyamus niger* L. and *Urtica pilulifera* L. respectively at very short photoperiods. It was investigated whether spinach too might show a reduced inhibition at very short photoperiods.

Two cvs 'Va' and 'N', 20 and 28 days old from emergence in SD, were placed in the spinatron at photoperiods of $3+0$, $3+3$, $3+6$ and $3+10$ h for 11 days.

TABLE 26. Number of LD cycles to stem elongation and flowering of 2 cultivars after 11 days at 6 photoperiods

Photoperiod	LD cycles to			
	Stem elongation		Flowering	
	'Va'	'N'	'Va'	'N'
$3+0$	–	21.9	15.2	37.7
$3+0$	–	23.1	15.2	39.8
$3+3$	–	25.2	16.0	41.1
$3+6$	–	24.7	15.8	40.5
$3+6$	–	24.9	17.1	41.9
$3+10$	–	21.9	12.8	36.5

The temperature was 15°C. Per treatment 15 plants were used. The $3+0$ and $3+6$ h treatments were duplicated.

At the end of the 11 days the plants were yellowish-green. 'Va' had already started to elongate before the photoperiodic treatments started. Table 26 shows that the inhibition of stem formation of 'N' was greatest at $3+3$ and $3+6$ h. The flower formation of both cvs also showed a maximal inhibition at $3+3$ and $3+6$ h. Although the differences are small, they are significant.

Since spinach like *Hyoscyamus niger* and *Urtica pilulifera* showed a decreased inhibition of the flower formation process at very short photoperiods, this effect seems to be of a more general nature.

2.9. DISCUSSION

From the experiments the conclusion can be drawn that spinach is a quantitative LD plant, although some genotypes behave like a qualitative LD plant at relatively high light intensities and temperatures (15–25°C).

Stem and flower formation both appear to be LD processes, of which the former seems easier to modify than the latter. In the so-called qualitative LD cvs stem formation is the qualitatively-reacting LD process. The stimulation of stem formation in SD by, for instance, low light intensity, GA_3, or temperatures of ca 10°C, results in a low rate of flower formation. From a study of the apex in SD the differentiation process, i.e. the formation of axillary bud primordia giving the flower clusters, appears to proceed at a much lower rate than in LD, until the stage is reached at which in LD stem formation starts. In SD the further differentiation is stopped. Apparently growth of caulescent tissue is needed for the continuation of the differentiation process. This is substantiated by the observations that flower formation is blocked if SD is given after a considerable progress to flowering has taken place in a preceding LD, while flower formation proceeds if GA_3 is given in the intercalated SD.

From these observations the following hypothesis is put forward. The flower formation consists of at least 2 processes: Firstly, a differentiation process regulating the initiation and further differentiation of the generative tissues, and secondly, a process, regulating the growth of caulescent tissue of the differentiating flower clusters. Both processes would be under control of different growth hormones. A *floral stimulus* as postulated for other flowering plants (40), would regulate the differentiation process, while the growth of caulescent tissue would be under control of gibberellins. This then is essentially the same hypothesis as put forward by CHAILAKHYAN (61), who supposes that the *floral stimulus* consists of an anthesin and gibberellin, the former needed for flower formation, the latter for stem growth.

SACHS et al. (63, 64, 65) indeed showed that gibberellins activate stem growth. They observed that there are 2 meristems in the growing tips of plants. The apical meristem is the site of shoot organization and the ultimate source of the entire shoot. This meristem would be responsible for the differentiation process. Separated from the apical meristem by a block of tissue showing less meristem-

34   *Meded. Landbouwhogeschool Wageningen 67-2 (1967)*

atic activity, the subapical meristem is found. This meristem is largely responsi-
ble for stem formation in caulescent as well as in rosette plants. Gibberellins
or native gibberellin-like substances, appear to regulate the activity of this sub-
apical meristem and could therefore be called stem-growth hormones as Lang
and Reinhard (41) proposed. Lang (39) suggests an even more general action
of gibberellins, which would be involved in the regulation of meristematic acti-
vity in general and not in the regulation of differentiation. Apical meristems in
rosette plants would then be activated at much lower levels of gibberellins than
the sub-apical meristems.

Within the regions of apical and sub-apical activity Wardlaw (78) distin-
guishes a number of growth centres giving rise to the various plant organs.

The simultaneous need for differentiation and growth of caulescent tissue is
not surprising, if it is realized that the flower clusters are, in fact, transformed
side-shoots. The axillary bud primordia giving rise to these flower clusters are
therefore likely to consist of a pattern of growth centres of apical and sub-
apical origin, which would need floral stimulus and gibberellins respectively.

Not only in spinach, but also in other LD plants, the stem growth process
may be the limiting factor in flower formation. In table 27 some LD plants, re-
presenting different reaction types, are given. In SD they do not elongate (ex-
cept the caulescent LD plants) nor flower. If GA_3 is applied in SD, stem growth
can occur enabling the differentiation process to proceed at its own rate. This
is high for *Rudbeckia bicolor* (10), low for *Spinacia oleracea* 'Nobel' and annual
Hyoscyamus niger (79) and zero for *Silene armeria* (79) and *Centaurea calcitra-
pa* (48). The caulescent species (40) do not react to GA_3, which is understand-
able because stem growth already occurs in SD without GA_3. The daylength
requirements for stem growth and differentiation may therefore be quite
different. The rosette plants are qualitative LD plants for the stem growth
process, but vary with respect to the differentiation process, *Rudbeckia bicolor*
being about day neutral, *Spinacia oleracea* and annual *Hyoscyamus niger* quan-
titative LD requiring and *Silene armeria* and *Centaurea calcitrapa* qualitative
LD requiring. The caulescent species are qualitative LD requiring only with
respect to the differentiation process.

TABLE 27. Effect of treatment with GA_3 on the rate of flower formation in SD in 7 LD plants

Species	Rate of flower formation		
	SD		LD
	$-GA_3$	$+GA_3$	$-GA_3$
Rudbeckia bicolor	−	+++	+++
Spinacia oleracea 'Nobel'	−	+	+++
Hyoscyamus niger, annual strain	−	+	+++ rosettes in SD
Silene armeria	−	−	+++
Centaurea calcitrapa	−	−	+++
Anagallis arvensis	−	−	+++ caulescent
Sedum telephium	−	−	+++ in SD

The results indicate that the daylength responses of the stem growth and the differentiation process in spinach are not inductive. The rate of stem growth, as well as the rate of differentiation, react to the actual daylength given, irrespective of the preceding daylength treatments. Only at an advanced stage of flower formation does the dependence on daylength of the flower formation seem to decrease, although the data were certainly not conclusive. This lasting dependence on daylength suggests that the formation of floral stimulus and gibberellins is not induced, but directly regulated by the daylength.

Temperature on the other hand has clearly an inductive effect. Low temperatures ranging from slightly above zero up to 8 °C induce a change in the photoperiodic requirements of both processes, while slightly higher temperatures (9–13 °C) have only an inductive effect on the stem formation process. The changed photoperiodic response of vernalized plants, as presented in fig. 7 on p. 20, can best be described as follows: for a given flowering response +V plants require a shorter photoperiod than –V plants. But the +V plants do also react to the actual photoperiod given. When they are transferred from LD to SD, the rate of development is adjusted to the SD. This strongly suggests that the vernalization effect is a condition rather than a substance. This was also proposed by BARENDSE (1) for *Cheiranthus allionii*. Hort. The condition would involve a changed regulation of the formation of floral stimulus and gibberellins by daylength. CHAILAKHYAN (9, 10) indeed observed that the level of gibberellin-like materials was high in LD for vernalized winter cvs of wheat *(Triticum vulgare)*, rape *(Brassica napus)* and rye *(Cecale cereale)*, but low if the cvs were not vernalized. DOORENBOS (80) suggested that the leaves formed by the vernalized meristem react differently to the daylength. According to CROSSIN and MATHON (11) many LD plants show, like spinach, a decreased daylength requirement after vernalization.

The decreased inhibition of stem and flower formation at very short daylengths, however, forms a separate problem. Like several other photoperiodic plants, SD as well as LD ones (7, 16, 23, 43), spinach flowers also in continuous darkness (17, 81). Like *Hyoscyamus niger* (43) and *Urtica pilulifera* (47) spinach shows less inhibition at photoperiods of 3 h than at 8–10 h. From the investigations of LOCKHART (44), LOCKHART and GOTTSCHALL (46), GORTER (18), KENDE and LANG (31) and KÖHLER (34, 35), it seems that in peas light exercises an inhibiting effect on stem growth, which is overcome by the production of gibberellins. LOCKHART (45) showed that the light inhibition in dwarf peas is mediated by the phytochrome system, red light being most effective in causing suppression of stem growth. R. L. JONES, according to VAN OVERBEEK (56), found that dwarf peas contained more dormin than tall ones, while there was no difference in gibberellin content. The production of dormin seemed to be linked to the phytochrome system. This suggests that the above-mentioned light inhibition is caused by the production of dormin. With the flowering of spinach the same observations have been made. Spinach plants of the cv. 'Indian Thorny', germinated and grown in the dark (81) formed a pronounced stem, bearing flower parts; this was also found by GENTSCHEFF and GUSTAFSSON

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(17), who used another cv. In SD (8–12 h) stem formation is inhibited completely in qualitative LD cvs like 'Nobel' and only partly in quantitative LD cvs like 'Indian Thorny'. In analogy with the stem growth of peas this inhibition could be a light inhibition via the production of dormin, this inhibition being removed by the production of gibberellins in LD. In continuous darkness, stem formation proceeds because no light inhibition (dormin) is present, although there is no gibberellin either. When small amounts of light (short photoperiods or low light intensity) are given, the amount of light is not sufficient to give complete suppression of stem growth, as has been found in the preceding tables 13, p. 24 and 26, p. 33. However, flower formation reacts approximately in the same way as stem formation, which suggests that the differentiation process is not suppressed in the dark, and is maximally suppressed at photoperiods of 6–10 h. It is therefore thought that for the differentiation process a similar light inhibition exists, which can be overcome by the floral stimulus production in LD. In the dark no floral stimulus production occurs, but there is no light inhibition either, so that the flower formation proceeds at a more or less normal rate.

SALISBURY (66) has demonstrated the great diversity of response in flowering. Although only a rather limited number of LD plants have been investigated thoroughly enough to warrant more detailed conclusions, it is clear that even within this group of plants a great diversity exists. It should, however, be noted that a great diversity in flowering response does not necessarily mean great differences in biochemical pathways. From the LD-plants which have been studied more extensively, *Rudbeckia*, endive and lettuce have a flowering response rather similar to that of spinach and will be discussed in some more detail.

Rudbeckia bicolor Nutt reacts to LD like spinach. Stem formation occurs only in LD and stops in SD, while flower formation also needs LD until a rather advanced stage is reached (52). SD is only inhibitory if the light intensity is high (SAMYGIN, 1948 mentioned by CARR, 5) as found for 'Nobel' spinach. Gibberellin applied in SD results in rapid flowering (10), while spinach shows only a low rate of flower formation under such conditions. As in spinach, stem growth is a LD process in *Rudbeckia* and determines the flowering response. The differentiation process, however, seems independent of daylength, which deviates from the spinach response.

Endive (*Cichorium endivia* L) is a quantitative cold-requiring LD plant. It flowers ultimately under all conditions where growth occurs (21), but the flower formation is very slow, unless low temperatures have been given (59). Gibberellins can replace the vernalization treatment, resulting in flower formation in SD as well as in LD, but faster in the latter daylength (60). Differentiation of the growing point appears to occur at the moment the plants start to bolt (19, 20). In gibberellin-treated plants, stem formation preceded differentiation considerably (19). The data suggest strongly that flower formation can only proceed if stem growth occurs, as in spinach. With vernalization both stem and flower formation seem to be stimulated (20). Like the stem formation

process in spinach, bolting is stimulated in endive by temperatures up to ca. 12 °C.

Lettuce (*Lactuca sativa* L.) is a quantitative LD plant, low temperatures accelerating flowering to some extent (3, 61). As in spinach, the cvs vary considerably in their daylength response, but the relation between earliness and daylength response is quite different. In spinach the daylength response curve tends in general to be more dayneutral, the earlier the cv. In lettuce this is just reversed, the late cvs being almost dayneutral (3, 61). This has also its consequences on the effects of vernalization. Like in spinach and other crops the lettuce cvs become earlier after vernalization. The daylenght response then tends to become more dayneutral in spinach and less so in lettuce. This is realized by a vernalization effect being strongest in SD for spinach and in LD for lettuce (61).

3. RELATION BETWEEN GROWTH AND DEVELOPMENT

3.1. INTRODUCTION

Two important aspects of the growing of spinach will be dealt with in this chapter, namely those of earliness and yield which both may vary with the growing methods used.

As in chapter 2, it is necessary to define the meaning of a few concepts used in this chapter.

Y i e l d is the fresh or dry weight of the shoots (stem and leaves). The yield after different treatments can be compared in 2 ways:

    1. At the same moment. Yields measured in this way give an indication of the average rate of growth.

    2. At the same stage of development.

E a r l i n e s s is determined by the time lapse between sowing and readiness for harvest. The latter is a rather unspecified concept and therefore difficult to apply to research, but in order to have a working-definition for this purpose, the crop is said to be ready for harvest when the average stem length is 5.0 cm. This definition of earliness is not always identical with earliness as it is used by the growers, but it is a good approximation.

3.2. LITERATURE

About the influence of growing methods on yield a lot of practical knowledge has been accumulated by the growers. But pertinent data are rather scanty in the literature. Only cv trials have been carried out and reported in great numbers. Most of these trials, however, are difficult to interpret as the harvest criterium is not described. Often the cvs are neither harvested at comparable stages, nor on the same day.

VAN OORSCHOT (55) showed that while the growth rate was higher at longer photoperiods, the highest yields were ultimately obtained at the shorter ones. THOMAS (70) observed a pronounced stimulation of leaf growth after transferring spinach seedlings from SD to continuous light for 2–6 days. REINHOLD (62) compared 8 cvs at different daylengths in the field during the spring and summer. The optimal daylength for growth tended to be shorter for the early cvs than for the late ones. TRONICKOVA (72) found that in spring-sown spinach yields increased when bolting was slower. Earlier sowings or later cvs therefore give higher yields.

BOSWELL (2), studying 7 cvs at 17 different sites and sowing dates in the U.S.A., found that the order of earliness was invariably the same. According to IWAMA (24) is the optimum temperature for vegetative growth 15–20°C. Several authors (26, 50, 74) state that yields increase when GA_3 sprays are applied. They probably compared the different treatments at the same moment and not at the same stage.

ZINK (85) observed that the yields increased at an accelerated rate, due to the

accelerated rate of leaf area expansion, as the leaf number increased at an almost constant rate from emergence to harvest.

3.3. MATERIAL AND METHOD

In the following chapters, a number of trials carried out in the field or glasshouse are described. Most of these trials were field trials carried out in 1963, 1964 and 1965 at 2 sites, a rather heavy clay near Duiven and a light sandy clay near Groessen, both like Wageningen, at a latitude of approximately 52°. All these trials were sown with a precision seed-drill machine, one row at a time. The seed rate was generally between 50 and 60 kg/ha, resulting in a plant density of 40–70 plants per meter-row. With plant densities of more than 30–40 plants the yield no longer responds significantly to plant density under normal growing-conditions. Practically all trials were sown at a row distance of 0.20 m. To estimate the yield and the day of harvest at a stem length of 5.0 cm, at least 2 and generally 3 or more harvests per plot were taken. The stem length and plant density were measured from 'one-meter-row' samples cut with a knife at soil level at the time of harvest. The rest of the sub-plot to be harvested was cut with a scythe or, if the sub-plots were smaller than 1 m², with a knife. The successive samples were always taken at stem lengths varying from approximately 3 to 8 cm. Using the linear regressions of yield and harvest day on stem length, the yield at a stem length of 5.0 cm and the day on which this was reached could be estimated.

With cv trials sown on the same day, the plots were separated from each other by leaving open one row of 0.20 m. This is enough to avoid cv interactions. In the trials of this type no guard rows were used, as all plots, except those at the sides, grew under the same conditions. The side plots were bordered by guard plots. In the case of replicated plot trials, including different sowing-dates, the outer rows of each plot were used as guard rows.

The other trials, including those carried out in the glasshouse, will be described in the appropriate paragraph.

3.4. THE INFLUENCE OF GENOTYPE

3.4.1. *Earliness*

In chapter 2 it was shown that stem elongation and flowering are closely linked processes. The expectation therefore is that earliness, determined by the moment at which the crop reaches a stem length of 5.0 cm, is largely controlled by the same factors that control the flowering behaviour. In paragraph 2.4 it was shown that especially the response to daylength may vary considerably with cvs. Hence, the daylength response of a cv may probably influence its earliness to a great extent. This was studied in the following experiment.

Four cvs were sown in a heated glasshouse on 10th February and in the field on 11th March 1965. At each site the cvs were randomized within blocks with 2 replicates. The row distance was 0.25 m in the glasshouse and 0.20 m in the field. The plant number per meter-row varied from 45–70. The day on which

each cv reached a stem length of 5.0 cm was recorded. The photoperiodic response of these 4 cvs was determined in the spinatron. 'Va', 'VR', 'N' and 'Nr' were sown in plastic pots. After emergence in SD, the seedlings were transferred to a range of 6 photoperiods (11 + 1 up to 11 + 6 h) in the spinatron at a temperature of 13–15 °C. After 27 days the plants were placed in a LD after-treatment (19 h) together with vegetative controls. The progress to flowering made at the different photoperiods was estimated as described in paragraph 2.3. From these data the number of days to flowering at the photoperiods of 14 and 16.5 h could be estimated.

Comparing the earliness data (2nd and 3rd column of table 28) of the 2 sowings the much wider range in the glasshouse attracts attention and this may be attributed to the higher temperature, lower light intensity and/or shorter daylength conditions in the glasshouse compared with those in the field. It is, however, unlikely that the higher temperatures or the lower light intensities caused the greater range in earliness in the glasshouse. A higher temperature, on the contrary, would decrease the differences in earliness, as it increases the development rate. Lower light intensities, too, tend to decrease the differences in earliness, as will be discussed later (paragraph 3.6, table 37). The 3rd environmental factor, daylength, was therefore thought to be the causative factor.

TABLE 28. Earliness and flowering in days from 'Va' of 4 cultivars at 2 sowing dates and 2 photoperiods respectively

Cultivar	Earliness at sowing		Flowering at photoperiod	
	Glasshouse	Field	14 h	16.5 h
'Va'	0	0	0	0
'VR'	14	0	27	–1
'N'	27	10	52	11
'Nr'	37	19	–	21

The daylength during the growth period of the glasshouse sowing (emergence on 20th February)was considerably shorter than the daylength during the growth period in the field (emergence on 30th March), the average difference in daylength being approximately 2.5 h. The days to flowering at 2 photoperiods, differing by 2.5 h, are given in the 4th and 5th column of table 28. The glasshouse sowing shows an order of earliness very similar to the order of flowering found at the shortest photoperiod. The earliness of the four cvs as found in the field sowing follows the same course as the flowering at the photoperiod of 16.5 h. This close agreement between the earliness data of the 2 sowings and the flowering data of the 2 photoperiods warrants the conclusion that the order of earliness in the glasshouse and field sowings in late winter and spring is largely determined by the daylength requirements of the cvs.

Together with the 4 cvs of the above-mentioned experiment a large number of cvs was sown in the glasshouse on 10th February and in the field on 11th March 1965. Table 29 gives the earliness data of 17 cvs sown in the glasshouse and in the field, including the 4 cvs of the first experiment. In the glasshouse

sowing the cvs 'Artex' to 'Spica' show a range in earliness of 23 days, while these cvs all have about the same earliness in the field. The later cvs show an increase in lateness at both sowings. The increase from 'Spica' to 'Noordland' was 16 days in the glasshouse and 17 days in the field.

TABLE 29. Earliness in days from 'Va' (=0) at 2 sowing dates of 17 cultivars

Cultivar	Earliness	
	Glasshouse	Field
'Artex'	–2	0
'Virtuosa'	0	0
'Huro'	2	1
'Protecta'	7	2
'Spartan B'	8	0
'Vital R'	10	0
'Aspi'	10	0
'Spinoza'	11	0
'Indures'	12	0
'Spartan A'	12	0
'Vroeg Reuzenblad'	14	0
'Eskimo'	15	1
'Winterreuzen'	19	2
'Spica'	21	2
'Münsterländer'	22	3
'Nobel'	27	10
'Noordland'	37	19

The long range in earliness in the glasshouse sowing can easily be explained by differences in daylength requirements of the cvs. The earliest cv then has a very low daylength requirement, resulting in a rather high development rate from emergence onward. The later cvs, with a more pronounced LD require-ment, have a lower development rate at the relatively short daylengths just after emergence. The latest cvs (from 'Spica' onward) have such a high daylength requirement that they remain vegetative for a while and start their development as soon as the daylength has become long enough. The first group (earlier than 'Spica'), although having different daylength requirements, has the same earliness in the field (emergence 30th March) because the daylength has then become long enough to enable them to develop at more or less their maximal rate which for all these very early cvs does not appear to vary greatly. The latest cvs show differences in earliness at both sowings, these differences being of the same order. This suggests that the critical daylength of cvs like 'Nobel' and 'Noord-land' is not reached before the end of March.

If the daylength is the major factor in determining the earliness of a cv in the spring, with the increasing daylength, this will be also the case in late summer and autumn with the decreasing daylengths. Nine of the glasshouse-sown early cvs, mentioned in table 29, were also included in field trials sown on 9th and 17th august 1965. Row distance and plant density were the same as in the field

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sowing in the spring. The average stem lengths were measured on 12th and 13th October respectively. The results are shown in table 30. Within each sowing date the stem length, which is a measure for the bolting rate, decreased regularly, the later the cv was in the glasshouse sowing.

TABLE 30. Earliness in days from 'Artex' (=0) in the glasshouse sowing in the spring and stem length in cm at harvest of 2 sowings in the autumn of 9 cultivars.

Cultivar	Earliness in spring	Stem length in autumn	
		Sown 9 August '65	Sown 17 August '65
'Artex'	0	–	5.1
'Huro'	4	–	4.0
'Protecta'	9	5.5	2.5
'Spartan B'	10	–	1.5
'Vital R'	12	3.1	–
'Aspi'	12	2.8	–
'Spinoza'	13	–	0.8
'Indures'	14	2.6	–
'Spartan A'	14	2.2	–

The midseason and late cvs also show a good relation between the order of earliness in the spring and the stem length at harvest in the autumn, as is shown by the following trials. Of 11 mid-season and late cvs belonging to the 'Nobel' and 'Noorman' groups the earliness data obtained from several field sown cv trials, carried out in the spring of 1964 and 1965 have been averaged. Stem length data have been collected from 5 autumn trials sown in 3 successive years. Per sowing date, stem lengths have been measured simultaneously. Table 31 shows for each sowing date a clear trend of the stem length to decrease, the later the cv. This means a lower bolting rate, the later the cv for a given sowing date.

This general trend, shown in the tables 30 and 31, of the bolting rate to

TABLE 31. Earliness in days from 'Noorman A' (=0) in spring and stem length in cm at harvest of 5 sowings of autumn spinach for 11 cultivars

Cultivar	Earliness	Stem length at sowing of:				
		15 July '63	6 Aug. '63	28 July '64	13 July '65	20 July '65
'Noorman A'	0	5.7	1.2	7.0	–	–
'Nobel A'	4	5.6	1.1	3.4	–	–
'Noorman B'	4	–	–	–	3.8	–
'Nobel B'	5	–	–	–	–	2.4
'Nobel C'	5.5	6.2	0.9	1.9	–	–
'Noorman C'	5.5	–	–	–	2.3	1.8
'Nobel D'	6	3.3	0.5	2.2	–	2.3
'Noorman D'	6.5	–	–	–	2.0	1.5
'Noorman E'	8	4.1	0.2	–	–	1.4
'King of Denmark A'	10	–	–	–	1.8	–
'Noorman F'	11	2.6	0.0	–	1.5	–

decrease, the later the cv, can be explained as follows: The daylength require-
ments increase, the later the cv, which means a decreased development rate at
a given daylength. For a given sowing date the daylength conditions are the
same for all cvs and the bolting rate will therefore be lower, the higher the day-
length requirements of the cv.

In spring, as well as in autumn, the spinach is generally grown under day-
length conditions permitting a certain degree of development. Overwintered
spinach, sown in September and harvested in April/May, has, however, a very
long growing period with relatively short days. The question then arose how the
earliness after overwintering is related to the earliness as measured in the spring
sown spinach. Some of the cvs also used in the glasshouse and field sowings
mentioned earlier in this paragraph, were sown on 22nd September 1964 on
a light sandy clay soil. The row distance was 0.25 m. The plant number per
meter row varied from 40–60 before and from 20–40 after the winter. Table 32
gives the earliness data of 8 early and 6 late cvs for the glasshouse, spring sown
and overwintered spinach. In the early group the order of earliness after over-
wintering deviates strongly from that of the glasshouse sowing and that of the
spring sowing. In the late group the order of earliness is approximately the
same for all 3 sowings. Since the order of earliness in the glasshouse sowing is
largely determined by the daylength response of the cvs, the deviations in the
order of earliness in the overwintered group of early cvs must be due to another
factor. This factor is probably the ability to recover rapidly after periods of
growth stagnation, or to go on growing under adverse weather conditions dur-
ing the winter months. All the early cvs have a fairly low daylength requirement,
enabling them to make some progress to stem elongation and flowering at
short days, as met during the period November to March. If, in this period,
temperatures are high enough for some growth, progress to stem elongation
and flowering will also be made. However, the growth rate under these rather
adverse conditions and recovery after growth stagnation may vary with the cvs.
In fact, this seems to be the case, as it is a general observation in practice that
the most winterhardy cvs recover considerably faster than the less winterhardy
ones after the winter is over. Early cvs which grow faster and resume growth
earlier after periods of growth stagnation in the winter will also make more
progress to stem elongation and flowering, which results in being relatively
early in the spring. The cvs 'Müma', 'Eskimo A' and 'Winterreuzen', shown in
table 32, are well-known winterhardy cvs and are indeed relatively early after
overwintering compared with their glasshouse earliness.

For the late cvs, belonging to the 'Nobel' or 'Noorman' group, the situation
is quite different. Firstly, the cvs of these 2 groups do not differ much in winter-
hardiness and therefore have a fairly similar growth pattern during the winter
months. Secondly, the rate of development during the winter months will be
small for the late cvs, as they all have a quite pronounced daylength require-
ment. The progress to stem elongation and flowering made by the different late
cvs during the winter will therefore be small and fairly similar. This results in

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TABLE 32. Earliness of 14 cultivars in days from 'Wolvex' (=0)
at a glasshouse, a spring and an overwintered sowing

Cultivar	Earliness at sowing:		
	Glasshouse	Spring	Overwintered
Early			
'Wolvex'	0	0	0
'Protecta'	0	1	7
'Müma'	3	−1	−2
'Spinoza'	4	−1	2
'Indures'	5	−1	4
'Eskimo A'	8	1	−2
'Eskimo B'	8	0	6
'Winterreuzen'	12	1	0
Late			
'Nobel A'	20	9	16
'Nobel D'	23	12	19
'Noorman G'	25	13	24
'Noorman H'	25	14	25
'Noorman F'	30	18	28
'King of Denmark A'	31	17	27

an order of earliness which agrees very well with those found in the glasshouse and spring sowings.

In the foregoing, it has been shown that the daylength response of a cv is predominantly responsible for its earliness. In fig. 3 on p. 11 the photoperiodic response curves of 7 cvs were shown. In general, a cv is earlier, the greater the progress to flowering at a given photoperiod. This would mean, according to fig. 3, that the order of earliness of some cvs is not always the same; for instance, the curve of 'Ji' crosses that of 'VA'. This indeed has been observed. 'It' is the earliest cv at any sowing in the glasshouse or in the field. 'Ji', on the other hand, is later than 'Va' if sown early in the glasshouse, but earlier if spring-sown in the field, and thus growing at longer days.

Within the late cvs, too, such daylength interactions may take place. Such interacting situations would, however, occur at daylengths of 16 h and more, which are longer than the daylengths met during the growth period of the spinach in spring and autumn. This may be the reason why no clear earliness/ sowing date interactions have been found among the late cvs.

Although other factors, such as temperature and light intensity, have a pronounced influence on the development, this is, in the first place, an indirect effect. Low temperatures and low light intensities in LD retard flowering because they retard all growth processes. Besides this indirect effect there is also a direct effect on the daylength response, as has been described in paragraphs 2.5 and 2.6. Temperatures of 8 °C or lower change the daylength requirements of both the stem and the flower formation processes, while slightly higher temperatures do so only for the stem formation process. All cvs, however, appear to react in a similar way to temperature. Lowering the light intensi-

ty also results in a changed daylength response, which goes in the same direction for all cvs, but is more pronounced, the later the cv. (paragraph 2.6).

As the cvs react in a parallel or only slightly divergent way to temperature and light intensity, which both increase with daylength in the late winter and spring, it is not likely that they will influence the order of earliness as determined by the daylength response of the cvs. Only in the case of overwintered spinach do temperatures seem to influence the order of earliness considerably, due to a differential growth and recovery rate during and just after the winter months.

The formation of the stem and of the flowers are themselves growth processes too. The rate of development may therefore be related to the general growth rate, as was shown in paragraph 2.6.

The growth rate as well as the rate of flower formation were retarded at the lower light intensities, the former more than the latter (table 11, p. 23). This relation between development and growth rate appeared to be present only when the rate of development was relatively high. In other words, the rate of flower formation is determined by a number of factors, including the general growth rate. Only when the latter factor becomes limiting for the rate of flower formation, which may occur at high rates of flower formation, can a relation between growth and development exist.

This may have some implications on the earliness of the cvs. If, for instance, 2 cvs have identical daylength requirements, they may nevertheless form flowers at different rates depending on their growth rate. The one with the highe growth rate will flower earlier than the slower-growing one under conditionsrpermitting a high rate of flower formation. The daylength response curve. i.e. the relation between daylength and rate of flower formation, includes this growth rate element. The faster growing cvs will therefore have a steeper response curve than the slower-growing ones. This, however, does not imply that the reverse is also true, namely, that the slope of the response curve is an indication of the growth rate of the cv. Each daylength response curve includes the actual daylength requirement together with the growth rate component. This, in fact, gives a partial explanation for the fact observed by breeders that selection for later bolting is closely correlated with a selection for slower growth. 'N' and 'Nr' may form an example of 2 cvs with rather similar daylength requirements, but with a different growth rate, 'Nr' growing considerably more slowly.

3.4.2. *The influence of earliness on yield*

Since earliness is a very important characteristic of a cv, the influence of earliness on yield has to be discussed. Cvs differing in earliness have been compared at the same moment and at a similar stage of development. The yields, measured at the same moment, give an indication of the average growth rate as influenced by the earliness, while the yields measured at a similar stage of development relate to the yields as they are obtained in practice. In the latter case, the yield is determined by the relation between growth rate and development rate. If, for instance, due to changed conditions the growth rate increases

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less than the development rate, the yields will be lower, whereas if the growth rate is accelerated more than the rate of development, the yields will be higher (13).

To compare the yield in relation to earliness, the data of 30 cvs sown without replications on 12th April 1963 were used. The row distance was 0.25 m, the plant density 60–70 plants per meter-row. On 28th May from each plot a one-meter-row sample was taken, of which the fresh weight and the average stem length were determined. The 30 cvs were divided into 5 groups each of 6 cvs, according to their earliness. Table 33 shows a clear relationship between earliness, measured as stem length, and fresh yields. The later the group, the lower the yield. This is not surprising as it is generally known by growers and breeders that spinach grows more slowly, the later the cv. In this experiment only fresh yields were measured. Dry weights would very likely have shown smaller differences between early and late groups as the growth rate is, in general, negatively correlated with the dry matter content (13).

TABLE 33. The average stem length and relative yields on 28th May of 5 groups of cultivars from early (I) to late (V)

Group	Stem length (cm)	Relative yields
I	6.2	100
II	3.6	94
III	1.8	86
IV	1.2	59
V	0.8	58

To compare the actual yield in relation to earliness, 28 cvs, sown in duplicate on 14th April 1964, were compared at a similar stage of development. The row distance was 0.20 m, with a plant density of 40–60 plants per meter row. From each plot, 5 m² in size, 4 or 5 successive 'one-meter-row' samples were taken with a 1–2 day interval. From these successive samples, of which fresh weight and average stem length were measured, the day on which each cv would reach a stem length of 5.0 cm and the yield on that day were estimated. Fig. 10 gives the yields of the 28 cvs in relation to their earliness. The yield increases strongly, the later the cv, the latest cvs yielding 2–3 times more than the early ones. Using the yields of the successive samples, estimates could be made of the growth rates of the early and late cvs. The ratio between the growth rates of early and late cvs was 1.1. The ratio between the development rates, however, was 1.3. Due to this higher ratio of the development rates, the earlier cvs yield less than the later ones.

Of course also differences in yield within groups of cvs with the same earliness are found. The deviations in yield from the regression line of yield on earliness, as shown in fig. 10, are only partly due to the trial error, the other part being caused by cv differences in yielding capacity independently of the earliness effect. The existence of such differences scarcely needs to be stated.
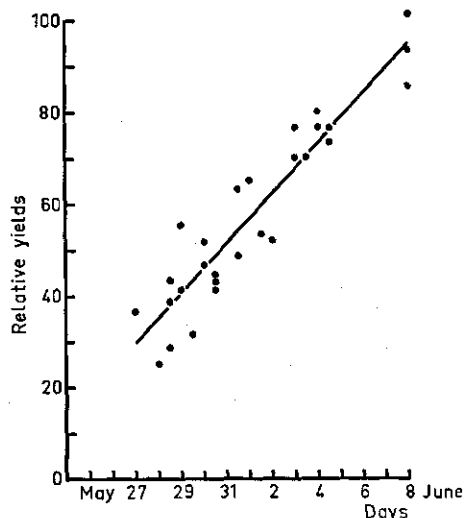
FIG. 10. Relative yields and harvest days of 28 cultivars sown on 14th April 1964 and harvested at a stem length of 5 cm. Each dot represents a cultivar

3.5. TEMPERATURE

The influence of temperature on yield is complex. Vernalizing temperatures, normally met in the early spring, induce an increased development rate through a lowered daylength requirement. This might mean reduced yields. Higher temperatures, although no longer affecting the daylength response, may still have a considerable effect on yield, namely, when growth rate and rate of development are differently affected by temperature. VAN DOBBEN (13) indeed observed that for crops adapted to cool climates an increase in temperature resulted in an increase of the development rate, which was greater than the increase in growth rate, thus causing lower yields. This may mean that temperature conditions for spinach exist which result in a maximal yield, lower and higher temperatures both reducing it. In paragraph 3.9 this will be discussed more fully under the spring-sowing date trials.

The following experiment was carried out to investigate the influence of temperature on yield under LD conditions. Two cvs, an early and a late one, were sown in wooden boxes, 2 per treatment. The germination was done at 5°C for 18 days preceded by 20°C for one day (+V) or by 20°C for 6 days (−V). At the moment of emergence the boxes were transferred to the spinatron. After emergence 40–50 plants per box were retained. The 2 temperatures in the spinatron were 11–14°C (average 12.5°C) and 17–19°C (average 18°C). The duration of the main light period was 15.5 h at the lower temperature and 17.5 h at the higher one while the photoperiod was 17.5 h in both cases. The fresh and dry yields were measured at a stem length of approximately 5 cm. The percentage of plants with visible flower buds was also determined at harvest.

Table 34 gives the fresh and dry weights. The −V treatments yielded considerably more than the +V ones, the difference being greater at 12.5°C than at 18°C. Moreover, the temperature during the growth appeared to be very

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TABLE 34. Fresh and dry weights in g at a stem length of 5.0 cm of 2 cultivars germinated at high (–V) or low (+V) temperatures and grown in LD at 12.5 and 18 °C respectively

Cultivar	–V		+V	
	12.5 °C	18 °C	12.5 °C	18 °C
		Fresh weights		
'Va'	216	159	170	135
'N'	238	153	193	148
Sum	454	312	363	283
		Dry weights		
'Va'	11.0	8.3	7.7	5.9
'N'	12,4	8.6	8.2	6.7
Sum	23.4	16.9	15.9	12.6

important; the yields at 12.5 °C were much higher than at 18 °C, the difference being greater for the –V treatments than for the +V ones. For both factors the yield is reduced when the development rate is accelerated (+V versus –V and 18 °C versus 12.5 °C). The temperature effects are the same for both cvs.

In paragraph 2.5 it was shown that spinach flowers on longer stems at lower temperatures. This was again clearly shown in the present experiment. The percentage of plants with visible flower buds was much lower at 12.5 °C (22%) than at 18 °C (85%) at a stem length of 5 cm. The +V plants tended to have a slightly higher percentage of plants with flower buds than the –V plants, this also agreeing with the results described in paragraph 2.5. Vernalized plants flowered on slightly shorter stems than unvernalized plants.

To see whether the yield/temperature relation also holds for shorter photo-periods, 2 early cvs were grown under the natural light conditions of the winter months at 4 temperatures. The seeds were planted in wooden boxes, 'IT' on 11th and 'Va' on 13th January 1966. 'IT' was sown 2 days earlier, since its emergence is slower than of 'Va'. Just after emergence the boxes (3 per treatment) were transferred to 4 temperatures in the phytotron at natural daylight and daylength conditions. The daylength increased during the growth period from 8.5 to 13 h. Per box 90 plants were kept. After 2 harvests, 18 and 33 days from emergence each consisting of 30 plants, the remainder was kept to be harvested at the moment that 10% of the plants flowered. Fresh and dry weights were determined at all 3 harvests. At the last harvest stem length and percentage of flowering plants were also recorded.

Table 35 gives the yield data of the 1st and 2nd harvest and the ratio between the weights of the 2nd and the 1st harvest, which is a measure for the growth rate in that period. This growth rate increased with temperatures up to 18.5 °C for both cvs. At the highest temperature, yield and growth rate decreased. This was most likely due to the rather low light intensity in the winter months, becoming limiting at higher temperatures. Although the growth rate of both cvs was approximately the same, 'Va' yielded much more than 'IT'. This was ap-

TABLE 35. Dry weights in g after 18 (1) and 33 (2) days and the ratio between the weights of the second and the first harvest of 2 cultivars at 4 temperatures

Cultivar	Temperature (°C)	Weights		Ratio
		1	2	
'IT'	14	0.10	0.46	4.6
	16	0.11	0.63	5.7
	18.5	0.12	0.71	5.9
	21	0.12	0.64	5.7
'Va'	14	0.26	1.12	4.3
	16	0.28	1.56	5.6
	18.5	0.29	1.77	6.1
	21	0.22	1.30	5.9

parently due to a much better start of 'Va'. The cotyledons of 'Va' are much bigger at emergence.

The yield data of the third harvest are given in table 36. As in the first experiment described in this paragraph, the yield decreased strongly with higher temperatures. As this experiment was carried out at relatively short daylengths, as opposed to the LD conditions of the first experiment, the present temperature effect on growth rate and rate of development is, at least largely, independent of the daylength response. It is therefore of a completely different nature than the effect of vernalizing temperatures, which induce a changed daylength requirement, resulting in a higher development rate and therefore in lower yields at a given daylength. As in the first experiment, the plants tended to flower on shorter stems, the higher the temperature.

TABLE 36. Days from emergence to harvest, relative dry weights, stem length in cm and percentage of flowering plants of 2 cultivars at 4 temperatures

Cultivar	Temperature (°C)	Days	Relative weights	Stem length	% flowering
'IT'	14	52	100	5.8	9.5
	16	47	83	3.1	10.5
	18.5	45	69	4.4	8.5
	21	42	39	2.0	9.5
'Va'	14.5	73	480	14.0	10.5
	16.5	69	370	9.6	10.5
	18.5	66	320	9.6	10.0
	21	59	187	8.6	28.5

3.6 LIGHT INTENSITY

In paragraph 2.6 it was shown that with decreasing light intensities the rate of flower formation in LD tends to decrease less, the earlier the cv. REINHOLD (62), carrying out shading experiments with a number of early and late cvs, concluded, that early cvs required less light than late ones. His conclusion,

50   *Meded. Landbouwhogeschool Wageningen 67-2 (1967)*

however, is questionable as the plant density was affected by light intensity. The later the cv, the lower the plant density at the lower light intensities. This was possibly caused by a differential damping-off and would agree with our glasshouse experience. In winter the later cvs usually show a higher incidence of damping-off, probably by their slow growth.

The following shading experiment was carried out in a glasshouse at Duiven. Three cvs were sown in duplicate on 4th March at 3 light intensities, namely approximately 25, 50 and 100% of the natural light intensity in the glasshouse. This was achieved by covering the respective plots with 2 or 1 layers of cheese cloth, while the 100% light treatment remained uncovered. Each plot consisted of 2 rows, 1.0 m long and 0.25 m apart. Per row 2 g of seed (ca 200 seeds) were sown. After emergence the plots were thinned to ca 50 plants per row to obtain equal plant densities. All treatments were harvested on 25th April. Fresh and dry weights, plant number and average stem length were determined. The plant number at harvest varied between 50 and 60, independently of the treatments. The dry weights cannot be mentioned as a number of samples were treated wrongly. From the remaining samples it could be concluded that the dry matter content tended to increase with light intensity, while the cvs did not show any appreciable differences. The fresh yields are therefore also fairly representative of the dry weights. According to table 37, the fresh weights decreased strongly with decreasing light intensities. This decrease tended to be largest for the earliest cv 'Va', which is widely used in glasshouses during the winter months. If earlier cvs need less light than late ones, as REINHOLD (62) supposed, 'Va' would have shown the smallest decrease in yield with decreasing light intensities.

TABLE 37. Fresh weights in g and stem lengths in cm on 25th April, and earliness in days from 'Va', 100% light, at a stem length of 5.0 cm of 3 cultivars at 3 light intensities

Light intensity %	Fresh weight			Stem length			Earliness		
	'Va'	'Sp'	'N'	'Va'	'Sp'	'N'	'Va'	'Sp'	'N'
100	278	178	210	3.7	1.1	0.0	0	3	17
50	71	86	102	4.2	3.1	2.0	-3	-2	8
25	41	38	37	4.1	3.3	2.1	-3	1	2

The stem length increased with decreasing light intensity, this increase in stem length being greater the later the cv. The earliness, being determined by the rate of stem formation, correspondingly decreased with lower light intensities, this effect being greatest for the latest cv. The difference in earliness between 'Va' and 'N' was 17 days at 100% light, 11 days at 50% light and only 5 days at 25% light. This agrees with the findings of paragraph 2.6, that the daylength requirement decreased with lower light intensities, this effect being greater the later the cv.

It may therefore be concluded that there is no reason to accept the view, that

earlier cvs require less light than late ones. In the following paragraphs 3.8 and 3.9 the much better growth of early cvs in winter, in comparison with that of late cvs, will be ascribed to daylength.

3.7 GIBBERELLIC ACID

Several authors (26, 50, 74) have reported increased yields after spraying with GA_3. However, they measured the fresh yields by harvesting at one and the same moment. In fact, they measured an increased growth rate. Yields measured at the same stem length can be expected to be lower, as especially stem formation is accelerated by GA_3.

To investigate this, two cvs were sown in duplicate on 4th March and 15th April in the glasshouse at Duiven. Each plot consisted of 2 rows, 1.0 m long and 0.25 m apart. The GA_3-treated plots twice received a spray with 500 ppm GA_3. The sprays were given 14 and 28 days after emergence, for the first sowing, and 10 and 20 days after emergence, for the second sowing. One row of each treatment was harvested on 14 th April (1st sowing) and 13th May (2nd sowing) respectively, to compare the yields at the same moment. The other row was harvested at a stem length of 6 cm. Fresh and dry weights and average stem lengths were measured.

Through the application of GA_3, the fresh weights, measured at the same moment, had increased by an average of 10.5%, the dry weights by 2.0%, thus showing that the growth rate had increased slightly. The stem elongation, however, was strongly accelerated, resulting in considerably lower yields at a stem length of 6.0 cm, for both cvs and sowing dates, as is shown in table 38. This effect was stronger for 'N', the late cv, than for 'Va', which is understandable. The later cv has a lower rate of stem formation than the early cv. GA_3 can therefore accelerate the stem formation more with such a late cv. This apparently happened, as the difference in earliness between $-GA_3$ and $+GA_3$ was greater for 'N' than for 'Va'.

TABLE 38. Fresh and dry weights in g at a stem length of 6.0 cm and the earliness of 2 cultivars at 2 sowing dates without ($-GA_3$) or with gibberellic acid ($+GA_3$)

Cultivar	Sown 4 March		Sown 15 April	
	$-GA_3$	$+GA_3$	$-GA_3$	$+GA_3$
	Fresh weight			
'Va'	1130	640	1100	620
'N'	5150	1950	3100	1410
	Dry weight			
'Va'	59	32	75	38
'N'	360	112	225	88
	Earliness in days from 'Va', $+GA_3 = 0$			
'Va'	5	0	4	0
'N'	28	12	13	5

Gibberellic acid seems therefore of no immediate advantage for commercial use. Only in special cases may it have some value. For instance, in late summer a spinach crop may have emerged too late, due to drought, resulting in a rather slow growth and too early a yellowing of the lower leaves. After an application of GA_3 the plants may bolt and grow more vigorously to give, after all, a good crop.

3.8 DAYLENGTH

VAN OORSCHOT (55) already observed that the growth rate was higher at longer photoperiods, although the highest yields were ultimately obtained at the shorter ones. THOMAS (70) found a considerable stimulation of leaf growth after transferring spinach to continuous light for 2–6 days.

Three cvs were sown on 24th March 1964 at photoperiods of 9, 13, and 17 h. The photoperiods were obtained by rolling the flat lorries on which the spinach was sown, into the open each day at 8 a.m. At 5 p.m. they were transferred to light-tight sheds where the day-light period was extended with weak incandescent light for 0, 4 and 8 h respectively.

The spinach was sown in rows 1.5 m long and 0.10 m wide. Each plot consisted of 3 rows. Within each lorry, subsidiary each photoperiod, the 3 cvs were replicated 4 times. The middle row of each plot was harvested on 12th May. On 19th May the second harvest was taken by cutting one of the border rows. The third row was not harvested due to deterioration of the plants at the longest photoperiod. The plant number per row varied from 72–81, independently of the treatments. At each harvest fresh and dry weights were determined. The rate of dry weight increase in the week of 12–19th May amounted 3.3, 3.7 and 3.8 at the photoperiods of 9, 13 and 17 h respectively, indicating that the growth rate increased with the photoperiod. The average dry matter content, on the other hand, decreased with increasing photoperiod, being 6.9, 5.8 and 4.9 respectively. This agrees with the conclusion of VAN DOBBEN (13) that in general an increased growth rate is accompanied by a decreased dry matter content. VAN OORSCHOT explained this increased growth rate as being due to the increased rate of leaf area expansion. The latter, in turn, would be due to the cell elongating effect caused by the very low light intensities of the day-light extension. If this be true, the growth rate would not in fact be stimulated by increasing daylengths. However, several field trial data to be discussed in paragraph 3.9, suggest strongly that the vegetative growth of spinach is also stimulated when the generative development is stimulated. The 3 cvs in this experiment do indeed show such behaviour. The stimulation of growth is related to the stimulation of flower formation, as can be seen in table 39. 'VR' shows the greatest yield increase between the photoperiods of 9 and 13 h; for 'Nr' this occurs between the photoperiods of 13 and 17 h, while 'N' takes up an intermediate position. This runs parallel with the increase in the progress to flowering. This means that early cvs show this increased growth rate at shorter daylengths than do late cvs as their daylength requirements for generative develop-

ment were found to be lower (Fig. 3 on p. 11). When the increase in the growth rate was solely due to the cell elongating effect of the very low light intensities during daylight extension, this relation between earliness and stimulation of growth would not exist.

The increases in fresh or dry weights with longer daylengths are rather small compared with the increases in the progress to flowering. This means that the yields, measured at the same stage of development will be lower at longer day-lengths, as already observed by VAN OORSCHOT (55).

TABLE 39. Fresh and dry weights and progress to flowering on 19th May of 3 cultivars on 3 photoperiods

Cultivar	Photoperiod in h		
	9	13	17
	Fresh weights in kg		
'VR'	1.3	2.0	2.3
'N'	1.3	1.9	2.5
'Nr'	1.1	1.4	2.2
	Dry weights in g		
'VR'	96	122	125
'N'	92	123	130
'Nr'	78	92	108
	Progress to flowering (%)		
'VR'	0	75	100
'N'	0	26	82
'Nr'	0	5	73

To study the LD effect on growth, independently of the possible 'low light intensity' effect of daylight extension, another experiment was carried out, following WAGENAAR (76), who showed that a light break in the middle of an otherwise non-inductive long night resulted in flower formation. An optimal result was obtained with a light break of 20 minutes with a light intensity of ca 23,000 erg. cm^{-2}. sec^{-1}. Higher or lower light intensities gave a lower rate of flower formation.

Eight wooden boxes with vegetative 'N' plants, 7 days old from emergence, were exposed to 9 h of light. Four boxes were given the 9 h uninterrupted (– light break), the other 4 boxes received 8 h 40' of light and 20' of light in the middle of the long night (+ light break). The light treatments were given in the spinatron at a temperature of 16–17°C. Together with TL light of an intensity of 29,000 erg. cm^{-2}. sec^{-1}, weak incandescent light was given. At the start of the light treatment 15 plants per box were collected to determine fresh and dry weights. This was repeated after 7 and 14 days. After the third harvest the boxes, each with 5–6 remaining plants, were transferred to LD after-treatment to-gether with vegetative controls, to determine the moment of flowering.

The control and –light break plants flowered 9.0 and 8.7 days later than the

+light break plants, indicating that the light break had a pronounced LD effect. The fresh and dry weights, 7 and 14 days after the start of the light break treatment, are given in table 40. The fresh weights, and to a lesser degree the dry weights, tend to increase faster in the +light break treatment. Extrapolating the fresh weight data, the difference between both treatments would have been 50% after 28 days.

TABLE 40. Fresh and dry weights in g of 60 plants after 0, 7 and 14 days from the start of 2 light treatments

Days from the start	Fresh weights		Dry weights	
	– break	+ break	–break	+ break
0	6.9	6.9	0.14	0.14
7	23.0	25.4	1.10	1.15
14	76.2	92.9	3.84	4.19

It may therefore be concluded that a stimulation of the stem and flower formation is accompanied by a stimulation of the growth. This, however, does not mean that the yields will be increased; on the contrary, the yields may decrease, as has indeed been found.

3.9. GENOTYPE, TEMPERATURE, AND DAYLENGTH IN RELATION TO THE GROWING OF SPINACH

In paragraphs 3.4, 3.5, 3.6 and 3.8 the separate influences of genotype (cultivar), temperature, light intensity and daylength on yield have been discussed. By using a glasshouse in the winter and by varying the sowing date it is possible, within certain limits, to choose the required combination of the above-mentioned factors. To determine in how far each of them contributes to the results of spinach growing, several sowing date/cultivar trials have been carried out in a glasshouse and in the field.

In 1963, 2 cvs were sown in the field in duplicate on 10 sowing dates ranging from 28th March to 14th May. In 1964 a similar trial was conducted with 3 cvs and 12 sowing dates. Each plot consisted of one row, 5.0 m in length. The row width was 0.50 m, which is wide enough to avoid row interactions. In 1963 each plot was harvested when 10% of the plants flowered; in 1964, this was done at a stem length of 5 cm. The plant density varied from 50–70 plants per meter row in 1963 and from 40–60 in 1964.

The relative yields are shown in table 41. In each year the highest yield was given a score of 100. The highest yields (underlined) were obtained in the first half of April, earlier and later sowings giving lower yields. The optimal sowing date appears to be earlier, the later the cv. The lower yields at the earliest sowing dates are probably due to vernalization, since the average temperature in March increases from about 3 to 7°C. The decreasing yields after the maximum has been reached are presumably due to the combined effect of the

TABLE 41. Relative fresh weights of 2 cultivars on 10 sowing dates in 1963 and of 3 cultivars on 12 sowing dates in 1964

Sowing date	1963		Sowing date	1964		
	Cultivar			Cultivar		
	'Hybrid 425' (early)	'Viking' (mid season)		'Vital R' (early)	'Nobel' (mid season)	'Noordland' (late)
–	–	–	3/3	31	45	43
–	–	–	10/3	29	51	78
–	–	–	17/3	39	60	59
28/3	40	71	24/3	33	46	59
4/4	65	<u>100</u>	31/3	39	63	82
8/4	74	87	7/4	44	79	<u>100</u>
11/4	<u>95</u>	58	10/4	47	<u>81</u>	83
15/4	59	59	14/4	<u>71</u>	61	78
18/4	36	56	17/4	53	56	72
22/4	45	22	21/4	44	67	59
25/4	44	37	24/4	45	43	59
29/4	41	19	28/4	33	32	56
2/5	18	24	–	–	–	–

increasing temperatures and daylength. The reason why later cvs have an earlier optimal sowing date will be discussed in connection with the results of the following experiment.

To obtain more information about the influence of temperature in spring sowings, 'N' spinach was compared after 3 sowing dates in the field and in a glasshouse in 1966. The sowing dates were 9th March, to secure natural vernalization, 7th April, this being approximately the optimal sowing date, and 9th May, to be fairly sure that no natural vernalization would occur. The seeds were planted in soil-blocks of $5 \times 5 \times 5$ cm, which were placed in the field (nV = naturally vernalized). On 23rd March, 18th April and 17th May these soil-blocks were planted in the field, together with seeds germinated in similar soil-blocks at 20 °C (–V) for 4 days and at 5 °C (+V) for 18 days. Although emergence did not occur on the same day, as planned, the difference was never more than 2–3 days. The –V and +V treatments were also planted in the glasshouse on each of the above-mentioned planting dates. Each plot in the field or in the glasshouse consisted of a double row of soil-blocks one meter long. The row distance was 0.50 m. The number of replicates was 4 in the field and 2 in the glasshouse. The plots were harvested at a stem length of about 7 cm. At harvest the fresh and dry weight, the plant number, and the average stem length were determined. The average daily temperatures in the glasshouse were kept above 12 °C to avoid possible vernalization effects. The number of plants at harvest varied from 38–41 per plot in the glasshouse and from 29–31 in the field. The average stem length at harvest of the different treatments ranged from 6.7–7.5 cm, with an average of 7.1 cm. Table 42 gives the fresh and dry weights

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and the earliness in days relative to the –V treatments. When we first consider the yields in the glasshouse, we find a strong decrease in yields with later sowing for the –V and +V treatments. This is undoubtedly due to the combined effect of increasing temperatures and increasing daylength, both resulting in lower yields, as was shown in paragraphs 3.5 and 3.8. The average daily temperatures between planting and harvesting for the 3 sowings of the –V treatments were 15.1, 18.3 and 22.6 °C respectively. The +V treatments yielded much less than the –V treatments, which confirms the results given in paragraph 3.5. This yield decrease was stronger, the earlier the sowing date, apparantly because the difference in earliness was greater, the earlier the sowing. The reason for this effect may be twofold. At the later sowings some devernalization may have occurred, as the temperatures after planting in the glasshouse were higher at the later sowings. Another reason may be the fact that the vernalization effect on flower formation, and therefore on yield, is greater at shorter photoperiods, as was shown in paragraph 2.5.

TABLE 42. Relative fresh and dry weights at a stem length of 7 cm, and earliness of unvernalized (–V), field germinated (nV) and vernalized (+V) 'N' seed, grown in the glasshouse and in the field at 3 sowing dates

Treatment during germination	Glasshouse			Field		
	Sowing date					
	9 March	7 April	9 May	9 March	7 April	9 May
Relative fresh yields						
–V	100	50	15	100	112	64
nV	–	–	–	70	83	66
+V	27	20	7	71	64	46
Relative dry yields						
–V	100	44	13	100	148	70
nV	–	–	–	78	103	77
+V	28	20	8	77	70	48
Earliness in days from –V						
–V	0	0	0	0	0	0
nV	–	–	–	–3	–4	–1
+V	–15	–10	–4	–5	–12	–5

The yield data of the field sowings showed a different pattern. The –V and the nV treatments give the highest yields at the 2nd sowing. This agrees nicely, as far as the nV treatment is concerned, with the results of the experiment of table 41. The yields of the nV treatments were equal to the +V at the earliest sowing, intermediate at the second and equal to the –V at the third sowing. This leads to the conclusion that natural vernalization indeed occurs and that the extent of vernalization gradually decreases with later sowings. In the earliest sowing the vernalization seems to be optimal, the germination period being the most sensitive. At the second sowing some natural vernalization

occurs, but apparently less than at the first sowing, resulting in a yield inter-
mediate between those of –V and +V. As was expected, no natural vernaliza-
tion could be observed at the last sowing.

The +V treatments showed a decrease in yield with later sowing, as now the
later sowings too are vernalized. Here the increasing temperatures and day-
lengths were responsible for the yield decrease. That a maximum yield was
found for the –V and nV treatments at the second sowing is therefore explained
as follows. The yields will have a clear tendency to decrease with later sowing
due to the combined effect of increasing temperatures and increasing daylength.
However, with later sowing the vernalization effect becomes smaller, resulting
in a tendency to increase the yields. These effects, combined together, result
in an initial increase in yield until the yield increase due to the decreasing ver-
nalization effect becomes smaller than the yield decrease due to the increasing
temperatures and daylength. This means that at the optimal sowing date some
vernalization still occurs. In table 41 it was shown that this optimal sowing date
is later, the earlier the cv, which now can be explained too. The daylengths in
April are rather long for the early cvs, as was shown in paragraphs 2.4 and
3.4.1. The daylength effect on yield with later sowing is therefore presumably
small. Since, on the contrary, the daylength in April is close to the critical
one for the late cvs, the daylength effect on yield with later sowing can be ex-
pected to be strong. This would imply that the decrease of yield with later sow-
ing is predominantly due to increasing temperatures for early cvs and largely
due to the increasing daylength for late cvs, which then results in an optimal
sowing date varying with earliness.

In the experiment discussed above, it was shown that the yields of spring-
sown glasshouse spinach decrease with later sowing. A cultivar/sowing date
experiment carried out in the same glasshouse indicated that the sowing date
also has a considerable influence on the growth rate.

Four cvs were sown on 5 sowing dates. In one part of the experiment each
plot consisted of only one row, 1.0 m long. The rows were hand sown using 2 g
of seed per row. The row distance was 0.25 m. Within each sowing date the 4
cvs were quadruplicated, using a latin square design. After emergence the plots
were thinned to approximately 40 plants per row. All treatments within one
sowing date were harvested on the same day, just before the earliest cv started to
cover neighbouring rows. At harvest the fresh and dry weight, the average stem
length and the plant number per meter-row were determined. The other part
of the experiment was carried out in the same way, except for the plot size.
Each plot now consisted of 3 rows, which were harvested successively at inter-
vals of one or a few days. Harvesting started when the stem length was ca 3 cm.
At each harvest the average stem length was determined. Using the linear re-
gression of harvest day on stem length, the day at which the plots reached an
average stem length of 5.0 cm was estimated giving the earliness.

The results of both parts are summarized in table 43. Within each sowing date
the yields relative to 'Va' are given. As the yields between sowing dates are
not quite comparable, 'Va' is given a score of 100 at each sowing, although the

yields were not the same. The yields (fresh and dry weights), measured on the same day, give an indication of the average growth rate, as the emergence date was practically the same for the 4 cvs. At the earliest sowing date this growth rate decreases considerably, the later the cv. The later the sowing date, the smaller this tendency becomes. At the fourth and fifth sowing the differences between 'Va', 'Sp' and 'N' disappears, only 'Nr' still growing somewhat more slowly. The tendency for the differences in growth rate to become smaller, the later the sowing, runs parallel with the differences in earliness, which themselves also become smaller at the later sowings. This suggests a relation between the sowing date effect on growth and on earliness. In paragraph 3.8 it was shown that growth is stimulated when spinach becomes generative, and this seems to confirm these results.

TABLE 43. Relative fresh and dry weights, earliness, differences in dry matter content and stem length of 4 cultivars at 5 sowing dates

Cultivar	Sowing date				
	9 Febr.	4 March	25 March	15 April	20 May
Relative fresh weights					
'Va'	100	100	100	100	100
'Sp'	59	49	90	95	115
'N'	44	39	83	100	109
'Nr'	27	29	44	74	80
Relative dry weights					
'Va'	100	100	100	100	100
'Sp'	76	71	90	92	106
'N'	59	60	84	94	96
'Nr'	37	51	58	75	74
Earliness in days from 'Va'					
'Va'	0	0	0	0	0
'Sp'	15	5	0	0	0
'N'	33	19	12	5	4
'Nr'	41	28	18	11	7
Difference in dry matter content between 'Va' and the other three cultivars (%)					
'Va'	0.0	0.0	0.0	0.0	0.0
'Sp'	1.8	0.8	0.0	−0.3	−0.6
'N'	2.1	1.2	0.1	−0.4	−1.0
'Nr'	2.4	2.0	2.0	0.1	−0.6
Stem length in cm at harvest					
'Va'	6.2	5.9	7.9	8.5	6.5
'Sp'	0.9	1.6	4.6	6.6	6.5
'N'	0.0	0.0	0.8	2.1	2.4
'Nr'	0.0	0.0	0.0	1.5	1.8

Together with the decreasing differences in growth rate and earliness with later sowing, the differences in dry matter content also decrease. The lower the

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fresh weights relative to 'Va', the higher are the dry matter contents relative to 'Va'. This would mean that the increase in growth rate at later sowings is accompanied by a decrease in dry matter content, which again confirms the results discussed in paragraph 3.8.

In paragraph 3.6 it was shown with the cvs 'Va', 'Sp' and 'N' that there was no tendency for the earlier cv to grow relatively better at lower light intensities. From the experiment described here, together with this light intensity experiment, it can be concluded that when spinach is grown in glasshouses during the winter months, early cvs like 'Va' should be used, because their growth rates remain relatively high, since the daylength requirements of these early cvs are low enough to allow a moderate rate of development at the short days in winter, as was already mentioned in paragraph 3.6.

If such a sowing date/growth rate effect exists under glasshouse conditions in the spring, it will also exist in the late summer, as the temperature and daylength conditions are then rather similar, but decrease, instead of increase, with later sowing. This, in fact, appears to be so. Seven cvs of the 'Nobel' and 'Noorman' type were sown quite early and rather late in 1963. The plots consisted of 6 rows, 0.20 m apart and 5 m long. The trial comprised 4 replications. Of each sowing all plots were harvested on the same day. At harvest the fresh weight, the plant number and the average stem length were determined. As the yields were measured at the same time, they will give an indication about the average growth rate. The stem length at harvest can be used as a measure for the differences in development rate; the longer the stem length, the higher the development rate. The plant number per meter-row varied from 45–60. Table 44 gives the relative yields and the stem lengths. The cultivars, arranged according to their earliness (stem length), show no relation between yield and stem length at the first sowing but do so at the second one. At the early sowing all cvs readily formed a flowering stem. At the late sowing the latest cvs remained in the rosette stage. This indicates, as in the former experiment, that the growth rate depends on the daylength response of the cv. At the early sowing all cvs developed at a fair to high rate, resulting in similar growth rates. The late sow-

TABLE 44. Relative fresh yields and stem lengths in cm at harvest of 7 cultivars at 2 sowing dates

Cultivar	Sowing date			
	15 July		6 August	
	Relative yields	Stem length	Relative yields	Stem length
'Noorman A'	100	5.7	100	1.2
'Nobel A'	100	5.6	105	1.1
'Noorman H'	91	4.5	95	1.1
'Nobel D'	111	3.3	85	0.5
'Noorman E'	101	4.1	68	0.2
'Noorman J'	100	3.9	72	0.0
'Noorman F'	108	2.6	61	0.0

ing was apparently more critical; the early cvs still had a moderate development rate, while the late ones behaved as if they were in short day. This resulted in a lower growth rate, the later the cultivar.

Because of this relation between the growth and development rates and the daylength response of a cv it is expected that in the late summer each cv will have its own optimal sowing date. This has been investigated in the following experiment carried out in 1964.

Three cvs were sown in 4 replications on 3 to 5 sowing dates. Each plot consisted of 5 rows, 0.20 m apart and 15 m long. The plant density varied from 40–60 per meter row. The sowing of 20th August, however, had a slightly lower plant number, 35–45. From each plot 5 or 6 harvests were successively taken. Each harvest consisted of 2 samples, which were obtained by cutting the 3 inner rows over a length of 1.0 m. Of each sample the fresh weight and average stem length were measured. At the same time it was determined when the crop on the field became unacceptable because of deterioration, predominantly because of yellowing of the lower leaves. From these data the optimal yields for each treatment were estimated. This was either the yield at a stem length of 5.0 cm or the yield on the day the crop became unacceptable. Using the stem length data of the successive harvests, an estimate could be made of the increase in stem length per day at the time of the optimal yield.

Table 45 gives the results. It can be seen that the optimal sowing date, that is the one giving the highest yield, is later, the earlier the cv, as was expected. The stem length at harvest is usually shorter, the later the sowing for each cv. The increase in stem length at harvest becomes smaller with later sowing and comes to a complete stop for 'N' and 'Nr'. This agrees nicely with the data discussed in paragraph 2.8.3, namely that the stem and flower formation is stopped (qualitative LD cvs) or slowed down (quantitative LD cvs) when the plants are trans-

TABLE 45. Relative fresh yields, stem length in cm and increase in stem length per day in cm at optimal harvest time of 3 cultivars at 5 sowing dates

Cultivar	Sowing date				
	23 July	3 Aug.	13 Aug.	20 Aug.	28 Aug.
	Relative fresh yields				
'Va'	54	67	72	79	92
'N'	97	108	84	53	–
'Nr'	100	95	74	–	–
	Stem length				
'Va'	5.0	5.0	5.0	4.9	4.0
'N'	5.0	1.7	0.9	1.0	–
'Nr'	3.0	1.2	0.7	–	–
	Increase in stem length per day				
'Va'	1.6	1.4	1.0	0.4	0.3
'N'	0.1	0.1	0.0	0.0	–
'Nr'	0.1	0.0	0.0	–	–

ferred from LD to SD. At the optimal sowing date the stem length increases only very slowly in the harvest period, while the stem length has not yet reached 5 cm. This indicates that the optimal sowing date for a cv is that date which causes initially a fairly high rate of development, coming almost to a stop when the stem has reached a length of only a few (2–4) cm. In that case, the yield profits from the initial high growth rate, while the crop can be harvested when the growth rate and the development rate decrease.

In conclusion, the development rate decreases with later sowing, which results in higher yields, if measured at the same stage of development. This is apparently only valid for the sowing dates earlier than the optimal one. At later sowings deterioration of the crop is the cause of earlier harvesting and therefore of lower yields.

It is quite clear from the field observations that the later the sowing date, the lower the growth rate and development rate and the higher the sensitivity to deterioration become. This latter effect, which is the cause of the lower yields at relatively late sowings, is clearly related to the daylength response of the cultivar. 'Nr' and 'N' showed this deterioration quite markedly at the second and third sowing respectively, while 'Va' did not do so even at the latest sowing.

3.10 Discussion

The yield measured at a certain stage of development depends on the rate of growth and the rate of development, both being affected by genotype, temperature, light intensity and daylength. The growth rate and the development rate, however, are not generally influenced to the same extent. If, due to changed conditions, the development rate increases more than the growth rate, the yield will drop.

In the preceding paragraphs it was shown that the yields were relatively high when the conditions favoured a relatively low development rate, these conditions being late genotypes, non-vernalizing, but relatively low temperatures, high light intensities and relatively short daylengths. The development rate is apparently a very important factor and as the development rate determines the earliness of the crop, the earliness is of equal importance.

Earliness: The genotype has a profound influence on the earliness, this influence being strongly determined by the daylength response of the cultivar, as was shown in 3.4.1. The daylength requirements of the cvs vary greatly, the early ones having a less pronounced LD response than the late ones.

Temperature in general, although influencing the rate of stem and flower formation considerably, has no appreciable effect on the order of earliness, since the rates of stem and flower formation are changed in a similar way for all cvs. Vernalizing temperatures induce a change in the daylength requirement of the cvs, resulting in earlier bolting and flowering. Vernalization, therefore, has an effect on earliness, acting through the daylength requirement of the cvs.

Light intensity has some effect on earliness, as shown in 3.6. At least an appreciable part of this light intensity effect on earliness is due to its effect on

the daylength requirement of the cvs. Lowering the light intensity results in a tendency to day-neutrality, this tendency being stronger, the later the cv, as shown in 2.6.

The daylength itself has, of course, a strong effect on earliness. Sown in late winter in the glasshouse, the cvs show a wide range of earliness, wich depends largely on the daylength requirements of the cvs. The lower the daylength requirement, the earlier the cv. If sown later in the field, a rather large number of early cvs, differing considerably in the glasshouse sowing, have about the same earliness, as was shown in 3.4.1. This is most likely due to the fact that the daylength at this later sowing has become long for those early cvs, resulting in a similar development rate.

To summarize, the factors: genotype, temperature, light intensity and especially photoperiod, exert their influence on earliness largely through the daylength requirement of the cv.

As the flower formation itself is a growth process, it is understandable that a relatively high rate of flower formation is not independent of the rate of growth, as was shown in 2.6. Two cvs having exactly the same daylength requirement may therefore differ in earliness if they differ in rate of growth. The slower growing cv then will be the later one. It is therefore conceivable that the earliness of a spinach cv is largely determined by both its daylength requirement and its rate of growth.

Growth rate: Not only does daylength influence the bolting and flowering of spinach, but also its growth rate. It was shown that a stimulation of flower formation is accompanied by a stimulation of the growth rate. This has been observed in other LD plants too. *Lactuca sativa* L. seems to grow faster when the development rate is increased (22, 71). *Mentha piperita* L. cv *vulgaris* showed a faster growth when flower formation was caused either by LD or by a light break in the middle of the otherwise non-inductive long night (42). A *Festuca pratensis* HUDS. strain from North Sweden failed to flower in the south, because of inadequate photoperiod, while its vegetative growth vigour was poor too. Other LD herbage grasses, however, did not show this relation (58).

The relation between growth and development, together with the variation in daylength requirement among the cvs, has important consequences for the growing of spinach. If the rate of development is zero or very low, the growth rate is low too, while the crop becomes more liable to deterioration. This can easily be observed when late cvs are sown too late in the summer or are sown in the winter in the glasshouse. If, on the other hand, the rate of development is high, as in early summer, the crop must be harvested before a reasonable yield is obtained, although the growth rate is high too. It is therefore important to grow the spinach in such a way that the rate of development is moderate. This gives a relatively high growth rate and enough time to obtain good yields. To grow spinach successfully over the greater part of the year, with its varying daylengths, the development rate can only be kept moderate by using a range of cvs differing in their daylength requirements. This, in fact, is now done by the

growers, who use cvs with a low daylength requirement in the glasshouses during the winter, while in late spring and summer cvs are used with a high daylength requirement. To obtain optimal yields it is therefore necessary to choose for each sowing date the cvs with the most suitable daylength response. The later the sowing in spring, the later the cv should be (the higher its daylength requirement). However, for early summer sowing no commercial cvs are available, which bolt slowly enough to be of economic value. In late summer spinach can be sown successfully. The optimal sowing date for a cv then again depends strongly on its daylength requirement; the earlier the cv the later it should be sown. It was demonstrated that the optimal sowing date is that date which initially results in bolting, this being almost brought to a standstill at the time of harvest. In this case the crop has initially a high growth rate, which later decreases, because bolting stops. Earlier sowing would result in the bolting proceeding too fast, later sowing in too slow a growth.

The cvs used in the glasshouses during the winter are therefore not chosen because they grow so well at low light intensities, as is often thought, but because they grow well under SD conditions. They bolt at a moderate rate in these short days, and this is accompanied by a relatively high growth rate.

Breeding: From the foregoing discussion two conclusions can be drawn, which are very important for spinach breeding. In the first place, the breeding of a non-flowering cv is of no use, apart from the problem of how to obtain seed from it. Secondly, it will be next to impossible to breed fast-growing late cvs. All spinach breeders have observed that selection for late bolting in a population of mainly fast-growing plants almost inevitably leads to slower growth. If, on the other hand, selection for fast-growing plants is carried out in a population of mainly late-bolting plants, the population will become earlier. This strong relation between growth rate and earliness has two causes:

1. Because the growth rate also depends on the daylength requirement of the cv. It has been shown that the growth rate increases when the development rate is increased. Since at a given set of daylength conditions, as for instance met at a spring sowing, the development rate of a cv (or its earliness) depends largely on its daylength requirement, the growth rate too will depend on this daylength requirement. The lower its daylength requirement is, the faster (or earlier) it will bolt and the faster it will grow, or conversely, the higher the daylength requirement, the lower (or later) the bolting and the slower the growth.

2. Because the growth rate itself also has an influence on the earliness. If 2 cvs have identical daylength requirements, but differ in growth rates, they will also differ in earliness. The slowest growing cv will be the latest. It should be realized that the growth rates of these 2 cvs, although differing, both depend on their daylength requirements. The growth rates will vary with the development rates, but at different levels.

This implies that within a population of plants bolting at the same time, 2 types of plants may be found. The first type combines a high daylength requirement with a relatively high growth rate, while the second type has a much lower daylength requirement, but grows more slowly too. In spring the first

type will start its development later than the second type, but will reach the harvesting stage at the same time because of its faster growth. This first type of plant will have a steep daylength response curve, like 'VR' in fig. 3, (p. 11) while plants of the second type have a less steep curve like 'Va' in fig. 3. The two types cannot be separated in the field, but it is possible to do so with the aid of artificial daylengths. When lines are exposed to both a rather short and a relatively long daylength, those of the first type will bolt later at the short daylength and earlier at the long daylength than those of the second type.

The production of spinach seed creates quite different problems. Conditions favouring a rather high rate of flower formation will result in good seed yields. Late cvs yield, in general, less seed than early ones, while the modern cvs, bred for leafiness, also tend to yield less seed than the older cvs (37). The sowing should be done as early as possible, presumably to secure vernalization. Selection for vigorously growing, late-bolting plants will therefore lead to lower seed yields, unless selection for a better seed production is applied simultaneously.

Other leafy vegetables: On comparing other LD requiring leafy vegetables, like endive (*Cichorium endivia* L.), chervil (*Anthriscus cerefolium* HOFFM.) and lettuce (*Lactuca sativa* L.), it is striking that fairly small differences in the requirements for temperature and daylength have such profound effects on the growing methods. Endive (21, 60) and chervil (57) are both quantitative cold-requiring LD plants. At higher temperatures flower formation proceeds slowly in LD. After vernalization both species behave like quantitative LD plants. Both crops (4, 57) show increasing yields with later sowings in spring, bolting becoming limited or not occurring at all from the beginning of June onward. This increase in yields results from the increasing temperatures met with in later sowings. These higher temperatures result in a lower degree of vernalization and thus a decreased rate of bolting. This is the same effect as met with in March sowings of spinach. At later sowings the daylength effect on bolting becomes more important in spinach, resulting in decreasing yields. With endive and chervil the temperature requirement is more important than the daylength requirement. The latter, in fact, can only be expressed fully when the plants have been exposed to low temperatures.

Lettuce is a quantitative LD plant (3, 61), in which seed vernalization acts only as an accelerating factor (61) like in spinach. This means that the yield/ sowing date relation in lettuce is mainly daylength dependent, as is also the case in spinach. The data of KRICKL (36) confirm this. He observed in Germany, that the head weight of lettuce decreased with later sowing in the field from 10th February onward until a minimum was reached at the end of May, after which the yields increased. As in spinach, the daylength requirement of a cv largely determines its growing period. The early cvs used in winter and spring have a pronounced daylength response, flowering earlier, the longer the daylength. The late cvs, used in summer, on the other hand, are almost day neutral. On comparing these two types at both a short and a long daylength, it was observed (3) that the early cvs flower later in SD (winter) and earlier in LD (summer) than the late cvs.

Quite surprising is the fact that in spinach and lettuce, in both of which the daylength requirement is dominating, a wide range of earliness exists within the assortment of cvs, while this is considerably smaller for endive and chervil, both with a very important temperature requirement. The reason for this is not quite clear, but may depend on whether a relation exists between growth and development. In crops like spinach and lettuce a large range of cvs differing in daylength requirement would be needed to grow these crops successfully in different seasons. This is because flower formation proceeding at a moderate rate is essential for vigorous growth. Endive and chervil, where the relation between generative development and vegetative growth does not seem to be so marked can be grown well under conditions not suitable for flower formation. In these crops the need for a wide range of cvs adapted to different daylength conditions, is not so compelling. More important for these crops is that bolting should not proceed too readily, and this has been achieved by selection for a higher cold requirement, involving later bolting.

4. SUMMARY

Introduction

The importance of spinach for the deep-frozen food industry in Western Europe has increased fast in the last decade. As the bolting behaviour is a very important character in spinach growing, research has been carried out into the developmental factors influencing growth and development.

Influence of environmental factors on development

The influence of genotype (cultivar), temperature, light intensity, GA_3 and daylength on stem elongation and flowering has been investigated.

Most cultivars of spinach react quantitatively to LD, both for stem and flower formation. The daylength requirements of the cvs vary from almost day neutral to an absolute need for LD.

Temperatures of 2–8 °C accelerate stem and flower formation through an induced lowering of the daylength requirement. Temperatures of 9–12 °C accelerate only stem formation. All cvs, irrespective of their daylength requirement, show a similar temperature effect.

Decreasing the light intensity results in a lowering of the daylength requirement, this effect being stronger, the later the cv.

GA_3 accelerates stem formation strongly, flower formation only slightly. The promotive action on flower formation is indirect via the strong effect on stem growth.

There is no juvenile phase for the temperature nor for the daylength response. The daylength effect is direct and hence not inductive. Even after stem growth and flower differentiation have started in LD, regression of the flower formation may occur in a qualitative LD cv after transferring to SD.

The slowest rate of flower formation occurs at photoperiods of 6–10 h. At shorter and longer photoperiods it increases.

A qualitative LD cv like 'Nobel' remains vegetative in SD only, if stem formation is inhibited. The formation of axillary bud primordia giving rise to the flower clusters, occurs much more slowly in SD than in LD. In SD this differentiation stops at an early stage unless growth of caulescent tissue is made possible by low temperatures, low light intensities or GA_3. This agrees with the hypothesis of CHAILAKHYAN, that for flower formation in LD rosette plants, both gibberellins and floral stimulus are needed for stem growth and differentiation respectively. The formation of both hormones would depend on the daylength.

Influence of environmental factors on yield

The time of harvest is generally determined by the bolting behaviour of the spinach crop, hence by the factors affecting stem elongation and flower formation.

The earliness of a cv is predominantly determined by its daylength requirement and its growth rate. The lower the daylength requirement and the higher

the growth rate, the earlier the cv. The order of earliness is not affected by temperature and light intensity, because these factors influence all cvs in a similar way via their daylength requirements. Early cvs may have a different growth and recovery rate during and after the winter. This may cause deviations in the order of earliness after overwintering.

The temperature has a twofold effect on yield. Vernalizing temperatures increase the development rate, resulting in a decreased yield. Increasing temperatures influence the rate of development more than the growth rate and hence lower the yields. This temperature effect is independent of vernalization and daylength.

Lowering the light intensity results in a strong decrease in yield. This effect does not vary considerably with the earliness of a cv.

GA_3 results in strongly reduced yields because of its pronounced effect on the development, especially stem formation.

A stimulation of the generative development is always accompanied by a stimulated growth rate. Increasing the daylength increases the development rate more than the growth rate, resulting in decreasing yields. Under strictly or nearly vegetative conditions the growth rate is low, and the plants are susceptible to deterioration. A non-flowering cv would therefore be of no interest. The growers have discerned this and for each growing period cvs are used which allow a moderate rate of development.

In spring the yields are higher, the later the cv. The yields of all cvs increase with later sowing until a maximum is reached in the first half of April, after which a pronounced decrease follows. This optimal sowing date is slightly earlier, the later the cv. The lower yields of the earliest sowings are due to vernalization, the decreasing yields at later sowings to the combined effects of increasing temperatures and daylength.

In the autumn, the optimal sowing date is earlier, the later the cv. This is mainly a daylength effect. Too early sowing of a cv gives lower yields because of too high a development rate, too late sowing because of too low a growth rate, accompanied by greater susceptibility to deterioration.

ACKNOWLEDGEMENT

SAMENVATTING

DE INVLOED VAN UITWENDIGE FACTOREN OP DE GROEI EN ONTWIKKELING VAN SPINAZIE CULTIVARS (*SPINACIA OLERACEA L.*)

Inleiding

Diep gevroren spinazie is een snel in betekenis toenemend produkt in West-Europa. Aangezien stengelvorming en bloei zeer belangrijke eigenschappen voor de teelt zijn, werd een onderzoek uitgevoerd naar de invloed van milieu-factoren op de groei en ontwikkeling van spinazie cultivars.

Invloed van milieufactoren op de ontwikkeling

De invloed van genotype (cultivar), temperatuur, lichtintensiteit, GA_3 en daglengte op stengelstrekking en bloei werden onderzocht.

De meeste cvs vertonen een kwantitatieve LD reactie, zowel voor stengel-groei als voor bloemvorming. De daglengtereactie van de cvs varieert van bijna dagneutraal tot absoluut LD-behoeftig.

Temperaturen van 2–8 °C versnellen stengelstrekking en bloei door middel van een geïnduceerde vermindering van de daglengtebehoefte. Temperaturen van 9–12 °C versnellen alleen de stengelgroei. Alle cvs, onafhankelijk van hun daglengtebehoefte, vertonen eenzelfde temperatuureffect.

Verlaging van de lichtintensiteit heeft een verminderde daglengte-behoefte tot gevolg. Dit effect is sterker naarmate de cv later is.

GA_3 versnelt de stengelgroei sterk, bloemvorming slechts weinig. Het posi-tieve effect op de bloemvorming is indirect via het sterke effect op de stengel-groei.

Er is geen jeugdfase voor de temperatuur-, noch voor de daglengte-reactie. Het daglengte-effect is direct en dus niet inductief. Zelfs nadat stengelstrekking en bloemvorming in LD zijn gestart, kan regressie van de bloemvorming in KD optreden bij kwalitatieve LD cvs.

De remming van het bloemvormingsproces is het grootst bij fotoperioden van 6–10 uur. Bij kortere of langere fotoperioden wordt de remming geringer.

Een kwalitatief LD cv, zoals 'Nobel', blijft alleen dan vegetatief in KD, wanneer de stengelgroei wordt verhinderd. De vorming van laterale knop-primordia, die uit kunnen groeien tot bloemen, gebeurt veel trager in KD dan in LD. Dit differentiatieproces stopt in KD in een vroeg stadium, tenzij stengel-strekking mogelijk is, door bv. lage temperaturen, lage lichtintensiteiten of GA_3. Dit is in overeenstemming met de theorie van CHAILAKHYAN, dat voor bloemvorming in LD-rozetplanten zowel gibberellinen als bloeistimulus nodig zijn voor respectievelijk groei van caulescent weefsel en differentiatie. De vorming van deze hormonen zou afhankelijk zijn van de daglengte .

Invloed van milieufactoren op de opbrengst

De oogstdatum is in het algemeen afhankelijk van het schieten van het

gewas en dus van de factoren, die stengelstrekking en bloei beïnvloeden.

De vroegheid van een cv wordt voornamelijk door zijn daglengtebehoefte en groeisnelheid bepaald. Hoe lager de daglengtebehoefte, hoe vroeger de cv. De volgorde van vroegheid wordt niet gewijzigd door temperatuur en lichtintensiteit, aangezien deze factoren alle cvs op dezelfde wijze beïnvloeden via hun daglengtebehoefte. Vroege cvs kunnen verschillende groeisnelheden en herstellingsvermogens hebben tijdens en na de winter. Dit kan wijziging in de volgorde van vroegheid na overwintering ten gevolge hebben.

De temperatuur heeft een tweeledig effect op de opbrengst. Vernaliserende temperaturen verhogen de ontwikkelingssnelheid, waardoor een geringere opbrengst wordt verkregen. Toenemende temperaturen doen de ontwikkelingssnelheid sneller stijgen dan de groeisnelheid met als gevolg dalende opbrengsten. Dit laatste temperatuureffect is onafhankelijk van vernalisatie en daglengte.

Verlaging van de lichtintensiteit geeft een sterk verminderde opbrengst bij alle rassen.

GA_3 veroorzaakt eveneens een sterke opbrengstreductie, daar het de ontwikkeling en met name de stengelgroei zeer sterk versnelt.

Een stimulering van de generatieve ontwikkeling blijkt steeds gepaard te gaan met een stimulering van de vegetatieve groei. Een toenemende daglengte doet echter de ontwikkelingssnelheid meer toenemen dan de groeisnelheid, met dalende opbrengsten als resultaat. Wanneer de ontwikkelingssnelheid zeer laag of nul is, is de groeisnelheid eveneens laag, terwijl de plant gevoeliger is voor kwaliteitsvermindering. Een 'nietbloeiende' cv is daarom van geen belang. De telers hebben dit in feite al onderkend, en telen slechts spinazie onder omstandigheden, die een matige ontwikkelingssnelheid toelaten.

In het voorjaar zijn de opbrengsten hoger naarmate de cv later is. De opbrengsten van alle cvs stijgen met later zaaien tot een maximum is bereikt bij zaai in de eerste helft van april, waarna de opbrengsten snel dalen. Deze optimale zaaidatum is vroeger naarmate de cv later is. De lagere opbrengsten bij de vroegste zaaidata zijn een gevolg van vernalisatie, de lagere opbrengsten bij late zaaisels worden veroorzaakt door de hogere temperaturen en langere dagen.

In de herfst is de optimale zaaidatum vroeger naarmate de cvs later zijn. Dit is voornamelijk een daglengte-effect. Te vroeg zaaien van een cv geeft lagere opbrengsten door een te snel schieten, te laat zaaien door een te lage groeisnelheid, gepaard met een grotere gevoeligheid voor kwaliteitsverlies.

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PHOTO 1. Spinach cv 'Nobel'; flowering female plants

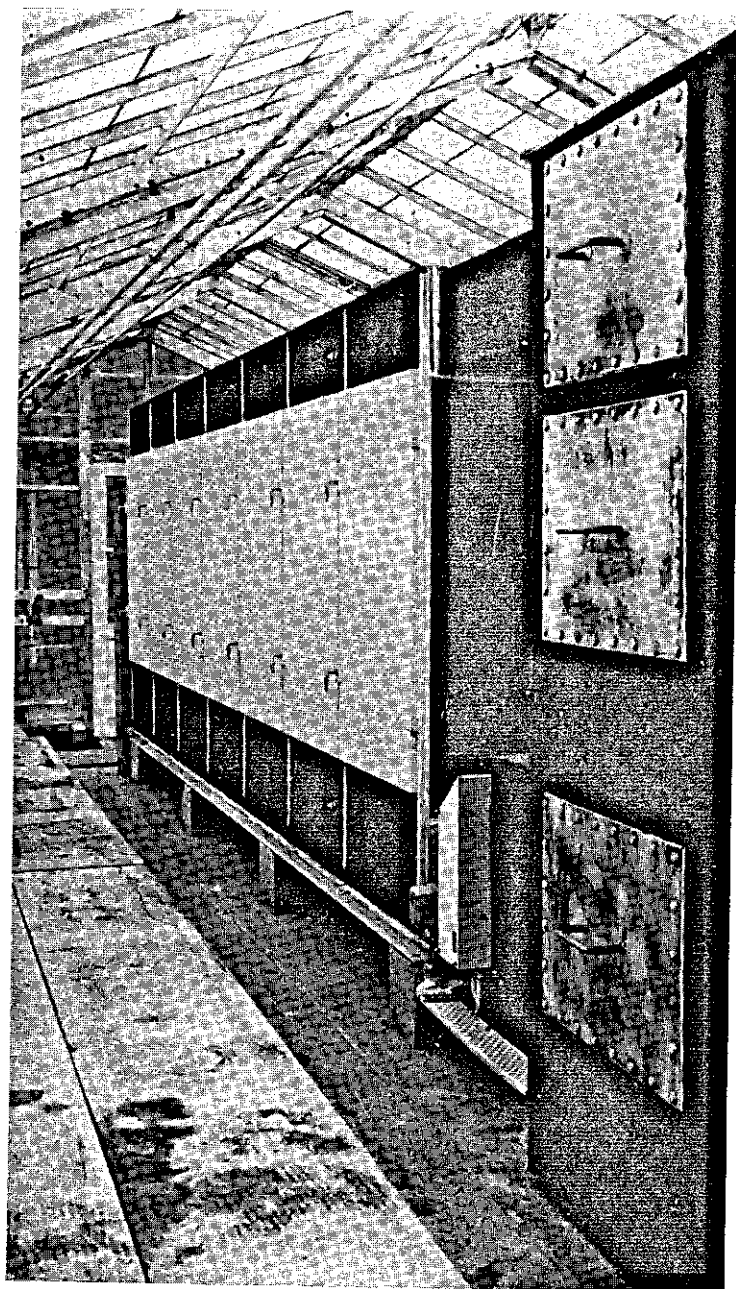PHOTO 2. Spinach cv 'Nobel'; flowering male plants

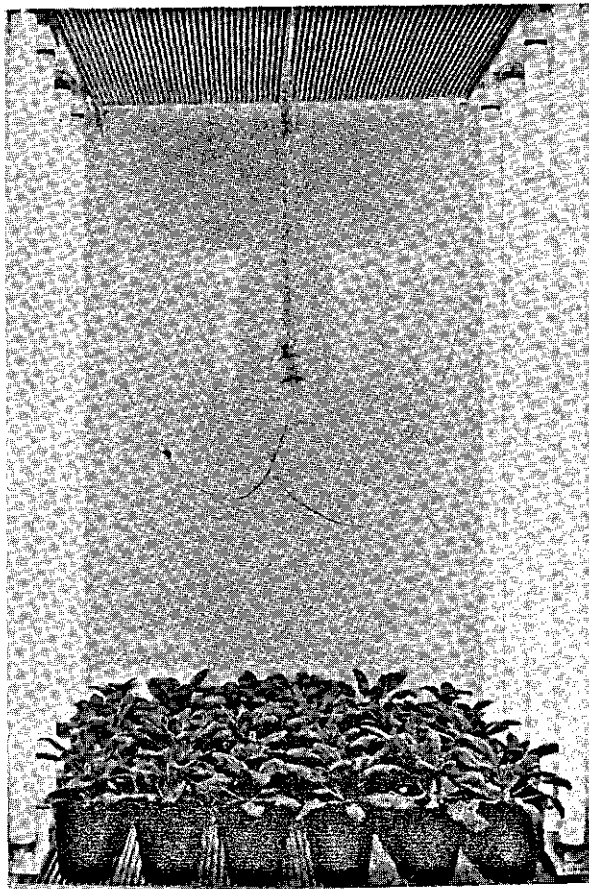PHOTO 3. Frontview of the spinatron, with its seven cabinets and on the right the cooling unit

PHOTO 4. One of the cabinets of the spinatron