THE INDUCTION OF FLOWERING IN SWEDES

by

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ABSTRACT OF THESIS

Early sowing of swedes increases yield but also increases the risk of bolting. The aim of the study was to investigate vernalisation, that is, low temperature flower induction, of swedes.

In a series of experiments, plants of different ages and cultivars were given low temperature treatments of varying duration and temperature.

Swedes were vernalised by temperatures of 11° and below, the optimum being around 5° to 6° for Wilhelmsburger and 3° to 6° for Doon Major. High temperatures following low temperature treatment and interruptions of treatment with periods at higher temperature were devernalising, reducing the numbers of plants flowering and decreasing the rate of flowering. Stem extension and response of stem growth to gibberellic acid were less affected by devernalisation than flowering.

Plants grown at around 15° were found to have a juvenile stage of under 4 days, some cultivars having a shorter stage of 2 days or less.

Low light intensities during vernalisation reduced the number of plants flowering but mature swedes could be vernalised in the absence of light.

There was considerable variation in susceptibility to vernalisation in the cultivars used in the experiments. In order of decreasing susceptibility they were Pentland Harvester, Della, Wilhelmsburger and Marian, Harrietfield, Doon Major and Ruta Otofte. There was evidence of differences in within cultivar variation, early and late flowering selected Wilhelmsburger lines differing more from the parent population in susceptibility to vernalisation than selected Doon Major lines.

The longer the duration of low temperature the more plants flowered and the earlier they flowered.

The normal site of vernalisation was found to be the growing point although axillary buds could be vernalised in the presence and absence of the growing point. There was no evidence of a translocatable flowering stimulus.

Methods of selection and shortening the reproductive cycle are described.

DECLARATION

I declare that this thesis is my own composition and that the work it describes was carried out by myself.

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1. INTRODUCTION

1.1 The induction of flowering in swedes

The study is an examination of flower induction in swedes *Brassica napus* L. var. *napobrassica*), a biennial plant grown as an annual crop chiefly for animal fodder but also for human consumption. The swede has a requirement for vernalisation, that is, flowering is induced by a period of growth at low temperatures.

The present farming practice in Britain is to sow swedes in May or June (Rodger 1975), but experiments have consistently shown greater yields with earlier sowing of swedes (Doling and Willey 1968, Rodger 1975) as with sugar beet (*Beta vulgaris* L.) (Davey 1957, Scott and Bremner 1965, Hull and Webb 1969, Scott and Wood 1972, Draycott, Webb and Wright 1973). Apart from possible difficulties in seedbed preparation, or timing with respect to other crops, the main risks associated with early sowing of swedes are the possible increased attacks of powdery mildew (*Erysiphe polygoni* D.C. or *E. crucifarum* Junell) and bolting - premature stem and flower formation. Although with current late sowing practice bolting is not a serious problem, if earlier sowing is adopted, in cold seasons bolting may considerably reduce the yield advantage of the earlier sowing, especially as there has been very little selection of cultivars for bolting resistance, nor any selection in commercial seed production. Sugar beet cultivars now have greatly improved bolting resistance compared with older cultivars and earlier sowing of sugar beet has now been widely adopted (Longden, Scott and Tyldesley 1975).

The means of the daily mean temperature during April in Scotland 1901 - 1930 were around 5.5° - 7°C (Meteorological Office 1952) which is within the vernalising range of temperature, and the means

of the daily maximum temperature in April were 8.5^{o} - 12^{o}, marginally vernalising or neutral, so that earlier sowing would expose seedlings to vernalising temperatures.

During flowering, the stem and root (the harvestable enlarged hypocotyl) of the swede become progressively more lignified. Assimilates from the leaves are used for stem and flower production rather than root growth, and the bolting plant tends to shade neighbouring non-bolting plants and reduce their growth. The tall bolted plant is more difficult to harvest mechanically and the woody root has a high fibre content (Lysgaard and Nørgaard Holm 1962). Experiments in Denmark with very early sown swedes (mid-March in some years) showed that in early sown plots with many bolters there was a lower root dry matter yield and bolting plants had a lower nitrogen content and higher crude fibre content. Different bolting percentages over the five years of the experiments were related to the particular spring temperatures, colder weather just after emergence being associated with more bolting (Lysgaard and Nørgaard Holm 1962).

Flowering swedes are often divided into early bolters which flower rapidly, during July and August, and late bolters which extend but may not even flower before harvest. Early bolters develop little root and are usually dead with seed set by harvest, whereas late bolters flower more slowly, in some cases only non-flowering stems being produced, and are normally green with well-developed roots at harvest, although the digestibility of the root is reduced compared with vegetative plants.

Differences in the frequency of bolting were found in Canadian swede cultivars (Peto 1934), and data from 1967 N.I.A.B.

swede variety trials at The Edinburgh School of Agriculture provide the following evidence on bolting resistance (Bell 1968). Purple top swedes were most resistant to bolting, with an average of 4 per cent bolters; bronze tops had an average of 9 per cent bolters; and the few green top cultivars in the trial had 11 per cent bolters on average. The highest percentage bolting in the trial was 20 per cent. Within each colour group, cultivars with an acceptable level of bolting resistance and yield were selected, including the purple tops Pentland Harvester and Doon Major, the bronze top Harrietfield, and the green top Wilhelmsburger. The swede is an amphidiploid of *B. oleracea* (cabbage group) and *B. campestris* - formerly *B. rapa* (turnip group) (McNaughton and Thow 1972), and within both species there is a range of susceptibility to bolting (Ito and Saito 1961, Heide 1970 on cabbages; Wester and Magruder 1937 on turnips).

To select for bolting resistance in swedes, Lysgaard (1974) sowed plants early enough in spring to give 75 per cent bolting. In autumn 100 non-bolters were selected and their progeny gave fewer bolters compared with the original seed.

The eventual aim of this study is to provide information on the response of swedes to low temperature in terms of flowering and bolting so that accurate methods of screening for bolting resistance can be devised and used in plant breeding. If more bolting resistant cultivars were available, early sowing of swedes could be more safely encouraged. Breeding of bolting resistant sugar beet cultivars has already resulted in the general adoption of earlier sowing, although more resistant cultivars are still being sought (Kimber 1976).

1.2 Other species which can be vernalised

Many plant species are affected by vernalisation. In some, it is an obligate requirement for flowering, in others it enhances the flowering process but is not essential for it. Often vernalisation and photoperiodic requirements, usually long day, can replace each other to some extent.

Species with an obligate requirement for vernalisation which is enhanced by long days include *Allium cepa* (onion) (De Mille and Vest 1974), *Beta vulgaris* (beet) (Margara 1960), *Campanula medium* (Canterbury bell) (Chouard 1959, Wellensiek 1960, 1962a) and *Daucus carota* (carrot) (Fisher 1956), and *Hyoscyamus niger* (henbane) (Thomas 1956, Salisbury 1963) in which long days are essential for flowering.

Some strains of *Chrysanthemum* require vernalisation for flower induction but like other chrysanthemums and unlike most other vernalisable species, short days are required for normal flower development (Schwabe 1951).

Scrofularia vernalis, a species with an obligate cold requirement, is day-neutral (Chouard 1959). Stokes and Verkerk (1950) claim that sprouts are day-neutral, perhaps because they cannot be induced to flower by photoperiod alone, and Miller (1929) found no effect of extending daylength with low intensity light after low temperature treatment of cabbages, but Heide (1970) found a marked response to long days after low temperature treatment in stem growth and earliness of flowering of Norwegian cabbage cultivars. This difference is likely to be because of the cultivars used, the Norwegian cultivars responding more to daylength than the American cultivars, bred in lower latitudes with shorter summer daylength.

Among other species with an obligate requirement for vernalisation are *Apium graveolens* L. (celery) (Thompson 1928), biennial strains of *Centaurium minus* (centaury) (Michniewicz and Lang 1962), *Cynosurus cristatus* (crested dog's tail) (Purvis 1961), *Digitalis purpurea* (foxglove) (Brian 1958), *Lunaria biennis* (honesty) (Wellensiek 1961), *Myosotis alpestris* (a forget-me-not) (Michniewicz and Lang 1962) and *Oenothera biennis* (evening primrose) (Chouard 1959).

Some perennial species, such as *Cichorium intybus* (chicory) (Brian 1958), late strains of *Lolium perenne* (perennial rye-grass) (Cooper 1951) and *Petroselinum crispum* (parsley) (Lang 1957, Brian 1958), will not flower unless they have been vernalised. No stimulus is passed from the vernalised flowering stems to the perennating part of the plant, which will continue to grow vegetatively till it itself is vernalised.

Many species with a wide range of environmental requirements for flowering respond to vernalisation of seed or plant with more rapid flower development, flowers appearing at lower nodes on the plant, or more flowers being produced per plant. Among these are *Agrostemma githago* (corncockle) (Purvis 1961), *Avena sativa* (winter oat) (Spector 1956), *Brassica juncea* (mustard) (Sen and Chakravarti 1941), *Cichorium endivia* (endive) (Harrington, Rappaport and Hood 1957), *Hordeum vulgare* (winter barley) (Spector 1956), *Lactuca sativa* (lettuce) (Knott, Terry and Anderson 1937), *Lilium longiflorum* (a commercial lily) (Lin, Wilkins and Angell 1974), *Linum usitatissimum* (linseed or flax) (Chakravarti 1954), *Lolium italicum* (Italian rye-grass) (Cooper 1951), *Lolium rigidum* (Wimmera rye-grass) (Cooper 1951), *Pisum sativum* (pea) (Haupt 1969), *Raphanus sativus* (radish) (Suge and Rappaport 1968), *Sinapis alba* (mustard) (Bernier 1969), *Spinacia*

oleracea (spinach) (Verkerk and Volosky Yadlin 1959), *Triticum aestivum* (winter wheat) (Spector 1956) and *Vicia villosa* (winter vetch) (Purvis 1961). In some of these species vernalisation and long days are complementary and can replace one another to some extent but in others specific daylength requirements are irreplaceable.

Within species there is frequently a wide range of response to different flower-inducing stimuli. The wide variety of plant types within the species *Brassica oleracea* have responses ranging from the non-essential but enhancing effects of plant vernalisation on flowering of long day broccoli (Fontes, Ozbun and Sadik 1967, Fontes and Ozbun 1971) through the variety of requirements for curd formation and flowering in cauliflower (Sadik 1966) to the obligate requirements for vernalisation in cabbage (Miller 1929) and brussels sprouts (Stokes and Verkerk 1950).

In *B. campestris*, the turnip species, there is a similar range. Sakr (1944) suggests that turnips (*sic* no species name given) have an obligate requirement for vernalisation, but in the Handbook of Biological Data (Spector 1956) it is suggested that *B. rapa*, considered an identical species to *B. campestris*, is a quantitative long day plant with no known response to vernalisation. Friend (1969) has shown that a strain of *B. campestris* is a quantitative long day plant without an obligate cold requirement.

In *B. napus* there are spring cultivars of oil seed rape which require chiefly long days for flowering, winter cultivars with a greater need for vernalisation (Mendham and Scott 1974) and the swede, *B. napus* var. *napobrassica*, which has a high requirement for vernalisation (Peto 1934).

Other crucifer species, such as *Arabidopsis thaliana* (Thale cress) (Napp-Zinn 1969) and *Matthiola incana* (stocks) (Post 1936, Heide 1962), have different races with and without an obligate requirement for vernalisation.

Cereals - barley, oats, rye and wheat - have winter cultivars that respond to vernalisation, and spring cultivars that do not (Spector 1956). Similarly, in *Trifolium subterraneum* (subterranean clover) (Purvis 1961) there are early and late cultivars, the latter having an obligate vernalisation requirement.

Sugar beet strains range from those that can be induced solely by continuous high intensity light (Stout 1944) to those in which cold treatment is essential (Chroboczek 1933).

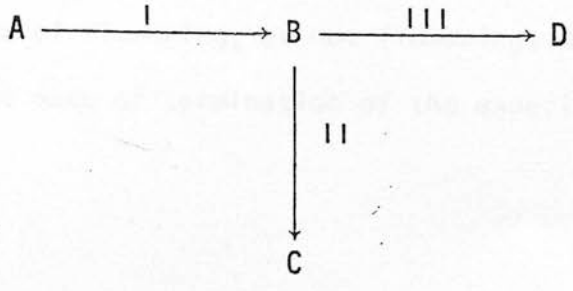
A large part of the variation in response to vernalisation within a species is genetic in origin. Occasionally ripening of seed at low temperatures will vernalise the seed giving rise to differences in bolting resistance between seed stocks of the same genetic constitution (Whyte 1948, Longden *et al* 1975).

The wide range of response types within *Brassica* species suggests a large number of genes are involved, but in some species the genetics of vernalisation are relatively simple. In rye the difference between winter cultivars (obligate vernalisation) and spring cultivars (no response to vernalisation) (Purvis 1948) and in henbane (*Hyoscyamus niger*) between biennial and annual races is only one gene (Purvis 1948, Salisbury 1963). More genes are involved in wheat (Salisbury 1963), and in *Arabidopsis thaliana* the inheritance is more complex with different numbers of genes involved in different races (Napp-Zinn 1969).

1.3 Theoretical models of vernalisation

Various theoretical systems have been put forward to describe the reactions involved in vernalisation, all based on the effects of environmental stimuli on flowering behaviour. None of them incorporate evidence of internal changes during vernalisation, as this kind of information is not available, apart from some hormone and carbohydrate level studies. The systems, quoted in Purvis 1961, of Gregory and Purvis for rye, Van de Sande Bakhuyzen for cereals and Napp-Zinn for *Arabidopsis* (see also Napp-Zinn 1969), are based on daylength and temperature effects peculiar to each species and are not generally applicable. Within the normal range of temperatures for plant growth, enzyme activity always increases with temperature although at different rates for different enzymes. Vernalisation seems to be a quantitative process and presumably involves the depletion of an inhibitor or accumulation of some substance or substances at low temperature that induce the flowering process.

Inhibitors including ABA (abscissic acid) tend to induce or maintain bud dormancy, which can be broken by chilling or application of gibberellins (Wareing and Phillips 1970), but there is no evidence for the involvement of inhibitors in vernalisation. All the systems devised assume the more probable accumulation of some substance. That of Lang and Melchers for *Hyoscyamus niger* (Purvis 1961, Salisbury 1963), also suggested by Gregory (Salisbury 1963), offers a simple mechanism to explain how a substance can accumulate at low temperature when all reactions are slowed down (see below).



The scheme can be modified so that reaction II is B to A, and there is no by-product C. Because reaction I has a low Q10, reaction II a high Q10, and reaction III a medium Q10, at moderate to high temperatures B will be rapidly converted to C. At low temperatures reaction I will still proceed at a moderate rate but very little B will be converted to C, and B will accumulate and be slowly converted to D. The longer the plant remains at low temperatures the more D accumulates. If the plant is transferred to moderate temperatures, D and C will be formed, but if to high temperatures, mostly C will be produced, and little D, as reaction II will proceed faster than III.

The scheme incorporates the main features of vernalisation but there is no experimental evidence to confirm that vernalisation does operate like this. It seems probable that vernalisation is chemically mediated, involving the different temperature responses of enzymes (Q10 s). Other factors, such as daylength, are often involved and the vernalisation response and flowering of any species is probably very complicated, and may be very different between and even within species.

There are several difficulties peculiar to the study of vernalisation. Unlike photoperiodism, when as little as one or two days may be required for flower induction, vernalisation occurs over a period of weeks and subsequent bud development may take months, especially after marginal treatments. The age of the plant exposed to low temperature changes during treatments; when induction is completed, the plant begins the process of flowering, even at low temperatures, confounding measures such as plant age or time to flower with duration of low temperature treatment. Measuring the qualitative change of flowering, or not flowering, avoids this difficulty but the date of termination of the experiment arbitarily

determines how many plants are included as flowering. In theory there is a minimum duration of low temperature longer than which all plants will flower normally but due to the genetic variability of the experimental material, an increasing proportion of plants will flower as the duration of low temperature treatment increases.

The vernalisation process may not be identical in all the species in which it occurs but it is likely that there is some type of reaction resulting in the formation of a flowering substance at low temperature common to all species. Photoperiodic requirements, however, vary considerably although long days enhance flowering in the majority of vernalisable species.

The range of genetic variability in the response to low temperature treatment differs between species, in rye and henbane the requirement for vernalisation being under the control of one gene, but in *Brassica* species the response is probably affected by a large number of genes, as there is considerable variability within *Brassica* species.

1.4 Temperature of vernalisation

Vernalisation normally takes place at temperatures between 0° and 10° although a few species which will not flower at high temperatures (25° and over) can be induced to flower at moderate temperatures, for example a Japanese cabbage cultivar grown at 17° for 20 days formed flower buds (Ito and Saito 1961), and *Hyoscyamus niger* is induced at 14° (Hillman 1969). Broccoli is not considered to have an obligate requirement for vernalisation but will not flower (Fontes and Ozbun 1971) or only slowly (Fontes *et al* 1967) if grown at 24° and above.

Temperatures between 1° and 7° are equally effective for vernalisation of cereal seeds and even at -4° and +12° vernalisation proceeds very slowly (Purvis 1961). Optimum temperatures for plant vernalisation are generally in the range 4° to 9°. Six degrees to 9° (Stout 1944) and 5° to 7° (Wellensiek and Verkerk 1954) are given as optima for sugar beet, 7° for sprouts (Verkerk 1954), 4°, 7° (Heide 1970) and 9° (Ito and Saito 1961) for different cabbage cultivars and 7° rather than 2° for onions (De Mille and Vest 1974).

The upper limit for vernalisation is around 12° or 13° for most species. Sugar beet grown at 16° and above for over three years never flowered (Chroboczek 1933, Margara 1960), but 60 per cent of sugar beet plants flowered, after winter storage at 13°, although no sugar beet plants flowered at 15° (Chroboczek 1933). Cabbages grown at 10° to 16° eventually formed buds and flowered, but development was very slow (Miller 1929) and in an experiment of Heide's (1970) some of a group of cabbages flowered, after growth at 12° for six months. Growing turnips (*sic*) at 10° to 16° for 124 days resulted in 70 per cent flowering (Sakr 1944) and 12 per cent of swedes grown at 11° average temperature for 50 days from sowing flowered (Peto 1934).

Vernalisation above 10° is normally very slow and the temperature range 10° to 15° is generally considered 'neutral'. An experiment of Heide's (1970) in which interrupting a fixed six weeks duration of low temperature (5°) with eight hours every day at 12° reduced the number of cabbages flowering (Heide 1970) compared with an uninterrupted six weeks, led him to suggest that 12° can be both vernalising and devernalising.

If Lang and Melchers' general scheme is accepted, at every temperature all the different reactions will be proceeding and the

specific temperature will only alter the balance of reactions so that no temperature can truly be called neutral.

Temperatures from 4^{o} to 9^{o} appear to be most effective for vernalisation of plants, and vernalisation over 10^{o} is so slow as to be of little importance in many *Brassica* species and in sugar beet.

1.5 Juvenility

Many plant species cannot be induced to flower during their earliest stages of growth. The inability of the young plant to respond to the environmental conditions that are normally sufficient to cause flowering is called juvenility. The juvenile phase is frequently several years in woody species and commonly there are morphological differences, such as leaf shape, or spininess, between juvenile and adult plants. In herbaceous plants the juvenile phase is much shorter or not present at all.

The cold induced species and varieties beet (Margara 1960), broccoli (Warne 1961, Fontes *et al* 1967), brussels sprouts (Stokes and Verkerk 1950), cauliflower (Sadik 1966), henbane (Salisbury 1963) and honesty, *Lunaria biennis* (Wellensiek 1963) have all been shown to have a marked juvenile phase, with young plants subjected to low temperature treatment failing to flower, while older plants did flower.

There is also a more quantitative effect of plant age, older plants responding more than younger plants in increased proportion of plants flowering or reduced time to anthesis when subjected to the same low temperature treatment. This has been observed in beet (Chroboczek 1933, Margara 1960), broccoli (Fontes *et al* 1967, Fontes and Ozbun 1971), brussels sprouts (Stokes and Verkerk 1950, Verkerk

1954), cabbage (Miller 1929, Ito and Saito 1961, Warne 1961, Heide 1970), cauliflower (Warne 1961, Sadik 1966), celery (Thompson 1928), henbane (Salisbury 1963), kale (Warne 1961), kohlrabi (Warne 1961) and stocks (Heide 1962).

Wellensiek (1961, 1963) found in honesty (*Lunaria biennis*) that leaf cuttings from juvenile plants (six weeks old) were less readily vernalised than leaves from adult plants (12 weeks old) and suggested that juvenility is a character of the whole plant, but later work with honesty by Pierik (1967) showed that juvenility is located in the buds, and, as in woody plants (Brink 1962), (Leopold and Kriedemann 1964) juvenile and adult tissue can exist in the same plant. Pierik also found that regeneration could result in rejuvenation which may explain many of the previous failures to vernalise cuttings.

Some morphological differences between juvenile and adult plants have been found in herbaceous species. In brussels sprouts the change from juvenile to adult is accompanied by an increase in stem diameter and an enlargement of the terminal meristem (Stokes and Verkerk 1950), and generally cabbages smaller than 5 to 6 mm stem diameter at the beginning of vernalisation cannot be induced to flower (Ito and Saito 1961).

In the absence of obvious morphological changes, juvenility has to be measured by subjecting plants of different ages to an inductive treatment and measuring the subsequent response to the treatment often as proportion of plants flowering. This measure will be affected by the uniformity of the population in terms of susceptibility to flowering, with homogeneous populations having an apparently steeper response to plant age than heterogeneous populations.

With cold induced species there is the further complication that plants continue to grow during the long treatment period and, passing out of the juvenile phase to adulthood, may receive sufficient low temperature treatment once out of the juvenile phase to induce flowering. It is difficult, therefore, to determine the exact length of the juvenile phase and whether the change from juvenility to adulthood is abrupt or gradual. This change, however, does appear to be more sudden in *Lunaria biennis* than in sprouts or sugar beet (Leopold and Kriedemann 1975).

In some strains of *Arabidopsis thaliana* (Napp-Zinn 1969) and in stocks (Post 1936) there is evidence of an optimum age for vernalisation: 45 - 90 days and 42 - 56 days respectively, susceptibility to cold induction declining beyond the optimum age, but in most species older plants are never less susceptible to low temperature treatment than younger plants.

Plant size has been shown to affect susceptibility to vernalisation. Larger turnips (Wester and Magruder 1937) and larger cabbages (Ito and Saito 1961) were observed to bolt earlier than smaller plants of the same age but Ito, Saito and Hatayama (1966) found that stem cuttings of cabbage responded only slightly less well than whole plants, to low temperature treatment. When cabbages of the same size but different ages were subjected to the same low temperature treatment, the older cabbages produced more bolters than the younger plants (Ito and Saito 1961).

Wellensiek (1962a) suggests that high light intensity during early growth can shorten the juvenile phase and Napp-Zinn (see Purvis 1961) found that growing *Arabidopsis thaliana* at higher light intensity reduced the age of minimal response to vernalisation from 46 to 7 days.

1.6 Seed vernalisation

Ripening seeds, including those still on the mother plant (Longden *et al* 1975) imbibed seeds, or seeds with the radicle beginning to show, can be vernalised. In a few species seed vernalisation can replace an obligate plant vernalisation requirement. Some races of *Arabidopsis* can be seed vernalised, and turnips can be, provided that seeds have germinated by the end of vernalisation (Wiebosch 1965). In many species with no obligate cold requirement, such as endive (Harrington *et al* 1957), lettuce (Knott *et al* 1937), mustard (*Sinapis alba*) (Bernier 1969) and spinach (Verkerk and Volosky Yadlin 1959) flowering or bolting is enhanced by seed vernalisation. Seed vernalisation increases percentage flowering in the following cold requiring plants, some cultivars of carrot (Chouard 1959), *Lunaria biennis* (Wellensiek 1962a), sugar beet (Owen, Carsner and Stout 1939, Wellensiek and Verkerk 1954, Wiebosch 1965, Longden *et al* 1975) and turnip (Wiebosch 1965), but failures of seed vernalisation have been reported in sugar beet (De Roubaix and Lazar 1947, Purvis 1961), *Oenothera biennis* (Chouard 1959), rape (*sic*) (Whyte 1948), turnip (*sic*) (Whyte 1948) and sprouts (Stokes and Verkerk 1950).

The successful seed vernalisation of species with a juvenile phase, such as *Lunaria biennis* and sugar beet, suggests that seed vernalisation is not exactly the same process as plant vernalisation. The successes and failures with seed vernalisation, often with the same species, have led to the suggestion that seed vernalisation acts in most species with an obligate cold requirement by enhancing flowering caused by subsequent plant vernalisation, rather than directly causing flowering (Pierik 1967). In experiments with sugar

beet in California (Owen *et al* 1939), seed vernalisation increased the percentage bolters when the crop was sown in March, but not when the crop was sown in May or October, either because the seed was devernalised at the higher May or October temperatures, or, more probably, because the crops received no vernalisation in the field during the adult plant stage.

Juvenility, which ensures that plants will not flower when younger than a critical age, occurs in many plants including *Brassica* species. Younger plants may not respond to low temperature treatment at all, or may respond less than older plants especially when the vernalisation period is long. Seed vernalisation appears to be unaffected by juvenility in several species possibly because the imbibed seed which can be affected by low temperature induction is not juvenile, and their juvenility is acquired during or after seed germination. Juvenility is closely related to plant age, although plant size can have similar effects, larger plants being induced more readily than smaller ones, but juvenility is not simply an effect of plant size.

1.7 Devernalisation

At higher temperatures, especially over 15°, devernalisation, that is reversal of vernalisation, occurs in seeds and plants. The higher the temperature the greater the reversal of vernalisation, whether in terms of increased time to flower or reduced number of plants flowering. In cabbages, three days at 36°, six days at 30°, or 12 days at 24° immediately following low temperature treatment completely reversed six weeks at 5° which would normally have caused complete flowering (Heide 1970).

Frequent short interruptions of low temperature treatment by periods at higher temperatures devernalise more effectively than less frequent and longer interruptions even when the total time at higher temperatures is the same (Purvis and Gregory 1952).

Even during stem extension plants can revert to the vegetative state and produce a rosette of leaves at the top of the elongated stem if temperatures following low temperature treatment are too high. This has been observed in beet (Owen *et al* 1939), brussels sprouts (Stokes and Verkerk 1950), and swedes (Peto 1934), especially after marginal low temperature treatment. In cabbages, devernalisation reduced flowering much more than stem extension (Heide 1970).

Devernalisation has been made use of in vegetative propagation of cauliflower. Curd formation is induced by suitable cold treatment, and apical meristems which are at an early stage of floral development (bud stage 2, Figure 2.1) are removed. They are grown in aseptic culture at 20° to 24° to make them revert to the vegetative state (Crisp and Walkey 1973). According to Margara (1960), *in vitro* culture in the absence of sugar, of extending stem tips of sugar beet can cause reversal to the vegetative state. Similarly, broccoli curd cuttings with flower buds kept at temperatures from 4.5° to 21° formed normal flowers whereas those transferred to 15.5° to 27° aborted their flower buds and developed bracts (Haine 1951).

Devernalisation can only occur for a short while after the low temperature period and, once securely induced, plants will flower at high temperatures and so the stimulus produced by the plant as a result of low temperature treatment must become stabilised with time. This stabilisation ('setting' or 'fixing') corresponds in the theoretical model (page 8) to a conversion of all B, the intermediate

substance, into D the stable flowering substance. Growth at moderate temperatures, 10° to 15°, or 20° for *Hyoscyamus* (Purvis 1961) for a few days up to a fortnight limits or prevents subsequent devernalisation by high temperatures. This 'setting' of vernalisation has been observed in rye (Purvis and Gregory 1952, Purvis 1961), *Hyoscyamus niger* (Purvis 1961), and radish, but exceptionally, *Arabidopsis thaliana* cannot be 'fixed' (Purvis 1961). In rye one additional week of low temperature treatment reduced the devernalising effect of three days at 35° by half (Purvis 1961) showing that vernalisation can be 'fixed' at vernalising temperatures. This result was found for two to eight weeks low temperature treatment when rye responds linearly to increased duration of treatment so that the effect of decreasing devernalisation by greater 'fixing' cannot be clearly separated from the effect of increasing duration of low temperature treatment. The 'setting' or 'fixing' therefore is something which occurs at low and moderate temperatures, and possibly at higher temperatures as well, but devernalisation may reduce the level of vernalisation considerably before 'fixing' is complete.

Devernalisation of chrysanthemum at high temperatures only occurs at low light intensity, 20 to 25 foot candles (215 to 269 lux) (Schwabe 1956). In all other species studied high temperature alone is sufficient, although light has been shown to increase the stabilisation of vernalisation in rye (Purvis 1961), that is, reduce its susceptibility to devernalisation.

Stem extension appears to be less affected by devernalisation than flowering (Heide 1970), suggesting that it is caused by somewhat different reactions in the plant.

In the theoretical model of the vernalisation process (see 1.3, page 8) at high temperatures reaction II (B to C) will proceed most rapidly so that little flowering substance (D) will be formed, and any B formed during low temperature and not yet converted to D will be rapidly converted to C. At moderate or low temperatures after vernalisation, however, the accumulated B will be converted more to D than to C, 'fixing' the effects of vernalisation.

In natural conditions although temperatures may frequently be vernalising, a short daily period of higher temperature will reduce the vernalisation, so that any calculation of the likely outcome of a period of cold weather on flowering will have to take into account not only the sum of the hours at low temperature but also the reversing effect of periods at high temperature.

1.8 Duration of treatment

Unlike photoperiodic induction which is often complete with only a few days of the appropriate daylength, vernalisation occurs very gradually. Plant metabolism is slow at vernalising temperatures and the lower limits to vernalisation are probably determined more by metabolic rate than by the temperature requirements of the vernalisation reactions.

Only a proportion of treated plants will flower after marginal durations of low temperature treatment and flower development will be slow. Sometimes plants extend and form leaves of the shape associated with reproduction but do not flower. As the duration of low temperature increases, the proportion of plants flowering, and the rate of, and completeness of flower development increases.

The requirements of different species range from two to nine weeks low temperature in general, although the vernalising effect of any duration of low temperature depends to a large extent on the particular conditions of the treatment. Turnips (Sakr 1944) are highly susceptible to bolting, and 15 days at 5° to 10° produced 22 to 80 per cent flowering plants, and 30 per cent of celery plants flowered after exposure to 9° for 20 days (Thompson 1928). Exposing swede plants to 50 days at 11° mean temperature gave 12 per cent flowering (Peto 1934). At temperatures below 10° (Thow 1974), 28 days gave almost complete flowering of swedes, and 42 and 56 days gave 100 per cent flowering and faster flower development than after 28 days. In sugar beet 30 to 31 days at 5° gave 30 per cent flowering (Margara 1960) but in other experiments gave 0 to 90 per cent (Chroboczek 1933) depending on post treatment growth temperatures (15° to 21°, or 21° to 27°). Fifty per cent of cabbage plants eventually formed flower buds after 21 days at 5° (Heide 1970) but 42 and 63 days gave 100 per cent flowering within the time of the experiment. In experiments with six Japanese cabbage cultivars only 30 days at 9° resulted in complete flowering (Ito and Saito 1961). Eight weeks at 5° is considered necessary for flowering of *Lunaria biennis* (Wellensiek 1963). No brussels sprouts flowered after three weeks at 3° (Stokes and Verkerk 1950) but some plants bolted. Six weeks gave some flowering, and nine weeks gave 100 per cent flowering (Verkerk 1954).

Stem growth is more rapid after longer vernalisation as shown in sprouts *Lunaria biennis*, *Campanula medium* and *Cheiranthus cheiri* (Verkerk 1954). Japanese cabbage cultivars had a shorter minimum duration for bolting than for flowering (Ito and Saito 1961), and

in sprouts the minimum durations for bolting and flowering varied in different plants (Stokes and Verkerk 1950).

Some species require only a few weeks low temperature treatment for complete flowering, others require months. Within a species there is also considerable variation in response, as durations of low temperature shorter than those required for 100 per cent flowering result in a proportion of plants flowering, because of either genetic or environmental differences within the group. By exposing plants to less than optimum duration of low temperature, or a range of durations of low temperature, treatments can be compared by the proportion of plants flowering.

1.9 Nitrogen

In the past, nitrogen has been suggested as a possible cause of premature bolting (Miller 1929), but this claim was not based on any clear evidence. Nitrogen has no effect on the response of winter rye to vernalisation (Purvis 1961), Miller (1929) observed no bolters in an N P field experiment on cabbages and Chroboczek (1933) found large applications of nitrate produced no bolters in beet plants grown at 16° to 21°. In beets given a low temperature treatment, however, nitrate treated plants were larger and flowered earlier (Chroboczek 1933). In another experiment with beet (Chroboczek 1933) plants were stunted before low temperature treatment by restricting water, restricting nitrate, or restricting water and nitrate. The number of flowering plants in the no nitrate, plentiful water group was only 12 out of 20, compared with 16 or 17 out of 20 in the other groups, suggesting that nitrogen deficiency

rather than stunting had caused the slight reduction in flowering. The main effect of nitrogen is likely to be on plant growth after low temperature treatment when temperatures will permit active uptake and growth rather than during treatment.

Suggestions that checks to plant growth, such as drought (Hannah 1959) may cause bolting have been put forward but there is no evidence to support this, and in celery (Thompson 1928) treatments applied to restrict growth - drought and freezing - delayed flowering.

Early claims that nitrogen causes bolting now seem unfounded, although shortage of nitrogen may restrict flowering induced by low temperature probably chiefly by reducing rate of growth.

1.10 Light and daylength

Most species with an obligate requirement for vernalisation will flower in any daylength although the appropriate daylength enhances flowering. As an exception, *Hyoscyamus niger* (Salisbury 1963) requires long days after low temperature treatment for flowering and winter rye only forms rudimentary flowers in the sheath during short days (Purvis 1961).

Continuous light replaced the cold requirement for flowering of beet, grown at 12° to 15° (Chroboczek 1933) and of a selected strain of sugar beet (Stout 1944) but sugar beet grown at 16° and above for 1¾ years in continuous light did not flower (Margara 1960).

Exposing carrots to short days just before low temperature treatment, especially in marginal conditions, enchanced flowering (Fisher 1956). In rye, short days followed by long days can induce flowering, the short days replacing vernalisation to some extent (Purvis 1961).

Light during vernalisation is not essential for flower induction or flowering of mature sugar beet. When sugar beet roots were vernalised and then grown at higher temperatures in darkness all plants bolted, and nine per cent produced normal open flowers in the dark (Fife and Price 1953). Long days during vernalisation slightly enhanced stem elongation of cabbage (Heide 1970). In onions (De Mille and Vest 1974), continuous low intensity light during low temperature treatment resulted in faster flowering than only 12 hours light which in turn gave more rapid flowering than treatment in the dark. Low light intensity, 35 foot candles (377 lux) during low temperature treatment had an inhibitory effect in flowering of carrots, the effect being perceived by the apical meristem, unlike the normal photoperiodic response, although long photoperiods after vernalisation stimulated flowering (Fisher 1956). This is similar to the effect of low light intensity after vernalisation in enhancing devernalisation of chrysanthemums, and probably operates through a different mechanism from the photoperiodic response.

Cold nights and cold days have different effects on the flowering and development of stocks. A period of continuous cold causes stocks to grow into tall flowering plants with entire leaves. Plants grown in warm days and cold nights produced entire leaves but did not extend. Those grown in cold days and warm nights flowered but remained dwarf and continued to produce vegetative pinnately lobed leaves (Post 1936). This suggests that there are at least three processes involved in the normal flowering of stocks, flowering itself requiring light, or perhaps being reversed in warmth and darkness, the production of reproductive leaves occurring in darkness or being reversed in warmth and light, and stem extension which requires continuous cold.

Daylength after low temperature treatment usually has a greater effect on stem extension and flowering than daylength during treatment. Chroboczek (1933) found that 16 hours of full intensity light compared with 12 hours after low temperature treatment enhanced stem elongation and rate of flowering of beets. Heide (1970) found that extending an eight-hour day to 24 hours with low intensity light, after low temperature treatment, had the same effect on cabbages. Long days after treatment had a much greater effect on stem extension and earliness of flowering than long days during treatment (Heide 1970). Miller (1929), however, found no effect of extending daylength with low intensity light on seedstalk development in cabbage. This difference may be due to the different cultivars used (see 1.2, page 4).

Light appears to enhance vernalisation, or to replace it to some extent, although it is not essential for all species during vernalisation. Daylength, chiefly long days, affects flower development after vernalisation, in some species long days being essential for normal flowering. The effect occurs mainly after vernalisation and so probably acts on flower development rather than directly on the vernalisation process. The effect of short days followed by long days in replacing vernalisation has not been widely studied, but short days during vernalisation did reduce the effect of vernalisation in cabbage and therefore probably can only replace vernalisation in some species.

1.11 Plant hormones

1.11.1 Gibberellins

The plant hormone group, gibberellins, have variable effects on the flowering of photoperiod induced plants, sometimes enhancing

flowering (Michniewicz and Lang 1962, Jones 1973), especially of long day rosette plants, and occasionally inhibiting flowering (Zeevaart 1970).

In most vernalisable species, especially those which are mainly cold induced rather than daylength induced, gibberellic acid (GA) often causes stem elongation and enhances flowering but rarely causes flowering directly. Biennial *Hyoscyamus niger* bolts and flowers after applications of GA (Lang 1956, Brian 1958), but as vernalisation occurs at relatively high temperatures, e.g. 14° (Hillman 1969) and it has an obligate requirement for long days (Salisbury 1963), it is not a typical vernalisable species.

Applications of gibberellin to the following cold requiring plants, cabbage, kale (*Brassica oleracea*), swedes (*B. napobrassica sic*), turnip (*B. rapa*), carrot (*Daucus carota*), foxglove (*Digitalis purpurea*), daisy (*Bellis perennis*), stocks (*Matthiola incana*) and pansy (*Viola tricolor*), grown at 10° to 13° in long days resulted in flowering in all cases (Wittwer and Bukovac 1957). At 18°, gibberellin was much less effective, but control plants grown at 10° to 13° did not flower. Lang (1957) found that swedes grown at 17° and above and treated daily with GA only produced flower buds after six months of continuous treatment. GA caused flowering in radish (*Raphanus sativa*) (Suge and Rappaport 1968) and some flowering in endive (*Cichorium endiva*) (Harrington *et al* 1957) and enhanced flowering in unvernalised winter rye plants (Purvis 1961), in broccoli (Fontes and Ozbun 1971), in vernalised carrot (Globerson 1971) and vernalised iris stem disks (Pereira 1964), and enhanced bolting in spinach (Verkerk and Volosky Yadlin 1959).

Lockhart (1956) has suggested, from studies on peas, that the stem tip is the site of gibberellin formation. After an application of gibberellin the initial effects are to stimulate active divisions, especially transverse divisions, in the subapical region. Increases in stem length in the first few days after application are probably mainly due to these transverse divisions although cell length increases later (Sachs, Bretz and Lang 1958).

Most experiments involve GA 3 - gibberellic acid. When nine different gibberellins, one to nine, were tried on five species (Michniewicz and Lang 1962), differences in activity were found. GA 3 was as active as GA 7, the most active gibberellin, except on cold requiring *Myosotis alpestris* and long day *Crepis parviflora*. Failures to induce flowering in different species may occasionally be due to the use of an inappropriate gibberellin.

CCC (chlormequat chloride), an anti-gibberellin, applied to radish after seedling vernalisation reduced endogenous gibberellin levels and greatly reduced stem height but had no effect on flowering (Suge and Rappaport 1968). CCC sprayed onto sugar beet seed crops reduced stem height by 18 per cent but did not affect seed yield and presumably had not affected flowering (Longden 1974). High rates of CCC applied immediately after low temperature treatment delayed stem elongation of *Oenothera biennis* but had no effect on the numbers of flower buds (Picard 1967).

In sugar beet the response of seedlings to exogenous GA in terms of stem extension is positively correlated with the bolting susceptibility of the particular cultivar, and the mean length of the hypocotyl epidermis cells is also positively correlated with bolting, suggesting that susceptible cultivars respond more to both

endogenous and exogenous gibberellin. It has been suggested that hydrophobic membrane proteins interfere with the plant's response to GA. Lexander (1974) has shown that there is a positive correlation between the available sulfhydryl (-SH) and disulphide sulphur (-S-) content of seedlings and their susceptibility to bolting whereas in bolting resistant plants there is a high proportion of hydrophobic proteins in which -SH and -S- are bound and unavailable.

Exogenous gibberellins do not directly replace vernalisation, but appear to enhance the effects of vernalisation, especially stem extension, and the anti-gibberellin CCC has more effect on stem growth than on flowering.

1.11.2 Auxins

Auxins have variable effects on flowering. At high concentrations auxin always inhibits flowering (Leopold 1955) but promotive effects do occur, more commonly in long day species, and auxin can induce flowering in long day plants in threshold conditions (Lockhart 1961).

Plants maintain apical dominance chiefly by production and transport of auxin from the apical meristem, and plants with unbranched habits of growth tend to have high apex levels of auxin (Leopold 1955). When plants become reproductive there is often a reduction in apical dominance with axillary buds growing out and flowering. Auxin polar transport declines in mature tissues (Wareing and Phillips 1970) and this may explain some of the increase in branching as vascular tissue in flowering plants is more mature than in vegetative plants. Cabbages, given a longer low temperature treatment, grew into more branched flowering plants (Ito and Saito

1961). When chrysanthemum stem tips were chilled side shoots grew out (Schwabe 1954a), but this may simply be due to a reduction in translocation from the tip, due to low temperature rather than a change in vascular tissue, or a reduction in auxin supply.

De Zeeuw and Leopold (1955) applied auxin paste to brussels sprout plants with an 11-week juvenile stage. Fifty ppm NAA (napthalene acetic acid) increased flowering in the nine-week old group from one to six plants out of ten, and the authors suggest the auxin had reduced the juvenility of the plants. This rate of NAA reduced days to flowering in all age groups, however, and this is probably simply a case of auxin stimulating flowering in marginal conditions. In contrast, soaking seeds of *Linum usitatissimum* L. in NAA or IBA (indoylbutyric acid) during low temperature treatment reversed the effect of the treatment, but IAA (indoylacetic acid) had no effect (Chakravarti 1954). IAA or IBA applied to chrysanthemums after low temperature treatment encouraged vegetative diageotropic growth and delayed flowering. When plants were grown at 28^{o}, IAA or IBA reduced flowering from 100 per cent for controls to 33 per cent (Schwabe 1970).

An interaction between NAA and temperature has been found in long day Wintex barley, short day biloxi soybean and winter rye. If plants were grown at low temperatures, 10^{o} or 3^{o}, during or after treatment with NAA, most concentrations promoted flowering but at 18^{o} or 25^{o}, flowering was unaffected or inhibited by NAA (Leopold and Guernsey 1953).

The effects of auxin on flowering of vernalisable species are somewhat contradictory, although NAA applied before or during low temperature growth generally promoted flowering, except on *Linum*

usitatissimum seeds - a species without an obligate vernalisation requirement - and NAA or IBA applied at higher temperatures tended to reverse vernalisation or inhibit flowering.

1.11.3 Abscissic acid

Abscissic acid is a growth inhibiting plant hormone. It reduces RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) synthesis in some plant tissues, and frequently inhibits processes such as germination or breaking of bud dormancy which GA and cytokinins can stimulate (Wareing and Saunders 1971).

ABA (abscissic acid) has been shown to reduce the flowering of long day *Lolium temulentem* (Evans 1965, Wareing and El-Antably 1970) and spinach (Wareing and El-Antably 1970), and lily bulbs infused or injected with ABA before or after vernalisation were delayed in anthesis (Lin *et al* 1974). In blackcurrant, strawberry and *Pharbitis nil*, all short day plants in which GA inhibits flowering, applications of ABA promoted flowering, although it had no effect on other short day species (Wareing and El-Antably 1970).

These inhibitory effects of ABA may be due to a general suppression of growth, or to ABA's anti-gibberellin activity rather than an effect on a specific flowering substance.

1.11.4 Carbohydrate level

The carbohydrate content of a plant is important in relation to vernalisation both as a source of energy and because of possible hormonal type of action (Watson 1955).

A carbohydrate source and O_2 are essential for vernalisation (Purvis 1961). Cereal seed embryos can be vernalised, presumably because they contain sufficient substrate for vernalisation but

addition of a carbohydrate source to the medium reduces the number of days to anthesis (Purvis 1961).

Exogenous sucrose can alter flowering behaviour. Root cuttings of chicory (*Chicorium intybus*) can be vernalised *in vitro* but vernalisation can be replaced by raising the sucrose concentration in the medium (Evans 1971).

Grainger (1964), using the swede as his principle example, postulates that a minimum leaf number (10 to 13 for swedes) and minimum carbohydrate content (49 per cent for swedes) is necessary for flowering. He obtained his data for swedes by examining carbohydrate content and leaf number throughout the growth of the crop and recording them at the time of flower initiation. He made no attempt to alter experimentally either carbohydrate content or leaf number and examine the effect of such alterations on flowering. All crops had been exposed to low temperature and all flowered, as might be expected, and his evidence on carbohydrate level and leaf number at flower initiation is purely circumstantial.

Sugar and starch levels tend to rise at low temperatures, especially soluble sugars (Rutherford and Naiem 1974). The carbohydrate levels in the stem tips of broccoli (Fontes and Ozbun 1971) and cauliflower (Sadik and Ozbun 1968) were examined in relation to chilling and flower initiation. Sugar and starch levels were higher in plants grown at 5° than in those grown at 20° and over. Defoliation greatly reduced sugar levels and reduced starch levels slightly in broccoli, and flowering was greatly inhibited (Fontes and Ozbun 1971). The application of SADH (succinic acid 2,2 - dimethyl hydrazide) reduced sugar levels and flowering in broccoli, and GA increased flowering at 21° to 27°, and slightly increased starch

levels (Fontes and Ozbun 1971). One week of exposure to 5° resulted in 55 per cent flowering of cauliflowers (Sadik and Ozbun 1968) but the absence of light or CO_2 during the week at 5° reduced carbohydrate levels to those of plants grown at 20° to 26° and prevented flowering. Excluding light or CO_2 for the three days following the week at 5° reduced carbohydrate levels by a third, and flowering from 55 per cent (control) to 20 to 25 per cent. Three days at 33° or at 20° in darkness, following the 5° treatment also reduced carbohydrate levels, and prevented flowering (Sadik and Ozbun 1968). After low temperature treatment cabbages were distinguished as reproductive or vegetative by leaf shape, and the reproductive plants found to contain more sugars than the vegetative type plants (Miller 1929).

In these cases there is strong evidence of a correlation between carbohydrate level and flowering but as yet there is no evidence to suggest that the relationship is in any way causal.

In experiments with cabbage several treatments were used to lower carbohydrate levels. Growing cabbages at 30° for 30 days before low temperature treatment resulted in lower sugar levels and a poorer flowering response to the low temperature treatment than pre-treatment growth at 17° or 24° (Ito *et al* 1966). However, defoliation 30 days before low temperature treatment substantially reduced sugar and starch levels at the start of treatment, but the defoliation had very little effect on flowering.

This tends to suggest that flower induction and flowering are not controlled by carbohydrate levels, although minimum levels of sugar and starch may be necessary for both processes, and environmental factors affect carbohydrate levels and vernalisation similarly.

1.11.5 Endogenous hormones

Changes in hormone levels occur during and after vernalisation. Most studies are of levels in the shoot tip, the usual site of vernalisation and flower initiation (see 1.12, page 34).

Gibberellins are frequently found to increase after low temperature treatment of vernalisable species (Evans 1971). Higher levels of gibberellin-like substances were found in radish after seed vernalisation (Suge and Rappaport 1968, Suge 1970) and plant vernalisation (Suge 1970). Applications of CCC to radish reduced endogenous gibberellins and stem extension, but had no effect on flowering (Suge and Rappaport 1968). Hormone levels of cauliflower during and after low temperature treatment were studied (Thomas, Lester and Salter 1971). Auxin levels rose then dropped after vernalisation treatment to a level below that of the control plants, cytokinins were somewhat lower during treatment but gibberellin levels were much higher than in the controls, during and after treatment. In both cold treated and control plants there was a peak of gibberellin level just prior to curd initiation - the formation of very immature flower buds.

Unvernalised lettuce seed had twice as much IAA as in vernalised seeds, but vernalised seeds had slightly more of an unidentified growth substance (Rf. 0.7 - 0.86) (Fukui, Weller and Wittwer 1957). A substance with a similar Rf value to the 'unidentified growth substance' in vernalised lettuce seeds was found in other species. High levels of a substance with Rf 0.65 - 0.85 in 80 per cent isopropanol were found in long day *Rudbeckia speciosa* after three weeks of long day induction and in a vernalisable *Chrysanthemum morifolium* after three weeks low temperature. In a short day

C. morifolium the possible presence of the substance was masked by an inhibitor (Harada and Nitsch 1958).

Two different substances which promoted flowering in iris stem disks were found in iris scales maintained at 13°. The main substance, Rf. 0.3 in 80 per cent isopropanol, was inactive at 25°, the other was gibberellin-like (Rf. 0.5 and 0.78) (Pereira 1964).

The main identifiable effect of vernalisation on plant hormone levels is to increase gibberellins and gibberellin-like substances and there is some evidence that auxin levels are reduced.

1.11.6 Hormones - a summary

The initial effect of vernalisation is to bring about changes in the plant apex. The stimulus is probably immobile and as yet uncharacterised, and unlikely to be a known plant hormone. Hormone levels, however, do change during and after vernalisation and exogenous applications of hormones can alter the flowering behaviour of vernalisable species.

Gibberellin levels have frequently been observed to rise during and after vernalisation, and gibberellins are associated with stem extension at flowering, and promotion of flowering. Application of GA to unvernalised plants usually causes stem extension but does not readily cause flowering. Similarly, CCC applied to vernalised plants usually restricts stem growth but rarely affects flowering. However, gibberellin is the only plant hormone undoubtedly involved in vernalisation and subsequent flowering. The role of auxins in flowering of vernalisable species is not very clear, although high levels of applied auxin may be inhibitory. There is some evidence that auxin levels may decrease at low temperatures, and the branching of rosette species at flowering suggests a decrease in auxin

production or transport, although this may be partly an effect of plant age as well as flowering. The effect of other hormones is not well-known, but abscissic acid which has growth depressing and anti-gibberellin properties might be expected to restrict vernalisation and there is some evidence that it does.

Plant carbohydrates are associated with flowering and it is possible that a high concentration of sugars is necessary for flowering in some species but there is no evidence of a direct causal relationship.

The vernalisation stimulus probably alters plant morphology and development to some extent through the action of hormones but the stimulus itself is initially retained in dividing cells of the meristem. This effect is similar to the phase change described when cells change from adult to juvenile (Brink 1962), although the vernalised state is more reversible that the attainment of adulthood.

1.12 The site of action

Dividing cells and cell activity are necessary for vernalisation (Leopold and Kriedemann 1975). Most evidence suggests that the apical growing point is the site of vernalisation (Salisbury 1963).

In rye, the embryo and not the endosperm, perceives the vernalisation stimulus (Purvis 1961). In a small experiment, Purvis (1940) removed scutellum, roots or coleoptile from the embryo and found the embryo was vernalisable provided the shoot apex was present.

In plant vernalisation, experiments have shown that cooling the crown - growing-point and petioles - causes flowering in beet (Chroboczek 1933) and celery (Curtis and Chang 1930) whereas cooling

the root is ineffective. Placing only the tips of chrysanthemum plants outside a warm greenhouse into low ambient temperatures greatly reduced the number of days to budding, compared with plants grown totally in the greenhouse (Schwabe 1954a). Defoliating cabbage plants immediately before low temperature treatment had no effect on their response to treatment and 5 cm apical stem cuttings were also readily vernalised (Ito *et al* 1966), confirming that the growing point is vernalisable and leaves are not essential.

There is some evidence that other plant parts can be vernalised (Wellensiek 1961, 1962b). Leaf and root cuttings (1962b) of *Lunaria biennis* (honesty) readily form buds, and these cuttings can be vernalised, subsequently regenerating and growing into a flowering plant. Cuttings taken from whole plants after low temperature treatment of leaves that were actively dividing during treatment will also flower (Wellensiek 1963). If the petiole base of a leaf cutting, which is the part of the cutting that regenerates the roots and buds, is removed after low temperature treatment, new buds form but the plant does not flower (Wellensiek 1963). This demonstrates that the vernalisation stimulus is perceived and retained only in the tissue that was actively regenerating during vernalisation, and is not mobile.

In chrysanthemum, lateral buds and basal buds on underground stolons are vernalised, as well as apical buds. If plants are decapitated immediately after low temperature treatment lower shoots grow out and flower, but if the plant is left intact, lateral and basal buds remain dormant and become devernalised (Schwabe 1954b).

There is evidence that in vernalised rye not only are all buds in a vernalised condition, but remain so even when the plant is left

intact. When all shooting tillers were removed from vernalised rye up to six weeks from sowing (Purvis 1948) small tillers grew out and became reproductive. However, not all tillers of partially vernalised plants will flower (Purvis 1948).

In rosette plants and in seeds the growing point is probably the normal site of vernalisation but other plant tissue such as honesty leaves can also be vernalised, provided the leaves are in active division and regenerating buds. Lower buds and tillers can be vernalised, either directly or possibly because the tillers and buds develop from a vernalised main shoot.

1.13 Translocation of the flowering stimulus

The vernalisation stimulus induced in the apical bud remains immobile initially, growing up with the apical meristem. By the time flower development is well-advanced there is some evidence that a translocatable stimulus can be produced in the apex.

Bulbils of *Cynosurus cristatus* left on the vernalised mother plant flowered, whereas those removed grew vegetatively (Purvis 1961), suggesting that a mobile substance is eventually produced. In a similar experiment Schwabe (1954b) removed the main apex from chrysanthemums at intervals after low temperature treatment and, from the flowering behaviour of the remaining lateral buds, concluded that translocation of a flowering stimulus only occurred after full floral initiation of the main apex.

Margara (1964) grew buds from flowering stems of beet (*Beta vulgaris*) and rape (*Brassica napus*) *in vitro* and compared them with similar buds on the plant, from which stem tips had been removed to

encourage growth of lateral buds. All buds on the plant flowered whereas in the *in vitro* group only buds from high on the stem flowered, basal buds growing into vegetative plants. This suggests that a mobile flowering stimulus is produced at the growing tip and moves slowly down the stem. Studies of stem changes after photoperiodic induction show that a stimulus associated with flower initiation moves slowly down the stem, altering the activity of the stem cambium (Robers and Stuckmeyer 1948).

The effects of gibberellin on the plant are normally considered to be non-polar (Hess and Sachs 1972), and it is unlikely to be this flowering stimulus.

Grafting experiments with *Chrysanthemum morifolium* (Schwabe 1954a), *Cynosurus cristatus* (Purvis 1961) and cauliflower (Sadik 1966) have failed to show any transfer of a flowering stimulus, but there have been some successes with grafting vernalisable species. Grafting a vernalised scion onto unvernalised biennial henbane (*Hyoscyamus niger*) (Salisbury 1963) caused the stock to flower, but as annual henbane and short day tobacco were also effective the effect is more probably due to the transfer of hormones that stimulate flowering in henbane rather than demonstrating the transfer of a specific flowering substance. Scions grafted onto seed vernalised stocks of a Japanese radish cultivar were induced to flower (Purvis 1961). Although the cultivar had a strong cold requirement, radishes are chiefly long day, and that may explain the successful promotion of flowering in the scions. Curtis, Hornsey and Campbell (1964) grafted scions of unvernalised bolting resistant beet onto a well-vernalised beet stock already developing flower buds. Several scions flowered, demonstrating that the flowering stimulus produced by vernalisation can cross a graft union.

The failures in transfer of a flowering substance by grafting could be explained if a flowering stimulus is produced for only a short time so that unless scions are present at that time they will not receive the stimulus. It is also probable that more than one substance is produced during or after vernalisation. Auxiliary substances that assist the flowering process may be able to cross a graft union and encourage flowering especially in readily vernalised species, which can be induced to flower by applications of exogenous gibberellin or exposure to continuous light. Some other specific flowering substance may be necessary for flower induction in other species, but be immobile.

The failure to induce flowering on *Cynosurus cristatus* by grafting and the observation that attached bulbils in vernalised plants do flower, whereas isolated bulbils do not, could be explained by a requirement for flowering of both an immobile stimulus generated in all dividing tissue during vernalisation, and for mobile auxiliary substances produced only in the mother plant.

1.14 Apex changes at flower initiation

After flower induction changes occur in the apical meristem. In crucifers for instance, brussels sprouts (Stokes and Verkerk 1950) and *Arabidopsis thaliana* (Miksche and Brown 1964), in sugar beet (Chroboczek 1933) and other species the apical meristem initially changes from the flat triangular vegetative apex to a more raised and domed shape. In crucifers the apex then produces smaller leaves with enlarged buds in the axils, which tend to remain round the apex giving it a star shaped appearance. As more flower buds

are produced the leaf primordia become less prominent until the apex is surrounded by rounded flower primordia which develop sepals and stalks. The gradual change from reproductive to vegetative is accompanied by a change in leaf shape to a more simple strap-like leaf, often sessile near the top of the flowering stem (Hector 1936, Post 1936).

Some species, such as stocks (Heide 1962), require low temperature for flower formation and will produce abnormal flowers if moved to a high temperature before flower primordia are formed, but in most species a period of low temperature is required only for flower induction, the visible change from vegetative to reproductive occurring at some time after the low temperature period.

1.15 Conclusions

A wide range of species respond to vernalisation either as imbibed seeds or as plants. Vernalisation occurs only in actively dividing cells, the normal site of action being the shoot apex. Changes in the apex - normally a raising of the apical meristem - may occur during low temperature treatment but generally flowers are formed after treatment, often many weeks later. The vernalisation stimulus is at first immobile in the apex, but in some species a stimulus has been shown to be translocated to other parts of the plant when flower formation is well-advanced.

Optimum temperatures are generally from 4^{o} to 9^{o} although higher and lower temperatures are effective. The process requires weeks and even months, in resistant species, to be complete. Vernalisation is quantitative and partial treatment results in fewer

plants within a population flowering and a longer time for flower development. Many species have a juvenile stage during which plants cannot be vernalised and in most species older plants respond more to low temperature treatment than younger plants.

Other factors, such as daylength and light, during and after low temperature treatment, modify its effects, in most species long days promoting flowering. GA applications cause flowering in some vernalisable species, especially at lower temperatures, but auxins have less clear effects, in some conditions promoting flowering, in others reducing it.

Swedes are a vernalisable species: 11^{o} caused limited flowering (Peto 1934) but there is no evidence in the literature on the effect of lower temperatures. There are cultivar differences in bolting resistance (Peto 1934, Bell 1968).

The following chapters describe experiments designed to:

a) establish the effects of several factors, including plant age, duration of low temperature treatment, temperature of treatment and cultivar on the vernalisation process in the swede, with the aim of providing more detailed information that may be used by plant breeders to screen for bolting resistance;

b) study the site of action and translocation of the vernalisation stimulus.

2. EXPERIMENTAL

2.1 THE DEVELOPMENT OF THE REPRODUCTIVE APEX

The effect of duration of low temperature on the development of the apex from vegetative to reproductive.

Experiment 1

The morphology of the apex and the stem extension of swedes was examined during the change from vegetative to reproductive growth, during and after low temperature treatment. Seventy-five four-week old Pentland Harvester swedes were grown out of doors from the end of October to December at 6^{o} mean daily mean temperature for 3, 6 or 9 weeks, all treatments beginning on the same day, and 25 plants were grown at 14^{o} mean daily mean temperature as controls. Four weeks of low temperature treatment was known to have resulted in a high proportion of plants flowering, and six weeks resulted in 100 per cent flowering (see Thow 1974), and so three weeks was chosen as a duration shorter than that known to cause some flowering, six weeks was expected to result in near 100 per cent flowering and nine weeks to ensure that some plants flowered even if conditions were less suitable for vernalisation than in the experiment mentioned.

Two weeks after the start of low temperature treatment, two plants from each of the four groups were dissected and their apical buds examined and drawn. This was repeated every 10 days for 13 weeks.

Results

A scale of apical bud stages was prepared from the drawings of the buds (Figure 2.1). The vegetative, flat, triangular apex (stage 0) first becomes raised and rounded (stage 1) often during low temperature treatment. The first flower primordia then appear, initially in leaf axils (stage 2) and then in place of leaves (stage 3)

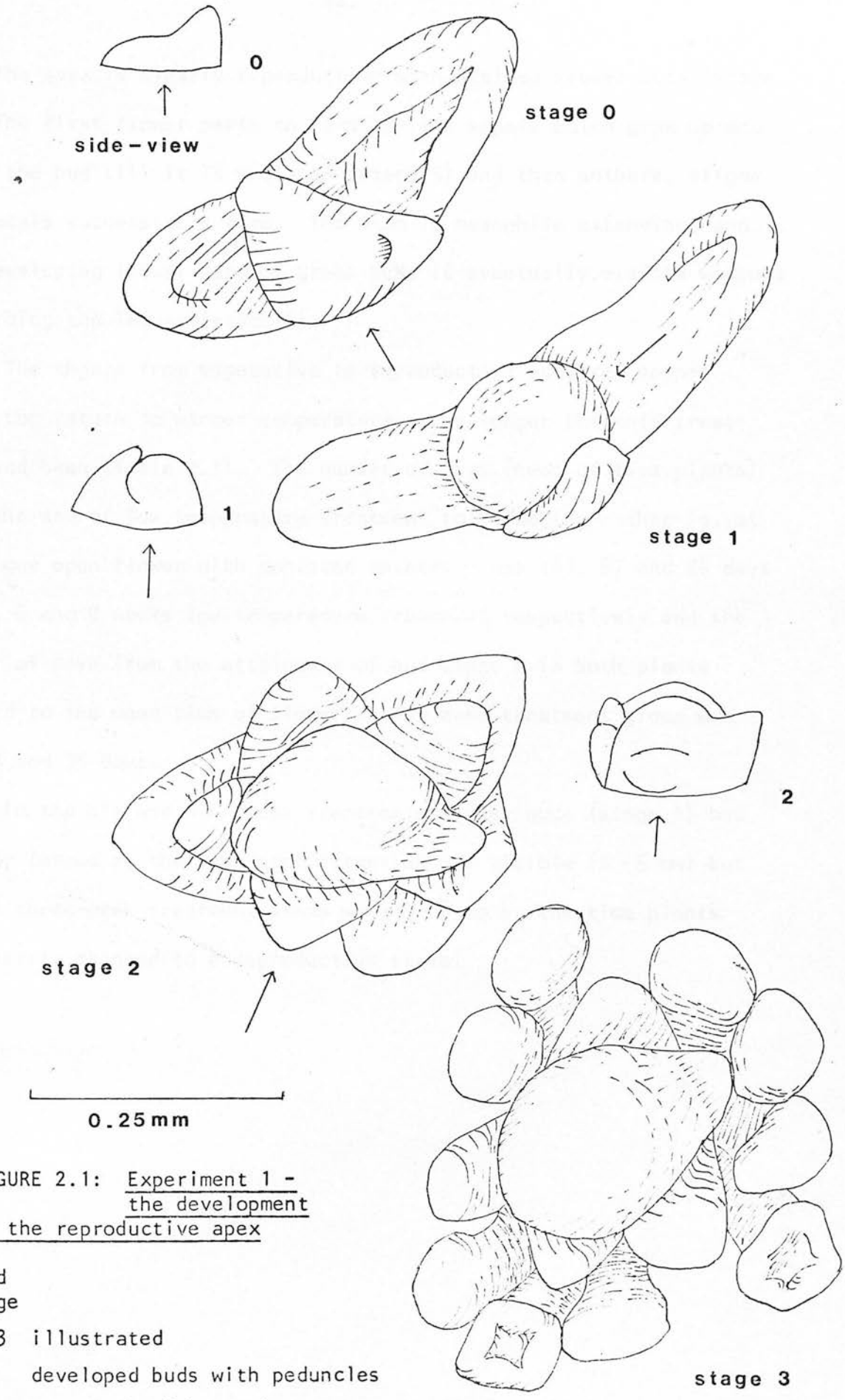


FIGURE 2.1: Experiment 1 - the development of the reproductive apex

Bud stage

0-3 illustrated

4 developed buds with peduncles

5 many buds with sepals

6 green buds visible without disturbing the leaves

till the apex is clearly reproductive with stalked flower buds (stage 4). The first flower parts to form are the sepals which grow up and round the bud till it is enclosed (stage 5) and then anthers, stigma and petals successively form. The stem is meanwhile extending, and the developing flower head of green buds is eventually visible without disturbing the leaves (stage 6).

The change from vegetative to reproductive occurred sooner after the return to warmer temperatures, the longer the cold treatment had been (Table 2.1). The number of days (mean of five plants) from the end of low temperature treatment to flowering - that is, at least one open flower with dehisced anthers - was 163, 67 and 54 days for 3, 6 and 9 weeks low temperature treatment respectively and the number of days from the attainment of bud stage 4 in both plants sampled to the mean time of flowering for each treatment group was 67, 39 and 35 days.

In the six and nine-week treatments flower buds (stage 4) had already formed by the time stem extension was visible (2 - 5 mm) but in the three-week treatment stems were 5 - 8 mm by the time plants had clearly changed to a reproductive state.

TABLE 2.1: Experiment 1 - the effect of duration of low temperature treatment on the development of the apical bud at 10 sequential samplings. Units of measurement are the scale of bud stages depicted in Figure 2.1

Plant age days	Duration of low temperature			
	0 weeks	3 weeks	6 weeks	9 weeks
44	0	0	0	0
54	0	0	0	0
65	0	1.5	1.0	0.5
76	0	0.5	1.0	0.5
85	0	1.0	2.0	1.0
97	0	1.5	3.5	1.0
105	0	1.0	5.0	2.0
115	0	2.0	5.0	5.0
125	0	1.0	5.0	5.0
135	0	2.0	5.0	5.0

Low temperature treatments were started at age 28 days and were finished at the age indicated by the double line in each column.

Discussion

The effect of low temperature on the swede is to initiate changes in the plant which result in flowering at a later time, rather than directly cause the development of flower buds. There was not a detectable change in the appearance of all plants sampled during and after low temperature treatment and the development of flower buds occurred some time after even the longest treatment period had ended.

Longer treatment not only increased the rate of change of the apex from vegetative and leaf producing to reproductive, producing flower buds, but also increased the rate of development of flower buds to open flowers.

After the shortest low temperature treatment stem extension was earlier relative to bud development than with six and nine weeks treatment, evidence that stem extension and flower formation are to some extent independent, and that stem extension probably requires a shorter low temperature period than flower formation.

2.2 THE JUVENILE STAGE

The effect of the age of the plant at the start of low temperature treatment, duration of treatment and cultivar, on the flowering of swedes.

2.2.1 Experiment 2

Many photoperiodic and many vernalisable species cannot be induced to flower until plants have attained a critical stage of development, the change from juvenile to adult. The experiment was carried out to determine whether the age of swede seedlings affects their susceptibility to vernalisation, that is, whether swedes, like many other brassicas, have a juvenile phase.

Two swede cultivars were used, Wilhelmsburger, a green skinned moderately high dry matter swede, and Doon Major, a purple skinned lower dry matter swede. Three durations of low temperature treatment were used, 20, 30 and 40 days at 7^{o} in a growth chamber, and an untreated control (0 days at 7^{o}). In experiment 1, six and nine weeks low temperature treatment had both resulted in 100 per cent flowering with little difference between them in days to flowering and so there appears to be no advantage in extending treatment beyond six weeks (42 days). Flower development after the three-week duration was much slower than after six weeks and so durations shorter than three weeks (21 days) are likely to give very slow, incomplete flowering and provide little information. The untreated control was included to check that conditions before and especially after the low temperature treatments were not vernalising so that any flowering could be attributed solely to the low temperature treatment.

Two-week old swede plants given four weeks low temperature treatment became reproductive (Thow 1974) and so any juvenile stage is likely to be below two weeks. Seeds were sown at intervals and raised at 16° mean daily mean temperature in a glasshouse so that they were 0, 1, 2, 4, 6, 8, 12 and 16 days old from sowing (see Figure 2.2) at the start of each duration of low temperature treatment, including the untreated control plants which were 0, 1, 2, 4, 6, 8, 12 and 16 days old when the other low temperature treatments ended. There were six plants per treatment (384 in total), plots being randomised within the low temperature chamber, and there were no replicates (see Appendix A).

The three durations of low temperature treatment ended on the same day and all plants, including the untreated control plants, were moved to a growth chamber at 13°, with warm white fluorescent light on 16-hour day/8-hour night for two weeks to reduce the risk of devernalisation by high post-vernalisation temperatures. The swedes were planted out in a field plot on 21 May at 15 cm spacing in the rows, 75 cm between the rows, the plot having previously been fertilised (see Appendix A).

The number of true leaves 1 cm and over, radicle length, hypocotyl height (from soil to cotyledons) and cotyledon width were measured where appropriate on each plant at the beginning and end of low temperature treatment. The date of first flowering was recorded of all plants flowering within 110 days of the end of the low temperature treatments. One hundred and thirty-four days after the end of low temperature treatment, plants were scored for flower stage on the scale set out below, a description of the whole plant.

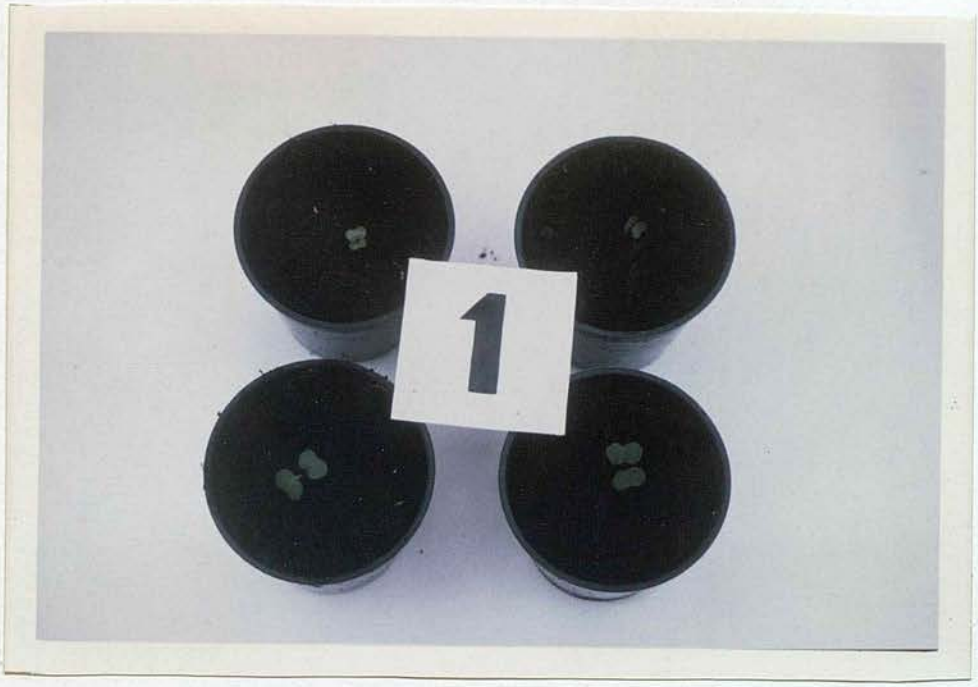
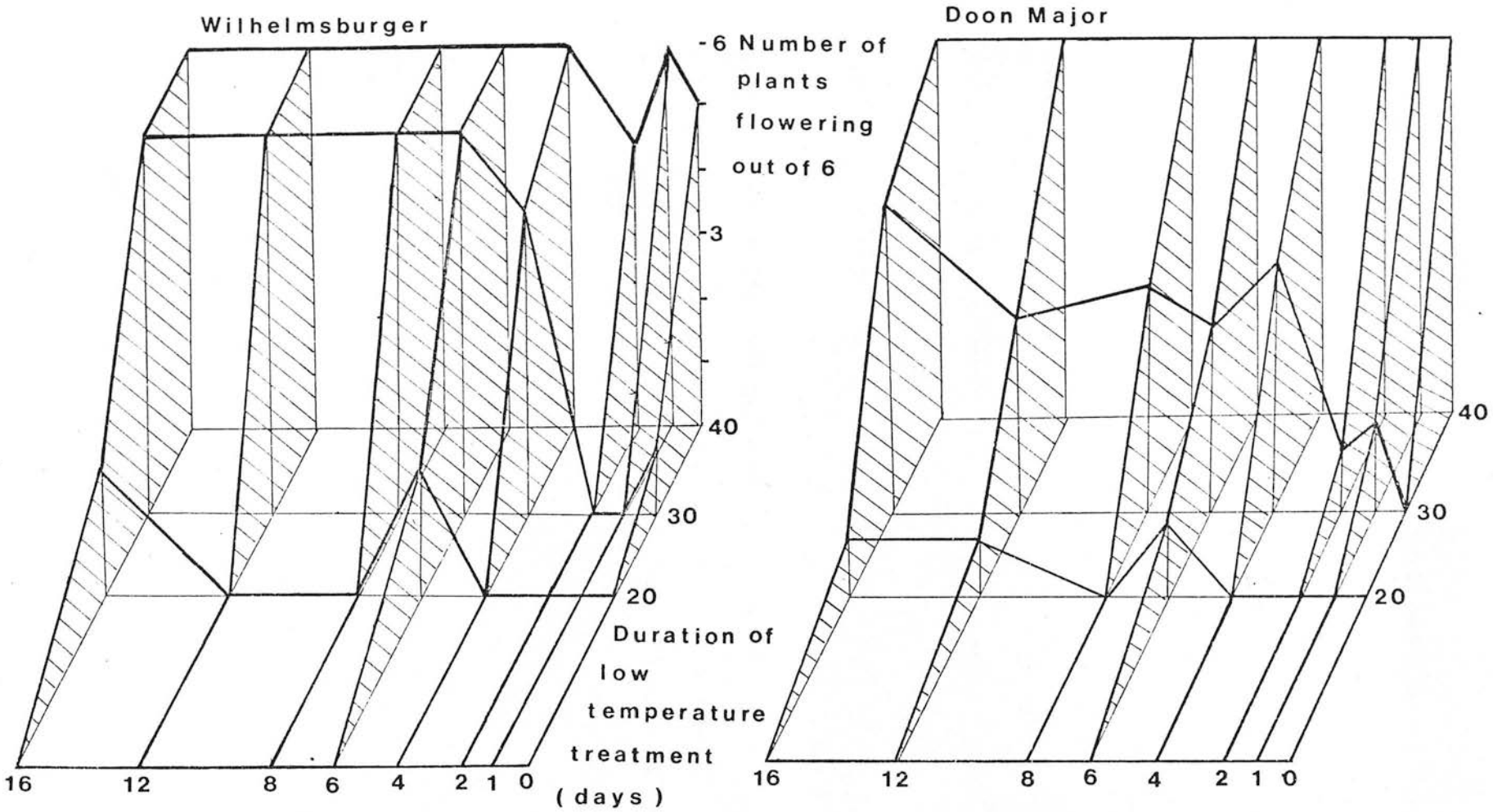


FIGURE 2.2: Experiment 2 - four-day old plants in the top pots, six-day old plants below. Wilhelmsburger swedes on the left, Doon Major on the right. Plant pots 7.5 cm in diameter

Ten-day old plants at the top, 16-day old plants below. Wilhelmsburger on the left , Doon Major on the right

FIGURE 2.3: Experiment 2 - the effect of duration of low temperature treatment, plant age at the start of treatment and cultivar on the number of plants flowering



Stages of flowering in the swede

Stage No.	Description
1	Rosette (vegetative)
2	Stem beginning to extend, internodes 1 cm or over
3	Stem extending, green flower buds just visible, the same as bud stage 6 (see Figure 2.1)
4	Flower buds visible, some of them yellowish in colour and almost open
5	Open flowers, but more buds than flowers on the central axis (main stem) and no seed pods set
6	Open flowers, flower buds still present, but more flowers than buds, and some seed pods set, on the central axis
7	Flowers and seed pods only on the central axis, no flower buds present except on side branches
8	No flowers on the central axis, but flowers still present on side branches
9	Only seed pods present on the whole plant

The flower stage measurement gave information on the vegetative or reproductive condition of all plants whether they had flowered within 110 days or not.

Results

The longer the duration of low temperature treatment the more plants flowered (Figure 2.3) and the shorter the time to flower (Table 2.2) with no plants flowering in the untreated control. Age of plant had less effect than duration of low temperature treatment but more older plants flowered than younger plants.

TABLE 2.2: Experiment 2 - the effect of duration of low temperature treatment, age of plant at the beginning of treatment and cultivar, on the mean number of days to flower of plants flowering within 110 days of the end of low temperature treatment

Plant age days	Wilhelmsburger				Doon Major				Mean of both cultivars
	Duration of low temperature (days)				Duration of low temperature (days)				
	20	30	40	mean	20	30	40	mean	
	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower
0	NF	91	86	87	NF	NF	90	90	88
1	NF	NF	81	81	NF	109	74	80	80
2	NF	NF	83	83	NF	92	81	83	83
4	NF	92	79	84	NF	97	69	80	82
6	106	86	70	82	103	101	62	78	80
8	NF	87	77	82	NF	92	80	84	83
12	NF	94	79	87	106	92	78	77	82
16	99	87	77	85	103	102	81	90	87
mean	102	89	79	84	104	97	76	84	
mean of both cultivars	103	93	78	84	103	93	78	84	84

NF = no plants flowering in the treatment

The following analyses with χ^2 were done by David Cooper of the ARC Unit of Statistics. Considering plants of both cultivars in the 30 days low temperature treatment, and comparing plants two days old and younger with plants four days old and over at the start of low temperature, there is a much greater number of plants flowering in the older group ($\chi^2 p<0.001$). If four-day old plants are included in the younger group, or if two-day old plants are included in the older group, the difference between the older and younger plants is decreased. With 20 and 40 days low temperature treatment the difference is not so obvious as very few plants flowered in the 20-day treatment, none aged less than six days, and almost all plants flowered in the 40-day treatment, but if all three low temperature treatments are included there is still a significant difference between the proportions of plants flowering in the 4 to 16 days group compared with the 0 to 2 days group ($\chi^2 p<0.001$). There was some evidence of an increase in flowering with age in Doon Major plants aged 6 to 16 days given 30 days low temperature treatment but on analysis the evidence of such an increase was found to be insufficient. Slightly more Wilhelmsburger than Doon Major plants flowered and if plants 4 days old and older in the 30-day treatment are compared, the proportion of Wilhelmsburger plants flowering is much greater ($\chi^2 p<0.005$).

The flowering data was also analysed using the GLIM program (General Linear Interactive Modelling) (see Appendix B). The flowering data can be expressed as a proportion of plants flowering out of the total number of plants in a treatment and the distribution of the data is binomial - a plant has either flowered or not flowered. A large number of the proportions of plants flowering in

each treatment are very small, near 0 per cent, or very large, near 100 per cent. Transformation of the flowering data to a logit scale - $\log_e(p/1-p)$ - where p is the probability of flowering in the treatment, makes small differences in the 0 per cent and 100 per cent ends of the scale larger.

The transformed data can then be fitted to treatment factors in a model (see Appendix B). If a treatment factor is on a linear scale, for instance duration of low temperature, 20, 30 and 40 days, a linear component of the deviance attributable to the factor can be examined. The mean deviance, that is, total deviance divided by the appropriate number of degrees of freedom for the factor or factor component, removed from the model by addition to the model of a factor or component of a factor is compared directly with χ^2 with the same number of degrees of freedom as the factor or component, and if greater than the appropriate χ^2, the effect of the factor is significant.

Duration of low temperature significantly affected flowering and the linear component of duration accounted for almost all of the deviance attributable to duration of low temperature treatment (total effect of duration, mean deviance 106.8, compare with χ^2 df 2; linear component of duration, mean deviance 211.71, compare with χ^2 df 1). Neither plant age nor cultivar affected flowering significantly.

A print out of the observed and fitted data for the previous model can be obtained with a linear predictor which gives the fitted data on a logit scale. The number of days required to cause 50 per cent flowering can be calculated from the linear predictor data. Around 50 per cent flowering the population will be responding most

rapidly and most linearly in increased flowering, to increased duration of low temperature and so it is at the 50 per cent flowering point that comparisons between separate response curves are most conveniently made. On the logit scale 50 per cent flowering is $\log_e$ (0.5/1-0.5), that is, $\log_e$ 1, which is zero, and so on a logit scale the 50 per cent flowering point occurs where the response line cuts the x-axis at y = 0. On fitted straight lines this point can be calculated from the equation for a straight line, y = mx + c and therefore at 50 per cent flowering y = 0 on the logit scale and x, the number of days of low temperature required to cause 50 per cent flowering, x = -c/m.

Fitting the data to the linear effect of duration only, this figure is 29.2 days at 7°. If the effect of plant age is included in the model and fitted lines derived for each age, the number of days at 7° required to cause 50 per cent flowering of both cultivars in each age treatment can be derived, see below:

Plant age at the start of low temperature treatment	Number of days at 7° required to cause 50 per cent flowering of Wilhelmsburger and Doon Major
0 days old	35.0 days at 7°
1 "	33.9 "
2 "	36.1 "
4 "	28.0 "
6 "	25.0 "
8 "	27.1 "
12 "	26.9 "
16 "	23.1 "

This analysis with GLIM has failed to show some of the effects which are obvious in the data itself and which are demonstrated in the analyses done by David Cooper (ARC Unit of Statistics) but the

50 per cent flowering data for the eight plant ages confirms the previous result that there is a large difference in susceptibility to flowering between plants younger than four days and those four days old and over.

Only the plants that flowered provided data on the number of days from the end of low temperature treatment to flowering and so there was an unbalanced number of plants contributing to each treatment mean, and analysis of the data by a standard analysis of variance was not possible. The data for days to flowering was analysed instead using the GLIM program (see Appendix B), this time with untransformed data and a normal distribution. There is a facility in the program by which the data of each plot mean can be weighted by the number of plants contributing to that mean so that in fitting the data to a model, plot means derived from few plants do not disproportionately affect the fit. The distribution of the data is normal and the significance of factors is found with the variance ratio and F tests.

The number of days to flowering was affected by the linear component of duration of low temperature (F test $p<0.001$) but plant age and cultivar had no effect (see Table 2.2).

As the experiment was unreplicated the deviance left after removing the three treatment effects - duration of low temperature, cultivar and plant age - the combined deviance of all the interactions, was used as the error term with the appropriate number of degrees of freedom for the calculation of variance ratios and F tests. None of the second order interactions were significant and there is no reason for expecting the third order interaction to be significant and so it is not unreasonable, although not strictly valid, to attribute this interaction deviance to error.

Flower stage (see Table 2.3), analysed in a standard analysis of variance, was affected by duration of low temperature, age and cultivar treatments in the same way as numbers of plants flowering, and plants that flowered earlier were more advanced in flower stage at the end of the experiment. The longer the low temperature treatment the more advanced the flower stage attained (F test $p<0.001$, with duration x cultivar x plant age interaction as the error term). The 20 and 30-day treatments and the 30 and 40-day treatments differed at the 0.1 per cent level (least significant difference) but the 20-day treatment only differed from the control plants at the 5 per cent level. Older plants were more advanced in flower stage than younger plants (F test $p<0.05$, effect of age) although there was little difference within the group 4 to 16 days, and within the juvenile group 0 to 2 days. All the untreated control plants were vegetative (flower stage 1) at the end of the experiment and were not included in the above analysis of variance.

The longer was low temperature treatment the greater was the production of leaves during the treatment (Table 2.4) (F test $p<0.001$, effect of duration), older plants produced more leaves than younger plants (F test $p<0.001$, effect of plant age) and Wilhelmsburger produced more leaves than Doon Major (F test $p<0.001$). The third order interaction was used as the error term as in the analysis of flower stage data.

Discussion

There is a short juvenile stage of under four days in both cultivars, although slightly more obvious in Wilhelmsburger. Longer durations of low temperature treatment resulted in a greater

TABLE 2.3: Experiment 2 - the effect of duration of low temperature treatment, age of plant at the beginning of treatment, and cultivar on the flower stage (see page 46) of plants 134 days after the end of low temperature treatment

Plant age days	Wilhelmsburger				Doon Major				Mean of both cultivars
	Duration of low temperature (days)				Duration of low temperature (days)				
	20	30	40	mean	20	30	40	mean	
	Flower stage	Flower stage	Flower stage	Flower stage	Flower stage	Flower stage	Flower stage	Flower stage	Flower stage
0	1.2	4.3	7.5	4.5	1.2	1.4	8.0	3.5	4.1
1	1.2	2.0	8.4	3.6	1.0	4.7	9.0	4.7	4.1
2	1.0	3.2	6.2	3.8	1.0	4.0	8.8	5.4	4.5
4	2.0	7.4	8.8	6.0	1.3	6.3	9.0	5.6	5.8
6	4.3	8.8	9.0	7.3	4.0	5.0	8.8	6.1	6.7
8	2.2	8.7	9.0	6.6	1.8	6.4	8.7	5.6	6.1
12	2.2	8.2	8.7	6.3	3.0	5.8	8.8	5.9	6.1
16	4.7	8.2	8.7	7.2	2.7	5.2	9.0	5.6	6.4
mean	2.4	6.3	8.3	5.7	2.1	4.9	8.8	5.3	
mean of both cultivars	2.3	5.6	8.5	5.5	2.3	5.6	8.5		5.5

For comparisons within the table	SE ± 1.49	For comparisons of duration of low temperature treatment means	SE ± 0.37
For comparisons of plant age treatment means	SE ± 0.61	For comparisons of cultivar means	SE ± 0.30

TABLE 2.4: Experiment 2 - the effect of duration of low temperature treatment, age of plants at the beginning of treatment, and cultivar, on the number of visible leaves produced during the low temperature treatment

Plant age days	Wilhelmsburger				Doon Major				Mean of both cultivars
	Duration of low temperature (days)				Duration of low temperature (days)				
	20	30	40	mean	20	30	40	mean	
	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves
0	0.00	0.00	0.83	0.28	0.00	0.00	1.00	0.33	0.30
1	0.00	0.33	1.67	0.67	0.00	0.00	1.00	0.33	0.50
2	0.00	0.33	1.67	0.67	0.00	0.83	1.00	0.61	0.64
4	0.00	1.33	2.33	1.22	0.00	1.50	1.83	1.11	1.17
6	1.67	2.50	3.67	2.61	1.00	2.17	2.00	1.72	2.17
8	1.83	3.17	3.83	2.94	0.17	2.17	2.83	1.72	2.33
12	2.50	4.00	4.00	3.50	2.17	3.17	3.00	2.78	3.14
16	1.83	3.33	3.00	2.72	1.83	2.50	3.00	2.44	2.58
mean	0.98	1.87	2.62	1.83	0.65	1.54	1.96	1.38	1.60
mean of both cultivars	0.81	1.71	2.29	1.60					

For comparisons within table	SE ± 0.348	For comparisons of low temperature treatment means	SE ± 0.087
For comparisons of plant age treatment means	SE ± 0.142	For comparison of cultivar means	SE ± 0.071

proportion of plants flowering and in earlier flowering, and Doon Major flowered slightly, but not significantly, earlier than Wilhelmsburger but fewer Doon Major plants flowered in total, suggesting that time to flower is not solely controlled by the factors that control the number of plants flowering.

The greater leaf production of older plants during low temperature treatment will be partly because very young plants had not even developed leaf primordia at the start of treatment but the growth rate of the older plants is likely to be greater than that of very young plants, initially.

Flower stage, a measure of the reproductive condition of all plants, was affected by treatments in the same way as numbers of plants flowering, and so the selection of a date when recording of flowering in the experiment was ended will not have distorted the results to any great extent.

During low temperature treatment plants are growing slowly and although they may have been juvenile at the start of low temperature treatment all plants had at least expanded cotyledons 8 to 23 mm wide by the end of low temperature treatment, that is, equivalent to plants eight days old grown in warmer conditions. In the 40-day treatment almost all plants had at least one leaf over 1 cm at the end of low temperature treatment. During a long period of low temperature, such as 40 days, seedlings will grow out of juvenility and may be vernalised and later flower so that it is difficult to determine the precise age at which juvenile plants become adult. The age at which the change occurs will also be affected by growth conditions and seedling vigour, and juvenility may be quantitative with plants responding increasingly to

vernalisation as they grow older, although the evidence from the experiment suggests that it is a sudden change and that there is no very marked response to increasing age once adulthood is reached.

2.2.2 Experiment 3

In experiment 2, 20 days low temperature treatment had resulted in very few plants flowering, and 40 days in almost 100 per cent flowering, and so a similar experiment was carried out with more duration of low temperature treatments between 20 and 40 days, in the range of maximum response in terms of flowering, to increased duration of low temperature. Six durations of low temperature were used, 20, 24, 28, 32, 36 and 40 days, all treatments ending on the same day.

The youngest plants used were two days old, as experiment 2 had indicated that this age was juvenile, and the oldest 10 days as there had been little or no effect of increasing age after four days old. There was some indication that six-day old plants flowered more readily than four or eight-day old plants (see Figure 2.2) and so these three ages were included. Plants were raised at 16° mean daily mean temperature in a glasshouse and were 2, 4, 6, 8 and 10 days old from sowing at the start of low temperature treatment. Wilhelmsburger and Doon Major cultivars were again used, and there were five plants per treatment in a completely randomised design.

The plants were treated at $5 \pm 0.5^{\circ}$ in a growth chamber and at the end of treatment the temperature was raised to $13 \pm 2.0^{\circ}$. After two weeks the plants were moved to a glasshouse compartment at 14.6° mean daily mean temperature. The plants were repotted into 12.5 cm

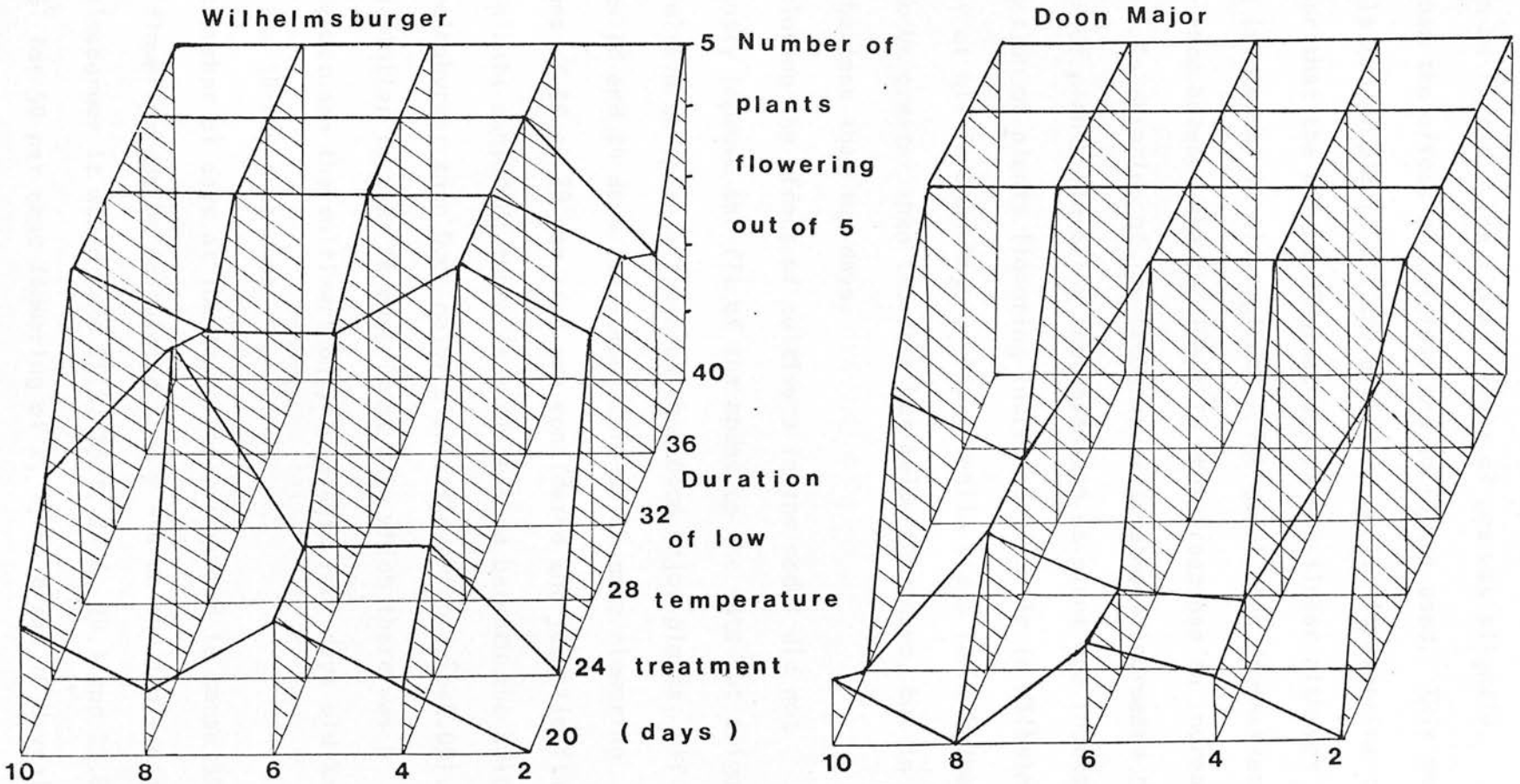
pots and remained in the compartment for four weeks before being moved back to the main glasshouse and grown on at 16.4° mean daily mean temperature. On 21 June, 16 weeks after the end of low temperature treatment, those plants that had not flowered were planted out in a previously fertilised field plot (see Appendix A). The experiment was terminated on 24 August, 176 days after the end of the low temperature treatment.

In the previous experiment no control plants had flowered and so a control was not included in this experiment as any vernalising conditions after low temperature treatment would affect all duration of low temperature treatments equally. Plant size, as in experiment 2, and the number of visible leaves (see Appendix A) were measured at the beginning, middle and end of low temperature treatment and a surplus plant from each pot was dissected at these times and the total leaf number, that is, including all leaf primordia, was recorded, giving a mean value of total leaves for each plot. The date of first flowering was recorded.

Results

Far fewer two-day old Wilhelmsburger plants flowered than older plants ($\chi^2 p<0.001$) but there was little effect of age on the flowering of Doon Major plants (see Figure 2.4). The proportion of plants flowering out of the total number of plants was transformed to a logit scale and a model fitted to it using the GLIM program. The linear effect of duration and the interaction between the linear effect of plant age and cultivar were both significant ($\chi^2 p<0.001$) and so the model that fitted the data best was the linear effect of duration, the linear effect of plant age and the interaction of the linear effect of plant age and cultivar. The program requires that

FIGURE 2.4: Experiment 3 - the effect of duration of low temperature treatment, plant age at the start of treatment and cultivar on the number of plants flowering



one of the factors in an interaction is fitted before the interaction can be fitted. The linear effect of age was slightly greater than the effect of cultivar and so it was used. This model, however, is too simple to fit the data very well. In experiment 2 it is clear that the effect of plant age is not linear although flowering is greater in older plants. The model does demonstrate the difference between the cultivars in their response to increasing plant age. As duration of low temperature treatment increased the proportion of plants flowering increased and as plant age increased the proportion of plants flowering increased slightly in Wilhelmsburger but hardly at all in Doon Major. The juvenile stage in Wilhelmsburger appears to be greater than two days but below four days, but in Doon Major to be less than two days.

Including the effect of cultivars in the model did not significantly improve the fit of the model to the data but slightly more Wilhelmsburger plants flowered than Doon Major plants. If the treatments 28 and 24 days low temperature which gave flowering percentages of 33 to 88 per cent are considered and juvenile (two-day old) plants excluded, there is a difference between the cultivars, more Wilhelmsburger than Doon Major plants flowering ($\chi^2 p<0.05$). This result is similar to that in experiment 2 in which there was a difference between the cultivars only in plants four days old and older in the 30-day treatment.

The number of days at low temperature required to cause 50 per cent flowering can be calculated from y = 0 on the logit scale. For Wilhelmsburger it would take 25.6, 25.1, 24.6, 24.1 and 23.6 days at 5° for 50 per cent flowering of 2, 4, 6, 8 and 10 day-old plants respectively and 24.6, 24.3, 24.0, 23.7 and 23.4 days at 5°

for 2, 4, 6, 8 and 10 day-old Doon Major plants. These estimates are not very satisfactory as the model from which they are derived did not fit the data very well.

Using GLIM, a model was fitted to the number of days to flowering, weighted by the number of plants contributing to each treatment and mean (the number of flowering plants) as in experiment 2 (and see Appendix B) (Table 2.5). The linear effect of duration and the linear effect of plant age were significant at the 0.1 per cent level (F test) and the effect of cultivar at the 5 per cent level. As duration of low temperature increased, the number of days from the end of treatment to flowering decreased, and younger plants were slightly faster in flowering than older plants. Doon Major flowered slightly earlier than Wilhelmsburger.

The production of visible leaves (Table 2.6) and the production of all leaves including leaf primordia (Table 2.7) during the low temperature period was greater the longer the duration of the low temperature treatment (F tests, effect of duration, $p<0.001$), and the older the plant at the start of treatment (F tests, effect of age, $p<0.001$). Wilhelmsburger produced more leaves and primordia than Doon Major (F test, $p<0.001$).

Discussion

From this experiment there is good evidence of a juvenile stage of under four days in Wilhelmsburger but no evidence of juvenility in Doon Major although it is likely that Doon Major has a juvenile stage under two days. A comparison of growth measurements in the two experiments show that some plants were slightly more advanced in experiment 2, more two-day plants having the seed-coat split,

TABLE 2.5: Experiment 3 - the effect of duration of low temperature treatment, age of plant at the beginning of treatment, and cultivar on the mean number of days to flower of plants flowering within 176 days of the end of low temperature treatment

Plant age days	Wilhelmsburger							Doon Major							Mean of both cultivars
	Duration of low temperature (days)							Duration of low temperature (days)							
	20	24	28	32	36	40	Mean	20	24	28	32	36	40	Mean	
	Days to flower							Days to flower							Days to flower
2	NF	NF	100	101	86	73	89	NF	116	105	98	68	62	89	89
4	154	141	129	72	98	71	99	154	101	88	72	66	61	77	88
6	151	156	115	93	78	73	100	100	105	96	68	68	75	79	90
8	147	133	134	94	82	75	104	NF	112	155	108	99	79	103	104
10	136	119	135	98	101	104	113	176	NF	133	105	98	75	103	109
mean	146	135	123	91	89	79	102	143	112	108	90	80	71		96
mean of both cultivars	145	126	116	91	84	75	96								

NF = no plants flowering in the treatment

TABLE 2.6: The effect of duration of low temperature treatment, cultivar and plant age at the start of low temperature treatment on the production of visible leaves during the low temperature treatment

Plant age days	Wilhelmsburger							Doon Major							Mean of both cultivars
	Duration of low temperature (days)							Duration of low temperature (days)							
	20	24	28	32	36	40	mean	20	24	28	32	36	40	mean	
	No. of leaves							No. of leaves							No. leaves
2	0.00	0.00	0.00	0.40	1.20	1.80	0.57	0.00	0.00	0.00	0.40	0.60	1.20	0.37	0.47
4	0.00	0.00	0.60	1.60	2.00	2.20	1.07	0.00	0.00	0.20	1.20	1.80	1.80	0.83	0.95
6	0.20	0.80	1.20	2.00	2.00	3.00	1.53	0.00	0.60	0.80	2.00	2.00	2.60	1.33	1.43
8	0.60	1.60	2.20	2.60	2.80	3.00	2.13	0.20	1.60	2.00	2.00	2.40	2.60	1.80	1.97
10	2.00	2.00	2.40	3.00	3.60	3.60	2.76	1.40	1.80	2.00	2.00	2.80	2.80	2.13	2.45
mean	0.56	0.88	1.28	1.92	2.32	2.72	1.61	0.32	0.80	1.00	1.52	1.92	2.20	1.29	1.45
mean of both cultivars	0.44	0.84	1.14	1.72	2.12	2.46	1.45								

For comparisons within table	SE ± 0.321	For comparisons of low temperature treatment means	SE ± 0.102
For comparisons of plant age treatment means	SE ± 0.093	For comparison of cultivar means	SE ± 0.059

TABLE 2.7: The effect of duration of low temperature treatment, cultivar and plant age at the start of low temperature treatment on the production of leaves and leaf primordia during low temperature treatment

Plant age days	Wilhelmsburger							Doon Major							Mean of both cultivars
	Duration of low temperature (days)							Duration of low temperature (days)							
	20	24	28	32	36	40	mean	20	24	28	32	36	40	mean	
	No. of primordia							No. of primordia							No. primordia
2	2.90	3.65	3.75	4.20	5.20	7.30	4.50	2.00	2.90	3.45	4.35	4.25	5.10	3.67	4.09
4	3.08	4.25	5.00	5.45	7.25	6.80	5.30	2.80	3.30	3.85	4.60	5.05	4.75	4.06	4.68
6	3.20	4.60	5.15	5.65	6.15	9.05	5.63	3.00	3.75	4.35	4.37	5.60	6.56	4.60	5.12
8	3.25	4.75	6.05	6.70	6.35	8.15	5.87	3.35	4.75	5.60	6.44	6.95	7.40	5.75	5.81
10	4.25	5.35	6.35	7.25	7.55	8.35	6.52	3.45	4.95	6.25	7.15	6.05	6.30	5.69	6.10
mean	3.34	4.52	5.26	5.85	6.50	7.93	5.57	2.92	3.93	4.70	5.38	5.58	6.02	4.76	5.16
mean of both cultivars	3.13	4.22	4.98	5.62	6.04	6.98	5.16								

For comparisons within table	SE ± 0.585	For comparisons of low temperature treatment means	SE ± 0.185
For comparions of plant age treatment means	SE ± 0.169	For comparison of cultivar means	SE ± 0.107

and six-day and older plants having larger cotyledons, but the four-day old plants were similar in radicle development in both experiments and so comparisons of the effects of plant age in both experiments are valid in terms of plant development.

Duration of low temperature affected vernalisation qualitatively, in numbers of plants flowering, and quantitatively, longer durations considerably reducing the time to flowering.

Duration of low temperature treatment, age of plant at the start of treatment and cultivar affected leaf and primordia production during low temperature in the same way as visible leaf production was affected by treatments in experiment 2, during low temperature.

2.3 TEMPERATURE OF VERNALISATION

The effectiveness of three vernalisation temperatures on the flowering of two swede cultivars.

2.3.1 Experiment 4

Temperatures in the range 4^{o} to 9^{o} induce flowering most effectively in the majority of vernalisable species (see 1.4, page 10). To find what temperature is most effective in inducing swede plants to flower, four-week old Wilhelmsburger and Doon Major swedes were grown for 3, 4, 5 or 6 weeks at 5^{o}, 8^{o} or 11^{o}, in three growth cabinets. The three temperature treatments were unreplicated but within the cabinets there were two blocks, one block in the better illuminated centre of the cabinet and one at the sides of the cabinet. There were seven plants per plot.

Differences in response of cabbage cultivars to different temperatures have been observed (Ito and Saito 1961, Heide 1970) and so two cultivars were used. Four durations of low temperature were used to give a range of flowering for each temperature. Air temperatures within the cabinets were recorded automatically every two hours, from thermistors. There were daily fluctuations in cabinet temperatures as outside air temperatures rose and fell, especially in cabinet 1 and least in cabinet 3. There was also some variation in daily mean temperatures, again greatest in cabinet 1 and least in cabinet 3.

Plants were raised for four weeks at 16.6^{o} mean daily mean temperature in a glasshouse bed so that they would be well into the adult stage by the start of low temperature treatment. At the end of treatment cabinet temperatures were raised to 13 ± 1.5^{o} and after

three weeks the swedes were planted in a fertilised field plot on 27 July with 15 cm between the plants and 75 cm between the rows.

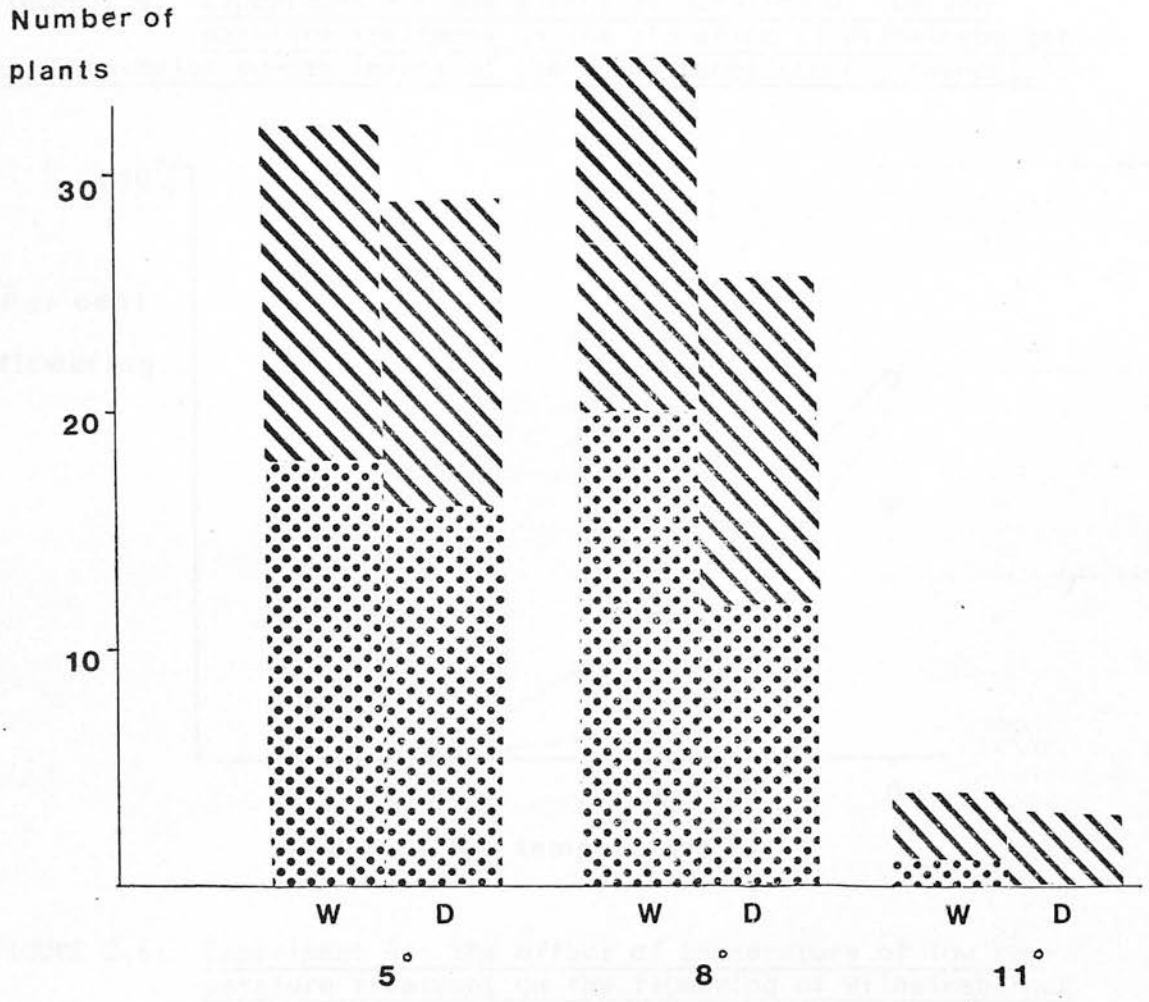
Visible leaf number was recorded immediately before and after low temperature treatment. The date of first flowering was recorded and the flower stage (see experiment 2) of all plants was assessed at the end of the experiment, 92 days after the end of low temperature treatment, to give a measure of the reproductive condition of all plants.

Results

Treatment temperatures of 5^{o} and 8^{o} resulted in almost the same numbers of plants flowering and the same number of plants with visible flower buds, flower stage 3. After six weeks at 11^{o} only one plant flowered and only seven plants had visible buds at the end of the experiment (see overleaf).

Duration of low temperature treatment had the largest effect on flowering, only one plant flowering after four weeks of treatment, compared with 21 plants after five weeks and 45 after six weeks but no plants had visible buds after three weeks treatment although 39 per cent had extending stems (flower stage 2).

The proportion of plants flowering (number of plants flowering over total number of plants in each plot) was transformed to a logit scale and using the GLIM program (see experiment 2 and Appendix B) a model was fitted to the transformed data. The linear effect of duration and the effect of temperature were significant at the 0.1 per cent level, the longer the duration of low temperature treatment the more flowering, and the effect of cultivar at the 5 per cent level, Wilhelmsburger flowering more than Doon Major. There was virtually no



The effect of cultivar (W - Wilhelmsburger, D - Doon Major) and temperature of treatment, 5°, 8° or 11°, on the number of plants flowering, and the number with visible flower buds (flower stage 3 and up, excluding flowering plants).

Plants with visible flower buds

Flowering plants

quadratic or other effect of duration but there was a significant linear and quadratic effect of temperature as flowering declined more steeply from 8° to 11° than from 5° to 8°. None of the interactions were significant and so the model that fitted the data best was linear effect of duration plus cultivar effect plus effect of temperature, shown in Figures 2.5 and 2.6. Calculating the duration of low temperature which will cause 50 per cent flowering in each treatment can be found from the point at which the fitted response

FIGURE 2.5: Experiment 4 - the effect of duration of low temperature treatment on the flowering of Wilhelmsburger and Doon Major swedes (means of the three temperature treatments)

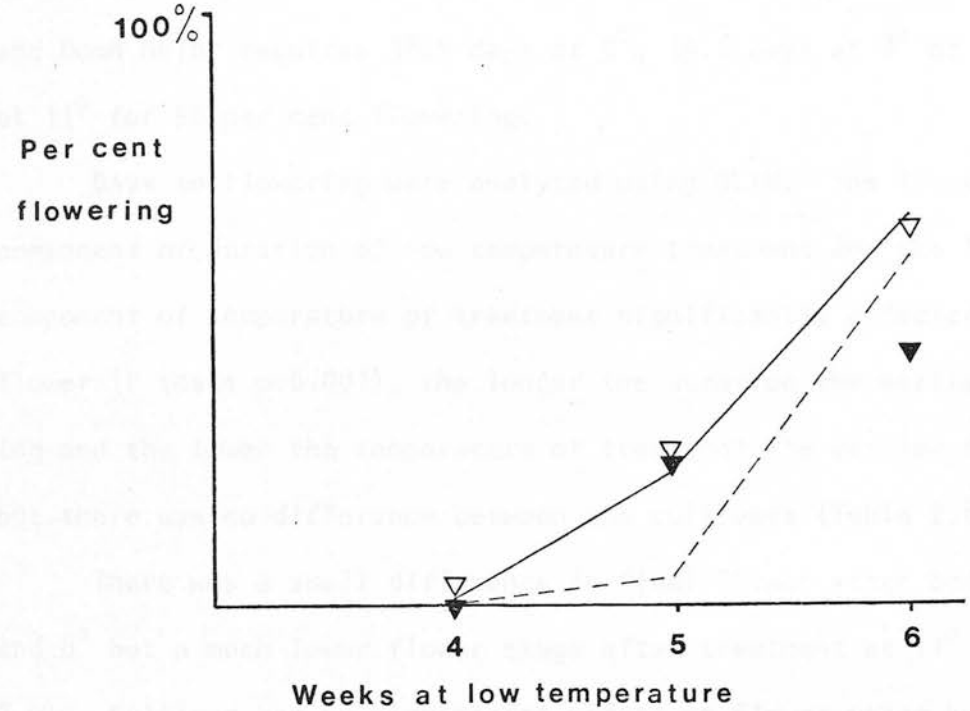
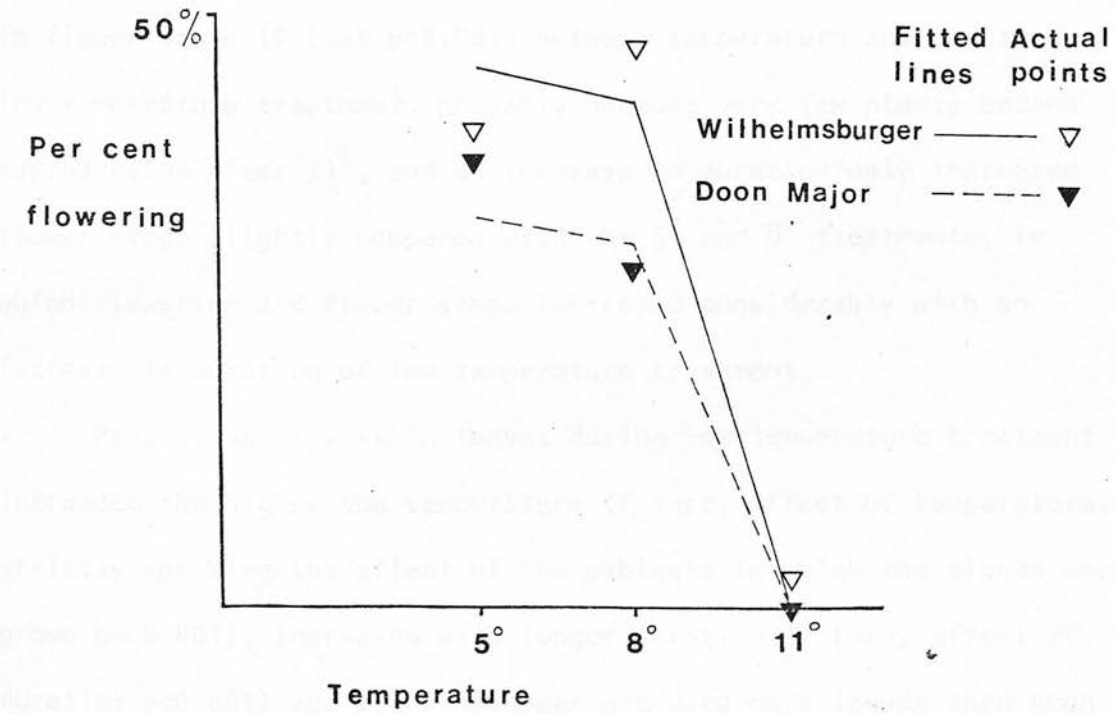


FIGURE 2.6: Experiment 4 - the effect of temperature of low temperature treatment on the flowering of Wilhelmsburger and Doon Major swedes (means of 4, 5 and 6 weeks low temperature treatments)



lines cut the x-axis at y = 0. Wilhelmsburger requires 35.7 days at 5^{o}, 36.2 days at 8^{o} or 51.0 days at 11^{o} for 50 per cent flowering, and Doon Major requires 38.5 days at 5^{o}, 39.1 days at 8^{o} or 53.8 days at 11^{o} for 50 per cent flowering.

Days to flowering were analysed using GLIM. The linear component of duration of low temperature treatment and the linear component of temperature of treatment significantly affected time to flower (F tests $p<0.001$), the longer the duration the earlier flowering and the lower the temperature of treatment the earlier flowering, but there was no difference between the cultivars (Table 2.8).

There was a small difference in final flower stage between 5^{o} and 8^{o} but a much lower flower stage after treatment at 11^{o} (Table 2.9). Cultivar had no significant effect on flower stage but the longer the duration of low temperature treatment the greater the final flower stage attained (F test, normal analysis of variance, effect of duration $p<0.001$). There was a significant interaction in flower stage (F test $p<0.001$) between temperature and duration of low temperature treatment, probably because very few plants became reproductive after 11^{o}, and an increase in duration only increased flower stage slightly compared with the 5^{o} and 8^{o} treatments, in which flowering and flower stage increased considerably with an increase in duration of low temperature treatment.

Production of visible leaves during low temperature treatment increased the higher the temperature (F test, effect of temperature, strictly speaking the effect of the cabinets in which the plants were grown $p < 0.001$), increased with longer duration (F test, effect of duration $p<0.001$) and Wilhelmsburger produced more leaves than Doon Major (F test $p<0.001$) (Table 2.10).

TABLE 2.8: Experiment 4 - the effect of temperature and duration of low temperature treatment, and cultivar, on the number of days from the end of low temperature treatment to first flowering

Duration of low temperature	Wilhelmsburger				Doon Major				Mean of both cultivars
	5°	8°	11°	mean	5°	8°	11°	mean	
	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower
3 weeks	NF	NF	NF	NF	NF	NF	NF	NF	NF
4 weeks	90.0	NF	NF	90.0	NF	NF	NF	NF	90.0
5 weeks	79.0	81.6	NF	80.9	81.0	81.3	NF	81.1	81.0
6 weeks	71.4	78.4	92.0	75.3	73.0	77.1	NF	75.1	75.2
mean	73.7	79.7	92.0	77.2	76.5	78.2	NF	77.2	77.2
mean of both cultivars	75.0	79.1	92.0	77.2					

NF = no plants flowering in the treatment

TABLE 2.9: Experiment 4 - the effect of the temperature and duration of low temperature treatment and of cultivar on the stage of flower development at the end of the experiment. The measurements are of flower stage (see Experiment 2).

Duration of low temperature	Wilhelmsburger				Doon Major				Mean of both cultivars
	5°	8°	11°	mean	5°	8°	11°	mean	
	flower stage	flower stage	flower stage	flower stage	flower stage	flower stage	flower stage	flower stage	flower stage
3 weeks	1.3	1.6	1.1	1.3	1.5	1.4	1.6	1.5	1.4
4 weeks	2.5	2.5	1.8	2.3	2.2	2.1	1.6	2.0	2.1
5 weeks	3.8	4.3	1.5	3.2	4.1	3.3	1.6	3.0	3.1
6 weeks	5.9	5.1	2.3	4.4	5.3	4.6	1.9	4.0	4.2
mean	3.4	3.4	1.7	2.8	3.3	2.8	1.7	2.6	2.7
mean of both cultivars	3.3	3.1	1.7	2.7					

For comparisons within tables	SE ± 1.49	For comparisons of temperature of treatment means	SE ± 0.68
For comparisons of duration of treatment means	SE ± 0.78	For comparison of cultivar means	SE ± 0.55

TABLE 2.10: Experiment 4 - the effect of temperature, duration of low temperature treatment, and cultivar on the production of visible leaves during low temperature treatment.

Duration at low temperature	Wilhelmsburger				Doon Major				Mean of both cultivars
	5°	8°	11°	mean	5°	8°	11°	mean	
	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves
3 weeks	1.36	1.71	2.57	1.88	1.00	2.00	1.71	1.57	1.73
4 weeks	1.57	2.57	3.57	2.57	1.07	1.79	2.43	1.76	2.17
5 weeks	1.57	2.71	3.71	2.67	1.29	2.00	2.79	2.02	2.35
6 weeks	2.57	3.86	4.50	3.64	2.50	2.93	4.64	3.36	3.50
mean	1.77	2.71	3.59	2.69	1.46	2.18	2.89	2.18	
mean of both cultivars	1.62	2.45	3.24	2.43					2.43

For comparisons within table	SE ± 0.56	For comparisons of temperature means	SE ± 0.20
For comparisons of duration means	SE ± 0.23	For comparisons of cultivar means	SE ± 0.16

Discussion

Five degrees and 8° are almost equally effective in inducing flowering. Temperatures between 5° and 8° may be more effective in flower induction of Wilhelmsburger plants or there may be little difference in the range 5° to 8°, but 5° is obviously more effective than 8° for Doon Major, and lower temperatures may be still more effective. Eleven degrees is only marginally vernalising but there is no evidence from the experiment on the lower temperature limits of vernalisation. Due to the fluctuations in treatment temperatures, the comparison is not strictly between 5°, 8° and 11° but between a range of temperatures, averaging 5°, 8° or 11°.

The number of days required to cause 50 per cent flowering are longer than might be expected from earlier experiments treated at similar temperatures but this experiment was terminated 92 days after the end of low temperature treatment and there was less time in which plants could flower. The estimated number of days required at 11° to cause 50 per cent flowering is not as reliable as the estimates for 5° or 8° as it is based on the flowering of only one plant.

As the temperature treatments were unreplicated, temperature effects cannot be separated from other effects of the cabinets, nor the immediate post treatment conditions, as plants remained for three weeks at 13° within the cabinets in which they were treated at low temperature. However, the effects of the cabinets are distinct and probably almost entirely due to the different low temperatures at which the cabinets were running during low temperature treatment.

The effect of duration - the longer the low temperature treatment the more plants flowered and the faster the rate of flowering - is the same as in previous experiments.

Although temperatures in the cabinets fluctuated around the mean, leaf production was greater the higher the mean temperature, and so the effects of the mean temperatures calculated from the hourly readings agrees with the expected effects of temperature on plant growth. It is possible that the more vigorous growth of Wilhelmsburger in terms of leaf production during the low temperature period, observed also in experiments 2 and 3, is associated with its greater susceptibility to flowering.

2.3.2 Experiment 5

Experiment 4 was repeated, with constant temperatures 3^{o}, 6^{o} and 9^{o}. Eleven degrees had been much less effective than 5^{o} or 8^{o} in experiment 4 and so a lower temperature, 9^{o}, was selected as the upper limit. Five degrees and 8^{o} had been almost equally effective and so a temperature in between, 6^{o}, was used and 3^{o} as the lowest temperature in the expectation that effectiveness of vernalisation might be declining at 3^{o}.

Wilhelmsburger and Doon Major plants were exposed to 3, 4, 5 or 6 weeks of low temperature, all treatments ending on the same day, as in experiment 4. As before, there were two blocks within each cabinet but only six plants per plot.

Plants were 15 days old at the start of low temperature treatment and after treatment the cabinet temperatures were raised to 12^{o} and all treatments randomised among the three cabinets. After a week the plants were moved to a cool compartment at 11.8^{o} mean daily mean temperature for three weeks before being repotted to 12.5 cm pots and moved back into the glasshouse bed at 17.2^{o} mean daily mean

temperature. Eighty-five days after treatment ended the plants were repotted to 19 cm pots.

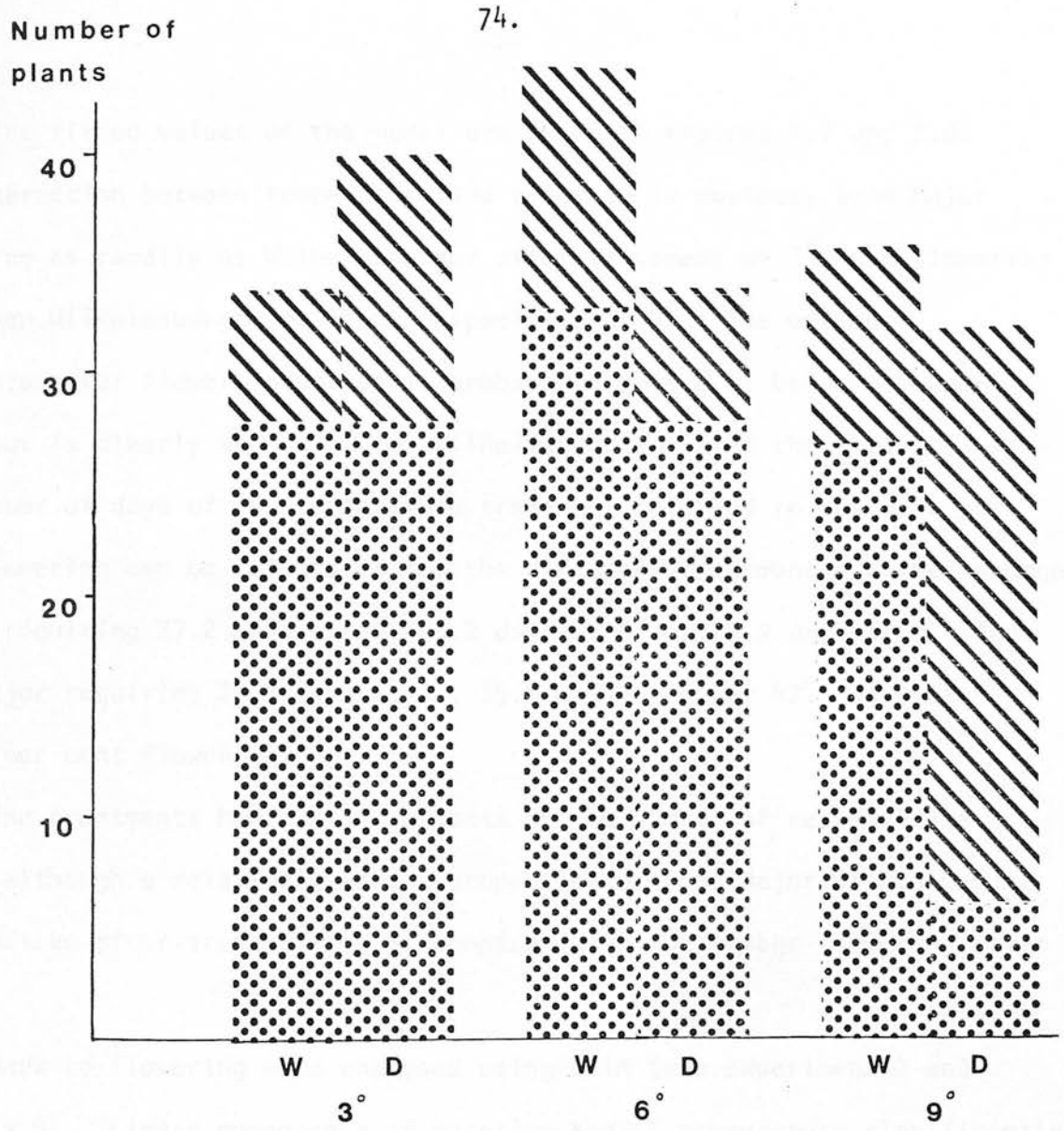
Visible leaf number was recorded immediately before and after low temperature treatment. Date of flowering and apical bud stage (see Figure 2.1) of all non-flowering plants were recorded 112 days after the end of treatment, all plants with bud stage 3 or more being classed as reproductive.

Results

More plants flowered after treatment at 6^{o}, and fewest after 9^{o}. Slightly more plants became reproductive after 6^{o} than 3^{o} and fewer plants were reproductive after 9^{o} but the difference was much less than for flowering plants.

Between 6^{o} and 9^{o} there was a significant difference in numbers flowering (χ^2 $p<0.001$) and reproductive (χ^2 $p<0.05$) but between 3^{o} and 9^{o} there was a significant difference only in numbers flowering (χ^2 $p<0.001$) and there were no significant differences between 6^{o} and 3^{o}.

The proportion of plants flowering in each treatment was transformed to a logit scale and a model fitted to it, using the GLIM program, as in experiment 2 (and see Appendix B). The model which fitted the data best was the linear effect of duration plus the effect of cultivar plus the effect of temperature plus the interaction of the linear effect of temperature with cultivar. All effects were greater than χ^2 at the 0.1 per cent level. The linear effect of duration accounted for almost all the deviance removed from the model by the addition of duration to the model, that is the longer the duration the higher the proportions of plants flowering (on a logit



The effect of cultivar (W - Wilhelmsburger, D - Doon Major) and temperature of treatment, 3°, 6° or 9°, on the number of plants flowering, and the number with apical buds at stage 3 or over, but excluding flowering plants.

Plants with apical buds at bud stage 3 and over, excluding flowering plants

Flowering plants

scale). There were three temperature treatments and therefore two degrees of freedom and two possible effects of temperature, a linear and a quadratic effect. The linear effect of temperature was much greater than the quadratic effect but both effects were significant at the 0.1 per cent level.

The fitted values of the model are shown in Figures 2.7 and 2.8. The interaction between temperature and cultivar is obvious, Doon Major flowering as readily as Wilhelmsburger after treatment at 3^{o}, but flowering less than Wilhelmsburger at 6^{o} and especially at 9^{o}. The optimum temperature for flower induction is probably around 3^{o} or below for Doon Major but is clearly around 6^{o} for Wilhelmsburger. From the fitted lines the number of days of low temperature treatment required to cause 50 per cent flowering can be calculated for the different treatments, Wilhelmsburger plants requiring 27.2 days at 3^{o}, 23.2 days at 6^{o} or 29.9 days at 9^{o}, and Doon Major requiring 27.2 days at 3^{o}, 29.6 days at 6^{o} or 42.5 days at 9^{o}, for 50 per cent flowering.

The treatments had similar effects on the number of reproductive plants although a relatively larger proportion of Doon Major plants became reproductive after treatment at 9^{o} compared with the number flowering after 9^{o}.

Days to flowering were analysed using GLIM (see experiment 2 and Appendix B). Linear components of duration and of temperature significantly affected days to flowering (F tests $p<0.001$). The longer the duration of low temperature and the lower the temperature of treatment the earlier was flowering and Wilhelmsburger flowered earlier than Doon Major (F test $p<0.025$) (Table 2.11). There was an interaction between the linear effect of temperature and the effect of duration of treatment (F test $p<0.005$) as there was a slight decrease in time to flower at higher temperatures in the three-week treatment but in the 4, 5 and 6-week duration treatments, the longer the duration, the greater the increase in time to flower as temperature of treatment increased.

The mean production of visible leaves during low temperature treatment was greater the longer the treatment and the higher the treatment

FIGURE 2.7: Experiment 5 - the effect of duration of low temperature treatment on the flowering of Wilhelmsburger and Doon Major swedes (means of the three temperature treatments)

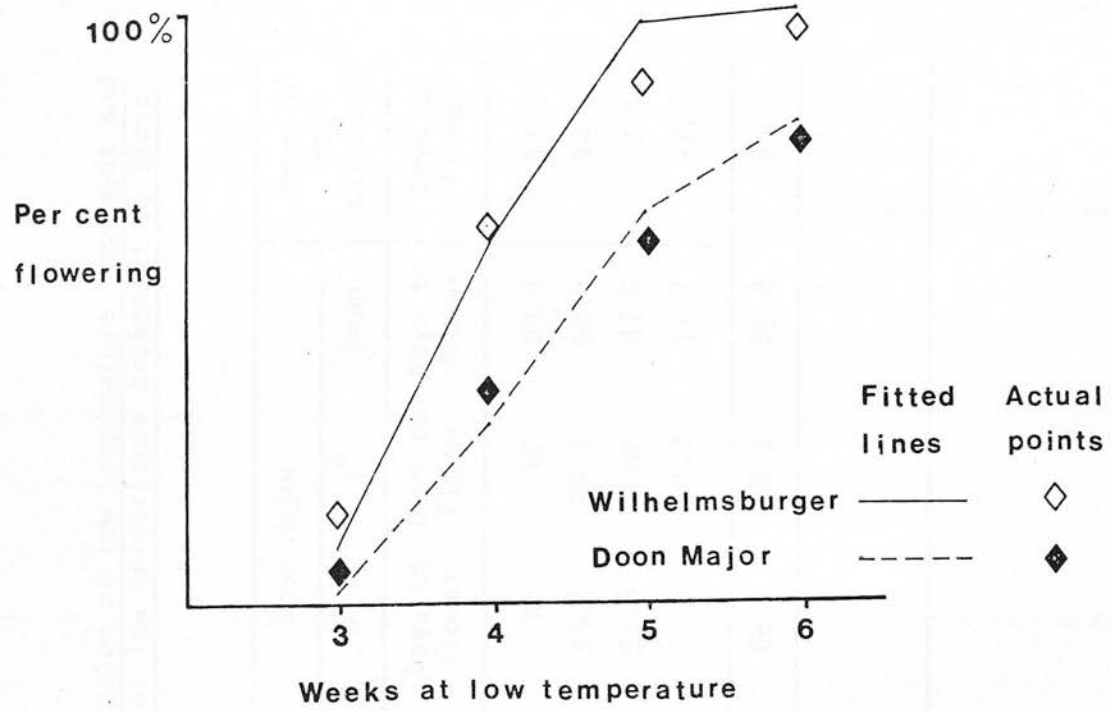


FIGURE 2.8: Experiment 5 - the effect of temperature of low temperature treatment on the flowering of Wilhelmsburger and Doon Major swedes (means of the four duration of low temperature treatments

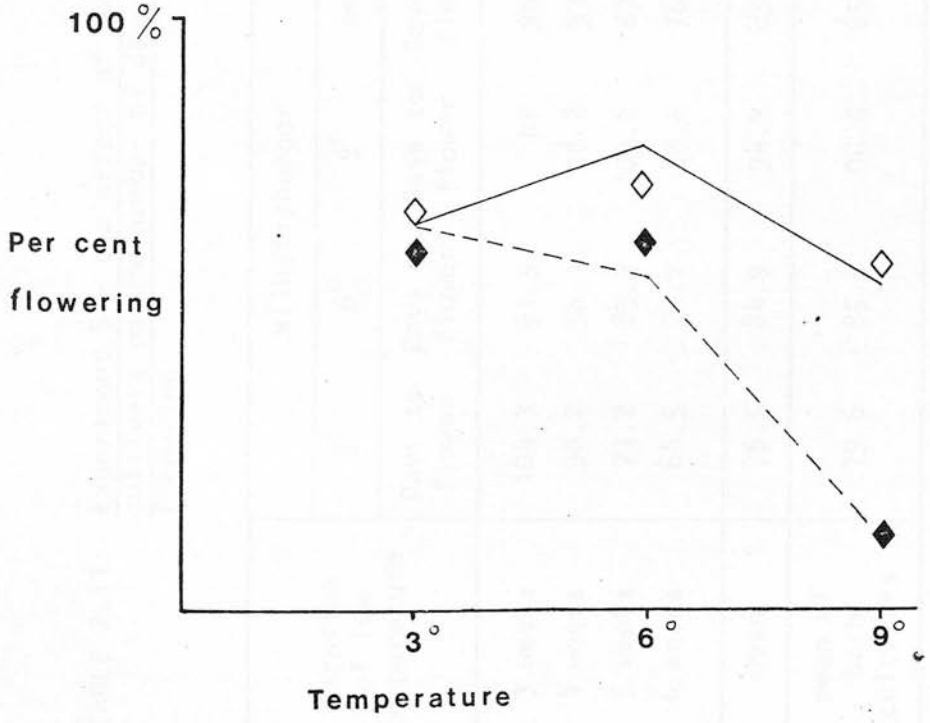


TABLE 2.11: Experiment 5 - the effect of temperature, duration of low temperature treatment and cultivars on the number of days from the end of low temperature treatment to first flowering

Duration of low temperature	Wilhelmsburger				Doon Major				Mean of both cultivars
	3°	6°	9°	mean	3°	6°	9°	mean	
	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower	Days to flower
3 weeks	104.3	91.5	NF	99.2	109.0	NF	NF	109.0	102.0
4 weeks	90.2	96.1	90.8	93.2	98.4	94.8	94.0	96.2	94.3
5 weeks	73.2	85.3	104.2	87.6	82.4	91.6	NF	87.0	87.4
6 weeks	64.5	74.2	88.6	76.1	71.4	77.1	99.0	78.7	77.3
mean	76.5	84.9	94.9	85.3	82.8	86.1	98.2	85.8	85.5
mean of both cultivars	79.6	85.4	95.5	85.5					

NF = no plants flowering in the treatment

temperature (F test, effect of temperature $p<0.001$, compared with within main plots error) (Table 2.12) and was slightly greater in Wilhelmsburger than Doon Major (F test $p<0.001$).

Discussion

The optimum temperature for inducing flowering in Wilhelmsburger was 6^{o}, but 3^{o} was more effective for Doon Major and it is possible that an even lower temperature might be more effective than 3^{o}. A much smaller proportion of Doon Major plants flowered after treatment at 9^{o} than Wilhelmsburger plants and this difference between the cultivars suggests that no one precise temperature is suitable for assessing bolting resistance of swedes. Doon Major will appear more resistant, relative to Wilhelmsburger, the higher the treatment temperature. These differences between the cultivars are also apparent when plants with developing flower buds (bud stage 3 and over) are included with the flowering plants.

The interaction of the linear effect of temperature with the effect of duration, in which the lower the treatment temperature the greater the response to increased duration of treatment in terms of reduced time to flowering, can be explained if it is assumed that the accumulation of substances that affect the rate of flower development (measured by days to flowering) is much faster at lower temperatures and so an increase in duration of a low temperature treatment has a much greater effect on the accumulation of these substances than a similar increase at a higher temperature, and so affects the rate of flowering more. In both Wilhelmsburger and Doon Major, 3^{o} was the optimum temperature for rate of flowering and so some other processes must be involved in determining the rate of flower development besides those governing whether a plant flowers or not.

TABLE 2.12: Experiment 5 - the effect of temperature, duration of low temperature treatment and cultivar on visible leaf production during low temperature treatment

Duration of low temperature	Wilhelmsburger				Doon Major				Mean of both cultivars
	3°	6°	9°	mean	3°	6°	9°	mean	
	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves
3 weeks	1.7	2.7	2.7	2.4	1.0	1.6	1.8	1.5	1.9
4 weeks	1.7	2.8	3.6	2.7	1.6	2.4	2.6	2.2	2.4
5 weeks	2.0	3.7	4.5	3.4	1.7	2.9	4.3	3.0	3.2
6 weeks	2.6	4.4	5.5	4.2	2.6	3.6	4.7	3.6	3.9
mean	2.0	3.4	4.1	3.2	1.7	2.6	3.4	2.6	2.9
mean of both cultivars	1.8	3.0	3.7	2.9					

For comparison within table	SE ± 0.21	For comparison of cultivar means	SE ± 0.06
For comparisons of temperature means	SE ± 0.07	For comparisons of duration means	SE ± 0.08

Increased leaf production during low temperature treatment caused by higher treatment temperatures does not appear to be related to the effectiveness of the treatment in causing flowering but the greater leaf production of Wilhelmsburger may be associated with its higher percentage of flowering plants.

The smaller number of days required for 50 per cent flowering in this experiment compared with experiment 4 is chiefly due to the experiment being extended for 20 days more than experiment 4 and to the higher temperatures in the glasshouse during the flowering period compared with experiment 4, which flowered out of doors, in September.

2.4 THE EFFECT OF POST-VERNALISATION TEMPERATURES

The effect of five different temperatures during one or two weeks immediately after low temperature treatment on the flowering of Wilhelmsburger and Doon Major swedes.

Experiment 6

The temperature immediately following low temperature treatment (post-vernalisation temperature) can have a large effect on flower induction, especially when the treatment period has been short (Heide 1970), high temperatures tending to reverse the effects of the low temperature treatment. The effect of a period of low temperature in the field or in experimental conditions cannot be predicted unless there is some knowledge of the post-treatment environment and its devernalising effect. To investigate the effects of post-treatment environment in swedes, 50 plants each of Wilhelmsburger and Doon Major were raised for seven weeks at 16° mean daily mean temperature, repotted to 12.5 cm pots and given a low temperature treatment for four weeks from mid-December to mid-January, at 8° mean daily mean temperature in an unheated glasshouse. A fairly short treatment period was used so that plants would be more susceptible to subsequent devernalisation. Immediately after treatment the plants were moved to five different environments, for one or two weeks with five plants per treatment.

1. Glasshouse bed: 18° daily mean temperature under sodium lamps supplementing daylength to 18 hours, in the glasshouse bed.
2. Glasshouse compartment: 19° mean daily mean temperature in natural daylight (short days).

3. Glasshouse, beside bed: 16^{o} mean daily mean temperature in natural daylight, protected by screens from glasshouse lights (short days).

4. Glasshouse compartment: 14^{o} mean daily mean temperature in natural daylight (short days).

5. Unheated glasshouse: 8^{o} mean daily mean temperature in natural daylight (short days).

At the end of the one or two-week periods plants were moved into the glasshouse bed (environment 1) and grown on for 18 or 19 weeks before being planted out in a previously fertilised field plot (see Appendix A) on 31 May. The experiment was replicated a week after the first replicate was started, and the second replicate was planted out on 2 June.

Date of flowering was recorded, and at the end of the experiment 31 or 30 weeks after low temperature treatment ended growing points of the remaining plants in replicates one and two were dissected and apical bud stage recorded (see Figure 2.1).

Results

High temperatures after low temperature treatment reduced the number of plants flowering, or becoming reproductive (bud stage 4 and over). Tables 2.13 and 2.14 give the percentages of plants flowering and reproductive in each treatment. The diagram on page 84 illustrates the differences between the proportions of flowering and reproductive plants in each treatment. A line joining treatments means that they are not significantly different at the 5 per cent level (χ^2 test).

TABLE 2.13: Experiment 6 - the effect of the post-vernalisation environment for one and two weeks on the percentage of plants flowering, and on the percentage of reproductive plants (bud stage 4 and over) of Wilhelmsburger and Doon Major swedes

Post vernalisation environment	Wilhelmsburger		Doon Major		Mean	
	% Plants flowering	% Plants reproductive	% Plants flowering	% Plants reproductive	% Plants flowering	% Plants reproductive
1 18° (glasshouse bed) 18 hr daylength	15.8	52.6	0	21.1	7.9	36.8
2 19° (glasshouse compartment) short days	10.5	63.2	5.9	17.6	8.3	41.7
3 16° (glasshouse) short days	29.4	70.6	11.1	22.2	20.0	45.7
4 14° (glasshouse compartment) short days	45.0	85.0	10.5	42.1	28.2	64.1
5 8° (unheated glasshouse) short days	66.7	77.8	31.6	68.4	48.6	73.0
mean	33.3	69.9	12.0	34.8	22.7	52.4

TABLE 2.14: Experiment 6 - the effect of the post-vernalisation environment of one or two weeks on the percentage of plants flowering, and on the percentage of reproductive plants (bud stage 4 and over) of Wilhelmsburger and Doon Major swedes

Post-vernalisation environment	One week		Two weeks		Mean	
	% Plants flowering	% Plants reproductive	% Plants flowering	% Plants reproductive	% Plants flowering	% Plants reproductive
1 18° (glasshouse bed) 18 hr daylength	see mean of one and two weeks				7.9	36.8
2 19° (glasshouse compartment) short days	5.9	23.5	10.5	57.9	8.3	41.7
3 16° (glasshouse) short days	11.8	41.2	27.8	50.0	20.0	45.7
4 14° (glasshouse compartment) short days	15.0	65.0	42.1	63.2	28.2	64.1
5 8° (unheated glasshouse) short days	25.0	55.0	76.5	94.1	48.6	73.0
mean	11.8	43.0	33.7	62.0	22.7	52.4

	least flowering				most flowering
Flowering plants	1	2	3	4	5
Reproductive plants	1	2	3	4	5

The low percentage of plants flowering in treatment 1 might have been due to the longer daylength but is more likely to be due to the effect of direct radiation on the plants heating them to above the 18° air temperature recorded in the bed by a thermohydrograph which was protected by aluminium foil from direct radiation. The higher percentage of flowering in treatment 5 is probably due mainly to the vernalising effect of 8° rather than the preventing of devernalisation. Plants in environments 2 to 5 that were moved to the glasshouse bed after only one week flowered less (χ^2 $p<0.005$) and fewer plants became reproductive (χ^2 $p<0.05$) than those given two weeks in the different environments before transfer to the glasshouse bed (Table 2.14). The glasshouse bed is the most devernalising environment, and delaying transfer to the bed by one week must reduce devernalisation. When the five post-vernalisation environment treatments are considered separately, only treatment 5 shows a significant difference in the proportions flowering (χ^2 $p<0.01$) and reproductive (χ^2 $p<0.025$) between the one and two-week groups. As the treatment was at 8°, the difference is more likely to be due to increased vernalisation in the two-week group than to reduced devernalisation. If treatments 2 to 4 only are considered there is no significant difference in the proportions of plants flowering or reproductive between the one and two-week treatments. Treatment 1 is omitted because it is the control, and the one and two-week treatments in it were exactly the same.

More Wilhelmsburger plants flowered (χ^2 $p<0.001$) or became reproductive (χ^2 $p<0.001$) than Doon Major plants.

Days to flowering increased with the post-vernalisation temperature (Table 2.15) and Doon Major was slightly later to flower than Wilhelmsburger. Days to flowering were slightly shorter after two-week post-vernalisation treatments than one-week treatments.

Discussion

Lower post-vernalisation temperatures increase the number of plants induced to flower by the vernalising low temperature treatment by reducing devernalisation, although if temperatures are 10^{o} or below, no distinction can be made between the effect of further vernalisation and the prevention of devernalisation. The effect of devernalisation is apparent both in the reduced number of plants flowering and in the longer time taken to flower after devernalisation.

In this experiment there was no clear advantage in increasing the period of moderate temperature from one to two weeks after low temperature treatment and it is possible that after a week, no further devernalisation takes place, although days to flowering were reduced slightly by prolonging the treatment for a further week.

TABLE 2.15: Experiment 6 - the effect of the post-vernalisation environment for one and two weeks on the number of days to flowering of Wilhelmsburger and Doon Major swedes

	Post-vernalisation environment	Days to flowering			Days to flowering		
		Wilhelms-burger	Doon Major	Mean	One week	Two weeks	Mean
1	18° (glasshouse bed) 18 hr daylength	203.7	NF	203.7	see mean of 1 and 2 weeks		203.7
2	19° (glasshouse compartment) short days	192.0	210.0	198.0	210.0	192.0	198.0
3	16° (glasshouse) short days	173.8	183.0	176.4	176.0	176.6	176.4
4	14° (glasshouse compartment) short days	181.4	156.0	176.8	180.7	175.4	176.8
5	8° (unheated glasshouse) short days	156.2	178.5	163.6	173.6	159.8	163.6
	mean	173.3	178.1	174.5	179.3	172.8	174.5

NF = no plants flowering in the treatment

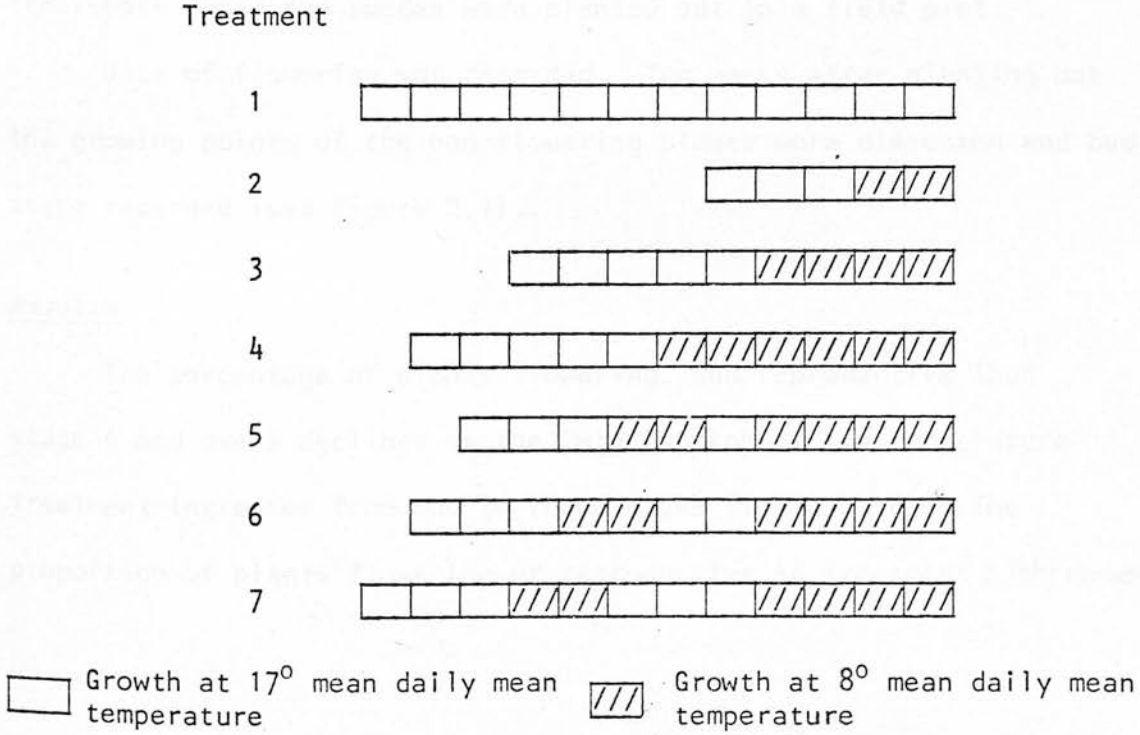
2.5 INTERRUPTED LOW TEMPERATURE TREATMENTS

The effect of interrupting a six-week low temperature period with one, two or three weeks at a higher temperature on the flowering of Wilhelmsburger and Doon Major swedes.

Experiment 7

In the field, periods of vernalising temperatures will often not be continuous, being separated by periods of neutral or devernalising temperatures. Interrupting a period at low temperature with periods at higher temperature reduces the inductive effect of the low temperature in other species (see 1.7, page 16). When the interruptions are long, in effect the plant is exposed to short periods of vernalisation, insufficient in themselves to cause flowering, but which may have an additive effect and together induce flowering.

To examine if interrupting low temperature treatment reduces flowering in swedes, and if separate periods of low temperature have an additive effect, five plants each of Wilhelmsburger and Doon Major swedes were given the following treatments from sowing:



Each square represents one week, and all treatments ended on the same day.

Treatments 5, 6 and 7 show the effect of one, two and three-week interruptions in six weeks low temperature treatment, compared with continuous treatment, treatment 4. In treatments 5, 6 and 7 the plants have 4, 5 or 6 weeks in total at 17°, before the final four weeks low temperature treatment, and so treatment 4 is given the mean value, five weeks growth at 17° before its low temperature treatment. Treatment 3, low temperature for four weeks only, is included so that the effect of two weeks additional treatment on the later four weeks in treatments 5, 6 and 7 can be examined. Treatment 2 is included to indicate whether two weeks alone can cause flowering or not, and treatment 1 is the untreated control.

The plants were grown at 8° in an unheated glasshouse during December and January with natural daylight (short days) only. Plants were repotted to 12.5 cm pots three weeks after sowing. All six treatments ended on the same day and the plants were moved into 17° beside the control plants. On 3 June, 17 weeks after low temperature treatments ended the swedes were planted out in a field plot.

Date of flowering was recorded. Ten weeks after planting out the growing points of the non-flowering plants were dissected and bud stage recorded (see Figure 2.1).

Results

The percentage of plants flowering, and reproductive (bud stage 4 and over) declines as the interruption of low temperature treatment increases from one to three weeks (Table 2.16). The proportion of plants flowering or reproductive in treatment 7 (three-week

TABLE 2.16: Experiment 7 - the effect of two, four and six weeks low temperature treatment, and of interrupting a six weeks low temperature treatment with one, two or three weeks at a higher temperature on the flowering of Wilhelmsburger and Doon Major swedes

□ one week at 17° ▨ one week at 8°

Low temperature treatment	Wilhelmsburger		Doon Major		Mean	
	% plants flowering	% plants reproductive	% plants flowering	% plants reproductive	% plants flowering	% plants reproductive
1 Control, no low temperature treatment	0	17	0	0	0	8
2 □□□▨▨	0	17	0	0	0	10
3 □□□□□▨▨▨▨	50	67	20	40	36	55
4 □□□□□▨▨▨▨▨▨	83	100	100	100	91	100
5 □□□▨▨▨□▨▨▨▨	100	100	80	80	91	91
6 □□□▨▨▨□□▨▨▨▨	33	67	67	67	50	67
7 □□□▨▨▨□□▨▨▨▨	50	50	0	33	25	42
mean	45	60	38	46	42	53

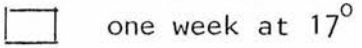
interruption) was significantly different from the proportion of plants flowering in treatments 4 (uninterrupted six weeks at 8^{o}) and 5 (treatment interrupted by one week) (χ^2 tests, treatments 5 v 7, reproductive plants $p<0.05$; 5 v 7 flowering plants, 4 v 7 flowering and reproductive plants $p<0.01$). Treatment 5 (two weeks at 8^{o}, one week at 17^{o}, four weeks at 8^{o}) is significantly different from treatment 3 (four weeks at 8^{o}) in numbers flowering (χ^2 $p<0.05$), and so there may be an additive effect of the extra two weeks low temperature treatment in treatment 5. There is no additive effect apparent comparing treatments 7 (two weeks at 8^{o}, three weeks at 17^{o}, four weeks at 8^{o}) and 3, and no significant effect comparing 6 (two weeks at 8^{o}, two weeks at 17^{o}, four weeks at 8^{o}) and 3. Only one plant became reproductive in treatment 2, as in the control, indicating that two weeks low temperature treatment was insufficient to cause flowering in most plants. The number of days to flowering was least with the treatments giving the most flowering, 4 and 5 (Table 2.17).

There was no significant difference between the cultivars in the proportions of plants flowering or reproductive, although more Wilhelmsburger plants became reproductive, and no difference in mean time to flower.

Discussion

Interrupting a period of low temperature treatment with a period at a higher temperature reduces the effectiveness of the low temperature treatment. Although there were few plants in each treatment it appears that increasing the duration of the high temperature interruption from one to three weeks reduces the vernalising effect of the total low temperature treatment period, that is devernalises more.

TABLE 2.17: Experiment 7 - the effect of four and six weeks low temperature treatment and of interrupting a six weeks low temperature treatment with one, two or three weeks at a higher temperature on the days to flowering of Wilhelmsburger and Doon Major swedes. Treatments 1 and 2 are not shown as no plants flowered in them



Low temperature treatment	Days to flowering		
	Wilhelms-burger	Doon Major	Mean
3	179.0	128.0	166.3
4	124.6	119.2	121.0
5	130.3	157.2	141.1
6	175.5	170.2	172.0
7	162.7	NF	162.7
mean	146.4	145.3	145.9

NF = no plants flowered in the treatment

Treatment 7 was no more vernalising than treatment 3 (four weeks low temperature treatment only), suggesting that the three week interruption in treatment 7 was sufficient to completely devernalise the vernalising effect of the previous two weeks low temperature treatment. There was evidence of an additive effect in treatment 5, and it is possible that if the temperature of the interruption had been lower, around 11° to 15°, and therefore much less devernalising, an additive effect might have been apparent in the other treatments, 6 and 7.

2.6 THE INTERACTION OF DEVERNALISATION AND GIBBERELLIN

2.6.1 The effect of gibberellic acid applications on the stem extension and flowering of unvernalised, vernalised and devernalised swedes.

Experiment 8

Interruptions of low temperature treatment with periods at higher temperature reduce or nullify the effect of the temperature treatment (see 1.7, page 16). More than one process is probably involved in vernalisation, and it is possible that the several effects of vernalisation, for instance in stem extension and flowering, are not reversed by devernalisation at the same rate.

Natural gibberellins are probably involved in stem extension, and applications of exogenous gibberellin cause stem extension in many plants (see 1.11.1, page 24). In a preliminary experiment gibberellic acid ('Berelex', 90 per cent biologically active isomer of gibberellic acid) was applied, 0.04 ml per day or 0.14 ml twice a week, at concentrations of 0, 0.01, 1, 100, 1000 and 10,000 ppm to Pentland Harvester swedes. Concentrations of 100 ppm and above caused stems to extend, but there was no sign of flowering by the end of the experiment, 50 days after the first application.

To examine whether devernalised plants return to the same state as unvernalised plants or retain some effect of vernalisation, gibberellic acid was applied as drops to the centre of the rosettes of vernalised, unvernalised and devernalised plants and their response in terms of stem extension and flowering was measured. A group of 20 four-week old Pentland Harvester plants were kept in a cool glasshouse at 14.2^{o} mean daily mean temperature as unvernalised

controls. Twenty similar plants were given a low temperature treatment out of doors in November to December at 4.6° mean daily mean temperature for 28 days and 20 plants were treated for a total of 28 days at 4.6° with one day each week in a growth cabinet at 20°, 16-hour day/8-hour night. All plants started treatment on the same day and at the end of the two low temperature periods, the two groups were moved into a growth cabinet at 15°, 16-hour day/8-hour night.

When the last low temperature treatment had finished, twice weekly applications of 0.14 ml gibberellic acid solution at concentrations of 0, 10, 100 or 1000 ppm were started and continued for nine weeks, giving total applications of 25.2 μg, 0.252 mg and 2.52 mg gibberellic acid for 10, 100 and 1000 ppm respectively. There were five plants at each level of gibberellic acid in every vernalisation group. Stem extension had begun almost as soon with 1000 ppm as 10,000 ppm in the preliminary experiment and so 1000 ppm was selected as the maximum level of gibberellic acid. There had been little difference between the effects of daily and twice weekly applications, and so the latter were chosen as being more convenient. The plants were then grown on at 15.5° mean daily mean temperature beside the control plants.

Dates of first elongation, that is when at least one internode is 1 cm or more, and of first flowering and stem height at first flowering were recorded. The experiment ended 200 days after the first application of gibberellic acid.

Results

Glasshouse temperatures were low, with mean minimum temperatures below 10° throughout the experiment although mean daily mean

temperature rose from 14^{o} to 20^{o} from January to June. All plants except six in the 0, 10 and 1000 ppm treatments in the unvernalised group had flowered by the end of the experiment, but there were differences between the groups in days to flowering. The comparison was strictly speaking between a weakly vernalised group (unvernalised group), a strongly vernalised group and a devernalised and then weakly vernalised group. The group of plants subjected to low temperature treatment with weekly interruptions was not completely devernalised as its flowering behaviour was slightly different from the unvernalised (weakly vernalised) group, although it was not the same as the vernalised group.

The stems of all plants, flowering and non-flowering, showed signs of extension but the weakly vernalised group started to elongate last (F test, effect of vernalisation group $p<0.001$, compared with within main plot error - main plots are vernalisation groups) (Table 2.18). Stem extension began earlier after gibberellic acid applications (F test, effect of gibberellic acid $p<0.001$) especially with the higher concentrations of gibberellic acid, and there was a significant interaction between vernalisation group and gibberellic acid level (F test. $p<0.001$), only the weakly vernalised group continuing to respond at the highest gibberellic acid level with a reduction in time to first elongation.

The strongly vernalised group flowered first (Table 2.18), the devernalised group next and the weakly vernalised group last (F test, effect of vernalisation groups $p<0.001$, compared with within main plot error). Gibberellic acid reduced the time to flowering slightly (F test, effect of gibberellic acid $p<0.001$) and there was a significant interaction between vernalisation and gibberellic acid, the devernalised group responding to gibberellic acid much less than the other two groups.

TABLE 2.18: Experiment 8 - the effect of gibberellic acid applications on the number of days to the first signs of stem elongation, days to flowering and stem height at flowering of vernalised, partially devernalised and partially vernalised Pentland Harvester swedes

Vernalisation treatment	Days to first elongation					Days to first flowering					Stem height at flowering cm				
	Gibberellin concentration ppm				Mean	Gibberellin concentration ppm				Mean	Gibberellin concentration ppm				Mean
	0	10	100	1000		0	10	100	1000		0	10	100	1000	
Weakly vernalised	91.6	48.2	35.0	20.4	48.8	125.0	107.5	110.4	104.3	111.4	47.9	48.5	54.8	62.9	54.7
Strongly vernalised	45.8	26.6	16.4	14.6	25.8	101.0	88.0	87.6	87.2	91.2	61.4	61.4	62.4	85.0	66.6
Strongly vernalised, partially de-vernalised	38.8	24.2	14.0	14.0	22.8	100.2	97.6	97.4	99.7	98.7	54.3	62.2	63.8	65.3	61.1
Mean	58.7	33.0	21.8	16.3	32.5	106.2	95.2	98.5	97.1	99.3	55.5	59.6	60.3	71.1	61.4
For comparisons within tables	SE ± 2.92					SE ± 2.65					SE ± 3.5				
For comparisons of GA treatments	SE ± 1.68					SE ± 1.53					SE ± 2.02				

The weakly vernalised group had the shortest stems at flowering (Table 2.18) then the devernalised group and the strongly vernalised group had the tallest stems (F test, effect of vernalisation group $p<0.001$, compared with within main plot error). Gibberellic acid increased stem height at flowering (F test $p<0.01$) chiefly due to the 1000 ppm treatment which had taller stems than the other three gibberellic acid treatments. There was a significant interaction between gibberellic acid and vernalisation group in stem height (F test $p<0.05$), the strongly vernalised group responding only to 1000 ppm while the other two groups responded to the lower rates.

Discussion

Devernalisation does not appear to be a uniform reversal of vernalisation. In days to flowering and stem height at flowering the devernalised group lay between the strongly vernalised and weakly vernalised groups as might be expected after a partial reversal of vernalisation. However, the devernalised plants elongated slightly earlier on average than the vernalised plants, suggesting that the cold-induced and possibly gibberellin-like factors that initially cause stem elongation were not reduced by the devernalisation treatment, unlike the flowering factors measured by days to flowering.

The devernalised and strongly vernalised plants responded to gibberellic acid application which reduced the time to the start of stem elongation and to flowering, and increased stem height at flowering, but the weakly vernalised treatment responded most to gibberellic acid, presumably because the level of cold-induced gibberellin-like substances within the plants was low. Devernalised plants responded less to exogenous gibberellic acid in all effects

measured, than strongly vernalised plants and started to elongate seven days earlier than the strongly vernalised plants in the absence of gibberellic acid. It is possible that devernalisation does not reduce gibberellin-like factors that cause stem elongation, as much as it reverses the factors that cause flowering.

2.6.2 The effect of gibberellic acid on the stem extension of unvernalised, vernalised, devernalised and strongly devernalised Wilhelmsburger and Doon Major swedes.

Experiment 9

Experiment 8 was repeated with modifications because the effects of the vernalisation treatments in experiment 8 were obscured by the subsequent low temperature in the glasshouse. Wilhelmsburger and Doon Major cultivars were used and only 0 and 100 ppm gibberellic acid, as in the previous experiment response to gibberellic acid declined at 1000 ppm. To examine devernalisation in more detail, an extra treatment was included, low temperature treatment for 28 days in total with two days interruption every week at 18°, besides the original treatments, control plants receiving no low temperature treatment, low temperature for 28 days and low temperature for 28 days in total with one day a week interruption at 18°, with 12 plants of each cultivar in each of the four vernalisation treatments.

Low temperature treatment was carried out in a growth chamber at 7° mean daily mean temperature, 18-hour day/6-hour night, and pre-, post- and de-vernalisation growth in a glasshouse bed at 18° mean daily mean temperature, with daylength extended to 18 hours. Plants

were seven weeks old at the start of low temperature treatment, all treatments starting on the same day, and 39 days later, one day after the longest devernalisation treatment was complete, gibberellic acid applications of 0.08 ml of 0 or 100 ppm gibberellic acid in water, applied twice a week to the centre of the rosette of leaves, were started and continued for eight weeks, giving total applications of 0.128 mg gibberellic acid per treated plant. The plants were completely randomised in the glasshouse bed.

Dates of first elongation and of flowering and stem height at the end of gibberellic acid treatment were recorded. The experiment ended 150 days after the start of gibberellic acid treatment.

Results

The vernalised group extended earliest and the unvernalised group last (F test, effect of vernalisation group $p<0.005$, compared with within main plot error - main plots are vernalisation groups) (Table 2.19), but the two devernalised groups were not significantly different from the vernalised although they differed from the unvernalised group (t tests $p<0.001$). Eleven out of 12 unvernalised plants, and four Doon Major plants in the other groups, all receiving 0 ppm gibberellic acid, did not extend. One unvernalised Doon Major plant did not extend after treatment with 100 ppm gibberellic acid. The difference between the proportions of plants extending in the four vernalisation groups was significant (χ^2 $p<0.001$) due almost entirely to the unvernalised group.

So that the days to first elongation could be analysed in a balanced analysis of variance, plants that had not started to extend by the end of the experiment were assumed to have begun to extend on

TABLE 2.19: Experiment 9 - the effect of gibberellic acid applications on the number of days to the first signs of stem elongation and on stem height after gibberellic acid treatment of unvernalised, vernalised and devernalised Wilhelmsburger and Doon Major swedes

Vernalisation treatment	Days to first elongation							Stem height (cms) at end of GA applications						
	Wilhelmsburger			Doon Major			Overall mean	Wilhelmsburger			Doon Major			Overall mean
	Gibberellin ppm		Mean	Gibberellin ppm		Mean		Gibberellin ppm		Mean	Gibberellin ppm		Mean	
	0	100		0	100			0	100		0	100		
Unvernalised	150.0	27.8	88.9	133.2	53.5	93.3	91.1	0	6.7	3.3	0.1	5.2	2.6	3.0
Vernalised	27.0	18.0	22.5	63.7	28.5	46.0	34.3	2.4	20.6	11.5	1.2	9.5	5.3	8.4
Devernalised one day per week	40.8	23.7	32.2	78.5	31.5	55.0	43.6	1.9	13.3	7.6	1.3	9.3	5.3	6.4
Devernalised two days per week	36.5	18.0	27.2	74.7	26.8	50.7	39.0	1.8	17.6	9.7	0.9	11.3	6.1	7.9
Mean	63.6	21.9	42.7	87.5	35.1	61.3	52.0	1.5	14.5	8.0	0.9	8.8	4.8	6.4
For comparisons* within tables	SE ± 11.04							SE ± 1.68						
For comparisons of GA cultivar treatments	SE ± 5.52							SE ± 0.84						

the last day of the experiment and given a score of 150 days. This was a conservative estimate as many of these plants would never have extended if allowed to grow on.

Gibberellic acid treatment shortened the time to the start of elongation (F test $p<0.001$) by 47 days on average and Wilhelmsburger elongated on average 20 days earlier than Doon Major (F test $p<0.001$). There was a significant interaction between gibberellic acid application and vernalisation group (F test $p<0.001$). Almost all the unvernalised plants did not extend and were given a score of 150 days so that applications of gibberellic acid causing plants to extend in mean 40.7 days reduced the time to elongation most in this group, and least in the vernalised group, which extended readily without gibberellic acid.

The days to first elongation data was also analysed using the GLIM program (see Appendix B), with the number of plants contributing to each mean (0 or 1) used as a weight. The data is not particularly suitable for such an analysis as the unvernalised plants receiving no gibberellic acid provided data on days to first elongation of only one plant, and the estimates fitted by the program were smaller (42 and 53 days Wilhelmsburger and Doon Major respectively) than would reasonably be expected, considering that many plants had not extended by 150 days. Vernalisation treatments (F test $p<0.05$), cultivar (F test $p<0.001$) and gibberellic acid application (F test $p<0.001$) all had a significant effect on days to first elongation and in the fitted values Wilhelmsburger elongated earlier than Doon Major, and the vernalisation groups elongated in the order vernalised group first, then the group devernalised two days a week, the group devernalised one day a week and the unvernalised group elongated last. Gibberellic acid reduced the time to elongation by 16.8 days (fitted values).

Stem height at the end of gibberellic acid treatment was greatest in the vernalised group and least in the unvernalised group (F test, effect of vernalisation group $p<0.05$, compared with within main plot error).

Gibberellic acid increased stem height in all treatments (F test $p<0.001$) and there was a significant interaction between gibberellic acid and vernalisation group (F test $p<0.01$). The unvernalised group responded least to gibberellic acid with a mean increase in stem height of 5.9 cm after gibberellic acid application compared with mean increases of 13.2, 9.7 and 13.1 cm for the vernalised, devernalised and strongly devernalised groups respectively.

Wilhelmsburger had taller stems than Doon Major (F test $p<0.001$) and there was a significant interaction between gibberellic acid application and cultivar, Wilhelmsburger responding much more to gibberellic acid than Doon Major.

The post-treatment temperatures, 18^{o} mean daily mean temperature, were much higher than in the first experiment, and only four plants flowered, all of them Wilhelmsburger receiving gibberellic acid. Three in the vernalisation treatment flowered in mean 142 days from the end of their low temperature treatment, one in the one day interruption treatment, in 151 days, and one plant in the two days interruption treatment reached bud stage 3 (see Figure 2.1) at the end of the experiment.

Considering the three vernalisation and devernalisation treatments only, there was a significant difference between the proportions of plants becoming reproductive in the two cultivars, Wilhelmsburger and Doon Major (χ^2 $p<0.05$) and in Wilhelmsburger, between plants with and without gibberellic acid treatment (χ^2 $p<0.05$)

Discussion

The results in this experiment tend to confirm the previous experiment, in this case all treatments being to some extent devernalised by post-low temperature treatment conditions. Although too few plants flowered for the differences between treatments to be significant, devernalisation reduced flowering compared with vernalised plants, especially the stronger devernalisation of two days a week. Days to elongation and stem height in the 100 ppm gibberellic acid treatments of the devernalised groups were much closer to those of the vernalised group than the unvernalised group, and were not significantly different from the vernalised group. The more extreme devernalisation, two days a week, was closest in extension to the vernalised group suggesting that devernalisation does not greatly decrease factors inducing stem extension.

No Doon Major plants flowered although in other experiments 28 days vernalisation resulted in at least a proportion of Doon Major plants flowering (44 per cent after 30 days in experiment 2, 80 per cent after 28 days in experiment 3 and 35 per cent after 28 days in experiment 5, mean of all temperature treatments). Many of the 36 Doon Major plants may have been induced to flower, but high post-vernalisation temperatures must have reversed the induction and caused the plants to return to the vegetative state. There were, however, very marked differences in stem extension and response to gibberellic acid between the three groups receiving vernalisation and the unvernalised group, presumably because stem extension factors are not reduced by devernalisation to nearly the same extent as flowering factors.

2.7 THE EFFECT OF NITROGEN ON FLOWERING

The effect of applications of nitrogen during and after low temperature treatment on the flowering of swedes.

Experiment 10

If nitrogen had a large effect on flower induction and flower development this would have to be considered in the commercial husbandry of the swede crop both as a root crop and for seed. There is some evidence for red beet (see 1.9, page 21) that shortage of nitrogen before low temperature treatment reduces the number of plants flowering, and shortage after treatment delays flowering. The effect of nitrogen nutrition during and after cold treatment of swedes on flower induction and development was examined.

Sixty Doon Major plants were raised in John Innes Number 2 compost made up without nitrogen (blood and bone meal) for four weeks and were then grown at 5^{o} mean daily mean temperature for 30 days. Four nitrogen treatments were each applied to 15 plants, with five plants per plot and three replicates:

1. No nitrogen applied;
2. nitrogen applied only during the 30 days low temperature treatment: total application of nitrogen per plant, 0.5 g NH_4NO_3 in solution;
3. nitrogen applied during and after the 30 days low temperature treatment: total application per plant, 1.2 g NH_4NO_3 in solution and 1.75 g NH_4NO_3 to the soil at planting;
4. nitrogen applied only after the 30 days low temperature treatment: total application per plant, 0.7 g NH_4NO_3 in solution and 1.75 g NH_4NO_3 to the soil at planting.

After low temperature treatment the plants were grown in a growth chamber at 13° for 12 days to reduce the risk of devernalisation.

The nitrogen treatment was 0.1 g NH_4NO_3 in 20 ml water applied weekly direct to the soil in each pot. Seven weeks after low temperature treatment ended the swedes were planted out in a field plot previously fertilised with P and K fertiliser (18.8 per cent soluble P, 1.2 per cent insoluble P and 20 per cent K at the rate 500 kg/ha) and 7 g per plant of 25 per cent N fertiliser (NH_4NO_3) was applied to each treated plot after planting out.

Visible leaf number was counted before and after low temperature treatment. The date of first flowering and flower stage (see experiment 2) at the end of the experiment, 154 days after the end of low temperature treatment, were recorded.

Results

All plants flowered and nitrogen applied after low temperature treatment reduced the time to flower significantly (F test $p<0.05$) with the nitrogen applied during and after low temperature treatment having the shortest time to flowering (Table 2.20). Nitrogen applications increased the flower stage at the end of the experiment (Table 2.21), but only nitrogen applied during low temperature treatment increased it significantly (F test $p<0.05$). Flower stage is related to time of flowering and as all plants flowered, gave an indication of the progress to seed development of the plants.

During low temperature treatment plants receiving nitrogen produced mean 1.7 leaves (0.057 leaves per plant per day), whereas those not receiving nitrogen produced mean 1.4 leaves (0.047 leaves per plant per day).

TABLE 2.20: The effect of nitrogen applied during and/or after low temperature treatment on the mean number of days to flowering from the end of 30 days low temperature treatment

Nitrogen treatments	Nitrogen applied during low temperature treatment	No nitrogen applied during low temperature treatment	Mean
	Days to flower	Days to flower	Days to flower
Nitrogen applied after low temperature treatment	91	93	92
No nitrogen applied after low temperature treatment	96	106	101
Mean	93	100	97
For comparisons within the table	SE ± 3.83		
For comparisons of means	SE ± 2.708		

TABLE 2.21: The effect of nitrogen applied during and/or after low temperature treatments on the flower stage (see experiment 2) 154 days after the end of low temperature treatment

Nitrogen treatments	Nitrogen applied during low temperature treatment	No nitrogen applied during low temperature treatment	Mean
	Flower stage	Flower stage	Flower stage
Nitrogen applied after low temperature treatment	8.7	8.6	8.7
No nitrogen applied after low temperature treatment	9.0	7.8	8.3
Mean	8.8	8.2	8.5
For comparisons within the table	SE ± 0.30		
For comparisons of means	SE ± 0.21		

Discussion

As all plants flowered, any effect of nitrogen on flower induction could not be measured, but nitrogen applied during and after low temperature treatment hastened flower development probably due to improved growth during and especially after low temperature treatment compared with treatments receiving less nitrogen.

Although there must have been sufficient nitrogen in the unfertilised compost and field plot soil for growth, the nitrogen treated plants were more vigorous with darker green leaves and produced more leaves during low temperature treatment, than untreated plants. Severe restriction of nitrogen supply (no nitrogen treatment) appeared to delay flowering and seed development, but the effect of nitrogen is unlikely to be of great importance with plants growing in normal conditions.

2.8 THE EFFECT OF LIGHT DURING LOW TEMPERATURE TREATMENT

2.8.1 The effect of the absence of light during low temperature treatment of Wilhelmsburger and Doon Major swedes on flowering.

Experiment 11

Swedes can be vernalised in short days, as in experiments 1, 6, 7 and 8 as well as in long days, for example experiments 2, 3, 4, 5 and 9. Mature sugar beet roots were vernalised, and flowered in the absence of light (Fife and Price 1953) but with cauliflower plants, the absence of light during one week at 5^{o}, prevented flowering (Sadik and Ozbun 1968). In cauliflower the levels of starch and sugar in the shoot tip were much lower than in the presence of light, whereas the sugar beet roots would have had large reserves of carbohydrates.

To investigate if vernalisation of swedes would occur during a low temperature period, in the absence of light, Wilhelmsburger and Doon Major plants were kept for 10 or 15 weeks in total darkness at 6^{o}. Ten-week old plants were used so that they would have a reserve of carbohydrates in the root. Four Wilhelmsburger and four Doon Major swedes were placed in each of six wooden boxes, 50 cm by 30 cm and 10 cm deep, filled with a layer of damp peat. The boxes were lined and covered with two layers of black plastic and a light meter placed in any box gave no reading. The boxes were placed in a refrigerator at 6 ± 2^{o}. The plants were watered infrequently and in darkness. Three boxes were removed after 10 weeks and the remaining three after 15 weeks. The plants were uncovered and placed in a glasshouse compartment at 14^{o} mean daily mean temperature for four weeks and then moved into a glasshouse bed at 17^{o} mean daily mean temperature.

Visible leaf numbers of each plant were recorded before and after low temperature treatment. Date of flowering was recorded.

Results

All plants flowered normally within 91 days of the end of low temperature treatment. One plant was dissected immediately after 15 weeks low temperature and was at bud stage 3 (see Figure 2.1). Flowering was much earlier after 15 weeks than after 10 weeks treatment (F test $p<0.001$) (Table 2.22), and Wilhelmsburger flowered slightly earlier than Doon Major (F test $p<0.05$).

TABLE 2.22: Experiment 11 - the effect of duration of low temperature treatment in the absence of light, and cultivar, on days to flowering of Wilhelmsburger and Doon Major swedes

Duration of low temperature	Days to flowering		
	Wilhelms-burger	Doon Major	Mean
10 weeks	82.3	85.3	83.3
15 weeks	38.5	42.2	39.6
Mean	62.0	65.4	63.0
For comparisons within table	SE ± 1.21		
For comparisons of duration or cultivar	SE ± 0.85		

Leaf number increased during low temperature treatment although all plants were yellow and etiolated by the end of treatment. After 10 weeks the mean increase was 1.9 leaves, and after 15 weeks 6.2 leaves (F test, effect of duration, $p<0.005$), but there was little difference between the cultivars, Wilhelmsburger having a slightly smaller increase in leaf number than Doon Major.

Discussion

Light was completely excluded from the plants during the entire time they were at low temperature but all plants flowered. Light, therefore, is not necessary during a period of low temperature for induction of flowering. It is possible that normal flowering and extension would not have occurred if light had been excluded after treatment but in the 15-week group one plant at least was beginning to develop flower buds (bud stage 3) by the end of treatment.

The time taken to flower after 10 weeks in darkness is similar to that in experiments 3, 4 (5^{o} and 8^{o}) and 5 (3^{o} and 6^{o}) after five weeks low temperature treatment in the light (16 or 18 hours daylength). and the mean leaf production of visible leaves during the low temperature period is similar, about two leaves.

The relatively greater increase in leaf production during 15 weeks compared with 10 weeks might be due to the acclimatisation of the plants to the growth conditions in the light-proof boxes or possibly to the difficulty in counting leaf scar numbers after treatment on pale etiolated plants so that the production of leaves in both 10 and 15-week treatments may have been greater than that recorded.

2.8.2 The effect of different levels of light intensity during low temperature treatment on the flowering of Wilhelmsburger and Doon Major swedes.

Experiment 12

Swedes flowered after 10 weeks low temperature treatment in the dark (see experiment 11) but a 10-week treatment period was

equivalent in terms of days to flowering to five weeks low temperature treatment in full light, suggesting that light intensity affects the rate of the vernalisation process. High levels of carbohydrates appear to be associated with flowering in some plants (see 1.11.4, page 29) and a high light intensity would tend to increase carbohydrate levels, even at low temperatures.

Seven-week old Wilhelmsburger and Doon Major swedes were subjected to three or six weeks low temperature treatment under four different light intensities, full artificial light, half light, quarter light and total darkness. Seven-week old plants were used so that plants treated in complete darkness would have an adequate supply of carbohydrate. The experiment was in a split-plot design, light treatments in two replicates being the main plots and cultivar and duration of low temperature the subplots with three plants per plot.

The plants were raised at 17^{o} mean daily mean temperature in natural daylight supplemented during most of the 18-hour day with sodium vapour light.

For the six-week low temperature treatment three Wilhelmsburger and three Doon Major plants were placed in each of eight wooden boxes similar to those used in experiment 11, half-filled with damp peat. Two boxes were left uncovered, two were shaded with two layers of muslin, two shaded with four layers of muslin and two were covered with two layers of closely woven dark cloth and a sheet of aluminium foil to exclude all light. The three-week treatment was placed in the same boxes three weeks later and both three and six-week treatments ended at the same time.

The plants were treated in a growth chamber illuminated with warm white fluorescent light on 18-hour daylength. Air temperature during treatment was $8.6 \pm 1^{\circ}$ although shade temperature in the boxes at soil level was slightly lower, 7.9° mean. Light intensity was measured at leaf level in the boxes at the beginning and end of low temperature treatment and was 11,800, 5000 and 2300 lux on average for full, half and quarter light respectively. A light meter placed in the dark treatment boxes gave no reading and any handling of these boxes was done in darkness. One replicate was placed at the two ends of the bench, where the light intensity was slightly less (1000 lux lower than the other replicate, on average).

At the end of low temperature treatment the plants were moved to a glasshouse compartment at 13.5° mean daily mean temperature, in which natural winter daylight was supplemented to 18 hours daylength with mercury vapour lamps (HPLR) giving a light intensity of 7000 to 11,000 lux around mid-day. After four weeks they were moved to the main glasshouse and grown on for 20 weeks at 16.5° mean daily mean temperature.

Visible leaf number was measured at the start and finish of the three and six-week low temperature treatments. Date of flowering was recorded. The remaining non-flowering plants were dissected at the end of the experiment, 175 days after the end of low temperature treatment, and their bud stage assessed (see Figure 2.1).

Results

More plants flowered after six weeks low temperature treatment the higher the light intensity (χ^2 $p<0.05$) (Table 2.23) and more became reproductive (bud stage 3 and over) (χ^2 $p<0.02$, three and six weeks low temperature treatment) (Table 2.24) as shown on page 116.

TABLE 2.23: Experiment 12 - the effect of light intensity, duration of low temperature treatment and cultivar on the number of plants flowering. There were six plants in each treatment

Light intensity	Wilhelmsburger			Doon Major			Total of both cultivars
	Duration of low temperature			Duration of low temperature			
	3 weeks	6 weeks	Total	3 weeks	6 weeks	Total	
	No. plants flowering			No. plants flowering			No. plants flowering
Full light 11,800 lux	1	6	7	0	3	3	10
Half light 5000 lux	0	5	5	0	2	2	7
Quarter light 2300 lux	0	4	4	0	0	0	4
Darkness 0 lux	0	1	1	0	1	1	2
Total	1	16	17	0	6	6	23
Total of both cultivars	1	22	23				

TABLE 2.24: Experiment 12 - the effect of light intensity, duration of low temperature treatment and cultivar on the number of plants becoming reproductive (bud stage 3 and over, Figure 2.1). There were six plants in each treatment

Light intensity	Wilhelmsburger			Doon Major			Total of both cultivars
	Duration of low temperature			Duration of low temperature			
	3 weeks	6 weeks	Total	3 weeks	6 weeks	Total	
	No. plants reproductive			No. plants reproductive			No. plants reproductive
Full light 11,800 lux	4	6	10	2	6	8	18
Half light 5000 lux	3	6	9	1	4	5	14
Quarter light 2300 lux	3	6	9	0	5	5	14
Darkness 0 lux	1	6	7	0	1	1	8
Total	11	24	35	3	16	19	54
Total of both cultivars	14	40	54				

Flowering plants in the six weeks low temperature treatment:

most flowering *least flowering*

full light half light quarter light dark

Reproductive plants in the three and six weeks low temperature treatment:

more plants reproductive *fewer plants reproductive*

full light half light quarter light dark

A line joining two treatments shows that they are not significantly different at the 5 per cent level (χ^2).

Wilhelmsburger flowered more than Doon Major (χ^2 $p<0.01$, six weeks low temperature treatment) and more Wilhelmsburger plants became reproductive (χ^2 $p<0.01$, three and six weeks low temperature treatments).

Slightly more plants, 13 compared with 9, flowered in the replicate with higher light intensity, and slightly more, 30 compared with 24, became reproductive, but the differences were not significant.

The full light treatment flowered more rapidly than the half light treatment (Table 2.25). The results for quarter light and darkness are less reliable as they are based on four and two plants respectively. Wilhelmsburger flowered earlier than Doon Major.

Light intensity affected the production of visible leaves during the low temperature period, the higher the light intensity, the more leaves were produced (F test, effect of light intensity, $p<0.05$) (Table 2.26). Wilhelmsburger produced more leaves than Doon Major, but the difference was not significant.

TABLE 2.25: Experiment 12 - the effect of light intensity during six weeks low temperature treatment and cultivar, on days to first flowering. In the three-week low temperature treatment only one plant, Wilhelmsburger, treated in full light intensity, flowered in 175 days

Light intensity	Days to flowering		
	Wilhelms-burger	Doon Major	Mean
	Days	Days	Days
Full light 11,800 lux	131.8	152.3	138.7
Half light 5000 lux	151.2	170.5	156.7
Quarter light 2300 lux	133.5	NF	133.5
Darkness 0 lux	126.0	129.0	127.5
Mean	137.9	154.5	142.5

NF = no plants flowering in the treatment

TABLE 2.26: Experiment 12 - the effect of light intensity, duration of low temperature treatment and cultivar on the number of visible leaves produced during treatment

Light intensity	Wilhelmsburger			Doon Major			Mean of both cultivars		
	Duration of low temperature			Duration of low temperature			Duration of low temperature		
	3 weeks	6 weeks	Mean	3 weeks	6 weeks	Mean	3 weeks	6 weeks	Mean
	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves	No. of leaves
Full light 11,800 lux	2.00	4.33	3.17	0.67	5.50	3.08	1.33	4.92	3.12
Half light 5000 lux	1.33	4.00	2.67	1.00	3.67	2.33	1.17	3.83	2.50
Quarter light 2300 lux	1.50	3.33	2.42	0.67	3.67	2.17	1.08	3.50	2.29
Darkness 0 lux	0.50	3.17	1.83	0.17	2.00	1.08	0.33	2.58	1.46
Mean	1.33	3.71	2.52	0.63	3.71	2.17	0.98	3.71	2.34

For comparisons within table	SE ± 1.329	For comparisons of duration of low temperature treatments	SE ± 0.470
For comparisons of cultivars	SE ± 0.470	For comparisons of light intensity treatments	SE ± 0.664

Discussion

Light intensity affects vernalisation, and reducing light to a quarter during low temperature treatment cut flowering by about a half. Leaf production was slower at lower light intensity although plants produced leaves even in total darkness. As the plants were fairly old with well-developed roots, the effect of light intensity was probably not as great as it would have been on smaller plants almost totally dependent on photosynthesis for carbohydrate supply.

2.9 THE BOLTING RESISTANCE OF DIFFERENT CULTIVARS

The effect of duration of low temperature treatment on the flowering of five cultivars.

Experiment 13

In experiments 2, 3, 4, 5, 6, 9 and 12 Wilhelmsburger was more susceptible to vernalisation than Doon Major, and in no experiment has it been less susceptible. There is also evidence of differences between cultivars in bolting resistance in experiments by Peto (1934) and in field trials (Bell 1968).

To examine the susceptibility of more cultivars, Wilhelmsburger, Doon Major, Pentland Harvester, Ruta Otofte and Harrietfield seedlings were exposed to a range of durations of low temperature, 20, 24, 28, 32, 36 and 40 days. Pentland Harvester was included as it is known to be susceptible to bolting, flowering quite readily after 21 days low temperature treatment (see experiment 1). Wilhelmsburger is also a susceptible cultivar, and it and Pentland Harvester were given a 16-day low temperature treatment in addition to the other six duration of low temperature treatments. Ruta Otofte was included as an example of a high dry matter dark purple skin and because it is one of the highest yielding cultivars presently available, and Harrietfield was included as an example of an intermediate dry matter bronze skin.

In experiments 2 and 3, 20 to 40 days of low temperature gave a range of flowering percentages from 5 to 10 per cent up to 100 per cent for Wilhelmsburger and Doon Major. Over this range of response, in terms of increased numbers flowering to increased duration of low temperature treatment, differences between cultivars should be most easily detected.

Seeds were sown in 5.5 cm 'Jiffy' compressed peat pots and raised for 14 days at 16.2° mean daily mean temperature so that plants would be well beyond the juvenile stage at the start of low temperature treatment. They were treated in a growth chamber at 6.1° mean daily mean temperature, all treatments ending on the same day when the temperature was raised to 11.6° mean daily mean temperature for 14 days. The plants were moved to a glasshouse compartment at 14° mean daily mean temperature for 10 days and were then planted out in a field plot on 14 May, at 15 cm spacing, 60 cm between the rows. Five blocks ran the length of the growth cabinet table, parallel with the fluorescent lights, with mean light intensities 8500, 18,500, 15,500, 12,500 and 6000 lux, and the same design was used in the field, each block being a row. Treatments were randomised within each block with four plants per plot.

Visible leaf number was recorded at the beginning and end of low temperature treatment. Date of flowering was recorded, and the experiment ended 150 days after the end of low temperature treatment.

Results

Pentland Harvester had the highest percentage of flowering plants, 96 per cent, then Wilhelmsburger with 87 per cent, both excluding the 16-day low temperature treatment for the purposes of comparison with the other three cultivars, Harrietfield 76 per cent, Ruta Otofte 70 per cent and Doon Major 68 per cent (Figure 2.9).

The following analyses were done by Michael Franklin of the ARC Unit of Statistics. Transforming the proportion of number of plants flowering to total number of plants in each treatment to a logit scale ($\log_e p/1-p$) lines were fitted against duration of low temperature

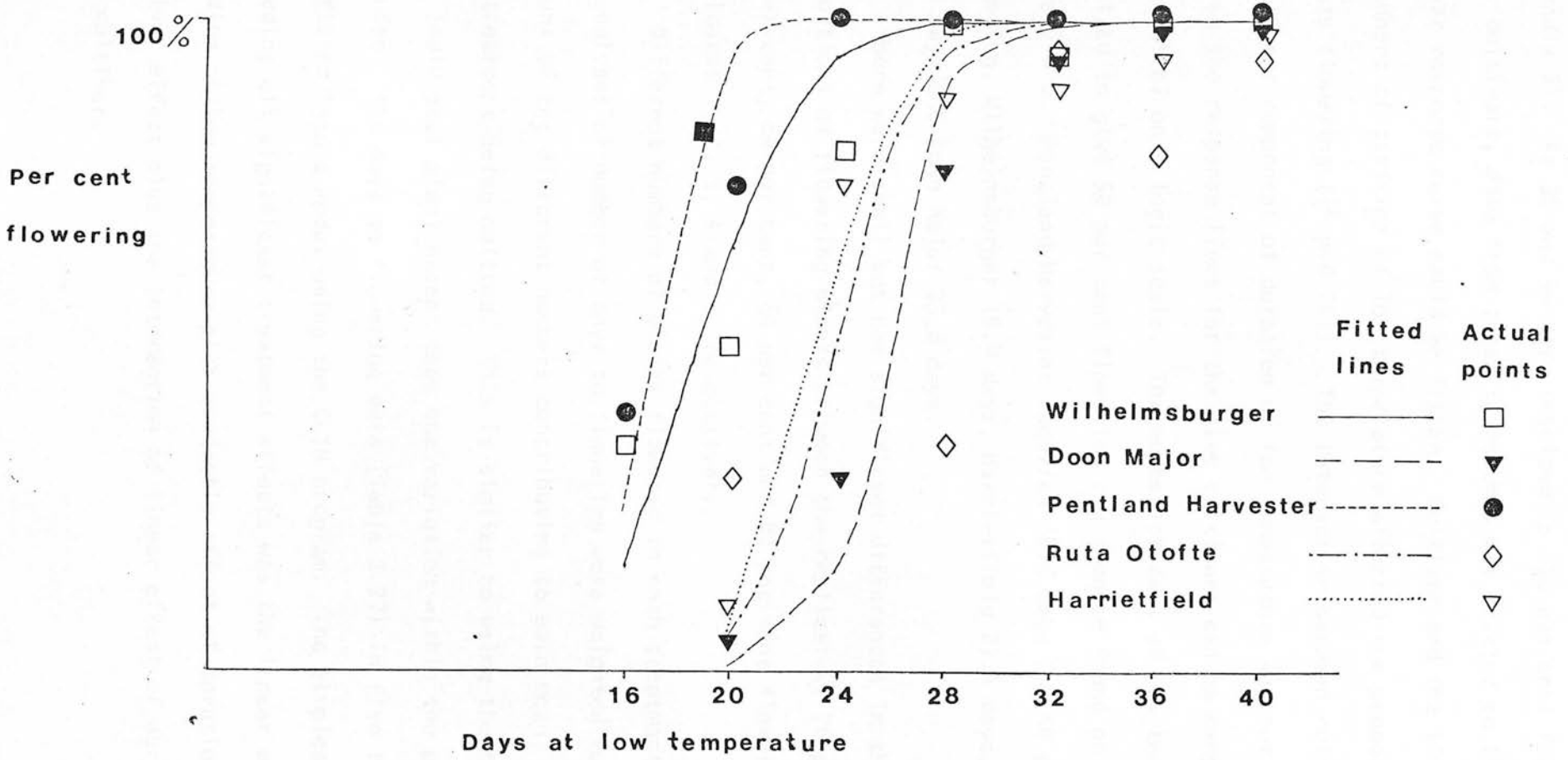


FIGURE 2.9: Experiment 13 - the effect of duration of low temperature treatment on the flowering of five swede cultivars. The fitted lines are from the GLIM model including the interaction of cultivar and linear component of duration

(in days) on the x axis, using the GLIM program (see experiment 2 and Appendix B). As 36 and 40 days resulted in 100 per cent flowering in most cultivars, data from these treatments was omitted so that a better linear response curve could be fitted. Cultivar and the linear component of duration of low temperature affected the proportions of plants flowering (χ^2 $p<0.001$). The interaction between cultivars and the linear component of duration of low temperature was not significant, and so the response lines for the five cultivars can be considered to be parallel on a logit scale. The number of days of low temperature required to give 50 per cent flowering can then be found on the x axis where $y = 0$. Pentland Harvester requires 16.8 days for 50 per cent flowering, Wilhelmsburger 19.4 days, Harrietfield 23.4 days, Ruta Otofte 24.0 days and Doon Major 25.8 days.

There were small but not significant differences in the proportions of flowering plants between the replicates, 76 per cent, 74 per cent, 84 per cent, 84 per cent and 80 per cent flowering in replicates 1, 2, 3, 4 and 5, respectively.

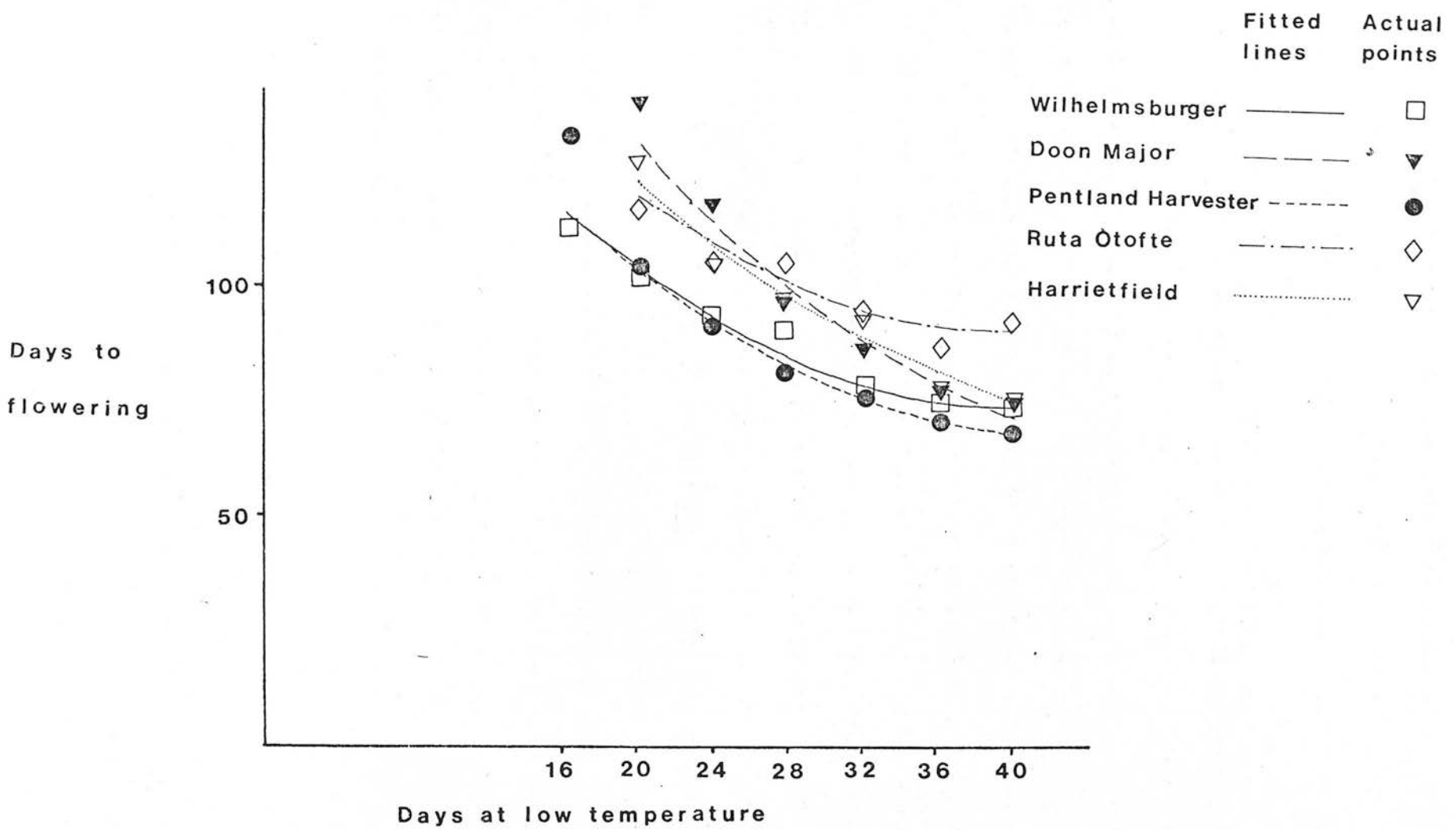
Different numbers of plants flowered in each treatment and so the analyses of number of days to flowering were weighted to take account of the different numbers contributing to each mean, the 16-day treatment being omitted. This is similar to using the data from each individual plant except that the variation within the plot is excluded. The days to flowering data (Table 2.27) in five replicates was fitted into a model using the GLIM program. The simplest model including all significant treatment effects was the linear effect of duration of low temperature plus quadratic effect of duration plus cultivar effect plus the interaction of linear effect of duration with cultivar.

TABLE 2.27: Experiment 13 - the effect of duration of low temperature on the number of days from the end of low temperature treatment to flowering of five cultivars, Wilhelmsburger, Doon Major, Pentland Harvester, Ruta Otofte and Harrietfield

Cultivar	Duration of low temperature (days)							Mean
	16	20	24	28	32	36	40	
	Days to flower							Days to flower
Wilhelmsburger	113.3	102.6	94.4	91.3	78.8	75.6	74.6	86.2
Doon Major	NP	153.0	117.5	97.3	87.7	77.6	73.9	86.6
Pentland Harvester	133.3	105.2	92.0	81.5	76.5	71.1	68.7	84.9
Ruta Otofte	NP	117.5	106.1	105.8	95.2	87.8	92.3	98.1
Harrietfield	NP	127.5	105.7	98.3	92.8	79.1	76.4	90.3
Mean	123.9	109.3	99.8	94.5	85.7	77.8	76.9	88.7

NP = no plants in this treatment

FIGURE 2.10: Experiment 13 - the effect of duration of low temperature treatment on the number of days from the end of low temperature treatment to flowering of five swede cultivars



Error mean square for this model was 186.68, with 19 df, and levels of significance in F tests were 0.1 per cent for cultivar and for linear effect of duration and 0.5 per cent for the quadratic effect of duration and the interaction of the linear effect of duration with cultivar.

The curves fitted to this model are shown in Figure 2.10. As duration of low temperature increases the time taken to flower decreases. The model is quadratic and so further increases in duration of low temperature should result eventually in increases in time taken to flower. This would be so if time to flower was calculated from the beginning of the period of low temperature, as very long durations of low temperature would slow up flower development. Time to flower is calculated from the end of low temperature treatment, however, and the real curves are probably asymptotic in nature, the asymptotic value being the minimum number of days at higher than vernalising temperatures required for the opening of flowers. As the fitted curves end at their low part, before the rise upwards again, the model fits the data reasonably well. Ruta Otofte was the slowest cultivar to flower, Harrietfield next, and Doon Major, Wilhelmsburger and Pentland Harvester taking about the same mean time to flower. Days to flowering of Doon Major and Harrietfield decreased more than in the other three cultivars as duration of low temperature treatment increased.

Production of visible leaves during low temperature treatment generally increased the longer the treatment (F test, effect of duration, $P < 0.001$) although the small size of the pots probably restricted growth in the longest duration treatment (Table 2.28). The five cultivars produced leaves at different rates (F test $p < 0.001$),

TABLE 2.28: Experiment 13 - the effect of cultivar on the mean number of leaves produced per plant during the low temperature treatment

Cultivar	Duration of low temperature (days)						Mean
	20	24	28	32	36	40	
	No. of leaves						No. of leaves
Wilhelmsburger	1.1	2.2	1.6	1.7	2.8	2.5	2.0
Doon Major	0.6	1.5	1.4	1.5	2.1	1.9	1.5
Pentland Harvester	1.3	2.0	1.8	2.0	2.7	3.0	2.1
Ruta Otofte	1.0	2.3	1.5	1.8	2.6	2.0	1.9
Harrietfield	0.9	1.9	1.7	1.8	2.5	1.8	1.8
Mean	1.0	2.0	1.6	1.8	2.5	2.3	1.9

For comparisons within table	SE ± 0.15	For comparisons of durations	SE ± 0.07
For comparisons of cultivars	SE ± 0.06		

with mean leaf production during low temperature treatment 0.071, 0.066, 0.062, 0.060 and 0.050 leaves per plant per day for Pentland Harvester, Wilhelmsburger, Ruta Otofte, Harrietfield and Doon Major respectively (mean of the mean daily production per plant in each of six durations of low temperature).

Discussion

The use of a range of durations of low temperature separates the five cultivars in terms of resistance to flower induction. There is not only variation between the cultivars but response to different durations of low temperature in Wilhelmsburger and Ruta Otofte was not very regular suggesting there is some variation within these cultivars.

Wilhelmsburger is an old variety and the seed stock used was not highly uniform, but Ruta Otofte is a recently bred cultivar.

The mean time taken to flower for each cultivar is related to bolting resistance in that weakly vernalised plants, that is exposed to shorter than optimum durations of low temperature, tend to flower later (see Table 2.27) and more resistant cultivars will have a higher proportion of weakly vernalised plants which will flower late and increase the mean time to flower of the cultivar. The relationship is not invariable as Ruta Otofte was very slow to flower although it had more flowering plants eventually than Doon Major, which flowered as rapidly as Wilhelmsburger and Pentland Harvester, the most susceptible cultivars.

The Pentland Harvester seed was older than that of the other cultivars, and germinated more slowly, but seedlings were only slightly smaller at the beginning of low temperature treatment.

Rate of production of leaves during low temperature treatment appears to be associated with susceptibility to flower induction, cultivars with higher rates of leaf production flowering more. Ruta Otofte flowered less than Harrietfield, and produced leaves at a greater rate, but there was little difference between the cultivars in either factor.

2.10 JUVENILITY IN FIVE CULTIVARS

The effect of plant age on the flowering of five swede cultivars.

Experiment 14

In experiment 2 juvenile plants (two days old and younger) of Doon Major flowered relatively better than Wilhelmsburger, 39.5 per cent compared with 30.6 per cent flowering for Wilhelmsburger, and in experiment 3 there was no evidence of juvenility in two-day old Doon Major seedlings whereas Wilhelmsburger two-day old seedlings showed strong juvenility. This suggests that Doon Major has a shorter juvenile phase than Wilhelmsburger. A small difference in the time taken to become adult and susceptible to vernalisation may affect flowering significantly if the flowering is in the range 20 to 80 per cent, caused usually by 20 to 30 days low temperature depending on other environmental conditions and on cultivar. If cultivars differ in the duration of their juvenile stages, comparisons of bolting resistance of adult plants will differ from comparisons of the same cultivars or lines if the plants are juvenile at the start of treatment.

The following experiment was carried out to test the relative importance of juvenility in the flowering of five cultivars. Wilhelmsburger, Doon Major, Pentland Harvester, Ruta Otofte and Harrietfield cultivars were used as their bolting resistance was known (see experiment 13) for adult plants. Two plant ages were compared, 20-day old plants, certainly adult, and 0-day old plants so that even very short juvenile phases would have an effect in the experiment. Long or short juvenile phases would be distinguished

quantitatively as plants attaining adulthood earlier would pass more of the low temperature period in a receptive state and therefore be more likely to flower. Two durations, 32 and 28 days of low temperature, were used to increase the chance of differentiating treatments. There were two blocks, one in the better illuminated centre of the growth chamber table, the second at the ends of the table, and seven plants per plot.

The adult plants were raised for 20 days in the glasshouse at 16° mean daily mean temperature and the juvenile (0 day old plants) were moved into low temperature immediately after sowing. Plants were grown at 8.5° mean daily mean temperature for 32 or 28 days, both treatments ending on the same day and then moved to a glasshouse compartment and grown for 14 days at 11.3° mean daily mean temperature. They were then moved to a glasshouse bed at 15.8° mean daily mean temperature, repotted to 12.5 cm pots and after 95 days repotted to 16.5 cm pots.

The date of emergence of the juvenile plants and the date of flowering were recorded. At the end of the experiment, 138 days after the end of low temperature treatment, the stem height of all remaining plants was measured, and the apices were dissected and bud stage assessed (see Figure 2.1).

Results

All cultivars showed a marked juvenile phase both in the numbers of plants flowering (χ^2 $p<0.001$) (Table 2.29) and the numbers of plants reproductive (bud stage 3 and over) (χ^2 $p<0.001$) (Table 2.30). Only six juvenile (0 day old) plants were reproductive by the end of the experiment, four Harrietfield and two Pentland Harvester, including one flowering plant of both of these cultivars.

TABLE 2.29: Experiment 14 - the effect of duration of low temperature and plant age at the start of low temperature treatment on the number of plants flowering of five cultivars (14 plants per treatment)

Cultivar	Duration of low temperature				Total of both durations		Overall total
	28 days		32 days				
	0 day old plants	20-day old plants	0 day old plants	20 day old plants	0 day old plants	20-day old plants	
	No. of plants flowering		No. of plants flowering		No. of plants flowering		No. of plants flowering
Wilhelmsburger	0	5	0	7	0	12	12
Doon Major	0	1	0	2	0	3	3
Pentland Harvester	0	7	1	13	1	20	21
Ruta Otofte	0	0	0	2	0	2	2
Harrietfield	0	0	1	5	1	5	6
Total	0	13	2	29	2	42	44
Total of both plant ages	13		31		44		

TABLE 2.30: Experiment 14 - the effect of duration of low temperature and plant age at the start of low temperature treatment on the number of reproductive plants (bud stage 3 and over) (see Figure 2.1) at the end of the experiment. (14 plants per treatment.)

Cultivar	Duration of low temperature				Total of both durations		Overall total
	28 days		32 days				
	0 day old plants	20-day old plants	0 day old plants	20-day old plants	0 day old plants	20-day old plants	
	No. of plants reproductive		No. of plants reproductive		No. of plants reproductive		No. of plants reproductive
Wilhelmsburger	0	6	0	12	0	18	18
Doon Major	0	5	0	10	0	15	15
Pentland Harvester	0	12	2	14	2	26	28
Ruta Otofte	0	2	0	3	0	5	5
Harrietfield	0	2	4	12	4	14	18
Total	0	27	6	51	6	78	84
Total of both plant ages	27		57		84		

Too few juvenile plants flowered for a comparison to be made between the cultivars, but if reproductive plants are included there are some differences in juvenility between the cultivars, Harrietfield in particular having relatively more reproductive juvenile plants than the other cultivars. The differences between the five cultivars in proportions of adult to juvenile plants becoming reproductive were not highly significant (χ^2 $p<0.1$). If either Pentland Harvester, the cultivar with most reproductive plants, or Ruta Otofte, the cultivar with least, are omitted, the probability of the differences being due to chance drops to $p<0.05$, and if Wilhelmsburger, Doon Major and Harrietfield are compared, the probability falls to $p<0.025$.

Pentland Harvester had the highest proportion of plants flowering, followed by Wilhelmsburger, Harrietfield, Doon Major and then Ruta Otofte, and the order was the same for reproductive plants. This is the same as in experiment 13, except the order of Doon Major and Ruta Otofte, which is the reverse.

Flowering was slower after 28 days low temperature (Table 2.31), Pentland Harvester flowered earliest, Harrietfield next, then Ruta Otofte, Wilhelmsburger and last Doon Major. The figures for Ruta Otofte and Doon Major are based on only two and three plants respectively and therefore carry less weight.

Stem height of plants at the end of the experiment, including stem height at flowering of plants which flowered is given in Table 2.32. Many plants had long stems, even though they had a vegetative terminal apex, suggesting that stem growth induction was greater than flower induction, or survived subsequent devernalisation better. Increased duration of low temperature increased stem height (F test $p<0.005$), adult plants had taller stems than juvenile plants (F test

TABLE 2.31: Experiment 14 - the effect of duration of low temperature treatment and plant age at the start of treatment on the number of days from the end of low temperature treatment to flowering of five cultivars

Duration of low temperature	Wilhelmsburger		Doon Major		Pentland Harvester		Ruta Otofte		Harrietfield		Mean of all cultivars		Overall mean
	Plant age		Plant age		Plant age		Plant age		Plant age		Plant age		
	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	
	Days to flower		Days to flower		Days to flower		Days to flower		Days to flower		Days to flower		Days to flower
28 days	NF	130.0	NF	127.0	NF	78.3	NF	NF	NF	NF	NF	101.9	101.9
32 days	NF	87.3	NF	131.0	117.0	85.8	NF	97.0	106.0	91.0	111.5	90.9	92.3
Mean	NF	105.1	NF	129.7	117.0	83.1	NF	97.0	106.0	91.0	111.5	94.3	95.1
Overall mean	105.1		129.7		84.8		97.0		93.5		95.1		95.1

NF = no plants flowering in the treatment

TABLE 2.32: Experiment 14 - the effect of duration of low temperature treatment and plant age at the start of treatment on the stem height, measured at the end of the experiment, of five cultivars

Duration of low temperature treatment	Wilhelmsburger		Doon Major		Pentland Harvester		Ruta Otofte		Harrietfield		Mean of all cultivars		Overall mean
	Plant age		Plant age		Plant age		Plant age		Plant age		Plant age		
	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	
	cm		cm		cm		cm		cm		cm		cm
28 days	18.2	54.4	14.2	24.3	22.7	68.7	14.2	18.2	17.1	20.4	17.3	37.2	27.1
32 days	19.4	71.7	16.9	43.4	31.4	100.6	14.1	30.5	26.3	63.1	21.6	61.8	41.7
Mean	18.8	63.0	15.5	33.8	27.0	84.6	14.2	24.4	21.7	41.7	19.5	49.5	34.5
Overall mean	41.3		24.7		55.8		19.3		31.7		34.5		34.5

For comparisons within table SE ± 4.94

For comparisons of cultivar means SE ± 2.47

For comparisons of plant age and duration of low temperature SE ± 3.50

$p<0.001$) and cultivar affected stem height (F test $p<0.001$), Pentland Harvester having the tallest stems, then Wilhelmsburger, Harrietfield, Doon Major and shortest, Ruta Otofte.

There was an interaction in stem height between plant age and duration of low temperature (F test $p<0.05$), adult plants responding more to increased duration than juvenile plants, and between cultivars and plant age (F test $p<0.025$), Wilhelmsburger and Pentland Harvester adult plants being considerably taller than juvenile plants, whereas in Ruta Otofte and Harrietfield plants there was much less difference in stem height between adult and juvenile plants. As some of these effects might have been due to the numbers of flowering plants in each treatment, these being much taller than non-flowering plants, a rough analysis was made of the stem heights of all non-flowering plants. The two blocks were pooled to increase the number of plants contributing to each mean, and the treatments compared against the third order interaction as the error term, but no attempt was made to weight the analysis. The duration of low temperature, age and cultivar treatments had similar effects on stem height of non-flowering plants as on stem height of all plants and the differences were significant (F tests $p<0.05$), but the interactions were not significant although similar in trend to those of stem heights of all plants. This suggests that differences in stem height of all plants are not largely due to the different numbers of flowering plants in each treatment but to treatment effects on the stems of all plants.

The mean times from sowing to emergence were 8.2 days, 8.5 days, 8.5 days, 9.1 days and 11.6 days for Wilhelmsburger, Doon Major, Ruta Otofte, Harrietfield and Pentland Harvester, respectively.

Discussion

All cultivars exhibited a juvenile stage, but that of Harrietfield is probably shorter than in other cultivars. The experiment unfortunately did not differentiate between the other four cultivars although evidence from experiment 3 suggests that the juvenile stage of Wilhelmsburger is longer than that of Doon Major. The Pentland Harvester seed was old (1972 compared with 1975 or 1976 for the other four cultivars), with emergence on average three days later, and it is possible that more Pentland Harvester juvenile plants might have flowered if they had emerged earlier. Harrietfield was slightly later to emerge than some other cultivars and so its apparently slightly shorter juvenile stage cannot be caused by earlier emergence. Similarly, the difference in juvenility observed in experiment 3 between Wilhelmsburger and Doon Major is unlikely to be an effect of time to emergence as there was little difference between the cultivars, if anything Doon Major being slower in development.

As in previous experiments, longer durations of low temperature resulted in more rapid flowering.

Stem height at the end of the experiment was much shorter in juvenile plants and there was some evidence of an interaction between cultivars and plant age to support the hypothesis that cultivars differ in the duration of their juvenile phases, with Wilhelmsburger having the greatest difference between adult and juvenile stem heights, Doon Major and Harrietfield next, and Ruta Otofte least, although Ruta Otofte is resistant to vernalisation and had the shortest stems overall. Pentland Harvester showed a marked difference in stem heights of adult and juvenile plants when all plants were included, but much less when flowering plants were excluded and so there is less evidence

of a difference between juvenile and adult plants in stem height when not linked with flowering, in Pentland Harvester.

2.11 DEVERNALISATION BY DAILY INTERRUPTIONS OF LOW TEMPERATURE TREATMENT

The effect of interruptions of vernalisation by a daily period at high temperature on the flowering of six swede cultivars.

Experiment 15

In field conditions the temperature is not constant and although night temperatures may be vernalising, during the day temperatures may rise high enough to devernalise. Any assessment of the effect of a period of natural low temperature must take into account the reversing effect of periods at a higher temperature. Daily periods of high temperature considerably reduced the effectiveness of low temperature flower induction in cabbages (Heide 1970) and winter rye (Purvis and Gregory 1952) but the sensitivity of swedes to daily interruptions of low temperature treatment is not known, although weekly interruptions of one or two days in a period of vernalisation considerably reduced flowering (see experiment 9).

Wilhelmsburger and Doon Major react differently to temperature of vernalisation (see experiments 4 and 5) and it is possible that they might respond differently to devernalising temperatures. If cultivars have different sensitivities to devernalisation, a treatment of continuous low temperature would not give a good indication of how cultivars would behave in normal field conditions. To test this possibility six cultivars were used, Wilhelmsburger, Doon Major, Ruta Otofte, Marian, Della and Seefelder. Ruta Otofte was used again as it is a high yielding swede, and Marian, a purple top medium high dry matter swede, Della, a green top high dry matter swede, and Seefelder,

a green top medium high dry matter swede, were used as they are all recently introduced cultivars with good yields.

Three low temperature treatments were used, 6° continuously, 6° with a daily four-hour interruption at 16°, and 6° with a daily four-hour interruption of 22°, each treatment occupying one growth cabinet. These treatments did not reproduce field conditions very realistically, but would indicate the extent to which a brief daily period of higher temperature can reduce vernalisation. Six durations of low temperature were used, 24, 30, 36, 42, 48 and 54 days, the two interrupted treatments starting each duration earlier than the continuous 6° treatment so that the number of hours spent at 6° would be the same in all three treatments and all treatments would end on the same day. Long durations of low temperature were included as it was expected that flowering would be considerably reduced by the interruptions at high temperature.

Plants were sown in 5.5 cm 'Jiffy' pots and grown for 10 days at 18° mean daily mean temperature in a glasshouse bed before the start of the treatments. There were six plants per treatment with no replicates, in a split-plot design, the temperature treatments (growth cabinets) being the main plots and duration of low temperature and cultivar treatments the sub-plots.

Daylength was 18 hours with the four-hour interruption occurring in the middle of the day, from 10.00 to 14.00.

At the end of low temperature treatment the temperature was raised to 13° in all cabinets and the three temperature treatments re-randomised among the three growth cabinets. After 12 days at 13° the swedes were planted out on the 27 May in a field plot in 75 cm rows, with 18 cm between the plants.

Date of flowering was recorded. The experiment ended 100 days after the end of low temperature treatment, and the flower stage of all non-flowering plants was assessed (see experiment 2).

To compare the growth of the six cultivars at 6^{o}, 36 four-week seedlings of each cultivar were grown for 27 days in the same cabinet in which the original continuous 6^{o} treatment was carried out. There were four plants per plot and nine replicates. Visible leaf number was measured at the beginning and end of the 27-day period of growth at 6^{o}.

Results

Interrupting the low temperature treatment with four hours daily at a higher temperature reduced the number of plants flowering especially when the interruption was at the higher temperature, 22^{o} (Figure 2.11).

The flowering (Table 2.33), transformed to a logit scale, was analysed with the GLIM program, as in experiment 2 and in Appendix B. Linear and quadratic components of the effect of duration of temperature treatment on flowering were significant (χ^2 $p<0.001$). The longer the duration, the greater the proportion of plants flowering, although the increase tailed-off after 42 days as treatments responded with virtually 100 per cent flowering, giving a quadratic shape to the curve. The cultivars differed significantly in proportion of plants flowering (χ^2 $p<0.001$) as did the three temperature treatments, plants treated in continuous 6^{o} flowering most and those receiving a daily four-hour interruption at 22^{o} flowering least (χ^2 $p<0.001$). The linear component of duration was much greater than the quadratic component. Between 24 and 36 days low temperature the curve is near

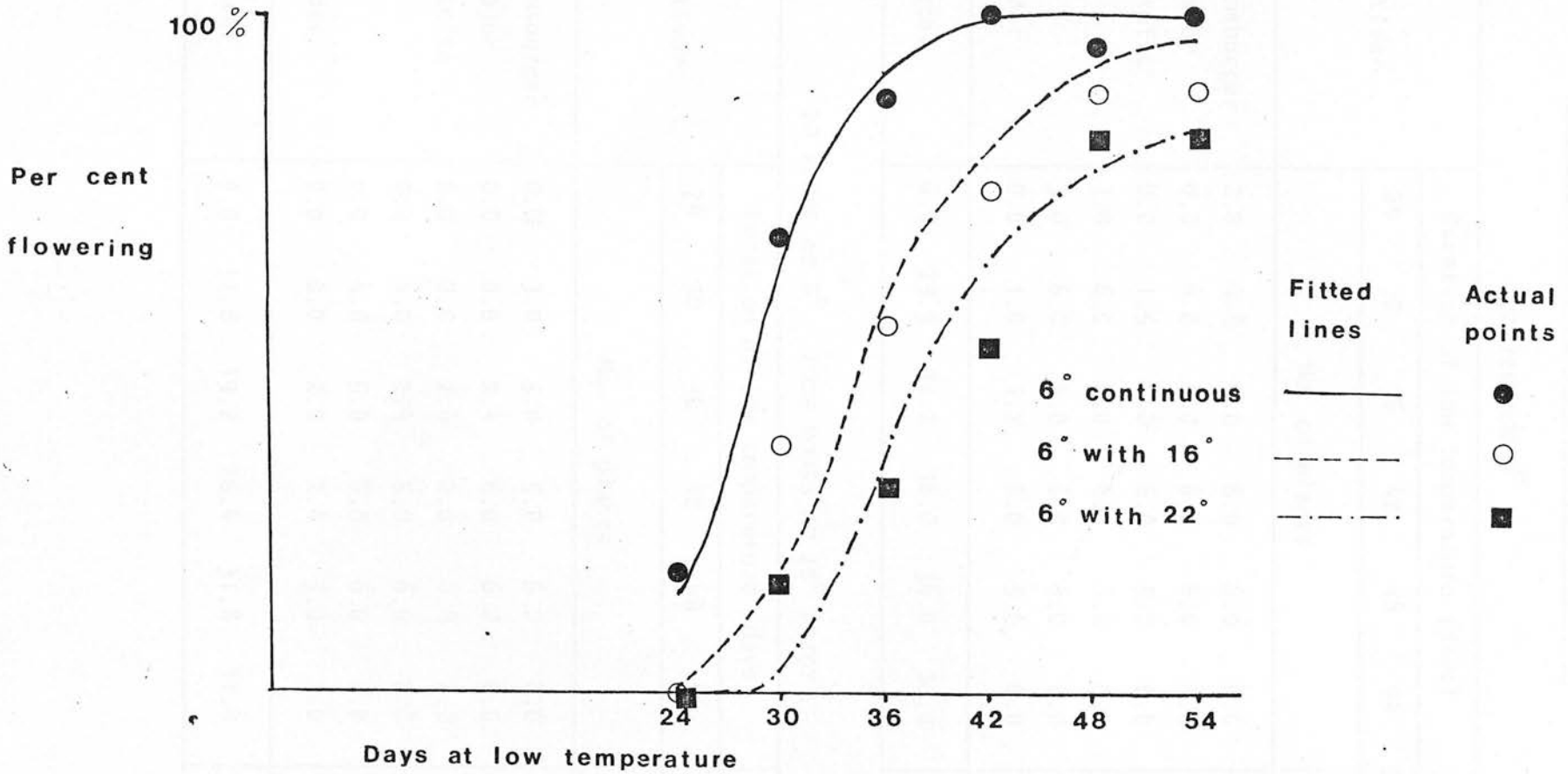


FIGURE 2.11: Experiment 15 - the effect of duration of low temperature treatment on the flowering of swedes in three temperature treatments, 6° continuous and 6° with daily four interruptions at 16° or 22° (means of six cultivars)

TABLE 2.33: Experiment 15 - the effect of daily interruptions of low temperature treatment with periods at higher temperature, cultivar and duration of low temperature treatment on the proportion of plants flowering out of six

CONTINUOUS 6°

Cultivar	Duration of low temperature (days)						Total
	24	30	36	42	48	54	
	No. of plants						No. of plants
Wilhelmsburger	2.0	4.8	6.0	6.0	6.0	6.0	30.8
Doon Major	0.0	4.0	6.0	6.0	6.0	6.0	28.0
Ruta Otofte	0.0	1.5	6.0	6.0	5.0	6.0	24.5
Marian	1.0	6.0	6.0	6.0	6.0	6.0	31.0
Della	3.0	6.0	6.0	6.0	6.0	6.0	33.0
Seefelder	0.0	1.0	1.2	6.0	5.0	6.0	19.2
Total	6.0	23.3	31.2	36.0	34.0	36.0	166.5

20 HOURS AT 6° FOUR HOURS AT 16° DAILY

Cultivar	Duration of low temperature (days)						Total
	24	30	36	42	48	54	
	No. of plants						No. of plants
Wilhelmsburger	0.0	3.0	5.0	5.0	6.0	6.0	25.0
Doon Major	0.0	0.0	2.4	6.0	6.0	6.0	20.4
Ruta Otofte	0.0	0.0	2.4	2.0	4.8	4.8	14.0
Marian	0.0	4.0	2.4	5.0	6.0	6.0	23.4
Della	0.0	4.8	5.0	6.0	6.0	6.0	27.8
Seefelder	0.0	0.0	2.0	2.4	3.0	3.0	10.4
Total	0.0	11.8	19.2	26.4	31.8	31.8	121.0

TABLE 2.33: continued

20 HOURS AT 6° FOUR HOURS AT 22° DAILY

Cultivar	Duration of low temperature (days)						Total
	24	30	36	42	48	54	
	No. of plants						No. of plants
Wilhelmsburger	0.0	1.5	2.0	3.0	6.0	6.0	18.5
Doon Major	0.0	0.0	1.2	4.0	6.0	6.0	17.2
Ruta Otofte	0.0	0.0	1.0	1.0	2.0	4.8	8.8
Marian	0.0	2.4	3.0	4.0	6.0	6.0	21.4
Della	0.0	2.0	4.5	4.8	6.0	6.0	23.3
Seefelder	0.0	0.0	0.0	2.0	4.0	1.0	7.0
Total	0.0	5.9	11.7	18.8	30.0	29.8	96.2

TOTAL OF ALL TEMPERATURE TREATMENTS

Cultivar	Duration of low temperature (days)						Total
	24	30	36	42	48	54	
	No. of plants						No. of plants
Wilhelmsburger	2.0	9.3	13.0	14.0	18.0	18.0	74.3
Doon Major	0.0	4.0	9.6	16.0	18.0	18.0	65.6
Ruta Otofte	0.0	1.5	9.4	9.0	11.8	15.6	47.3
Marian	1.0	12.4	11.4	15.0	18.0	18.0	75.8
Della	3.0	12.8	15.5	16.8	18.0	18.0	84.1
Seefelder	0.0	1.0	3.2	10.4	12.0	10.0	36.6
Total	6.0	41.0	62.1	81.2	95.8	97.6	383.7

to a straight line and the 50 per cent flowering point, where the curve on the logit scale cuts the x-axis, can be calculated from the predicted logit values for 24 and 36 days, assuming the curve is a straight line between these points. These values, the number of days of treatment at 6^{o}, 6^{o} with interruptions at 16^{o}, and 6^{o} with interruptions at 22^{o}, required to give 50 per cent flowering are:

Cultivar	6^{o} continuous	6^{o} with 16^{o} interruptions	6^{o} with 22^{o} interruptions
Wilhelmsburger	26.7 days at 6^{o}	32.9 days at 6^{o}	36.0 days at 6^{o}
Doon Major	28.8	35.0	38.0
Ruta Otofte	33.2	39.5	42.5
Marian	26.2	32.4	35.4
Della	23.5	29.8	32.8
Seefelder	35.4	41.6	44.6

In the interrupted treatments the number of days is that number of units of 24 hours at 6^{o}, with daily four-hour interruptions adding to the time shown, for instance for Wilhelmsburger interrupted with 16^{o}, 39.5 (39.48) days of 20 hours at 6^{o} and four hours at 16^{o}, are necessary for 50 per cent flowering, or 789.6 hours at 6^{o} which is 32.9 units of 24 hours at 6^{o}. The following table shows the number of calendar days, that is 24 hours including in the interrupted treatments, 4 hours at the higher temperature, in each treatment required to cause 50 per cent flowering. The relative susceptibilities of the cultivars to vernalisation remain the same in the three temperature treatments, and there was no interaction between cultivar and temperature treatment in the analysis. Della was the most susceptible cultivar, then Marian, Wilhelmsburger, Doon Major, Ruta Otofte and Seefelder the most resistant. Comparing the observed percentage of plants flowering,

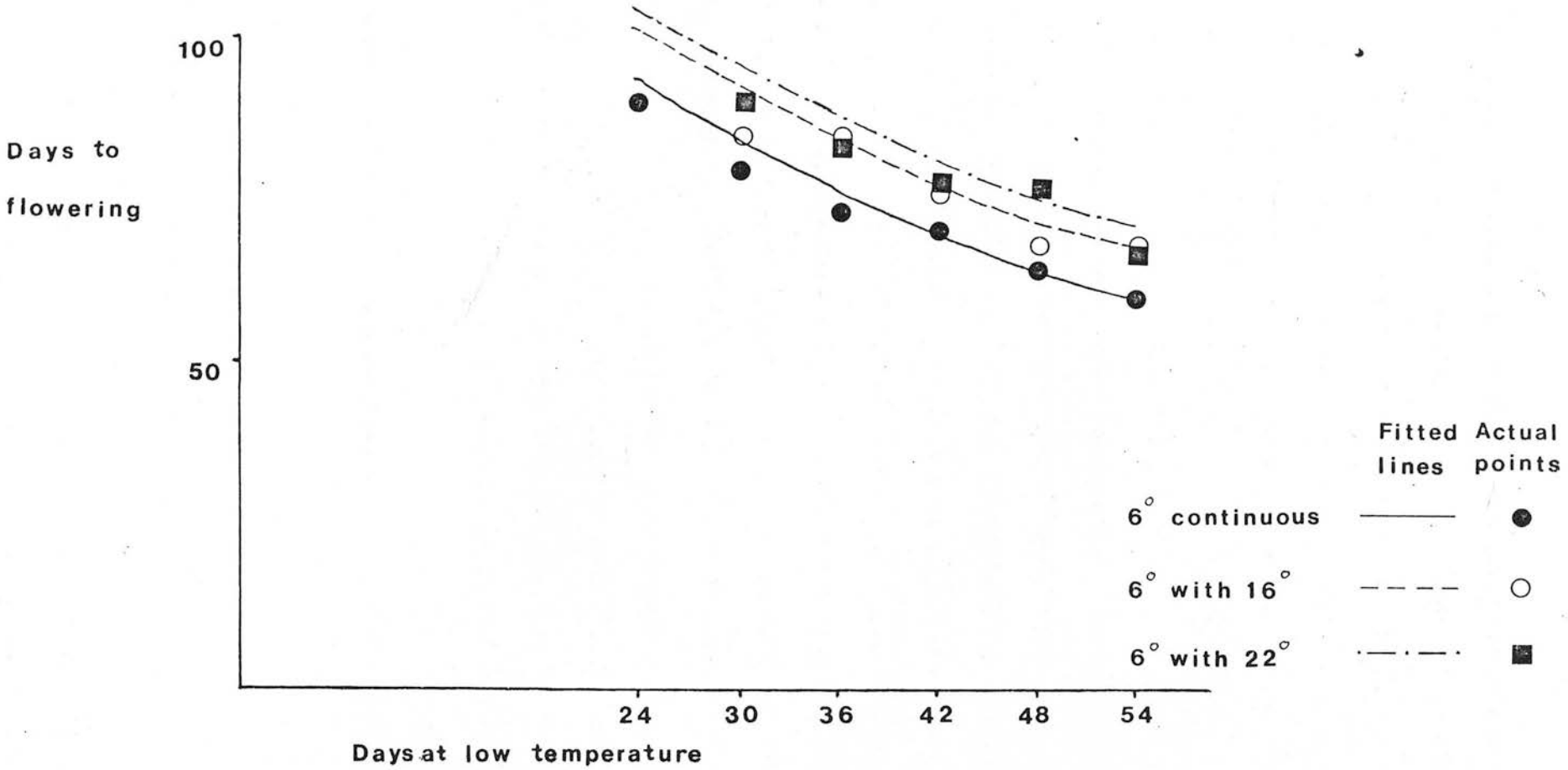
Cultivar	6° continuous	6° with 16° interruptions	6° with 22° interruptions
Wilhelmsburger	26.7 days at 6°	39.5 days at 6° and 16°	43.2 days at 6° and 22°
Doon Major	28.8	42.0	45.6
Ruta Otofte	33.2	47.4	51.0
Marian	26.2	38.9	42.5
Della	23.5	35.7	39.3
Seefelder	35.4	49.9	53.5

a slightly higher proportion of Wilhelmsburger plants flowered than Marian, but the differences between the two cultivars was very small, the observed percentages of all cultivars being 82, 73, 71, 70, 45 and 34 per cent for Della, Wilhelmsburger, Marian, Doon Major, Ruta Otofte and Seefelder.

When the number of plants with visible flower buds (flower stages 3 and 4, see experiment 2) 100 days after the end of low temperature treatment are included with the number of plants flowering the cultivars are still in the same order of susceptibility to flower induction, Della with 93 per cent, Wilhelmsburger 90 per cent, Marian 86 per cent, Doon Major 81 per cent, Ruta Otofte 64 per cent and Seefelder 60 per cent, and flower bud development and flowering is decreased by interruptions at high temperature, especially at 22°. Extending the time in which flowering was recorded would not therefore have altered the results of the experiment to any great extent.

Days to flowering were analysed as in experiment 2 (and see Appendix B) using GLIM, with the number of plants contributing to the mean used as a weight for that mean. The number of days to flowering from the end of low temperature treatment was affected by cultivar and temperature treatments (F tests $p<0.001$) and by the linear and quadratic components of duration of treatment (F tests $p<0.001$ for

FIGURE 2.12: Experiment 15 - the effect of duration of low temperature treatment on the number of days from the end of low temperature treatment to flowering of swedes in three temperature treatments, 6° continuous and 6° with daily four-hour interruptions at 16° or 22° (means of six cultivars)



the linear component, and $p<0.05$ for the quadratic component). Unlike experiment 13, there was no significant interaction between cultivar and duration of low temperature treatment, days to flowering decreasing as duration of low temperature treatment increased, at the same rate for all cultivars. With longer durations, the decrease in time to flower was smaller giving the slight quadratic shape to the line. Interruptions of the 6° low temperature treatment at 16° and especially at 22° delayed flowering (Figure 2.12 and Table 2.34).

Della flowered earliest, then Marian, Doon Major, Wilhelmsburger, Ruta Otofte and Seefelder, in both observed and fitted values.

The number of leaves 1 cm long and over produced by the six cultivars during 27 days at 6° was 0.085, 0.083, 0.088, 0.100, 0.090 and 0.077 leaves per plant per day for Wilhelmsburger, Doon Major, Ruta Otofte, Marian, Della and Seefelder respectively, and the effect of cultivar on leaf production was significant (F test $p<0.025$). When the means for each cultivar were compared using the least significant difference (0.00138 for $p<0.05$) only Marian was significantly different from most other cultivars, as shown below. Cultivars joined by a line are not significantly different at the 5 per cent level.

Marian Della Ruta Otofte Wilhelmsburger Doon Major Seefelder

Discussion

Interrupting a period of low temperature with four hours daily at a higher temperature reduced the proportion of plants flowering and increased the number of days to flowering, even although the number of hours spent at 6° was the same. Interruptions at 22° decreased flowering and rate of flowering more than 16°. The high

TABLE 2.34: Experiment 15 - the effect of daily interruptions of low temperature treatment with periods at higher temperature, cultivar, and duration of low temperature treatment on the number of days from the end of low temperature treatment to flowering

CONTINUOUS 6°

Cultivar	Duration of low temperature (days)						Mean
	24	30	36	42	48	54	
	Days to flower						Days to flower
Wilhelmsburger	93	90	75	75	61	58	72
Doon Major	NF	85	73	67	64	58	68
Ruta Otofte	NF	92	86	75	75	63	75
Marian	88	77	70	62	61	55	66
Della	89	69	65	62	58	53	64
Seefelder	NF	85	89	87	70	69	76
Mean	90	80	74	71	64	60	70

20 HOURS AT 6° FOUR HOURS AT 16° DAILY

Cultivar	Duration of low temperature (days)						Mean
	24	30	36	42	48	54	
	Days to flower						Days to flower
Wilhelmsburger	NF	84	88	74	72	71	77
Doon Major	NF	NF	82	75	75	65	73
Ruta Otofte	NF	NF	96	79	74	83	82
Marian	NF	84	78	84	61	67	73
Della	NF	86	75	63	60	57	67
Seefelder	NF	NF	100	96	70	77	83
Mean	NF	85	85	76	68	69	74

NF = no plants flowering in the treatment

TABLE 2.34: continued

20 HOURS AT 6° FOUR HOURS AT 22° DAILY

Cultivar	Duration of low temperature (days)						Mean
	24	30	36	42	48	54	
	Days to flower						Days to flower
Wilhelmsburger	NF	92	85	76	69	70	74
Doon Major	NF	NF	99	84	75	70	78
Ruta Otofte	NF	NF	88	80	92	73	81
Marian	NF	86	74	74	72	64	72
Della	NF	94	84	67	69	61	71
Seefelder	NF	NF	NF	95	94	95	95
Mean	NF	91	83	78	76	68	76

MEAN OF ALL TEMPERATURE TREATMENTS

Cultivar	Duration of low temperature (days)						Mean
	24	30	36	42	48	54	
	Days to flower						Days to flower
Wilhelmsburger	93	88	82	75	67	66	74
Doon Major	NF	85	78	74	71	64	72
Ruta Otofte	NF	92	89	77	78	72	78
Marian	88	81	72	72	65	62	70
Della	89	79	73	64	63	57	67
Seefelder	NF	85	96	91	78	74	82
Mean	90	83	79	74	69	65	72

NF = no plants flowering in the treatment

temperature must reverse some, but not all, of the effect of the previous 20 hours at 6°. In spring, daily temperatures are unlikely to reach 22° but might reach 16° fairly often, and a period of four hours each day at 16° reduced flowering by 24 per cent (38 per cent for 22°) given the same total number of hours at 6°.

There was a wide range of susceptibility to flower induction in the six cultivars, and over 11 days difference between the number of days at continuous 6° required to cause 50 per cent flowering in Della, the most susceptible cultivar, and in Seefelder, the most resistant cultivar. The more resistant the cultivar, the slower was flowering, except for Doon Major which flowered slightly earlier than Wilhelmsburger although having a lower percentage of flowering plants than Wilhelmsburger.

As in experiment 13, although there was initially a linear response to increasing duration of low temperature in decreasing days to flowering, at the longer durations the response curve became less steep. In experiment 13, analysed by Michael Franklin of the ARC Unit of Statistics, the longest durations, 36 and 40 days low temperature, were not included in the analysis of number of plants flowering and the response on a logit scale, to increasing duration of low temperature, was linear. When the longest durations 48 and 54 days were omitted in this experiment, the quadratic component of the effect of duration was still significant (χ^2 $p<0.01$) and so there was no advantage in discarding any duration of low temperature treatments.

The order of decreasing leaf production at 6° of the six cultivars was similar to the order of decreasing susceptibility to flower induction, with Marian and Della, two of the most susceptible cultivars, having the highest rate of leaf production and Doon Major

and Seefelder, two of the most resistant cultivars, having the lowest rate. The correlation between the rate of leaf production and the percentage of flowering for each cultivar was not, however, significant.

The experiment was unreplicated and so the residual variation, or deviance, left after subtracting the main effects duration, cultivar, and temperature treatment was used as the error term for the analysis of days to flowering. This error is really the second and third order interactions. Although it is possible that the third order interaction, which could not be tested, was significant, the deviance that could possibly be attributed to a third order interaction was much less than that attributable to the effects which were considered to be significant. The proportion of plants flowering was assumed to have a binomial error and the mean deviances removed by treatment effects compared directly with χ^2 with no reference to the residual deviance and so replicates would not improve the analysis. In this case the main treatments removed so much of the deviance in the proportion of plants flowering model that it was not possible that a third order interaction could be significant.

2.12 SELECTION OF RESISTANT AND SUSCEPTIBLE LINES

The effect of three durations of low temperature treatment on the flowering of progeny of early and late flowering Wilhelmsburger and Doon Major swedes, and their parents.

Experiment 16

In experiments 13, 14 and 15 cultivars that were most susceptible to flowering tended to flower earlier than more resistant cultivars. Selecting early or late flowering plants might be a convenient method of selecting for bolting susceptibility or resistance. It would have the advantage that the seed could be collected in that year whereas selecting non-bolters from a population of bolting plants involves exposing the plants to a further period of cold before seed can be obtained. Early flowering, however, is not invariably associated with susceptibility to flower induction, for instance in experiments 13 (fitted values) and 15, Doon Major flowered earlier than Wilhelmsburger, especially after longer low temperature treatments, although in experiments 6, 11, 12 and 14 Doon Major flowered later. Flowering is usually slower after shorter periods of low temperature and within a duration of low temperature treatment, the latest flowering plants might be the most resistant, that is those for which that period of low temperature is less effective.

Early and late flowering plants in experiment 3 were selected, covered in a muslin bag when the first flowers opened, and their seed collected. Age treatments were ignored as they did not show any marked effect on time to flower either in experiments 2 or 3 and so the two earliest flowering plants in each cultivar times duration of low temperature treatment were selected. The two latest flowering

plants were also selected, but if not all plants had flowered two non-flowering plants were left in the field for further exposure to low temperature and then moved to a cool glasshouse till flowering and seed ripening were completed.

Seed from the following 15 lines was used:

	Line	Duration of low temperature treatment	Date of flowering		Date of harvest
1	Early flowering Wilhelmsburger	20 days	19/7/76	2nd to flower	29/10/76
2	"	28 days	10/5/76	1st to flower	24/ 8/76
3	"	28 days	17/5/76	2nd to flower	24/ 8/76
4	"	32 days	10/5/76	2nd to flower	24/ 8/76
5	"	36 days	8/5/76	1st to flower	24/ 8/76
6	Late flowering Wilhelmsburger	32 days	2/8/76	2nd last to flower	29/10/76
7	"	40 days	8/7/76	2nd last to flower	24/ 8/76
8	"	40 days	26/7/76	last to flower	18/11/76
9	Early flowering Doon Major	28 days	6/5/76	1st to flower	24/ 8/76
10	"	28 days	10/5/76	2nd to flower	24/ 8/76
11	"	32 days	3/5/76	1st to flower	24/ 8/76
12	"	32 days	5/5/76	2nd to flower	24/ 8/76
13	"	36 days	30/4/76	1st to flower	24/ 8/76
14	Late flowering Doon Major	36 days	27/7/76	2nd last to flower	24/ 8/76
15	"	40 days	28/5/76	last to flower	24/ 8/76

These lines were compared with the parent populations, Wilhelmsburger (Garton's 1973) and Doon Major (McGill 1974) used in experiment 3.

Early flowering lines were taken from short duration of low temperature treatments as much as possible. It was assumed that only the most susceptible individuals would flower after a short duration of low temperature treatment, and late flowering plants were taken from the longest temperature treatments.

The seeds were germinated in three successive sowings in petri dishes at 18° in the light and planted out into 5.5 cm 'Jiffy' pots after four days, with four plants per plot and five replicates.

Plants were raised for 18 days from sowing at 18° mean daily mean temperature in a glasshouse bed, hardened off for three days at 12° and then moved outside for 4, 5 and 6 weeks during March and April at 5.3° mean daily mean temperature. The three low temperature treatments all ended on the same day and the plants were then moved into a glasshouse compartment at 12° mean daily mean temperature for 24 days, before being planted out in a field plot on 19 May, in rows 75 cm apart, 12.5 cm between plants in the rows.

Date of flowering was recorded. At the end of the experiment, 100 days after the end of low temperature treatment, flower stage (see experiment 2) of all non-flowering plants was recorded.

Results

Progeny from early flowering plants flowered more, and from late flowering plants flowered less, than the parent populations, especially in Wilhelmsburger (Figures 2.13 and 2.14).

The percentage flowering of the 15 lines and the two parent cultivars is given below. If the number of plants at flower stage 3 and 4, that is with visible flower buds at the end of the experiment, are included with the number of plants flowering, the relative susceptibilities to vernalisation of the different lines do not change.

The number of flowering plants for all early flowering Wilhelmsburger lines was combined, similarly the late flowering Wilhelmsburger lines and the early and the late flowering groups of Doon Major lines.

FIGURE 2.13: Experiment 16 - the effect of duration of low temperature treatment on the flowering of 5 early flowering Wilhelmsburger lines (EFW), the Wilhelmsburger parent population (W) and 3 late flowering Wilhelmsburger lines (LFW)

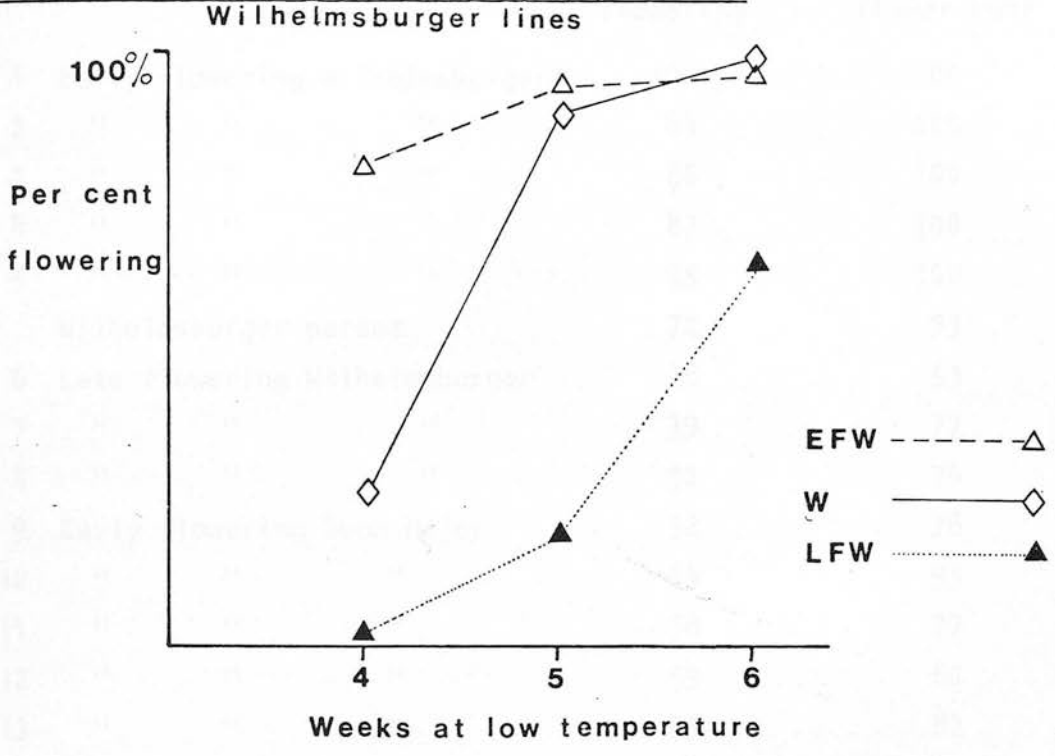
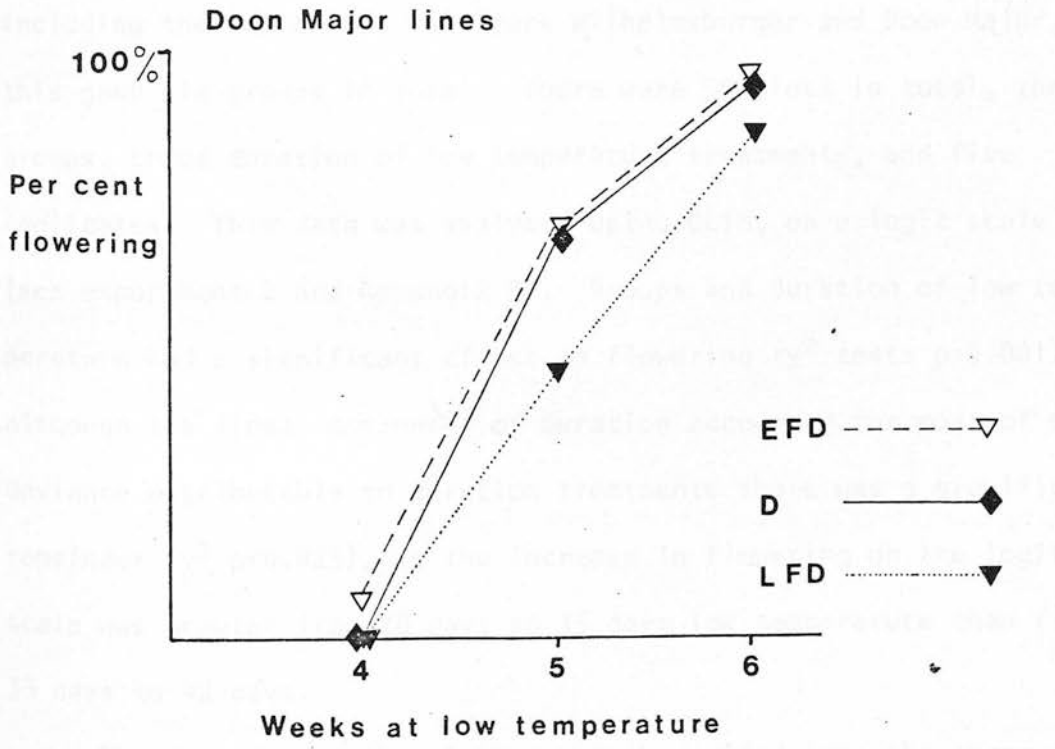


FIGURE 2.14: Experiment 16 - the effect of duration of low temperature treatment on the flowering of 5 early flowering Doon Major lines (EFD), the Doon Major parent population (D) and 2 late flowering Doon Major lines (LFD)



	Line	% plants flowering	% plants with flowers or visible flower buds
1	Early flowering Wilhelmsburger	100	100
2	" " "	84	100
3	" " "	88	100
4	" " "	87	100
5	" " "	95	100
	Wilhelmsburger parent	72	93
6	Late flowering Wilhelmsburger	18	53
7	" " "	39	77
8	" " "	32	74
9	Early flowering Doon Major	52	76
10	" " "	64	93
11	" " "	58	77
12	" " "	59	80
13	" " "	59	82
	Doon Major parent	53	73
14	Late flowering Doon Major	51	64
15	" " "	38	56

Including the two parent cultivars Wilhelmsburger and Doon Major, this gave six groups in total. There were 90 plots in total, the six groups, three duration of low temperature treatments, and five replicates. This data was analysed using GLIM, on a logit scale (see experiment 2 and Appendix B). Groups and duration of low temperature had a significant effect on flowering (χ^2 tests $p<0.001$) and although the linear component of duration accounted for most of the deviance attributable to duration treatments there was a significant remainder (χ^2 $p<0.025$), as the increase in flowering on the logit scale was greater from 28 days to 35 days low temperature than from 35 days to 42 days.

The data from both cultivars, each divided into the three groups, early flowering lines, late flowering lines and parent population, were

also analysed separately with GLIM. The groups were placed in the data in the order, early flowering lines, parent cultivar, late flowering lines, so that a linear component of the effect of selection on flowering could be examined. Factors significantly affecting the flowering of the Wilhelmsburger groups were the linear component of duration and the linear component of selection (χ^2 tests $p<0.001$), that is the flowering, on a logit scale, of the three groups, early flowering lines, parent, and late flowering lines could be arranged in a straight line and fit the data satisfactorily (mean deviance for total effect of lines, 116.81, compare with χ^2 df 2; mean deviance for linear effect of lines, 231.3, compare with χ^2 df 1). The linear component of selection had a significant effect on the flowering of the Doon Major group, although smaller than on the Wilhelmsburger group (mean deviance for total effect of Doon Major groups 6.56, compare with χ^2 df 2 and for linear effect of groups, 12.66, compare with χ^2 df 1) but the linear component of duration was not the only significant effect of duration, as flowering (on a logit scale) increased more rapidly from 28 to 35 days than from 35 to 42 days (χ^2 effect of duration, $p<0.001$). Although selection affected flowering in both cultivars the differences betwen the selected Wilhelmsburger lines and the parent population were much greater than the differences between the Doon Major lines and the parent.

The number of days at 5.3° mean daily mean temperature required to cause 50 per cent flowering in the various groups was calculated from the analysis in which all six groups were included using the data for 28 and 35 days low temperature, assuming a straight line between these two points, and also from the 28 and 42 days low

temperature treatments, when the data was fitted to a linear effect of duration. The number of days for 50 per cent flowering was also calculated from the fitted lines in the separate analyses of the two cultivars, in the case of Doon Major from the 28 and 35 days fitted points with the total effect of duration and from the 28 and 42-day points with the data fitted to the linear effect of duration. This gave three estimates of the number of days for 50 per cent flowering for the Wilhelmsburger groups, and four estimates for the Doon Major groups. The estimates generally varied by about one day, and at most by 2.6 days and so the means of the three or four estimates were taken, and are shown below:

Group	Number of days at 5.3° required to cause 50% flowering
Early flowering Wilhelmsburger lines	24.4 days
Wilhelmsburger parent cultivar	30.5 days
Late flowering Wilhelmsburger lines	39.3 days
Early flowering Doon Major lines	33.3 days
Doon Major parent cultivar	34.2 days
Late flowering Doon Major lines	35.9 days

Days to flowering (Table 2.35) were analysed with GLIM as in experiment 2 (and see Appendix B), using the number of plants contributing to each mean to weight that mean. The three Doon Major groups and the three Wilhelmsburger groups were analysed together, and separately.

In all analyses the number of days to flowering from the end of low temperature treatment declined linearly (F tests, $p<0.001$) as duration of low temperature increased. The early flowering Wilhelmsburger lines flowered 1 and 3 days earlier (observed and fitted mean

TABLE 2.35: Experiment 16 - the effect of duration of low temperature treatment on the number of days to flowering from the end of treatment of selected lines of Wilhelmsburger and Doon Major and the parent cultivars

Cultivar or group of lines	Duration of low temperature treatment			Mean
	4 weeks	5 weeks	6 weeks	
	Days to flowering			Days to flowering
Mean of five early flowering Wilhelmsburger lines	90	81	75	81
Wilhelmsburger cultivar	93	87	74	82
Mean of three late flowering Wilhelmsburger lines	94	96	86	89
Mean of five early flowering Doon Major lines	95	89	79	84
Doon Major cultivar	NF	88	74	80
Mean of two late flowering Doon Major lines	NF	92	82	85
Mean	91	86	78	83

values respectively) than the parent population and the late flowering lines 6 and 10 days later (observed and fitted values) than the parent, giving a significant linear effect of selection (F test $p<0.001$). In the Doon Major groups, although the effect of groups was significant (F test $p<0.05$) and the late flowering group was the last to flower by mean 1 and 3 days (observed and fitted mean values) the Doon Major parent was earlier in flowering than the early flowering group by mean 3 days.

Late flowering lines flowered less and flowered later than other lines. When the flowering percentages and days to flowering of all nine Wilhelmsburger lines, including the parent population,

were compared, there was a significant negative correlation ($r = -0.867$ $n = 9$ $p<0.01$) between flowering percentage and days to flowering. The higher the percentage flowering of any line, the earlier it tended to flower. There was no similar correlation in the eight Doon Major lines.

Discussion

Resistant and susceptible lines were successfully selected from Wilhelmsburger and Doon Major cultivars by taking seed from early and late flowering plants in the parent population. The first and second earliest and latest flowering plants were selected from cultivar times duration of low temperature treatments each containing 25 plants, and selected plants formed the extreme 8 per cent of each treatment population. Wilhelmsburger seed stocks used in previous experiments have always shown more variability in flowering than Doon Major stocks, and the selected Wilhelmsburger lines differed much more from the parent population than the selected Doon Major lines. Although Wilhelmsburger is normally more susceptible to flower induction than Doon Major the late flowering Wilhelmsburger lines were less susceptible than any of the Doon Major lines.

Earliness of flowering of the Wilhelmsburger lines was correlated with susceptibility to flowering but not in Doon Major although late flowering lines were slightly later in flowering than early flowering lines. In experiment 3 the variation in time to flower was similar in both cultivars, but presumably the genetic component of the variability was greater for Wilhelmsburger than for Doon Major as selection for earliness or lateness of flowering was much more effective in Wilhelmsburger. In general, however, the time to flower

of an individual plant within a population is related to its susceptibility to flower induction and this susceptibility is heritable.

The seed of the 15 lines was not ripened at one time. Low temperature during ripening has been shown to have an inductive effect on sugar beet (Longden *et al* 1975) and it is possible that lower temperatures in September and in the cool glasshouse may have partially vernalised seed of lines 1, 6 and 8 but even if this happened the flowering of two of these lines was still much less than that of other lines.

As the lines were selected on flowering time, concluding the experiment fairly early, at 100 days, might have discriminated against lines derived from late flowering plants which might have fewer flowering plants at that time although equally susceptible to vernalisation. Examination of the number of plants flowering and with visible flower buds show that this is not the case. Even if all plants in bud at 100 days had flowered, the early flowering lines would still have had considerably more flowering plants than the late flowering lines.

2.13 SITE OF VERNALISATION

2.13.1 The effect of cooling the growing point on the flowering of swedes.

Experiment 17

In two preliminary experiments all leaves over 1 cm were removed from Pentland Harvester swedes in the first experiment, and from Wilhelmsburger and Doon Major swedes in the second, during a six or nine weeks low temperature treatment respectively. Leaves were allowed to grow after the treatment period and all plants extended and flowered normally although the defoliated plants flowered more slowly, 6.2 and 10.5 days later than undefoliated plants in the first and second experiments respectively. Defoliation probably depleted plant reserves of carbohydrates during the low temperature period which may have reduced the efficiency of vernalisation and the rate of plant growth and development after the low temperature period. Defoliating cabbage plants immediately before low temperature treatment had no effect on their subsequent flowering (Ito *et al* 1966) but, in contrast, defoliation of broccoli plants at the beginning of a low temperature period lowered sugar levels greatly and prevented flowering (Fontes and Ozbun 1971). Broccoli is possibly more sensitive to carbohydrate levels, or more dependent on photoperiodic induction which requires the presence of leaves. Unlike cabbage and swedes, broccoli does not have an obligate requirement for vernalisation.

There is evidence that the crown of beet (Chroboczck 1933) and celery plants (Curtis and Chang 1930) and the growing point of the chrysanthemum (Schwabe 1954) is the site which perceives the effect of low temperature. To determine if the growing point alone can act

as the site of vernalisation in the swede, the growing points of a group of plants were cooled to a vernalising temperature while the rest of the plant remained at a higher, non-vernalising temperature.

Seven-week old Doon Major swedes in 7.5 cm pots were placed in the apparatus described below (Figure 2.15). Twelve mm lengths of 6 mm diameter copper rod with 6 mm deep V-shaped slots cut in them were brazed to 7 mm bore, 40 cm long copper pipes, four rods, 10 cm apart, to each of eight pipes. All copper parts were coated with polyurethane varnish. The eight pipes were held in a frame with the slotted rods pointing down. Four of these pipes were connected together with pvc (poly vinyl chloride) tubing and lagged with plastic foam. Water/antifreeze mixture at $-1.0 \pm 0.5^{\circ}$ was pumped through continuously from a constant temperature water bath.

Treatment was carried out in a laboratory at 18° mean daily mean temperature (measured with a maximum/minimum thermometer), and the two treatments, cooled or uncooled copper pipe, randomised in four blocks, parallel to the window. The plants were placed in trays under the frame holding the pipes, 16 with their growing points in contact with cold slotted copper rods, and 16 control plants with growing points in contact with copper rods at room temperature (Figure 2.16).

The plant surface temperature close to the growing point and on the leaves (mean of three leaves) of each plant was measured weekly with a thermocouple connected to an electronic thermometer.

The temperature on the surface of the 16 cooled growing points ranged from 6.5° to 8.8° (mean of seven weekly readings), 7.6° mean of all 16 plants. The individual readings ranged from 2.8° to 15.9° as copper/plant contact varied between plants, and over time. The growing points of the control plants were 16.7° to 18.1°, mean 17.4°,

FIGURE 2.15: Experiment 17 - apparatus for cooling the growing point, showing water-bath, pipes for circulating the water, control copper pipes and some plants *in situ* with growing points in contact with copper rods

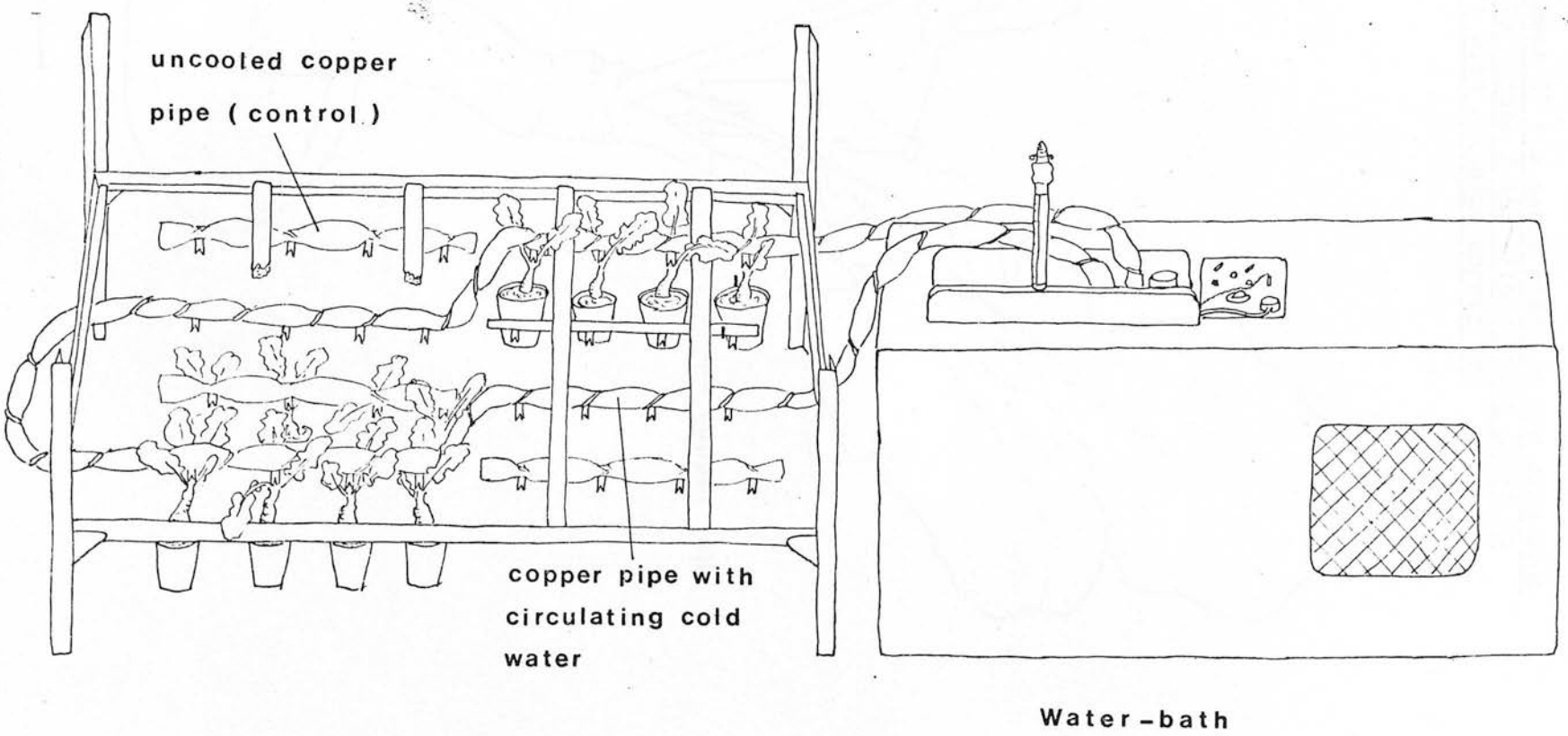
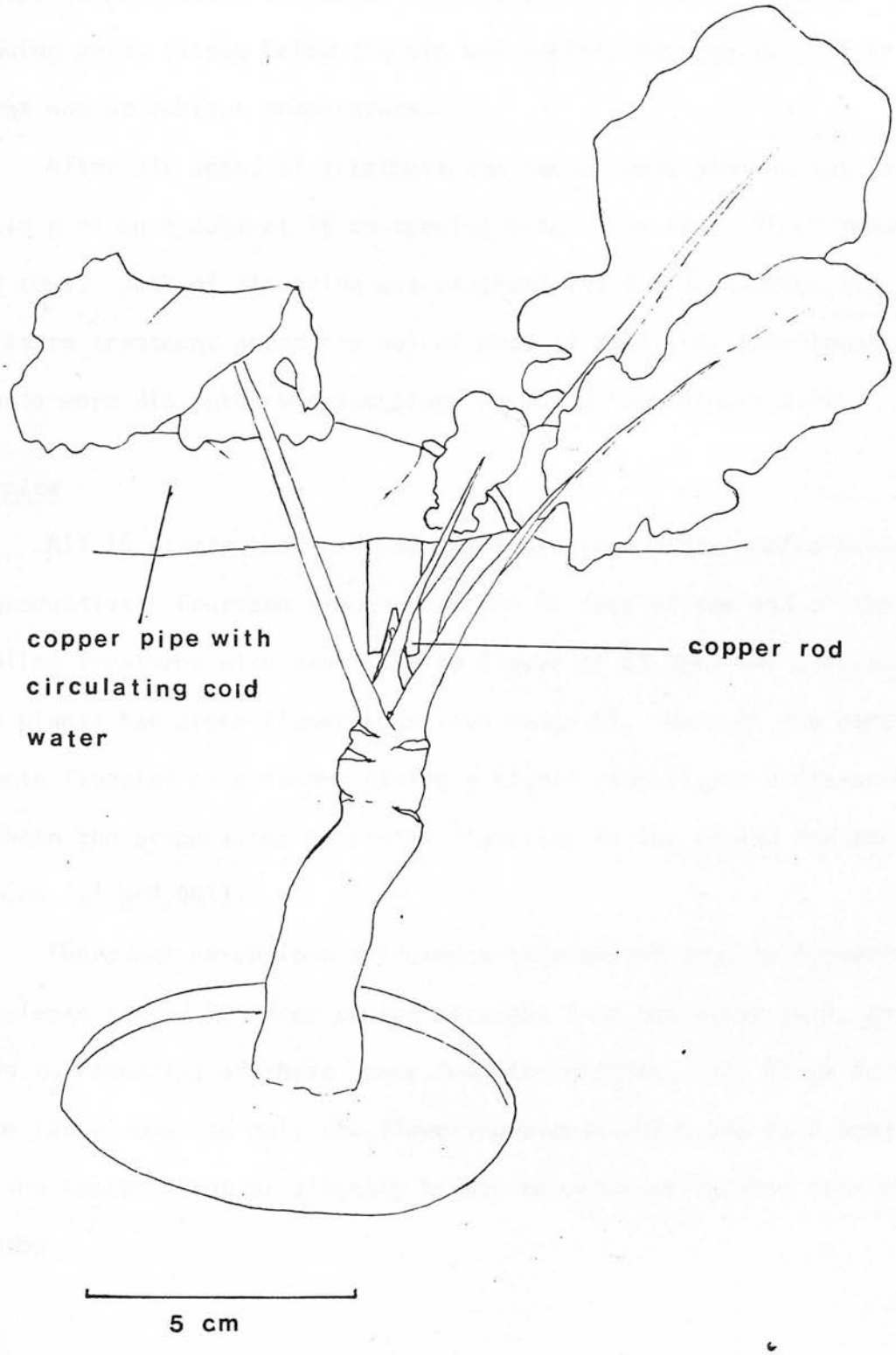


FIGURE 2.16: Experiment 17 - a swede plant in the apparatus shown in Figure 2.15, with growing point in contact with a cooled copper rod



with a range in individual readings of 13.7° to 26.8°. Mean leaf surface temperatures were 17.5° and 17.2° for the cooled and uncooled groups respectively, and never fell below 13.5°. Up to 2 cm of growing point tissue below the tip was cooled, but the rest of the plant was at ambient temperature.

After six weeks of treatment the swedes were planted out in a field plot on 6 June at 15 cm spacing within the rows, 75 cm between the rows. Date of flowering was recorded and 110 days after low temperature treatment ended the apical buds of remaining non-flowering plants were dissected and bud stage assessed (see Figure 2.1).

Results

All 16 plants that had had their growing points cooled became reproductive. Fourteen flowered within 85 days of the end of the cooling treatment with mean time to flower of 67 days and the remaining two plants had green flower buds (bud stage 6). None of the control plants flowered or extended giving a highly significant difference between the proportions of plants flowering in the cooled and control groups (χ^2 $p<0.001$).

There was no obvious difference between the days to flowering of plants cooled by water coming straight from the water bath, and days to flowering of those lower down the circuit. The block furthest from the window had only two flowering plants which may have been due to the poorer light or slightly higher temperature further from the window.

2.13.2 Experiment 18

Experiment 17 was repeated using six-week old Wilhelmsburger plants and re-randomising the two treatments in the four blocks. The air temperature was higher, 22° mean daily mean temperature, and to compensate for this the temperature of the water/antifreeze mixture was lowered to $-2 \pm 0.5^{\circ}$ and treatment extended to seven weeks.

Weekly plant temperature and daily maximum/minimum air temperature measurements were made as in experiment 17. The temperatures near the growing points of cooled plants were between 7.0° and 10.9°, mean for all plants 9.3°, and of uncooled plants 20.3° to 21.5°, mean 20.7°. Mean leaf temperatures were 21.2° for cooled plants and 21.0° for uncooled plants.

After treatment the swedes were planted in a field plot on 7 August and after a month lifted and replanted in a glasshouse compartment at 13° mean daily mean temperature, without supplementary light. After 16 weeks in the compartment, plant apices were dissected and the bud stage recorded (see Figure 2.1).

Results

Seven of the 15 surviving cooled plants were clearly reproductive (bud stage 3 and over) and six were at bud stage 2. One uncooled plant out of 14 surviving plants was at bud stage 2, possibly due to low temperatures in the compartment. Comparing proportions of plants at stage 3 and over the difference between the two treatments, cooled and uncooled, is significant at the 2 per cent level (χ^2 test) and including plants at bud stage 2, is significant at the 0.1 per cent level. There was no difference in bud development between the four blocks.

2.13.3 The effect of heating the growing point on the flowering of swedes.

Experiment 19

The previous two experiments have demonstrated that cooling the growing point only can induce flowering in swedes. To find out if other parts of the plant can be vernalised while the growing point is maintained at a non-vernalising temperature the same apparatus as in experiments 17 and 18 was used but with circulating hot water instead of cold water.

Thirty-two 11-week old Wilhelmsburger plants were placed under the apparatus in an unheated greenhouse for six weeks in October and November with the ambient temperature 6.5° mean daily mean temperature, range in daily mean temperature 3.3° to 12.2°, and no supplementary light. The two treatments, heated copper or copper at ambient temperature, were randomised in the four blocks and the circulating water was maintained at 48 ± 2° to heat the growing points of treated plants above vernalising temperatures.

The temperatures of the plant surface near the growing point, on the leaves, and on the root were measured on three occasions with a thermistor electronic thermometer. At the end of the experiment a test plant was set up and the temperature measured at various points in or on the surface of the plant.

The surface temperatures (mean of three readings) of the heated growing points were in the range 14.6° to 21.8°, mean 18.5°, and of the unheated growing points 7.7° to 8.8°, mean 8.1°. Leaf temperatures were 8.5° and 8.4°, and root temperatures 8.3° and 8.1° for the heated and unheated plants respectively. In the test plant only the tissue

within about 1 cm of the copper rod was maintained above 10° and tissue 3 cm from the rod was at ambient temperatures (Figure 2.17). All the lower buds, the root and leaves were at vernalising temperatures even when the growing point was heated to 15° to 20°.

At the end of six weeks treatment the plants were moved to a glasshouse compartment at 13° mean daily mean temperature for two weeks, and then to a glasshouse bed at 16.3° mean daily mean temperature for nine weeks. On 8 June the swedes were planted out in a field plot and the experiment ended 140 days after the end of low temperature treatment. Date of flowering was recorded, and buds of non-flowering plants were dissected and bud stage (see Figure 2.1) recorded at the end of the experiment.

Results

None of the plants whose growing points had been heated during vernalisation flowered. Fifteen of the 16 control plants had flowered 140 days after low temperature treatment with a mean time to flower of 88 days. The remaining control plant had visible flower buds (bud stage 6) at the end of the experiment, but all the treated plants had vegetative apical buds. The difference in proportions of plants flowering between heated and unheated plants was highly significant (χ^2 $p<0.001$).

2.13 *Discussion*

Cooling the growing point can cause flowering. The growing point may not be the only site where low temperature can induce flowering but it is as effective as the entire plant as in experiment 17 flowering was rapid and normal after treatment. The slow and less complete flower development in experiment 18 was probably chiefly due

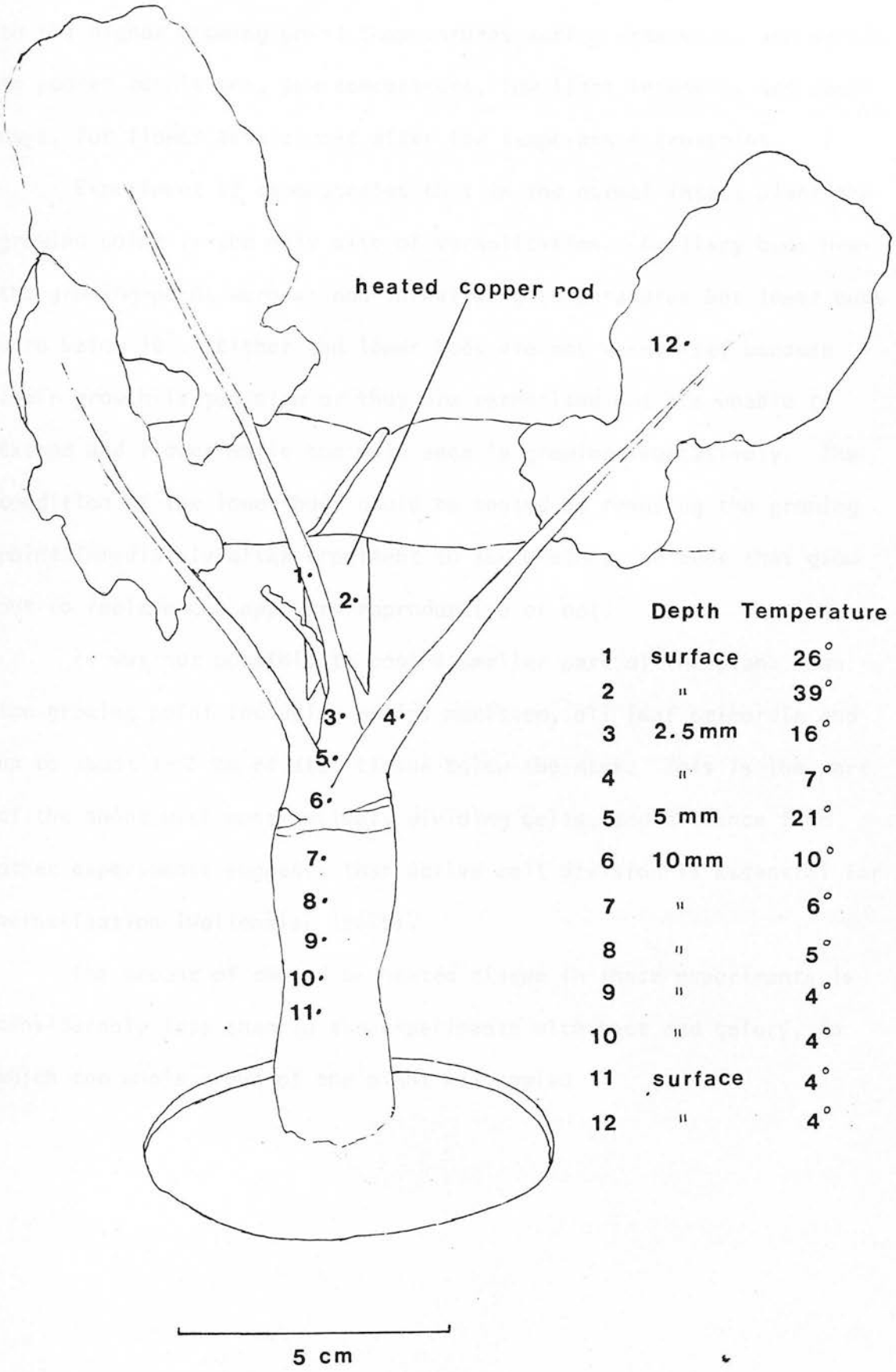


FIGURE 2.17: Experiment 19 - temperatures within the tissue and on the surface of a swede plant with its growing point in contact with heated copper rod

to the higher growing point temperatures during treatment, and partly to poorer conditions, low temperature, low light intensity and short days, for flower development after low temperature treatment.

Experiment 19 demonstrates that in the normal intact plant the growing point is the only site of vernalisation. Axillary buds near the growing-point were at non-vernalising temperatures but lower buds were below 10°. Either the lower buds are not vernalised because their growth is too slow or they are vernalised but are unable to extend and flower while the main apex is growing vegetatively. The condition of the lower buds could be tested by removing the growing point immediately after treatment to see whether the buds that grow out to replace the apex are reproductive or not.

It was not possible to cool a smaller part of the plant than the growing point including apical meristem, all leaf primordia and up to about 1-2 cm of stem tissue below the apex. This is the part of the shoot with most actively dividing cells, and evidence from other experiments suggests that active cell division is essential for vernalisation (Wellensiek 1962b).

The amount of cooled or heated tissue in these experiments is considerably less than in the experiments with beet and celery, in which the whole crown of the plant was cooled.

2.14 SITE OF DEVERNALISATION

The effect of heating only the growing point to devernalising temperatures, after low temperature treatment, on flowering of swedes.

Experiment 20

Experiments 17, 18 and 19 have established that the growing point is the normal site of vernalisation in an intact plant, and as the flowering stimulus induced by vernalisation is generally considered to be initially immobile (see 1.13, page 36), it is probable that the site of devernalisation is the growing point also. The same apparatus and methods as in experiments 17, 18 and 19 were used to investigate the site of devernalisation.

Twenty-four, 5-week old Wilhelmsburger plants were repotted to 12.5 cm pots, and placed out of doors on 15 October for 32 days at 7^{o} mean daily mean temperature. The fairly short low temperature period was used so that plants would be more susceptible to devernalisation. The plants were then placed under the same apparatus as in experiments 17, 18 and 19 in a compartment with mercury vapour lamps extending daylength to 18 hours, with three plants per plot. The compartment temperature was 12.3^{o} mean daily mean temperature and water at 56 ± 2.0^{o} was circulated to heat the growing points of the 12 treated plants to a devernalising temperature. Plant surface temperatures on the growing points and leaves were measured weekly with a thermistor electronic thermometer, with the lights off, and once with the lights on, as a comparison.

Mean growing point temperatures of heated plants (mean of three readings) were from 19.5^{o} to 24.5^{o} with mean 21.6^{o}, and of unheated plants 12.8^{o} to 13.8^{o}, mean 13.2^{o}. Mean leaf temperatures were 13.3^{o}

and 13.0° for heated and unheated plants respectively. While the lights were on, for 12 hours a day to lengthen the daylength, temperatures were slightly higher: 28.2° and 15.6° for heated and unheated growing points, and 16.2° and 16.6° for leaf surface temperatures of heated and unheated plants respectively.

The temperature of a test plant heated and unheated was measured at several points. Only the 2 cm of the plant growing point closest to the copper rod were heated above ambient temperature.

Treatment in the apparatus finished after three weeks and the plants were moved to a glasshouse bed at 17° mean daily mean temperature and two months later repotted to 19 cm pots. Date of flowering was recorded, and 160 days after the end of low temperature treatment the apical buds of the remaining plants were dissected and bud stage recorded (see Figure 2.1).

Results

None of the devernalised plants flowered or became reproductive, but four of the control plants flowered, and five more had flower buds at bud stages 4 to 6 by the end of the experiment. The difference in the proportions of flowering plants in the two treatments was not significant but the difference in numbers of reproductive plants (bud stage 4 and over) was highly significant (χ^2 $p<0.001$).

Flowering was very slow, mean days to flower being 140.7.

Discussion

Heating the growing point after low temperature treatment prevented flowering, which suggests that devernalisation occurs in the growing point. It also suggests that the flowering stimulus is immobile in the growing point as the stimulus appears to be destroyed by heating

the growing point after low temperature treatment, even while the rest of the plant is at ambient temperatures which did not prevent flowering of control plants. It is possible, however, that lower buds were induced but unable to extend and flower while the main apex was growing vegetatively.

2.15 VERNALISATION OF GROWING POINT CUTTINGS

The flowering and stem extension of plants grown from growing point cuttings previously subjected to a low temperature treatment.

Experiment 21

Preliminary experiments (see experiment 17) have demonstrated that leaves are not required for vernalisation and experiments 17 and 18 have shown that cooling the growing point alone can cause flowering. Apical stem cuttings of cabbage, 5 cm long, have been successively vernalised (Ito *et al* 1966). In this experiment detached growing points were given a low temperature treatment and grown into plants to examine whether the presence of leaves, root and fibrous roots are essential for vernalisation or not.

The growing points were cut from 65 Wilhelmsburger and 50 Doon Major 12-week old swedes and trimmed to three sizes, 7, 12 or 15 mm cubed, leaving the apical bud intact but with all leaves removed. The buds were placed in 5.5 cm 'Jiffy' pots filled with peat in three seed trays, covered with clear plastic and moved into an unheated glasshouse at 8^{o} mean daily mean temperature. One tray was moved into a compartment at 12^{o} mean daily mean temperature after seven weeks and the other two trays moved into the compartment after nine weeks.

As the cuttings took root and grew they were repotted. Date of flowering was recorded. The apical buds of non-flowering plants were dissected and bud stage (see Figure 2.1) recorded 90 days after the end of the nine-week low temperature treatment.

Results

Thirty-eight of the 106 cuttings that survived and grew into plants flowered, and 23 more had reproductive buds (bud stage 4 to 6). The larger the size of the cutting, the greater the proportion of flowering and reproductive plants (bud stage 4 and over) (χ^2, flowering plants 15 mm v 12 mm, $p<0.01$; reproductive plants 15 mm v 12 mm, $p<0.05$; 12 mm v 7 mm, $p<0.01$) (Table 2.36). The nine-week low temperature treatment resulted in greater proportions of flowering (χ^2 $p<0.05$) and reproductive plants (χ^2 $p<0.01$) than the seven-week treatment. There were no significant differences in proportions between the two cultivars.

Larger bud pieces flowered earlier than smaller pieces (Table 2.37), but there was no difference in flowering time between the cultivars. After the seven-week low temperature treatment flowering was slightly earlier than after nine weeks treatment, but only six plants flowered after seven weeks compared with 32 after nine weeks and so the mean values are more accurate for the nine-week treatment.

Discussion

Cuttings of only apical buds can be vernalised, and once established as plants flower and extend normally, confirming that the presence of other parts of the plant - leaves, root and fibrous roots - are not essential for vernalisation. Larger cuttings flowered more readily and earlier than smaller cuttings presumably partly due to more vigorous regeneration and growth after low temperature treatment but probably also because of the greater amount of tissue, better growth and larger food reserves during low temperature treatment.

TABLE 2.36: Experiment 21 - the effect of duration of low temperature treatment, size of apical bud cutting and cultivar (see below table) on the percentage of plants flowering and reproductive (bud stage 4 and over)

Duration of low temperature	Size of cutting	Number of plants flowering	Number of plants reproductive	Total number of plants	% of plants flowering	% of plants reproductive	Total of both durations	
							% flowering	% reproductive
7 WEEKS	0.34 cm^3	0	0	10	0	0	16	28
	1.73 cm^3	2	4	14	14	29	30	61
	3.37 cm^3	4	7	8	50	88	68	86
	Total	6	11	32	19	34	36	58
9 WEEKS	0.34 cm^3	5	9	22	23	41		
	1.73 cm^3	12	24	32	37	75		
	3.37 cm^3	15	17	20	75	85		
	Total	32	50	74	43	68		
OVERALL TOTAL		38	61	106	36	58		

% of plants flowering — Wilhelmsburger 34% ($\frac{21}{61}$); Doon Major 38% ($\frac{17}{45}$)

% of plants reproductive — Wilhelmsburger 52% ($\frac{32}{61}$); Doon Major 64% ($\frac{29}{45}$)

TABLE 2.37: Experiment 21 - the effect of duration of low temperature treatment, size of apical bud cutting and cultivar on the number of days from the end of each treatment period to flowering

Duration of low temperature	Size of cutting	Days to flowering			
		Wilhelms-burger	Doon Major	Mean of both cultivars	Overall mean
7 WEEKS	0.34 cm^3	NF	NF	NF	73.6
	1.73 cm^3	64.5	NF	64.5	62.8
	3.37 cm^3	56.0	66.3	63.8	57.9
	Mean	61.7	66.3	64.0	61.8
9 WEEKS	0.34 cm^3	75.7	70.5	73.6	
	1.73 cm^3	62.0	63.0	62.5	
	3.37 cm^3	58.6	53.2	56.4	
	Mean	62.6	59.9	61.4	
OVERALL MEAN		62.4	61.0	61.8	

NF = no plants flowering in the treatment

2.16 REMOVAL OF THE GROWING POINT

2.16.1 The effect of the removal of the growing point or shoot before or after low temperature treatment, on flowering of swedes.

Experiment 22

Previous experiments (17, 18, 19 and 21) have established that the apical growing point is the normal site of vernalisation. It is not certain whether lower buds on the plant are induced, or only the growing point, but it is generally supposed that the flowering stimulus is immobile, and if this is so, removal after low temperature treatment of the induced growing point would prevent flowering unless lower buds were induced.

The growing point or almost the entire shoot was removed from nine-week old Doon Major plants after low temperature treatment so that the flower buds would grow out and their condition, whether reproductive or vegetative, could be assessed. The two amounts of tissue, growing point or shoot, were removed so that the condition of both axillary buds near the growing point and the oldest buds on the plant could be examined.

To check that buds other than the growing point can be vernalised, the growing point or shoot was removed before low temperature treatment, so that axillary buds would be released from apical dominance and would be actively growing during low temperature treatment.

Forty-five plants were repotted to 12.5 cm pots and the following treatments each applied to nine plants:

1. Control plants left intact.
2. The apical bud (approximately 1 cm^3 of tissue, weighing about 1 g) removed on the day low temperature treatment started,

leaving four or five leaves, their axillary buds and lower axillary buds.

3. The shoot removed on the day low temperature treatment started, leaving no leaves, and only four or five axillary buds on the stem.

4. The apical bud removed on the day low temperature treatment ceased, leaving four or five leaves, their axillary buds and lower axillary buds.

5. The shoot removed on the day low temperature treatment ceased, leaving no leaves, and only four or five axillary buds on the stem.

All plants were given a six-week low temperature treatment at 8^{o} mean daily mean temperature in an unheated glasshouse during October and November and were then moved to a compartment at 14^{o} mean daily mean temperature for two weeks before being moved to a glasshouse bed at 18^{o} mean daily mean temperature.

The number of buds over 1 cm in length was counted after four weeks of low temperature treatment and bud number and leaves over 5 cm in length counted at the end of low temperature treatment. The date of flowering of the first bud to flower on each plant was recorded and 135 days after low temperature treatment finished the buds on the remaining plants were dissected and bud stage assessed (see Figure 2.1).

Results

One of the nine control plants left intact (treatment 1) flowered in 121 days, three others having unmistakable flower buds

(bud stage 4 to 6) and four of the eight surviving plants that had had their apical buds removed before low temperature treatment (treatment 2) flowered with a mean time to flower of 107 days, three others having unmistakable flower buds (Table 2.38). No other plants flowered or had flower buds, although two plants each in treatments 4 and 5 had internodes over 1 cm.

More plants flowered or were reproductive in treatments 1, 2 and 3, in which the buds growing actively during low temperature treatment were retained on the plant after treatment, compared with treatments 4 and 5, in which actively growing buds were removed after low temperature treatment (χ^2 $p<0.01$).

Four weeks after the start of low temperature treatment all plants in treatments 2 and 3 had at least one axillary bud over 1 cm (mean number 3 buds) and at the end of low temperature treatment the number of buds had increased slightly (Table 2.38).

Discussion

Development of flower buds only occurred in plants in which the buds that were actively growing during low temperature treatment were retained, that is the apical bud, or axillary buds fairly high on the stem released from apical dominance. If the apical bud was removed after low temperature treatment the plants did not flower, suggesting that the dormant axillary buds were not induced, and that no flowering stimulus was translocated from the apical buds. Plants with only lower axillary leaf and cotyledon buds present during low temperature treatment did not flower, probably because their bud growth during treatment was too slow. As few control plants flowered, vernalisation can only have been marginal. Although the mean temperature was 8°, the temperature frequently rose above 10°.

TABLE 2.38: Experiment 22 - the effect of the removal of plant organs before and after low temperature treatment on the number of reproductive plants (bud stage 4 and over)

Treatment No.	Time when treatment was applied	Plant parts removed	Plant parts retained	No. of buds over 1 cm at end of low temperature treatment	No. of leaves at end of low temperature treatment	No. of reproductive plants out of 9
1	-	-	all	1	8.0	4
2	before low temperature treatment	apical bud	all leaves, axillary buds, root, fibrous roots	6	3.5	8
3	before low temperature treatment	apical bud, all leaves, stem, axillary buds	four axillary buds on root, root, fibrous roots	4	0	0
4	after low temperature treatment	apical bud	all leaves, axillary buds, root, fibrous roots	0	6.0	0
5	after low temperature treatment	apical bud, all leaves, stem, axillary buds	four axillary buds on root, root, fibrous roots	0	0	0

2.16.2 The effect of removing the apical bud or almost the entire shoot 12 days and 0 days before low temperature treatment on the flowering of Wilhelmsburger swedes.

Experiment 23

In experiment 22 plants that had their entire shoot except for a few buds removed immediately before low temperature treatment did not subsequently flower, but this may have been due to insufficient vernalisation rather than an inability to be vernalised, as few control plants in the experiment flowered. To investigate if lower axillary buds can be induced to flower the following treatments were each applied to eight, 5-week old Wilhelmsburger swedes:

1. Control plants left intact.
2. The apical bud removed 12 days before the start of low temperature treatment.
3. The apical bud removed on the day low temperature treatment started.
4. All shoot tissue except the cotyledons and their axillary buds removed 12 days before the start of low temperature treatment.
5. All shoot tissue except the cotyledons and their axillary buds removed on the day low temperature treatment started.

Treatments 2 and 4 were included so that the axillary buds on these plants would be growing before low temperature treatment started, and would grow vigorously throughout the low temperature period. The plants were given a nine weeks low temperature treatment at 4.2° mean daily mean temperature in an unheated glasshouse, from

December to February, and then moved to a glasshouse compartment at 11.9° mean daily mean temperature for three weeks before being moved back to the glasshouse bed and grown on to flowering at 16.8° mean daily mean temperature.

Growing buds (those over 1 cm long) and all leaves over 5 cm long were counted before and after low temperature treatment, and date of flowering of the first branch to flower on each plant was recorded.

Results

All plants flowered within 62 days of the end of low temperature treatment and there was no difference in days to flower between the treatments (Table 2.39). Four plants that had had their cotyledon buds removed immediately before treatment died.

In treatments 2 and 4 in which tops were cut off 12 days before low temperature treatment plants had 2 and 1.9 growing buds respectively (mean of eight plants) at the start of low temperature treatment. After low temperature treatment the mean numbers of growing buds were 1, 4.5, 3.1, 2 and 1.7, and mean numbers of leaves at the end of treatment were 3.1, 4.6, 2, 4.6 and 3 for treatments 1, 2, 3, 4 and 5 respectively.

Discussion

During a long low temperature period both cotyledon and higher axillary buds are as readily vernalisable as the apical bud. Removing the apical buds 12 days before low temperature treatment so that axillary buds were growing by the time treatment started made no difference to flowering but considerably increased survival of plants if the whole shoot was removed. The greater number of leaves and buds

TABLE 2.39: Experiment 23 - the effect of removing the apical bud or the shoot, except for the cotyledon buds, 12 days or immediately before low temperature treatment on days to flowering

Treatment No.	Time when treatment was applied	Plant parts removed	Plant parts retained	Days to flowering
1	-	-	all	52.9
2	12 days before low temperature treatment	apical bud	all leaves, axillary buds, root, fibrous roots	54.5
3	at the start of low temperature treatment	apical bud	all leaves, axillary buds, root, fibrous roots	52.3
4	12 days before low temperature treatment	all leaves and most buds	cotyledon buds, root, fibrous roots	50.6
5	at the start of low temperature treatment	all leaves and most buds	cotyledon buds, root, fibrous roots	52.7
mean				52.6

present during low temperature treatment in the control plants and on those plants with only the growing point removed did not increase the rate of subsequent flowering.

Flowering probably did not occur in the previous experiment in the treatment with the shoot removed only because bud growth was slow whereas in this experiment a more susceptible cultivar was used, low temperature treatment was longer, and was at a lower, more effective temperature.

2.16.3 The effect of the removal of the apical meristem, or apical bud after low temperature treatment on the flowering of Wilhelmsburger swedes.

Experiment 24

In experiment 22 the removal of the apical bud or of most of the shoot immediately after low temperature treatment prevented flowering. The experiment was repeated with modifications to investigate how much apical tissue must be removed after low temperature treatment to prevent flowering. Thirty-six, 2-week old Wilhelmsburger plants were grown for eight weeks at 4.3^{o} mean daily mean temperature in an unheated glasshouse from December to February. On the day low temperature treatment ended, the following treatments were applied to nine plants each:

1. Control plants left intact.
2. The apical meristem removed, some leaves being damaged in the process.

3. Leaves damaged as in treatment 2 but the apical meristem left undamaged.

4. The apical bud removed as in treatment 4, experiment 22.

To remove the apical meristem leaves were cut away from one side and the meristem cut out with a scalpel. Treatment 3 was included so that any effect of damage to leaves could be separated from the effect of removing the apical meristem.

After treatment the plants were grown in a compartment at 11.8° mean daily mean temperature for two weeks before being returned to the glasshouse bed at 16.9° mean daily mean temperature, and grown on for a further 66 days.

Leaf number was recorded at the end of low temperature treatment before and after the four treatments, and date of flowering of the first branch to flower on each plant was recorded.

Results

All the plants flowered within 80 days of the end of low temperature treatment and mean days to flower did not differ significantly between the treatments (Table 2.40).

All treatments had a mean leaf number of 6 after low temperature treatment, and after the removal or damage of plant parts, leaf numbers were 4.7, 5.0 and 4.7 for treatments 2, 3 and 4 respectively.

The leaf primordia were counted in the apical buds removed in treatment 4 and mean total leaf number at the end of low temperature treatment was 13.7 leaves, a mean number of 9 primordial and small leaves having been removed from each plant. In treatment 4 the buds that flowered were the highest or next highest left on the stem, the fourth or fifth bud from the stem base (not including cotyledon buds),

TABLE 2.40: Experiment 24 - the effect of the removal of the apical meristem, or apical bud, or of damaging the leaves at the end of low temperature treatment, on days to flowering from the end of low temperature treatment

Treatment No.	Treatment	Days to flowering
1	Control, plants left intact	44.8
2	Apical meristem removed; most leaves, over 1 cm, axillary buds, root, fibrous roots retained	52.0
3	Leaves damaged as in treatment 2, but apical meristem retained	47.4
4	Apical bud removed; most leaves, over 1 cm, axillary buds, root, fibrous roots retained	49.4
mean		48.4
	For comparisons of treatments (means of 9 plants)	SE ± 3.51

but in treatment 2 higher axillary buds grew out in place of the apical meristem as the total number of axillary buds must have been around 12 to 15, and only the apical meristem was removed, although some leaves were damaged.

Discussion

Not only the apical bud, but axillary buds, both very near the apical meristem, and lower down, were vernalised by the long low temperature period. The failure of flowering of plants with apices removed after low temperature treatment in experiment 22 must have been due to inadequate vernalisation although there was no treatment in this experiment (24) to show the condition of the lowest three or four axillary buds and it is possible that they were not induced.

It is probable that the apical bud is the most easily vernalised as, in the intact plant, it will be undergoing most active growth, although in this experiment axillary buds flowered almost as rapidly as apical buds.

2.17 TRANSLOCATION OF THE FLOWERING STIMULUS

2.17.1 The flowering of upper and lower stem buds from flowering swedes, grown *in vitro*.

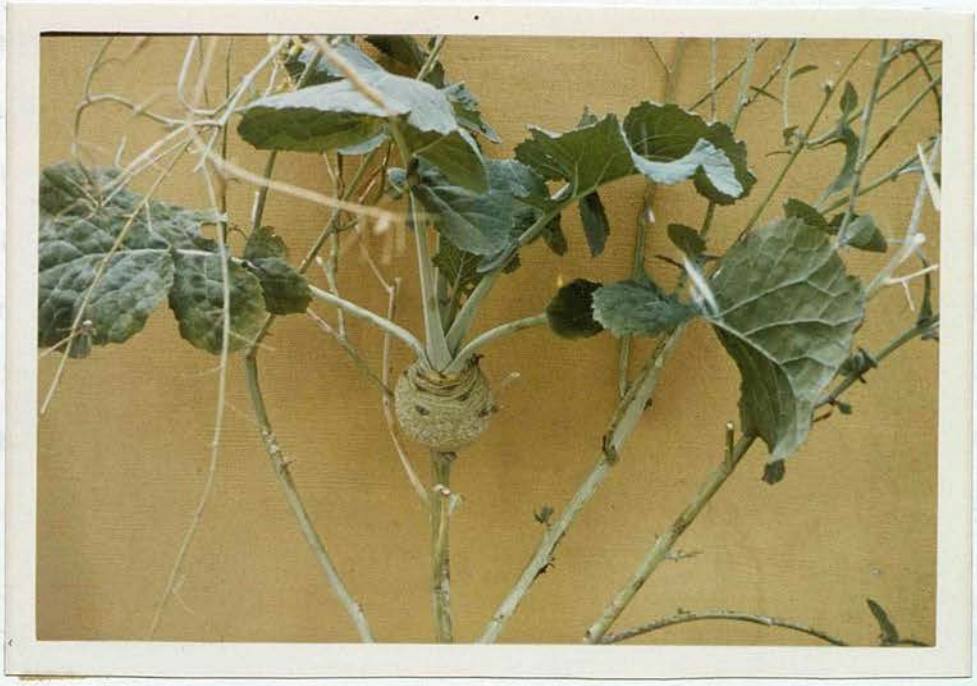
Experiment 25

Attempts were made to transmit a flowering stimulus by grafting. Seven and 14-day old seedlings of Wilhelmsburger and Doon Major swedes were grafted into the 'bark' of the stems of older swedes, sometimes in the place of an axillary bud. Young scions were used to increase the chances of vigorous growth and the graft taking, and so the stock had to be the flowering plant in the union. Flowering Wilhelmsburger and Doon Major swedes, and extending plants with visible green buds were used. At least 12 grafts took, but all the scions continued to grow vegetatively and showed no signs of stem extension (Figure 2.18). The failure to induce flowering in the scion suggests that there is no mobile flowering stimulus but the failure might also have been due to the stimulus only being translocated at a particular stage in flower development, to the grafts not forming a close cell union sufficiently early, or the paths of translocation in the plant not carrying any substances in the phloem from the stock to the scion, as the scions always had green leaves whereas the stock gradually died.

There is some evidence, however, that a translocatable flowering stimulus is produced. When a swede plant flowers, the axillary buds, starting near the top of the stem, develop as flowering branches. After a long low temperature treatment buds only a few centimetres above the base of the stem may grow out and flower.

Margara (1964) compared buds removed from flowering stems of sugar beet and rape (*Brassica napus*) grown *in vitro* with similar buds

FIGURE 2.18: Experiment 25 - vegetative Wilhelmsburger scions grafted onto flowering Wilhelmsburger swedes



left on the parent plant, and finding that lower stem buds removed from the plant did not flower, whereas those left on the stem did, suggested that the axillary stem buds are induced by a translocatable stimulus moving down from the apical bud.

In experiment 24 when the growing point was removed from the plant immediately after low temperature, the highest axillary buds left on the plant grew and flowered, and so must have been induced directly by the low temperature treatment or by a stimulus translocated from the apical bud during low temperature treatment, but no lower buds grew out and so there is no evidence on whether they were induced or not.

To examine whether lower axillary buds on the stems of flowering swedes are already induced at first flowering, stem buds were removed from flowering swedes to see whether or not they would flower when isolated from the parent plant.

The stems from one Wilhelmsburger plant which had flowered seven days previously and from two Wilheimsburger plants which had just flowered, and one Doon Major plant in bud, all having received six weeks low temperature treatment, were cut into sections each with a bud. Sections from the upper parts of the stems had visible flower buds and were discarded. Alternate sections were dissected and bud stage recorded, and the remaining 20 sections from all four stems were grown into plants. They were surface sterilised in 10 per cent 'Deosan', rinsed three times in sterile water and placed in 25 ml conical flasks containing 10 ml Long Ashton solution with 2 per cent agar, and stoppered with cotton wool and aluminium foil covers.

The flasks were kept under lights in the glasshouse at 18° mean daily mean temperature and each stem piece was potted out as soon as

it had formed roots. Flowering date or final apical bud stage (see Figure 2.1) 129 days after the stems had been cut up, were recorded.

Results

Five buds from the middle of the stem, two from the earliest plant to flower and one and two from the other two Wilhelmsburger plants, grew into flowering plants, but buds from near the base of the stems did not flower and were at bud stage 0 (vegetative) at the end of the experiment. The dissected alternate buds ranged from bud stage 4 near the middle of the stem to bud stage 0 at the base in the Wilhelmsburger plants but only from stage 1.5 near the top of the stem of the Doon Major plant. No cultured buds from the Doon Major plants flowered, the highest bud being at bud stage 2 at the end of the experiment.

The five flowering buds from the three Wilhelmsburger plants flowered in 72 and 91 days, 94 days, and 94 and 102 days from dissection and eight buds did not flower.

Discussion

Buds at the top and middle of flowering stems appear to be reproductive at the time of first flowering whereas those lower down are not. The absence of flowering in the buds from the Doon Major plant suggests that axillary buds are not induced before the main stem flowers. The flowering of the upper buds may be because reproductive axillary buds are only produced after the apex has reached a certain stage of flower development or there may be a flowering stimulus that moves slowly down the stem from the apical bud, inducing flowering in axillary buds. The evidence from the Doon Major plant tends to support the latter suggestion.

There is some evidence that *in vitro* culture tends to devernalise (Crisp and Walkey 1973) and lower buds might have flowered in other conditions.

2.17.2 The effect of removing different amounts of stem from flowering plants on the flowering of axillary buds remaining on the stem.

Experiment 26

In experiment 24 buds near the growing point were induced by the end of low temperature treatment. There was no evidence on the condition of the lowest buds, whether reproductive or not, but only stem buds from the middle stem and none from the lower stem flowered when removed from the parent plant and grown *in vitro* in experiment 25. Removing the stem above an axillary bud, allowing the bud to grow out, can also demonstrate whether it is induced or not.

Seventeen 9-week old Doon Major plants were selected, 10 having flowered that day, five one day previously and two two days previously, 31, 30 and 29 days respectively after the end of eight weeks low temperature treatment at 4.3^{o} mean daily mean temperature in an unheated glasshouse, from December to February. The plants had 16 to 22 buds in the axils of true leaves, mean 18.5, at the time of flowering. Part of the stems were removed so that the plants were left with two cotyledon buds, 1 true leaf bud, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 true leaf buds respectively for the 17 plants. The bud stage of the lowest axillary bud on the portion of stem removed was recorded.

The procedure was repeated two days later with 22 similar plants, 13 having flowered that day, and nine the previous day. Plants had 17 to 23 true leaf buds, mean 19.7, and stems were removed so that two plants were left with 2 cotyledon buds only, two plants each with 1, 2, 3 or 4 true leaf buds, and one plant each with 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 leaf buds.

Date of flowering was recorded, and the buds of non-flowering plants were examined 97 and 95 days after the removal of stems, and bud stage recorded (see Figure 2.1).

Results

Of the plants left with three or more leaf buds, all but one flowered. The non-flowering plant had had six leaf buds left after stem removal and had reached bud stage 5 by the end of the experiment. One plant with only one true leaf bud flowered (Table 2.41) but all other plants with fewer than three leaf buds did not flower and were vegetative at the end of the experiment. The bud stage of the lowest axillary bud removed decreased the more stem was removed, that is buds lower down on the stem were more vegetative (Table 2.41). Lower axillary buds took more days to flower from the time of dissection probably because the buds were less well-developed and took longer to form flowers.

Discussion

As in experiment 25, axillary buds higher on flowering stems flowered more readily than lower buds. Buds near the stem base flowered whereas they did not in the previous experiment but the stems in this experiment had fewer buds, and so basal buds were not so far from the apex as in experiment 25.

TABLE 2.41: Experiment 26 - the effect of removing different portions of the stem on the flowering of the buds left on the lower part of the stem, and days to flowering from the day when the portion of stem was removed

No. of buds left on stem	Total No. of plants	No. of plants flowering	Mean bud stage of lowest bud removed from stem	Mean days to flowering
2 cotyledon buds	3	0	0.7	NF
1 true leaf bud	3	1	0.7	45.0
2 "	3	0	1.0	NF
3 "	3	3	2.0	50.3
4 "	3	3	3.3	45.0
5 "	2	2	3.0	46.5
6 "	2	1	3.0	49.0
7 "	2	2	4.0	31.0
8 "	2	2	4.0	22.0
9 "	2	2	5.0	30.5
10 "	2	2	5.0	18.0
11 "	2	2	5.5	17.5
12 "	2	2	5.5	17.5
13 "	2	2	6.0	14.5
14 "	2	2	6.0	14.5
15 "	2	2	6.0	14.5
16 "	2	2	6.0	9.5
Mean or total	39	30	3.6	28.4

NF = no plants flowering

There is no evidence in this experiment to disprove the suggestion that the axillary buds are induced by a flowering substance, but if they are, the substance must be produced and translocated down the stem before flowers open as buds low down on the stem are already induced at first anthesis.

Total leaf number at the end of low temperature treatment was at least 4 on 15 plants sampled from the same group and so some of the flowering axillary buds were produced during low temperature treatment, but the majority after treatment, from the induced apex.

3. DISCUSSION

3.1 Introductory

The swede is induced to flower only by a period of low temperature. During three years of experiments only one plant grown at 13° and above developed flower buds (experiment 7) and two Wilhelmsburger and one Doon Major swede grown in a treated glasshouse for 17 months showed no signs of flowering or stem extension.

Low temperatures, generally below 10°, induce flowering, and high temperatures, over 15°, tend to reverse the induction. Other plant and environmental factors can influence the induction process and there is variation in the response of cultivars and within cultivars. The site of induction is in the growing point.

3.2 Temperature

3.2.1 Optimum temperature

The range of temperature 3° to 6° appears to be most effective for vernalisation, 5° to 6° being slightly better than 3° for Wilhelmsburger, but effectiveness dropping off above 6°, especially for Doon Major. The results from other experiments in which temperature control was reasonably good and post vernalisation conditions not strongly devernalising agree with the results in experiments 4 and 5. The results from experiments 2, 3, 4, 5, 13, 14, 15 and 16 are shown in Figure 3.1. Only Wilhelmsburger and Doon Major adult plants (4 days old and above at the start of treatment) flowering within 92 days of the end of low temperature treatment were included, and the results of all durations of low temperature from 20 to 42 days were pooled, as all experiments had evenly spaced treatments within this range. Flowering data in experiment 4 was only recorded up to 92 days and so this limit is set in the other experiments. The low

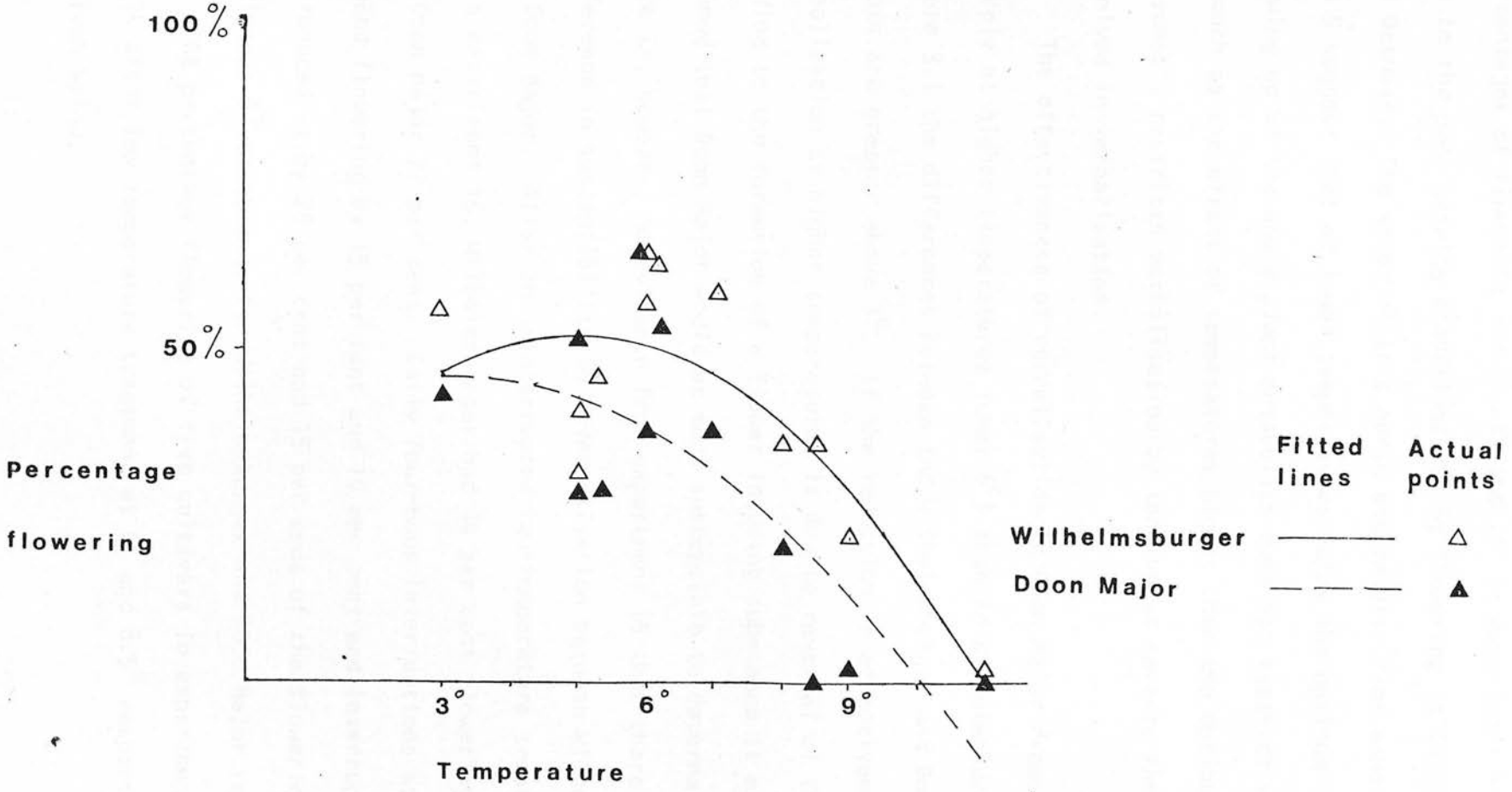


FIGURE 3.1: The effect of treatment temperature on the flowering of Wilhelmsburger and Doon Major swedes in experiments 2, 3, 4, 5, 13, 14, 15 and 16

percentages of flowering for 5°, 8° and 11° in experiment 4 are probably due to the poor growing conditions during flowering in September and October. The shape of this curve and results from experiments 4 and 5 suggest that at lower temperatures below the optimum range, the slowing up of the whole plant metabolism does not restrict vernalisation as much as the effect of temperatures higher than the optimum which presumably restricts vernalisation by tending to reverse the reactions involved in vernalisation.

The effectiveness of vernalisation in Doon Major drops off more sharply at higher temperatures (over 6°) than in Wilhelmsburger. In Figure 3.1 the differences between the Wilhelmsburger and Doon Major points are greater above 3°. If the reduction in effectiveness of vernalisation at higher temperatures is due to reversal of the reactions leading to the formation of a flower inducing substance it might be assumed that Doon Major would be more susceptible to devernalisation. There is, however, no evidence from experiment 15 that there is any difference in susceptibility to devernalisation between Wilhelmsburger and Doon Major. After an uninterrupted low temperature treatment at 6° in experiment 15, Wilhelmsburger had 86 per cent flowering plants and Doon Major 77 per cent. Daily four-hour interruptions at 16° reduced flowering by 15 per cent and 10 per cent and interruptions at 22° reduced it by 25 per cent and 15 per cent of the flowering of the continuous 6° treatment, for Wilhelmsburger and Doon Major respectively.

The percentage flowering of five cultivars in experiments 13 and 14 after low temperature treatment at 6° and 8.5° respectively is given below.

Cultivar	% flowering experiment 13	% flowering experiment 14
	6°	8.5°
Wilhelmsburger	87	43
Doon Major	68	11
Pentland Harvester	96	71
Ruta Otofte	70	7
Harrietfield	76	18

There is a much greater difference in flowering percentages after treatment at 8.5° than 6°, and this could be because more susceptible cultivars are induced relatively more easily at higher temperatures than more resistant cultivars. However, longer durations of low temperature treatment were included in experiment 13 and post-vernalisation conditions were better for flowering and this may partly explain the smaller differences between cultivars in this experiment. Where such a high proportion of all cultivars are flowering there is less chance of differences between cultivars being expressed.

If the greater differences between the cultivars at the higher temperature are due to differential responses to temperature of the five cultivars, as seems to be the case with Wilhelmsburger and Doon Major, this will be important in selection. Higher treatment temperatures around 5° to 10° will probably be most suitable for selecting out susceptible cultivars but the most suitable treatment is probably a fluctuating temperature, for instance with a daily cycle from 2° to 10°. This will resemble natural conditions better than one constant temperature and every strain and cultivar will be exposed to its own optimum temperature for vernalisation for at least some of the time.

3.2.2 Temperature measurement

In all experiments the temperature quoted is the shade air temperature as this is considered to be the most constant and will be most like temperatures within the plant tissue. Radiation raises the temperature of the leaf and exposed parts of the plant but the site of vernalisation, the growing-point and axillary buds, will be less affected by radiation. In experiment 20 the surface temperature of the growing-point of plants was 2^{o} higher when the lights to supplement daylight were illuminated, but the air temperature was also 1.5^{o} higher.

Temperature control was accurate to $\pm 0.5^{o}$ in experiments 3, 5, 12 and 15 but was also reasonably good in experiments 2, 4, 9, 10, 13 and 14, holding to $\pm 1.0^{o}$ while temperatures out of doors were low, but tending to rise during warmer periods although very rarely above 10^{o}.

Except when temperature control was very good, temperatures are expressed as the mean of the daily mean temperature. When this mean temperature is above the optimum for vernalisation it is a useful estimate of temperature, as the amount of time spent at temperatures higher than and less vernalising than the mean will be balanced by the time spent at lower more vernalising temperatures, and so a mean temperature of 8^{o} will have roughly the same vernalising effect as a continuous temperature of 8^{o}. When the mean temperature is in the optimum range, the time spent at temperatures higher and lower than the mean will be less vernalising than the mean, and so the effect of a mean temperature near the optimum cannot be compared with the effect of the same continuous temperature. In experiment 14 in which the mean of the daily mean temperature during treatment was 8.5^{o}, and in the constant 9^{o} treatment of experiment 5, both temperatures being above the optimum for vernalisation, flowering of adult plants 124 days after

low temperature treatment in the 28-day treatments was 50 per cent for Wilhelmsburger in both experiments and for Doon Major 14.3 and 8.3 per cent for experiments 14 and 5 respectively. Despite the greater variation in temperature in experiment 14 the effect was much the same as that of the similar constant temperature in experiment 5. Five degrees is near the optimum, however, and in experiment 4 the temperature in the 5^{o} treatment sometimes fluctuated between 2^{o} and 8^{o}. Flowering was only 28 per cent for all Wilhelmsburger and Doon Major treatments at 5^{o}, but in experiment 3 in which the treatment temperature was constant at 5^{o} flowering, was 51 per cent for all Wilhelmsburger and Doon Major treatments 4 days old and over at the start of treatment. Only the 20, 28, 36 and 40-day treatments were included and only plants flowering within 92 days of the end of low temperature treatment, to make a valid comparison with the 21, 28, 35 and 42 days treatments in experiment 4. In all four experiments, 3, 4, 5 and 14, post low temperature conditions were similar.

It is not possible to sum temperature over time during a period of low temperature to obtain a direct estimate of the vernalising effect of the period as the vernalising effect of temperature is not linear, unlike the effect of temperature on growth. It would be necessary to give each temperature a score of relative effectiveness and this score might vary depending on the cultivar being used, and then sum these scores.

3.3 Devernalisation

3.3.1 The effect on flowering

Beyond the range of optimum temperature for vernalisation the rate of the vernalisation process declines sharply till at 11° (see experiment 4) only one per cent of all plants flowered. As the temperature rises above 11°, the effects of low temperature in inducing flowering are progressively reversed.

In experiment 6 high temperature immediately following low temperature treatment reduced the number of flowering plants, and the higher the temperature the fewer plants flowered. As with other species (Stokes and Verkerk 1950, Heide 1970) this reversal only occurs immediately after low temperature treatment. In most experiments plants were grown in moderate temperatures (12° to 15°) immediately after low temperature treatment but in those experiments in which plants were moved directly to higher temperatures flowering was reduced. For instance, in experiment 9 Doon Major plants grown for 28 days at 7° and moved immediately to 18° did not flower but in experiment 2, 21 per cent of adult Doon Major plants flowered after 30 days at 7° followed by 14 days at 13°. The reactions involved in vernalisation must eventually produce a heat stable substance or change in the plant as swedes will flower in high temperatures, for example 18° mean air temperature at plant level in experiments 14, 23 and 24. In the theoretical model of vernalisation (see 1.3 page 8) reaction III is not considered to be reversible and product D is assumed to be but stable. This heat stable substance is not formed immediately during low temperature treatment but sufficient may be formed during a long period of low temperature so that subsequent high temperatures do not prevent 100 per cent flowering or greatly increase the time taken to flower.

In experiment 7 in which six weeks of low temperature treatment at 8° was interrupted after two weeks by one, two or three weeks at 17°, flowering was reduced more by the three-week interruption than the two-week, and more by the two-week than the one-week interruption which suggests that reversal of a two-week low temperature treatment can take up to three weeks at 17°. The three-week interruption completely reversed the effects of the initial two weeks at 8° so that there was no difference in flowering after four weeks at 8° and after six weeks at 8° with a three-week interruption at 17° after the first two weeks.

A short period of high temperature during a period of low temperature will tend to reverse the vernalising effect of the low temperature. The more frequent the interruptions the more effective is a given duration of high temperature in reversing vernalisation (Purvis and Gregory 1952). This fits the theoretical model of vernalisation. Substance B, produced from A by reaction I, will accumulate at low temperature and slowly be converted to D through reaction III, but if the temperature rises considerably most of the B formed at low temperature will be converted to C through reaction II. The more frequent are the interruptions of high temperature the less B will be converted to D assuming that reaction III proceeds more slowly at low temperature than reaction I.

3.3.2 *Quantifying devernalisation*

As would be expected from the model, a daily four-hour period at high temperature during a low temperature treatment reduced the proportion of swedes flowering although the total time spent at the low temperature was the same (see experiment 15). Interruptions at 22° reduced flowering more than interruptions at 16° and in the model

reaction II will be faster at 22° than 16° and more B will be converted to C during the four-hour interruption period. To quantify the effect of the high temperature interruptions, the number of calendar days required to cause 50 per cent flowering at 6° continuous, 6° with 16° interruption, and 6° with 22° interruption are compared. The number of calendar days for six cultivars are given in the table on page 143, and the mean of the number of calendar days required to cause 50 per cent flowering for the six cultivars is 28.97 days at 6° continuous, 42.23 days for 6° with 16° interruption, and 45.85 days for 6° with 22° interruption. One calendar day in the interrupted treatments consists of 20 hours at 6° and 4 hours at a higher temperature and so 42.23 calendar days with interruptions at 16° is composed of $42.23 \times 20 = 844.6$ hours at 6°, and $42.23 \times 4 = 168.9$ hours at 16°. This treatment, however, had the same vernalising effect as 28.97 days ($28.97 \times 24 = 695.3$ hours) at 6° continuous. The extra number of hours ($844.6 - 695.3 = 149.3$ hours) at 6° required in the 16° interrupted treatment, to cause 50 per cent flowering, must have compensated for the devernalising effect of the 168.9 hours at 16°. In other words, the vernalising effect of 149.3 hours at 6° equals, or neutralises, the devernalising effect of 168.9 hours at 16°. As the interruption periods were 4 hours in duration the devernalising effect of 16° is expressed in units of 4 hours as shorter or longer interruptions would probably not have a linear relationship with the effect of 4 hours.

$$-149.3 \text{ hours at } 6^\circ = +168.9 \text{ hours at } 16^\circ$$

$$\therefore \quad -3.54 \text{ " at } 6^\circ = +4 \text{ " at } 16^\circ$$

Similarly for 22° interruptions, 917.0 hours at 6° with 183.4 hours at 22° have the same vernalising effect as 695.3 hours at 6°

continuous, therefore 183.4 hours at 22° reverses 917 - 695.3 = 221.7 hours at 6°.

-221.7 hours at 6° = +183.4 hours at 22°

∴ -4.84 " at 6° = +4 " at 22°

This can be represented graphically (Figure 3.2). The line between 6° and 16° cuts the temperature axis at 11°. Above 11° the vernalising effect of a period at 6° is reversed, although at a very slow rate from 11° to 12°. The straight line from the temperature axis to 6° assumes that temperatures above 6° are not so effective in vernalising as 6°. The levelling off of the slope between 16° and 22° suggests that increasing temperature can only increase devernalisation to a certain extent. In the model this could be explained as a limited amount of B available to be converted to C, the rest of B having been converted to D during the low temperature period. Different durations of interruption would have different effects on vernalisation. If the interruptions were very short, it is possible that higher temperatures would be much more effective in devernalisation as the rate of conversion of B to C would be much greater, and the main limit on this conversion would not be the supply of B, but the duration of the interruption. The graph agrees with the findings of Heide (1970) for cabbages that interruptions at 12° did reduce vernalisation to a small extent.

Over the whole range of temperature the response to increasing temperature in terms of proportion of plants flowering or of vernalising effect will be a combination of Figures 3.1 and 3.2. Subsequent flowering will increase as vernalising temperatures rise above 0° and rate of plant metabolism increases, level off around 5° and then

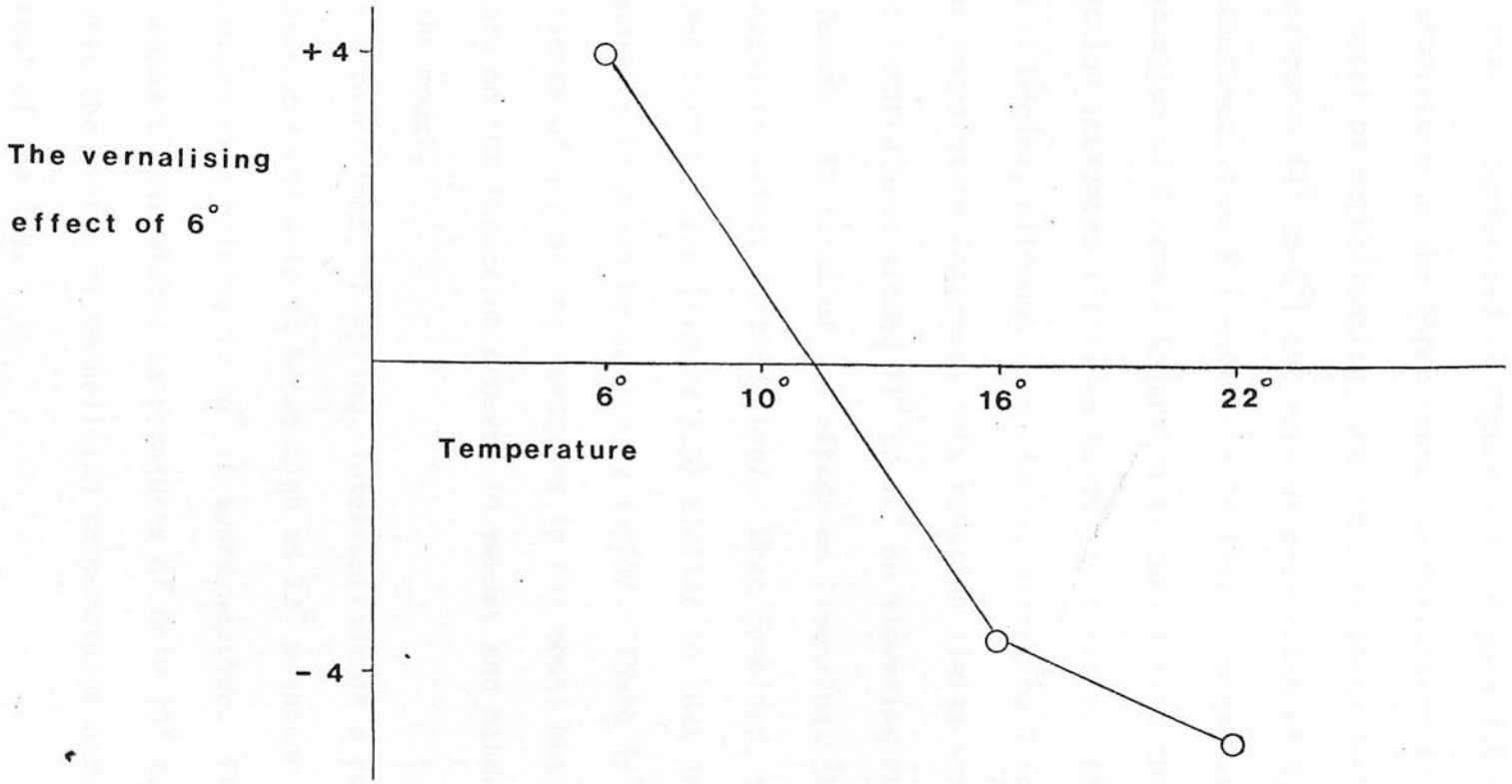


FIGURE 3.2: Experiment 15 - the devernalising effect of daily 4-hour interruptions at 16° and 22° on the scale of the vernalising effect of 4 hours at 6°

decline as temperature increases above 6°. Beyond the range of vernalising temperatures, about 11°, an increase in temperature will give a negative proportion of plants flowering, that is flower induction caused by lower temperatures is reversed at higher temperatures. This is summarised in Figure 3.3. Figure 3.4 shows the effect of temperature on the three reactions postulated in the theoretical model of vernalisation, and their response to temperature. At low temperatures (0° to 4°) the rate of production of D, the flowering substance, from B (reaction III) limits vernalisation. The rate of production of B from A (reaction I) then limits the process as this reaction increases little as temperature rises. Above 7° to 8° reaction II begins, although very slowly, diverting B to C, instead of D, and as temperature increases this reaction limits vernalisation more till at temperatures around 11° to 12° no flowering substance D is being formed. In terms of its effect on flowering, this reaction only has a negative effect (dotted line). When combined, these reaction lines form a curve (Figure 3.5) similar to that in Figure 3.3. A similar analysis is given by Salisbury (1963). There is no evidence for the existence of any of the reactions in the model but the effects of temperature on the induction process in swedes and other species agree with the model.

In field conditions, in spring, interruptions of a period of low temperature are unlikely to be as high as 22° although the plant temperature might rise briefly to 16° in sunny weather. From Figure 3.2 it even appears that midday temperatures of only 12° to 13° will tend to reverse the effect of vernalising temperatures occurring during the rest of the day.

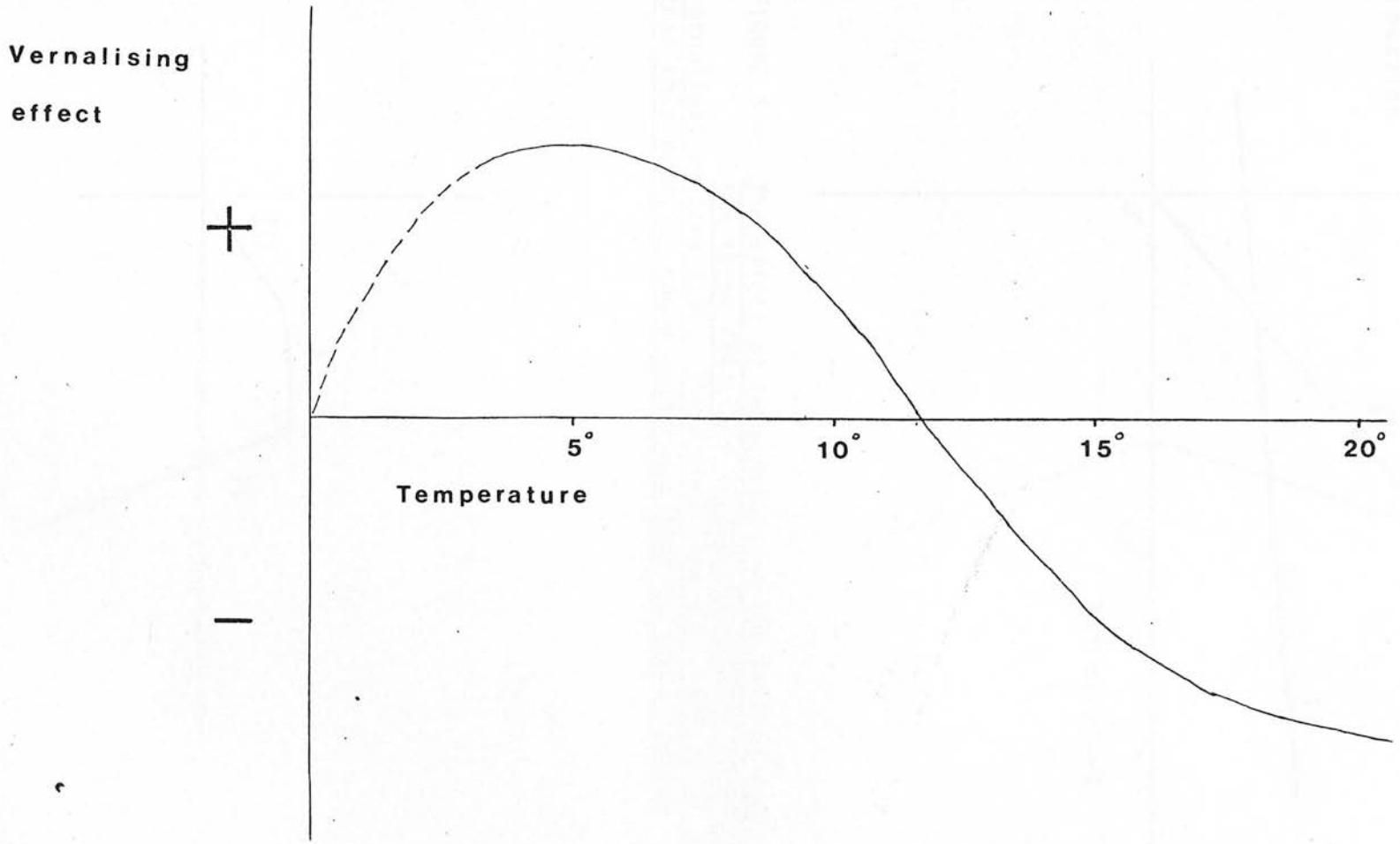


FIGURE 3.3: Summary of the effectiveness of temperature in vernalising or devernalising. The continuous line is based on experimental data, the dashed line is extrapolated.

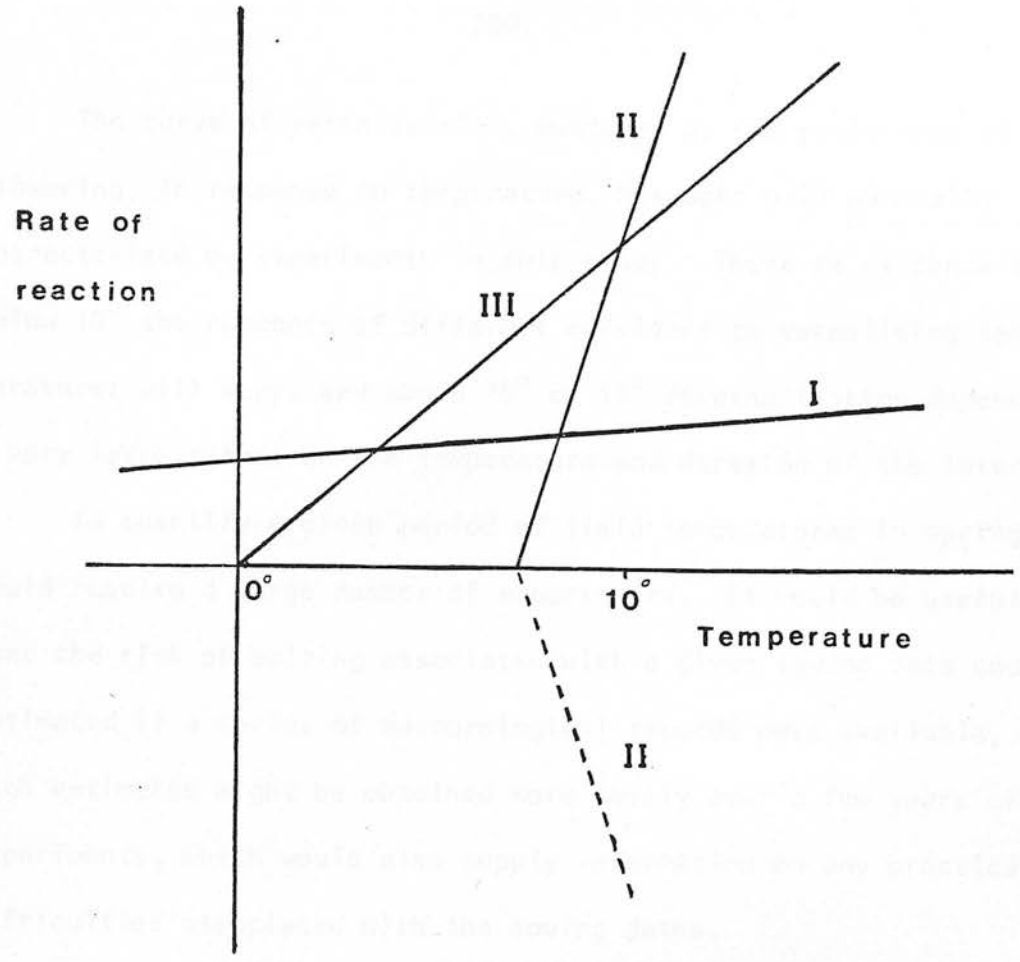


FIGURE 3.4: The effect of temperature on the rate of reaction of the three reactions in the theoretical model of vernalisation (see 1.3). The dashed line for reaction II indicates that this reaction has a negative effect on vernalisation

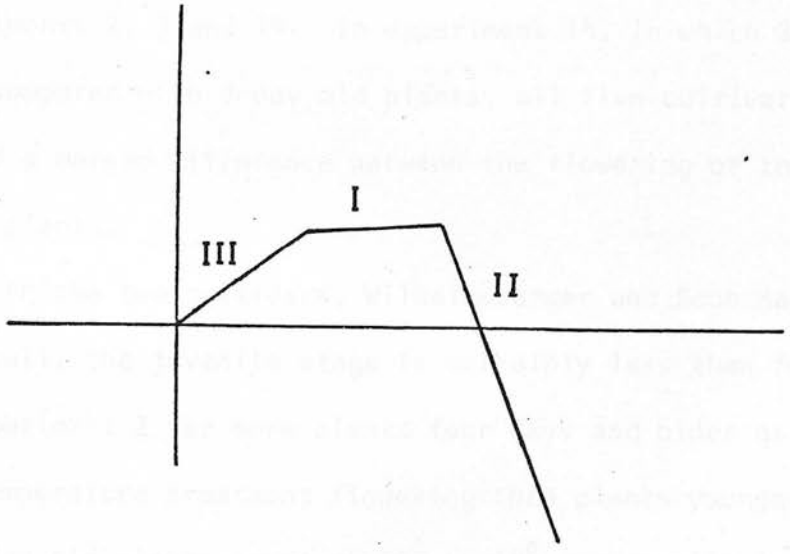


FIGURE 3.5: The combined effect of temperature on the three reactions in the theoretical model of vernalisation in limiting the overall rate of vernalisation

The curve of vernalisation, measured by the proportion of plants flowering, in response to temperature, has been only partially characterised by experiments in this study. There is evidence that below 10^{o} the response of different cultivars to vernalising temperatures will vary, and above 10^{o} or 11^{o} devernalisation depends to a very large extent on the temperature and duration of the interruption.

To quantify a given period of field temperatures in spring would require a large number of experiments. It would be useful in that the risk of bolting associated with a given sowing date could be estimated if a series of meteorological records were available, but such estimates might be obtained more easily over a few years of field experiments, which would also supply information on any practical difficulties associated with the sowing dates.

3.4 The juvenile stage

3.4.1 Differences between cultivars

There is a short juvenile stage in swedes as demonstrated in experiments 2, 3 and 14. In experiment 14, in which 20-day old plants were compared with 0-day old plants, all five cultivars examined showed a marked difference between the flowering of the juvenile and adult plants.

In the two cultivars, Wilhelmsburger and Doon Major, examined in detail, the juvenile stage is certainly less than four days, in experiment 2 far more plants four days and older at the start of low temperature treatment flowering than plants younger than four days. Four-day old plants grown at 15^{o} to 18^{o} are usually just emerging (see Figure 2.2) with radicle 0.5 to 2 cm and cotyledons sometimes

still in the seed coat. This was confirmed for Wilhelmsburger in experiment 3, far fewer two-day old plants flowering than older plants.

In experiment 2 the difference in percentage flowering between adult and juvenile Doon Major plants was not so great as for Wilhelmsburger but experiment 14, in which newly sown seed and 20-day old plants were compared, confirmed that Doon Major has a juvenile stage. In experiment 3, however, there was no difference in the flowering of two and four-day old Doon Major plants in any of the six durations of low temperature treatment, strongly suggesting that the juvenile stage is less than two days. If the change from juvenile to adult occurs at about two days, in Doon Major, a slight difference in vigour of germination between experiments 2 and 3 might affect whether two-day old plants were still juvenile, or just adult. Although four-day Wilhelmsburger plants are certainly adult, and two-day plants certainly juvenile the change from juvenility to adulthood may occur at 3 days, or $2\frac{1}{2}$ days, in which case there is only a small difference between the cultivars. In experiment 14, more differences between cultivars in juvenility were apparent, Harrietfield having a shorter or less marked juvenile stage than the other four cultivars examined, Wilhelmsburger, Doon Major, Pentland Harvester and Ruta Otofte.

3.4.2 Plant development

Whether chronological age or stage of development determines when adulthood is reached has not been determined but it seems more likely that juvenility is determined by plant development than purely by time from sowing.

During a low temperature treatment seedlings will grow out of juvenility and if the subsequent period of low temperature is long enough will be induced and later flower.

A longer duration of low temperature to cause a given proportion of plants to flower is required by plants that are juvenile at the start of treatment than by adult plants.

1. If plant development is the most important factor affecting juvenility the longer time at low temperature required by juvenile plants for a given proportion of flowering will simply be the time required for the plants to grow out of the juvenile phase during the low temperature.
2. The size of plants at this change from juvenile to adult can be estimated if the growth of the juvenile plants has been recorded during the low temperature period.
3. If the size of plants grown at higher temperatures has been recorded over time the estimated size of a plant at the change-over from juvenile to adult, found above, can be compared with that of plants grown at higher temperatures and the age, in terms of days of growth at higher temperatures estimated.

Calculation 1: In experiment 3 the proportions of Wilhelmsburger plants flowering, on a logit scale, were fitted to a model, using GLIM (see Appendix B), of the linear effect of duration of low temperature (χ^2 test $p<0.001$) and the effect of plant age. The number of days at low temperature (5°) required to cause 50 per cent flowering for each plant age could be calculated from these lines and were 29.17 days for two-day old (juvenile) plants, 23.28 days for four and six-day old plants and 21.39 days for eight and 10-day old plants. Assuming that the susceptibility to vernalisation of all adult plants is the same, the mean, 22.34 days, was compared with the figure for juvenile plants, 29.17 days. The difference, 6.83 days at 5°, must be the time required by the two-day old plants to reach adulthood.

Fitting the percentages of plants flowering against duration of low temperature treatment into separate regression lines for juvenile and adult plants, a very similar difference, 6.6 days at 5^{o}, was found between the number of days required to cause 50 per cent flowering in juvenile and adult plants.

Calculation 2: Total leaf number, that is including leaf primordia, is the only available measurement of plant development for the early part of low temperature treatment. From the dissections of sample plants before, once during and after each duration of low temperature treatment a line ($r = +0.977$, $n = 10$; $p<0.001$) can be plotted of total leaf number against days of growth at 5^{o} for plants that were two days old at the start of each duration of low temperature treatment. On this fitted line, using the data from the GLIM fitted lines, after 6.83 days at 5^{o}, plants that were two days old at the start of low temperature treatment have 0.8090 leaf primordia.

Calculation 3: To find what age of plant grown at 16^{o} has reached this stage of development (0.8090 leaf primordia), the total leaf number of all plants at the start of the six duration of low temperature treatments can be plotted against the age of these plants, from two to ten days old. On this line ($r = 0.946$, $n = 30$; $p<0.001$) a plant with 0.8090 leaf primordia would be 3.87 days old if grown at 16^{o}, that is Wilhelmsburger plants grown at 16^{o} attain adulthood at 3.87 days.

The 3.87 days agrees with other evidence from flowering in experiment 2 that the juvenile stage ends between 2 and 4 days in Wilhelmsburger. There is no evidence to suggest that the difference in flowering response to low temperature treatment of young and older

plants is due to anything other than the extra time required for juvenile plants to reach adulthood. In this analysis the lines of flowering response fitted to duration of low temperature treatment are the main sources of possible error as they are based on few plants and few duration points. Differences in flowering behaviour of only one or two plants, especially in the juvenile group might appreciably alter the estimate of the number of days required to cause 50 per cent flowering, and therefore the estimates of plant development and age (at 16°) at the change from juvenile to adult. The lines fitted using GLIM and the fitted regression lines for flowering (not shown) were very similar and it is the data itself, rather than the fitting of lines, which is likely to be the main source of error.

A similar analysis with Doon Major was not possible, as two-day old plants did not differ from older plants in their flowering response.

The difference between the number of days required to cause 50 per cent flowering for juvenile plants, and for adult plants is assumed to be the same as the number of days at low temperature required by juvenile plants to reach adulthood. In experiment 3 this difference is about 6.6 to 6.8 days for Wilhelmsburger and in experiment 2, 8.98 days for zero day old Wilhelmsburger and Doon Major plants, that is juvenile plants take longer to reach adulthood during a period of low temperature than at higher temperatures.

From experiments 2 and 3 it appears that juvenility ends just at, or slightly before, the emergence of the seed above soil level as emergence occurred at or after four days growth.

3.4.3 Importance of juvenility

In field conditions in April and May seedlings may remain juvenile for as long as two weeks if temperatures are low, and certainly longer than the four days required when plants are grown at a uniform temperature of 16°. The existence of a juvenile stage must reduce the incidence of bolting as it occurs at the earliest and generally coldest period of the growth of the crop. As the juvenile stage probably lasts from one to two weeks at low, vernalising temperatures it can reduce the duration of a period of vernalising temperature in the field by a significant amount, as only 20 to 30 days of low temperature are generally required to cause 50 per cent flowering.

Over five years of experiments with swedes, in which several sowing dates were used, Lysgaard and Nørgaard Holm (1962) found that a "relatively low temperature during a consecutive period after the plants had emerged" was the main factor affecting bolting percentage which agrees with the suggestion that the juvenile stage has ended by the time plants have emerged.

There is some evidence of variation between cultivars in the duration of the juvenile stage and it is possible that a longer juvenile phase could be selected for. If there is sufficient variability in the duration of juvenility for selection to be effective, a long duration of juvenility would be an advantageous plant character as it would provide resistance in the field to vernalisation at the time when a period of vernalising temperature is most likely to occur just after sowing and would not interfere with seed production if temperatures were reasonably warm during the early growth of the seed crop. In practice, however, selection for long juvenility would be

difficult as it requires two treatment factors in the testing of lines and cultivars - age of plants at the beginning of low temperature treatment as well as duration of low temperature treatment - instead of the one factor required in straightforward testing for bolting resistance. No morphological changes on attainment of adulthood have been detected and so there appears to be no easier way of measuring juvenility than by exposing a range of plant ages to low temperature treatment.

3.5 Stem extension

Stem height - the height of the main axis from soil level to the apical bud - was measured at flowering in all experiments but as stem extension always accompanied flowering and was generally affected by treatments in the same way as proportion of plants flowering, data on stem height has been omitted in the results of most experiments.

Like flower induction, the induction of stem extension occurs in the growing point and can occur in the absence of light. In experiment 5 the proportion of plants flowering was slightly greater after a low temperature period at 6° than at 3° and mean stem height of all plants was also greater after 6° (Table 3.1). The longer the duration of low temperature treatment the greater the stem height although after long durations - 40 days or over - stem height at flowering tends to decline with increasing low temperature treatment, probably because days to flowering are also reduced and flowering occurs earlier relative to stem extension.

More susceptible cultivars tended to have taller stems at flowering than more resistant cultivars but stem height was always

TABLE 3.1: Experiment 5 - the effect of temperature, duration of low temperature treatment and cultivar on the stem height of all plants, 112 days after the end of low temperature treatment

Duration of low temperature	Wilhelmsburger				Doon Major				Mean of both cultivars
	3°	6°	9°	Mean	3°	6°	9°	Mean	
	Stem height (cm)				Stem height (cm)				Stem height (cm)
3 weeks	38.7	43.3	9.1	30.5	21.6	12.2	8.3	14.0	21.9
4 weeks	60.7	87.9	63.2	70.7	49.8	70.9	24.7	47.1	58.7
5 weeks	97.3	96.4	92.4	95.3	86.4	89.2	29.1	66.5	80.9
6 weeks	89.2	96.1	96.1	94.1	84.7	95.2	74.7	84.8	89.3
Mean	72.1	80.4	65.8	72.8	59.5	66.2	34.2	52.9	62.6
Mean of both cultivars	65.4	73.4	49.7	62.6					

For comparisons within table	SE ± 9.77	For comparisons of temperature means	SE ± 3.45
For comparisons of cultivar means	SE ± 2.82	For comparisons of duration means	SE ± 3.99

more variable than days to flowering and the effects of treatments less clear.

Evidence from other species (Owen *et al* 1939, Stokes and Verkerk 1950, Heide 1970) suggests that stem extension is not always affected in the same way as flower induction by low temperature and other treatments.

Applications of exogenous gibberellic acid result in immediate stem extension in swedes (see experiments 8 and 9) but only result in flowering if temperatures are fairly low or treatment very prolonged (Wittwer and Bukovac 1957, Lang 1957). The accumulation of endogenous gibberellin has been found to occur during low temperature treatment of some brassica species and the stem extension of swedes after a low temperature treatment is probably caused by a gibberellin type of substance.

Devernalisation does not have exactly the same effect on stem extension as on the induction of flowering, although it does reduce stem height. Occasionally, when flower induction has been reversed by a period of high post vernalisation temperature the stem continues to extend so that the leaves and growing point eventually form a perched rosette on top of an extended stem (Figure 3.6). Stem extension substances must be less affected by devernalisation than flower induction factors rather than being produced more readily during low temperature treatment. If the latter were the case, plants that had received a period of low temperature too short to induce flowering, followed by cool, post vernalisation temperatures, would have extending stems. This has never been observed in any experiment.

FIGURE 3.6: Swedes with vegetative rosettes on the top of extended stems

In experiment 9 in which plants were devernalised by a weekly interruption of one or two days at high temperature during low temperature treatment, the stems of devernalised non-flowering plants extended more than stems of unvernalised plants. This difference in extension was particularly noticeable when gibberellic acid was applied after low temperature treatment, the devernalised plants extending much more in response to the gibberellic acid than the unvernalised plants. It is possible that gibberellin-like substances or gibberellin precursors produced during low temperature treatment are to some extent destroyed by devernalisation, like flower inducing factors, but the ability of the plant to respond to gibberellin is increased during the low temperature treatment, and not affected by devernalisation.

It is probable that stem extension inducing factors are produced by a similar set of reactions to those illustrated in the theoretical model of vernalisation, but the substances, and temperature relationships of the reactions are clearly not exactly the same as for flower induction.

If many plants with extended stems are present in a field it may make harvesting of swedes more difficult but lignification of root and stem is the main yield-reducing effect of flowering and it is associated with flowering not stem extension. Extended stems of non-flowering plants whether the extension was caused by gibberellin or a low temperature period, were never heavily lignified like the stems of flowering plants. As far as yield of digestible dry matter is concerned, stem extension in the absence of flowering is not of any great importance.

3.6 Light intensity

Although light is not essential for vernalisation of swedes, at least when plants have sufficient reserves to survive for a long period in darkness at low temperature, vernalisation occurs more rapidly at higher light intensity. The highest light intensity (11,800 lux) used in experiment 12 was lower than the saturation of photosynthesis (around 20,000 lux) (Seliger and McElroy 1965) and the lowest light intensity (2300 lux) was above the compensation point for photosynthesis (around 1000 lux) and so the three light intensities used in experiment 12 were in the range in which rate of photosynthesis increases as light intensity increases. The temperature was low, 8.5°, but leaf production was affected by light intensity and it is likely that photosynthesis was also.

In the field light intensities are much greater, for instance light June sunlight is around 100,000 lux (Seliger and McElroy 1965) and so light intensity is only likely to affect natural vernalisation when a plant is shaded by other plants and at the beginning and end of the day.

At a quarter of the highest light intensity in experiment 12 flowering was cut by a half but the number of reproductive plants (those with bud stage 3 or over at the end of the experiment) was only reduced to 78 per cent of those in the highest light intensity treatment. It is possible that light during low temperature treatment affects the subsequent development of flowers more than it affects the induction of flower buds. In one plant examined at the end of 15 weeks low temperature treatment in darkness, flower buds (stage 3, see Figure 2.1) had already formed. In this respect the swede

resembles sugar beet more than it resembles cauliflower as the former can be induced in darkness (Fife and Price 1953), but not the latter (Sadik and Ozbun 1968). In rye and other seed vernalised species light is not required for vernalisation. The reactions leading to flower induction of swedes do not require light but other effects of light such as improved supply of photosynthates and consequent increased growth are likely to affect the rate of vernalisation reactions.

3.7 Nitrogen

Plants receiving plentiful nitrogen whether before or after low temperature treatment flowered more rapidly than plants receiving a very restricted supply (see experiment 10), but whether additional nitrogen has any effect when plants have a moderate supply is uncertain. Comparisons of different experiments suggest that closeness of planting, or pot size, both factors liable to affect nitrogen supply to the plant made no observable difference to flowering, whereas differences in temperature during low temperature treatment or immediately after could result in large differences in flowering between similar experiments.

Nitrogen supply, however, did have a slight effect on flowering, and if the maximum possible rate of flowering is required, additional nitrogen might be useful.

3.8 Cultivars and selected lines

3.8.1 Variation between cultivars

There is a considerable range in susceptibility to vernalisation in the eight cultivars that have been studied and in the 15 lines selected from two cultivars. Taking the results from experiments 13, 14 and 15 (continuous 6° treatment only) together the cultivars are, in order of increasing resistance to vernalisation, Pentland Harvester, Della, Wilhelmsburger and Marian, Harrietfield, Doon Major and Ruta Otofte, and Seefelder most resistant.

Slightly more (four more) Ruta Otofte plants than Doon Major flowered in experiment 13 and this result was based on 120 plants per cultivar, but slightly fewer (one fewer and four fewer) Ruta Otofte flowered than Doon Major in experiments 14 and 15 (6° continuous) respectively and it was not possible to determine with certainty which is the least susceptible cultivar. Doon Major responded steadily to increases in duration of low temperature treatment but the flowering of Ruta Otofte was much more erratic probably because of genetic variation in the seed stock used. Della may be more susceptible to vernalisation than Pentland Harvester as they were not compared in the same experiment but Pentland Harvester flowered 175 and 110 per cent of the flowering of Wilhelmsburger in experiments 13 and 14 respectively while Della only flowered 111 per cent of Wilhelmsburger in experiment 15 (continuous 6° treatment). Similarly, although Marian and Harrietfield were not directly compared, Marian flowered 100 per cent of Wilhelmsburger in the continuous 6° treatment in experiment 15 and 97 per cent of Wilhelmsburger taking all treatments into account, whereas Harrietfield flowered only 50 and 87 per cent of Wilhelmsburger in experiments 13 and 14 respectively.

In experiment 13 and 15 the proportions of plants flowering in each duration of low temperature treatment were transformed to a logit scale and a curve fitted to these values, using GLIM. The interactions between cultivar and duration of low temperature were not significant and so it can be assumed that the response curves of the different cultivars are parallel.

The slope of the line is a measure of the genetic variability in resistance to vernalisation within the cultivar. If the cultivar was perfectly uniform, after a given duration of low temperature all plants would flower and the line would rise vertically from 0 to 100 per cent flowering at that duration. The response lines slope because a proportion of the population is more susceptible and flowers after short durations while a proportion of plants will only flower after longer durations. An estimate of the resistance to vernalisation within the population is the number of days of low temperature treatment required to cause 50 per cent flowering. The durations of low temperature which would have caused 50 per cent flowering were calculated from the parallel lines for flowering response fitted with GLIM and are given below:

	Cultivar	Days at 6° required to cause 50% flowering
Experiment 13:	Pentland Harvester	16.8 days
	Wilhelmsburger	19.4 "
	Harrietfield	23.4 "
	Ruta Otofte	24.0 "
	Doon Major	25.8 "
Experiment 15:	Della	23.5 days
	Wilhelmsburger	26.7 "
	Marian	26.2 "
	Doon Major	28.8 "
	Ruta Otofte	33.2 "
	Seefelder	35.4 "

The number of days is applicable only to the precise conditions of that experiment and the time at which recording of flowering ceases. This was 151 days in experiment 13 and 100 days in experiment 15 and so more plants flowered in experiment 13 and the number of days for 50 per cent flowering was generally shorter. The number of days for 50 per cent flowering gives a clear quantitative measure of the relative susceptibilities of the cultivars and demonstrates that a relatively short period of continuous low temperature, 2 to 3 weeks, can cause a damaging level of bolting in susceptible cultivars. When 50 per cent of plants are flowering a further proportion will be extending with developing flower buds and so more than 50 per cent of plants will contain a high percentage of fibre and be difficult to harvest.

Pentland Harvester and Wilhelmsburger, two of the most susceptible cultivars used in these experiments, have been described as having "a good yield with an acceptable level of 'bolting', when drilled in circumstances likely to favour bolting" (Bell 1968). Many cultivars tested by Bell were rejected on the grounds of poor bolting resistance, and if Pentland Harvester and Wilhelmsburger were acceptable, much improvement in the bolting resistance of cultivars, at least those available in 1968, is desirable.

3.8.2 Variation within cultivars

There is clearly a wide range of susceptibility to flowering among currently available cultivars and within the cultivars there is probably considerable scope for improvement. The progeny of early and late flowering Wilhelmsburger swedes differed considerably from the parent population in susceptibility to flowering, the late flowering

lines being more resistant than any of the similarly selected Doon Major lines and in comparable duration of low temperature treatments had a lower percentage of plants flowering at 100 days than Ruta Otofte at 100 days, in experiment 13 and slightly lower than Seefelder in experiment 15, although this difference was small and could easily have been due to the different experimental conditions. Temperatures in all three experiments were similar, mean daily mean temperature 6.1° in experiment 13, $6 \pm 0.5^{\circ}$ in experiment 15 and mean daily mean temperature 5.3° in experiment 16. The early flowering Wilhelmsburger lines are probably not as susceptible to flowering as Pentland Harvester or Della, although flowering percentages at 100 days after the end of low temperature treatment were very high and comparisons more difficult.

The late flowering Wilhelmsburger lines were less susceptible to flowering than any of the Doon Major lines which suggests that the ability of a cultivar to respond to selection for resistance to flowering depends not so much on the present average resistance of the cultivar as on the variability in response to low temperature treatment within the cultivar.

In previous experiments within a group of Wilhelmsburger plants there have frequently been a few plants which responded unusually, flowering more readily or less readily than the majority of the group whereas Doon Major plants have in general behaved very uniformly. Ruta Otofte has shown even more variability in response to low temperature treatment than Wilhelmsburger and it is possible that a highly bolting resistant strain could be selected from this already resistant cultivar.

Although the interaction between cultivar and duration of low temperature was not significant in experiment 13, the slopes of the cultivar response lines did differ when individually fitted, that is with an interaction term, those of Wilhelmsburger and Ruta Otofte being less steep than the others, suggesting greater variability within these cultivars.

Figure 3.7 shows the additional number of plants induced to flower in experiment 15 as duration of low temperature increases. The requirements of the individual plants for the appropriate period of low temperature to induce flowering form a normal distribution. The position of a distribution curve depends on the time at which flowering recordings are concluded, if early, the curve will be centred over longer durations, if late over shorter durations. At any line, however, the curve of more variable cultivars is wider than that of more uniform cultivars.

Even in different experimental conditions, the relative responses of the cultivars remain fairly constant. Wilhelmsburger and Doon Major were both used in 15 low temperature experiments (2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 21 and 25), and in none of these did Doon Major flower more, or have more reproductive plants than Wilhelmsburger and in experiments 2, 3, 4, 5, 6, 9, 12, 13, 14 and 16 Wilhelmsburger had significantly more flowering or reproductive plants. The response of a cultivar to low temperature is consistent and environmental factors affect the response of different cultivars similarly enough so that the differences between cultivars remain generally the same in different environmental conditions.

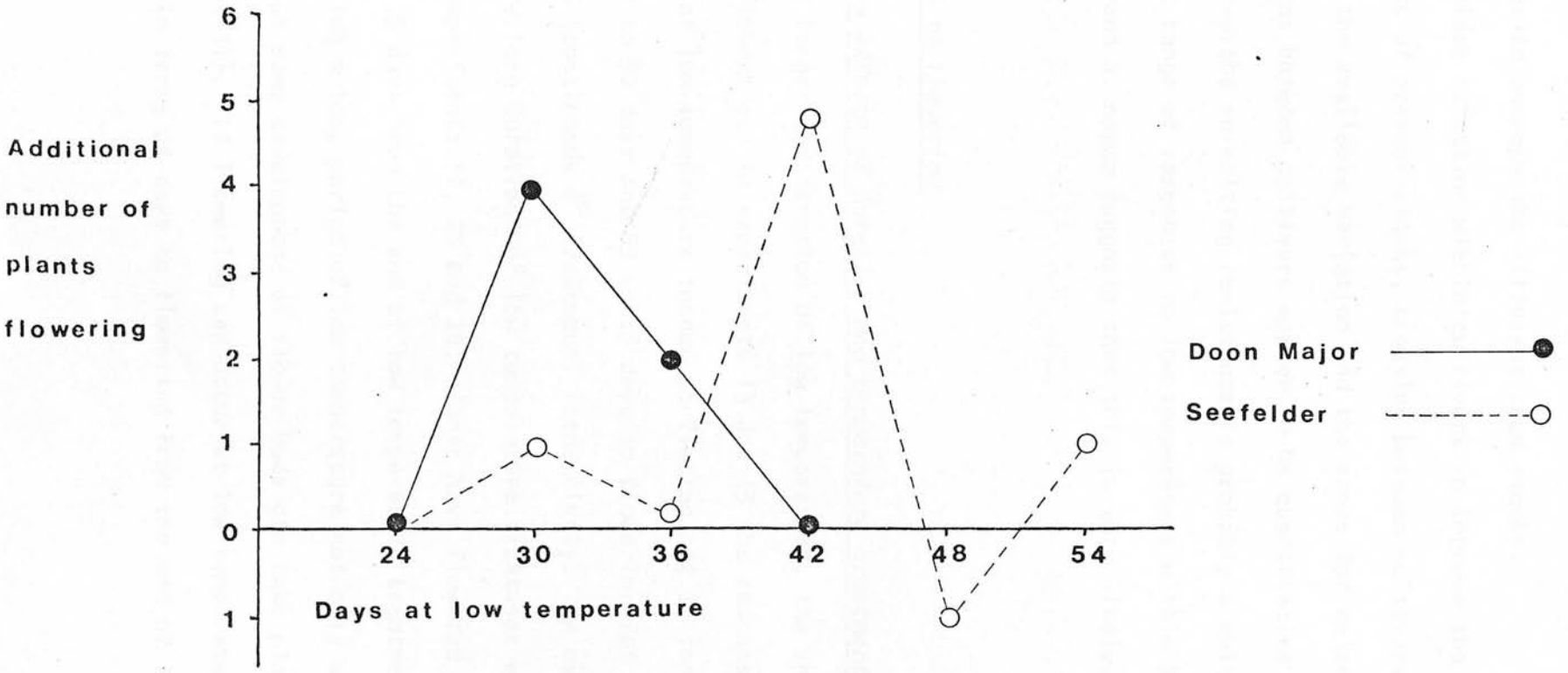


FIGURE 3.7: Experiment 15 - the effect of duration of low temperature treatment on the increase in numbers of plants flowering compared with numbers in the previous shorter treatment

Two seed stocks of Wilhelmsburger and Doon Major were used: Wilhelmsburger 1973 Garton's and 1976 Sharpes; and Doon Major 1974 Sinclair and 1976 Barclay Ross and Hutchinson, but no differences were observed between the different seed stocks.

Besides selection within cultivars to improve the bolting resistance of present stocks, crossing between cultivars might increase the available variation and the scope for selection. Differences between cultivars appear to be quantitative rather than qualitative and so bolting resistance is probably a multigenic factor. The great range of responses to low temperature within *Brassica oleracea* and *B. napus* suggests that this is very likely.

3.9 Days to flowering

3.9.1 The effects of duration and temperature treatment

The longer the duration of low temperature, the shorter the time to flowering. In experiments 13 and 15 the response to increased duration of low temperature tended to flatten off at long durations, around 70 to 90 days and 50 to 75 days to flowering for experiments 13 and 15 (continuous 6° treatment) respectively. In experiments in which very long durations of low temperature treatment were used, for example experiments 11, 23 and 24, plants have flowered in as little as 40 to 55 days from the end of low temperature treatment.

During a long period of low temperature not only are flowers induced but some development of flower buds can take place (see experiment 11). If flowering can occur at low temperatures the response in terms of days to flowering from the end of low temperature

treatment to increasing duration of low temperature treatment will decline to zero, but if a short period of higher temperature is required before flowers will open the response will decline to this asymptotic value.

Days to flowering are calculated from the end of low temperature treatment in the assumption that development of flowers is much slower below 10° than at normal growing temperatures (12° to 20°) and it would therefore not be valid to compare two values of days to flowering from the beginning of low temperature treatment, both, for instance, 110 days, one value including 30 days at low temperature, and the other 40 days. Temperature of treatment affects days to flowering, lower temperatures tending to result in more rapid flowering.

For the purpose of shortening the reproductive cycle, days to flowering from the start of low temperature treatment is more important than days to flowering from the end of treatment. The duration of low temperature treatment which results in the minimum number of days to flowering from the start of low temperature treatment will then be the appropriate duration of low temperature to select. In experiment 13 this minimum value for mean days to flowering occurs after 36 days low temperature treatment, in experiment 3 after 40 days treatment, and in experiment 15 (continuous 6° treatment) after 30 days low temperature treatment but the increase in days to flowering from the beginning of low temperature treatment was only 4 days from 30 days low temperature treatment to 54 days. Increasing the period of low temperature from around 36 days to around 50 days is unlikely to delay flowering measured from the start of low temperature treatment by more than 3 or 4 days and flowering of all plants will be

assured. The minimum number of days to flowering from the start of treatment varies among the experiments partly because of the different conditions for plant growth and flower development and partly because in the shorter treatments around 30 to 36 days not all plants flowered and those experiments in which the recording of flowering continues longer will have longer mean times to flowering, as there will be more late flowering plants included in the mean. Minimum times from the start of low temperature treatment to mean flowering time are:

	Low temperature treatment	Experiment
104 days	42 days	1
118 "	40 "	2
115 "	40 "	3
116 "	35	4
119.3 "	42	5
113.8 "	36 "	13
109.6 "	30 "	15

In experiments 2, 3, 4 and 5 Wilhelmsburger and Doon Major cutlivars were used, and in experiments 13 and 15, a range of cultivars, but in experiment 1 only Pentland Harvester - a very susceptible cultivar - was used, which explains the more rapid flowering.

The increase in the rate of flower development with the increase in duration of low temperature suggests that there is some substance, or substances which are produced during a period of low temperature which govern the rate of bud and flower development. In experiment 1 flower buds with sepals had formed within 130, 40 and 30 days of the end of low temperature treatment for 3, 6 and 9 weeks low temperature treatment respectively and this corresponds fairly well with the mean differences in days to flowering from the end of low temperature

treatment, 96 days difference between 3 and 6 weeks low temperature treatment, and 13 days difference between 6 and 9 weeks. It seems probable that the main effect of duration of low temperature is on the rate at which bud formation takes place rather than the rate of flower bud development after initiation of buds.

Devernalisation reduces the proportion of plants flowering and increases the number of days to flowering. In experiment 6 the higher the post vernalisation temperature the later was flowering and in experiment 7 the treatments with a one, two or three-week interruption of low temperature treatment flowered later than the uninterrupted treatment. Devernalised plants flowered later than plants which received an uninterrupted low temperature treatment in experiment 8, and in experiment 15 days to flowering were increased by a daily 4-hour interruption at 16^{o} or 22^{o} of low temperature treatment, although there was little difference between the effects of 16^{o} or 22^{o}. The time taken to flower seems to be an expression of the degree of vernalisation. After a long low temperature treatment whatever substance or substances are required for flower induction and development are probably present in large amounts, and flower bud initiation occurs promptly, and plants flower rapidly. After short low temperature treatments only small amounts of the substances are present and floral initiation is slow and erratic, only occurring in a proportion of plants.

There is a reduction in the variability of time to flower as duration of low temperature treatment increases. In experiment 13 the difference between the least and greatest number of days to flowering of individual plants for Doon Major, one of the more uniform cultivars, was 71, 64, 33, 26 and 10 days difference for 24, 28, 32,

36 and 40 days of low temperature treatment respectively. The differences between earliest and latest number of days to flowering of individual plants of all six cultivars in experiment 15 (6° continuous treatment) was 35, 31, 37, 27 and 19 days for 30, 36, 42, 48 and 54 days low temperature treatment respectively, and in experiment 3 (mean of the differences in two cultivars) was 120, 98, 101 and 58 days for 28, 32, 36 and 40 days low temperature treatment, respectively. In experiments 3 and 13 flowering was recorded for a longer time than in experiment 15 and so there is a greater range in days to flowering.

3.9.2 The effect of cultivar

Cultivars differ in their rates of flower development. To some extent this is a function of their susceptibility to flowering, especially after short durations of low temperature. In experiment 13 after 24 days low temperature treatment the earliest cultivar to flower is Pentland Harvester, then Wilhelmsburger, Harrietfield, Ruta Otofte and Doon Major last to flower but after 40 days low temperature treatment the order is, from earliest to last to flower, Pentland Harvester, Doon Major, Wilhelmsburger, Harrietfield and Ruta Otofte. The response, in terms of more rapid flowering, of Doon Major to increased duration of low temperature treatment, is steeper than that of other cultivars, the response of Harrietfield somewhat steeper but the responses of Wilhelmsburger, Pentland Harvester and Ruta Otofte being mutually parallel (see experiment 13, Figure 2.10).

In experiments 2, 3, 4, 5, 7 and 21 there was no real difference between the rate of flowering of Wilhelmsburger and of Doon Major. Only in experiments 6, 11 and 12 was Wilhelmsburger earlier in flowering.

In general, especially after short durations of low temperature treatment, rate of flowering of a cultivar corresponds fairly well to its susceptibility to flowering, readily induced cultivars flowering most rapidly. Both characters, rate of flowering and susceptibility to flowering, are heritable and there is probably some linkage between them as selected early and late flowering plants gave susceptible and early flowering and resistant and late flowering lines, respectively. The correlation between the percentage of plants flowering in each line, and the mean number of days to flowering for each line was high, $r = -0.867$ ($n = 9$, $p<0.01$) for the Wilhelmsburger lines, but very small, $r = -0.0053$, for the Doon Major lines ($n = 8$ NS). Selection was much less effective in Doon Major which probably explains the very low correlation as neither flowering percentages (from 38 to 64 per cent in Doon Major lines) nor days to flowering (80.5 to 85.9 days) varied between the Doon Major lines nearly as much as in the Wilhelmsburger lines (18 to 100 per cent flowering and 78.8 to 90.8 days to flowering).

3.9.3 *Measurement*

The mean number of days to flowering for a treatment depends partly on the date when the experiment is terminated. If recording of flowering continues for a long time, very late flowering plants will be included in the data and the mean number of days to flowering will be slightly increased. No experiment was terminated before the great majority of extending plants had flowered and in all experiments increasing the time during which flowering was recorded would only have included a few more plants in the data. All plants usually flowered in the longest duration of low temperature treatments and

so it is only the shorter durations with their more variable flowering that are affected by the date of termination of an experiment. Analyses of days to flowering data were weighted by the number of plants contributing to each mean and so the addition or exclusion of a single, or a few plants flowering later than the majority of plants would not greatly alter the result of the analysis. The effects of treatments are similar in experiments which are terminated early and in those that are continued for a longer time, for instance days to flowering in experiments 2 and 3, terminated after 110 and 176 days after the end of low temperature treatment respectively (see Tables 2.2 and 2.5).

A crop sown in mid-April would take at the very least 4 days to grow out of the juvenile phase, if temperatures were around 15^{o} at sowing and they are likely to be lower, and a further 24 to 30 days of low temperature (below 10^{o}) for a proportion of plants to be vernalised. Early bolting, that is flowering before the end of August, would occur within 100 to 110 days of the end of low temperature treatment. Harvest in October or November or cessation of crop growth due to low temperatures would be 50 or 60 days later, around 160 days. Experiments which finish around 100 days are recording early bolting only, and recording has to continue to around 160 days to include late bolters. In some experiments, such as 7, 12 and 20, short low temperature treatments or high post vernalisation temperatures resulted in very slow flowering and in these experiments recording of flowering continued for a longer time till all or nearly all plants showing signs of flowering had flowered.

In all experiments the number of plants flowering is used as the best indicator of susceptibility to vernalisation as flowering

is a clear and irreversible process. In some experiments the remaining plants were dissected and the presence of developing flower buds recorded. If the experiment had been prolonged these plants would presumably have flowered eventually. The number of reproductive but non-flowering plants provides further information on the effects of experimental treatments but has not been found to be affected differently from numbers flowering only, by experimental treatments. Plants in bud at the end of an experiment provide no information on the number of days to flowering except that the plants have not flowered by a certain date. Assessing the number of reproductive plants by dissecting the apical buds can shorten an experiment where treatments have been marginally vernalising and flowering very late, but it is not a satisfactory substitute for recording flowering.

3.10 Flower stage

Flower stage was recorded at the end of experiments 2, 4 and 10. It is an assessment of the state of flower or seed development of all plants at a given time and is therefore related to the number of plants flowering and the date of flowering, as early flowering plants will be further advanced in development of seeds than later flowering plants. Plants of all stages of flower development, before and after flowering, including vegetative plants, are taken into account, which is an advantage over simply recording flowering plants, but flower stage is assessed subjectively from a scale, unlike flowering, which is very easy and precise to record. When large numbers of plants are involved and regular recording of flowering cannot be made

it is a useful alternative to daily recording of flowering especially for the estimation of earliness of flowering.

3.11 Growth during low temperature treatment

3.11.1 Measurement and some effects of treatments

Even at temperatures below 10°, slow growth occurs in swedes. The most convenient non-destructive assessment of plant size or development is visible leaf number taken as being all leaves of 1 cm and over including true leaf scars but not cotyledon scars. The total number of leaves on a plant, visible leaves and primordial leaves, is related to the number of visible leaves. In experiment 3 the visible leaf number of plants in each treatment was measured at the end of low temperature treatment and the total number of leaves of a sample of plants from the same treatment was recorded at the same time. Over the range 0 to 4 visible leaves the total number of leaves increases linearly as the number of visible leaves increases ($r = +0.968$ for Wilhelmsburger, and $+0.92$ for Doon Major, $n = 30$ $p<0.001$) the equations of the fitted lines being y (total number of leaves) = $2.067x$ (number of visible leaves) + 3.745 for Wilhelmsburger and $y = 2.031x + 3.487$ for Doon Major. As the relationship is linear, measurements of production of visible leaves will bear a close relationship to the production of primordial leaves over the same period.

The longer the low temperature treatment the more visible leaves are produced during the treatment and as the increase in the number of visible leaves produced is apparently linear over increasing duration of low temperature (see Tables 2.6 and 2.12) there is no

evidence of acclimatisation to low temperature and consequent improved rate of leaf production during longer durations of low temperature treatment. Temperature of treatment also affects leaf production linearly, the higher the temperature the more leaves being produced (see Tables 2.10 and 2.12). Plant age at the beginning of low temperature treatment affected leaf production during treatment (see Tables 2.4 and 2.6), older plants producing more leaves than younger plants. There is some difficulty in measuring production of visible leaves in very young seedlings which have no visible leaves at the start of the measurement period. Leaf primordia, however, are present in very young seedlings, even two-day old seedlings and in experiment 3 the production of all leaves, including primordia during low temperature treatment was estimated from measurements of total leaf number in samples from each treatment before and after low temperature treatment. Younger plants produced fewer primordia than older plants (see Table 2.7). Plants that had a very limited supply of nitrogen during low temperature treatment produced fewer leaves during treatment and flowered more slowly (see experiment 10). Higher light intensity results in a greater production of leaves during low temperature treatment (see Table 2.26). The correlation between daily leaf production and percentage flowering in the four light intensity treatments was significant ($r = 0.953$, $p<0.05$).

3.11.2 *The effect of cultivar*

Wilhelmsburger produced 0.057, 0.054, 0.085, 0.101 and 0.080 leaves per plant per day during low temperature treatment in experiments 2, 3, 4, 5 and 12 respectively, and Doon Major produced 0.044, 0.043, 0.069, 0.082 and 0.069 leaves per plant per day in the same

experiments. The difference between the cultivars was not significant (F test) in experiment 12 but the differences were significant at the 0.1 per cent level in the others, and production of primordia in experiment 3 also differed (F test, $p<0.001$) between Wilhelmsburger with 0.186 leaf primordia per plant per day and Doon Major with 0.161 primordia per plant per day. Cultivars differed in their visible leaf production during low temperature treatment (F test, $p<0.001$) in experiment 13.

In experiments 4 and 5 it was apparent that lower temperatures were relatively more effective in causing flowering of Doon Major than Wilhelmsburger and Figure 3.8 shows the leaf production of the two cultivars at different temperatures using data from experiments 2, 3, 4, 5, 12, 13 and 15. The production of leaves by Doon Major is reduced less at low temperatures than that of Wilhelmsburger.

The order of decreasing leaf production, Pentland Harvester, Wilhelmsburger, Ruta Otofte, Harrietfield and Doon Major agrees fairly well with the order of decreasing susceptibility to flowering, Pentland Harvester, Wilhelmsburger, Harrietfield, Ruta Otofte and Doon Major, but the correlation between leaf production and flowering percentage of each cultivar ($r = +0.871$ $n = 5$) was not significant. The leaf production during low temperature of the six cultivars used in experiment 15 was measured for two-week old plants grown for four weeks at 6°. Leaf production was 0.100, 0.090, 0.088, 0.085, 0.083 and 0.077 leaves per plant per day for Marian, Della, Ruta Otofte, Wilhelmsburger, Doon Major and Seefelder, which agrees fairly well with the percentage flowering after treatment at 6°, 86, 91, 72, 86, 77 and 53 per cent for the same cultivars but the correlation between leaf production and flowering percentage ($r = +0.716$ $n = 6$) was not

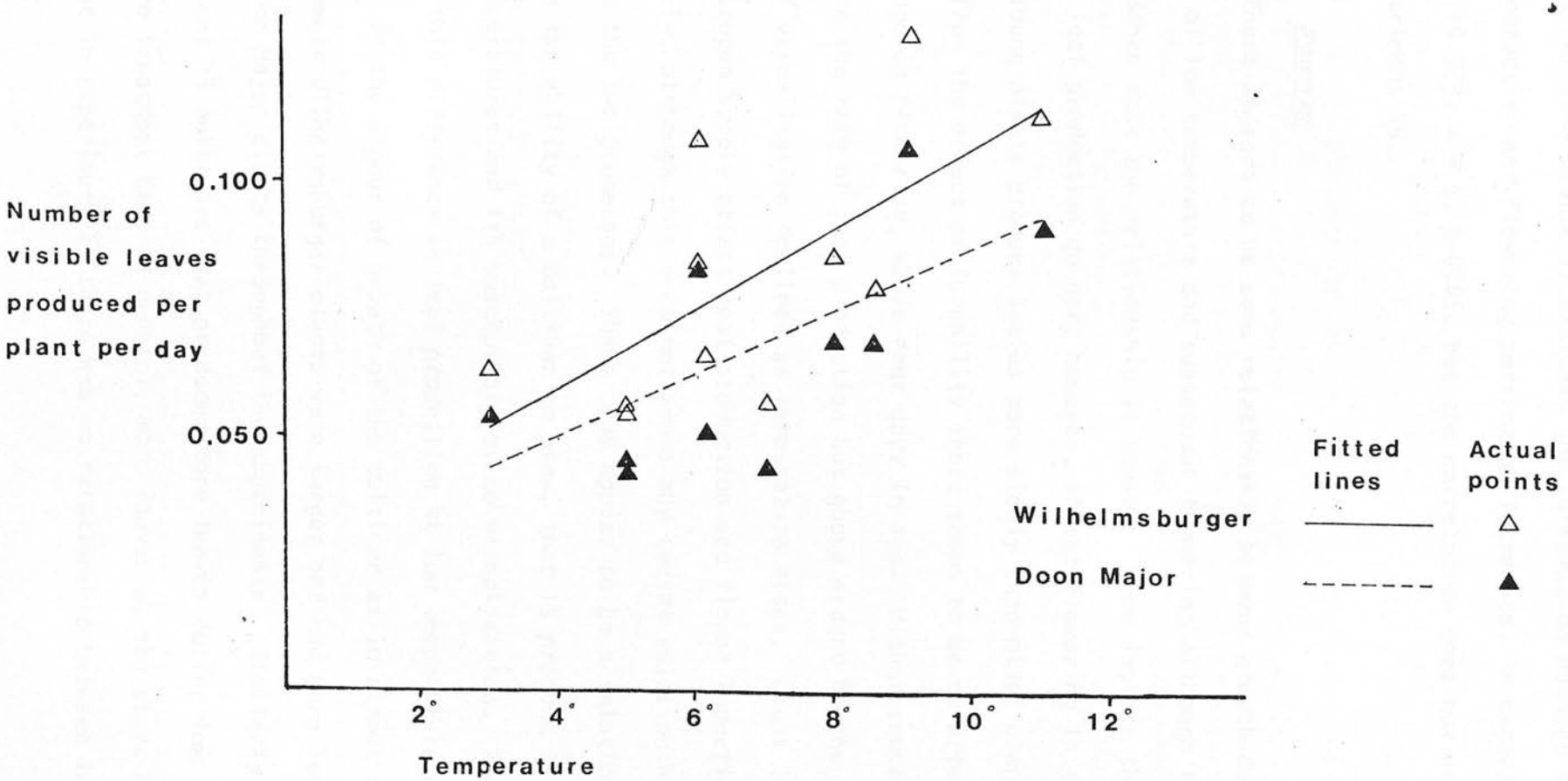


FIGURE 3.8: The effect of temperature of treatment on daily visible leaf production of Wilhelmsburger and Doon Major swedes in experiments 2, 3, 4, 5, 12, 13 and 15

significant. In both experiments 13 and 15 Ruta Otofte had a greater leaf production than would be expected from its flowering percentage. If data for Ruta Otofte is excluded the correlation between daily leaf production and flowering percentage increases, in experiment 13 to $r = +0.979$, $n = 4$, $p<0.05$, but the correlation does not increase in experiment 15.

3.11.3 Summary

There appears to be some relationship between growth during a period of low temperature and subsequent flowering although there is no evidence that the relationship is causal. Some factors that affect leaf production do not, however, affect flowering in the same way. Young plants produce leaves more slowly than older plants, but apart from the effect of juvenility there seems to be no effect of plant age on flowering, above four days in age. Higher temperatures increase the rate of leaf production but above around 6° the effectiveness of vernalisation declines as temperature rises. Light intensity and nitrogen supply affect leaf production and flower induction similarly, although this does not prove any causal relationship between the two processes. There does appear to be a relationship between the ability of a cultivar to grow, that is produce leaves, at low temperatures and its susceptibility to vernalisation. To some extent this difference in leaf production at low temperature may be related to the vigour of growth of the cultivar as in almost all experiments Wilhelmsburger plants were larger and had more leaves than Doon Major plants throughout the experiments. Similarly in experiment 15 cultivars that produced more leaves during low temperature treatment had, in general, more leaves at the start of treatment but in experiment 13 there was no relationship between leaf

number at the beginning of low temperature treatment and subsequent leaf production.

Cell division is essential for vernalisation and as the production of leaves is partly the result of cell divisions it is likely that leaf production will be associated with vernalisation. As a measure of growth, leaf production is crude, but as the apex is the site of vernalisation, cell division at the apex is probably the most important component of growth to be measured, and leaf production is a more appropriate indicator of this than, for instance, dry weight. For a more precise estimate, the number of leaf primordia produced during a given period can be measured by dissection of a suitable sample.

3.12 Selection methods

3.12.1 Direct methods

There is evidence of considerable variability in susceptibility to vernalisation between and within present cultivars (see 3.8). It would be easy to improve the bolting resistance of some cultivars such as Wilhelmsburger, Ruta Otofte and possibly Seefelder also, but not so easy to improve the resistance of Doon Major and Pentland Harvester which have been much more uniform in their responses to low temperature treatment. Within only eight cultivars used in these experiments there is clearly sufficient variability for considerably more resistant swede cultivars to be bred.

The most direct way of selecting for bolting resistance is to expose a population of swedes to a period of low temperature and discard the plants that flower. Lysgaard (1974) successfully selected resistant swedes by taking 100 non-bolters from a population of very

early sown swedes. The degree of selection can be varied by altering the duration of low temperature treatment whether by sowing date or, more accurately, in a controlled low temperature environment. Short durations, for example 20 to 24 days at the optimum temperature for vernalisation, will cause only the most susceptible 10 to 30 per cent of individuals to flower, and could be used to improve the bolting resistance of an existing strain or cultivar. After longer durations, such as 28 to 32 days, only the more resistant 10 to 30 per cent of plants will not flower. Some of the selected non-flowering plants may actually be induced but not yet extending, but it is possible to select plants which are certainly vegetative by examining the apical bud, and if vegetative (bud stage 0, see Figure 2.1) subjecting the plant to a further low temperature treatment and collecting seed from axillary shoots in the following year.

An alternative method is to select late-flowering plants which has the advantage that seed can be collected at the time of selection, but the disadvantage that if not all plants have flowered the most resistant plants in the population will not be included among the selected resistant plants.

The use of longer durations of low temperature will tend to correct this difficulty, as almost all plants will flower, but the variation in time to flower decreases as duration of low temperature increases, and selection will become less effective as differences in time to flower between individuals decrease. There is also some evidence that especially after long durations differences between cultivars in time to flower are affected by other factors besides resistance to vernalisation, in experiment 13 the five cultivars being affected differently in days to flowering as low temperature treatment

increased. A resistant cultivar is not necessarily slower to flower especially after long durations of low temperature and the same will be true of individual plants within a population, later flowering plants generally, but not always, being more resistant to vernalisation. However, selection of early and late flowering plants to obtain separate lines even within a small population (15 plants selected out of a population of 296 Wilhelmsburger and Doon Major plants) was successful in creating resistant and susceptible lines of both cultivars.

Selection of late flowering plants can be combined with selection of non-flowering plants. If too short a duration of low temperature is used, or there is less flowering than expected after treatment, there may be more non-flowering plants left than are required in the selection programme with no way of distinguishing between the resistance of the non-flowering plants unless they are beginning to bolt and show signs of flowering. However, if a longer duration is used there may be fewer non-flowering plants than are required, but the last plants to flower could be included to make up the required number of plants in the selected resistant population. Whether a plant is regarded as flowering or not will depend on when selection is carried out, the longer after low temperature treatment, the more plants will have flowered, but under normal conditions the bulk of flowering is usually over 100 days after the end of low temperature treatment.

As suggested in 3.2, a daily fluctuating temperature from, for instance, 2° to 10°, will be the most suitable during an artificial low temperature treatment for selection although a steady temperature is also effective, for example, 5° in experiment 3.

Selection by retaining non-flowering plants or taking early and late flowering plants is effective for obtaining resistant swede strains.

3.12.2 Indirect methods

Less direct methods, involving measurements of seedling and plant factors associated with bolting resistance, such as hypocotyl epidermis cell length in sugar beet (Lexander 1974), may be quicker and more convenient but are unlikely to be as successful.

There is some evidence that a high rate of leaf production during a period of low temperature treatment is associated with susceptibility to flowering. Measurements of leaf production during low temperature treatment may provide some information on the possible resistance of a cultivar or strain but is unlikely to enable the resistance of individual plants to be assessed. Measurements of the production of leaf primordia are more accurate but are destructive and therefore of no use for assessments of individual plants. The measurements themselves are time-consuming and the only advantage of the method is that the test can be done on plants in the seedling stage while they are receiving a low temperature treatment to induce complete flowering for bulking up of seed, or for crossing programmes.

The relationship between a rapid rate of leaf production at low temperature and susceptibility to flowering is not absolutely confirmed, and if it is not an invariable relationship it might result in bolting resistant plants with the valuable ability to grow rapidly at low temperature being discarded. Ruta Otofte, for instance, is resistant to flowering but has an average rate of leaf production at low temperature.

3.13 Shortening the reproductive cycle

The swede is a biennial, and normally the reproductive cycle takes almost a year to complete with young plants which are exposed to low temperature during the winter flowering and setting seed the following summer. When controlled environment growing conditions are available it is possible to shorten the cycle.

From the results of experiments in this study the following methods of shortening the reproductive cycle can be suggested. Plants should be grown for at least four days from sowing, and probably seven to eight days to ensure that all plants have outgrown the juvenile stage and should then be placed in a low temperature environment. In experiment 5 flowering time was slightly shorter (about seven days shorter) after 3° than 6° especially after longer durations of low temperature and so a temperature of 3° or possibly even lower is more suitable than a higher temperature. In experiments 1, 2, 3, 4, 5, 13 and 15 the minimum time from the beginning of low temperature treatment to mean flowering time occurred after 30 to 42 days low temperature treatment (see 3.9) but from the beginning of low temperature treatment to the time when the last Wilhelmsburger or Doon Major plant had flowered was longer, see below:

139 days (40 days low temperature treatment, adult plants only) in experiment 2

187 days (40 days low temperature treatment, adult plants only) in experiment 3

125 days (42 days 5° low temperature treatment but not all plants flowered) in experiment 4

132 days (42 days 3° low temperature treatment, one plant did not flower) in experiment 5

139 days (40 days low temperature treatment, Wilhelmsburger and Doon Major only) in experiment 13

114 days (54 days low temperature treatment, Wilhelmsburger and Doon Major only) in experiment 15

If it is necessary that every plant should flower promptly, for instance to avoid excluding very bolting resistant individuals from the breeding cycle, longer durations of low temperature than 30 to 40 days low temperature will be required. In experiments 23 and 24 in which low temperature treatment continued for 56 and 63 days respectively, all plants had flowered within 136 and 125 days of the start of low temperature treatment. Extending the low temperature treatment beyond 50 to 56 days may not be necessary, and if the use of the controlled low temperature environment is restricted, a shorter time, 40 to 50 days, could be used, depending on the resistance to flowering of the swedes treated.

The light intensity should be high during low temperature treatment and it is probable that a 24-hour daylength would promote more rapid flowering if only by increasing the amount of light received and plant growth during the low temperature period. A good nitrogen supply during treatment will also promote flowering and a sufficient supply of other nutrients may also be important.

After low temperature treatment the temperature should be raised to around 11° to 14° for at least a week, and preferably two weeks, to prevent or limit devernalisation, before the temperature is raised to a higher temperature, 15° to 18°, to encourage rapid growth and flower development. This study has provided no useful information on the effects on flower and stem development of daylength or light following low temperature treatment but it seems likely that

a long daylength, 24 hours preferably, and high light intensity will promote more rapid flowering. It is possible that applications of gibberellic acid will also increase the rate of flowering, but if conditions for vernalisation and growth after low temperature treatment are optimal, exogenous gibberellins may have no effect. In the conditions suggested, flowering of all plants should occur within 130 to 140 days of sowing which leaves 40 to 50 days for seed set and ripening, if a six-month reproductive cycle is to be achieved.

Apart from providing optimum conditions of temperature, light and nutrition, there does not appear to be any way of reducing the induction and flower development phases of the reproductive cycle, and the seed development and ripening part of the cycle may offer better opportunities for shortening the cycle.

3.14 Site of vernalisation

Expanded leaves are not necessary for vernalisation, although removal of leaves during low temperature treatment resulted in slightly delayed flowering but this was probably chiefly due to the growth check caused by defoliation. Stem cuttings (see experiment 21) without expanded leaves exposed to low temperature subsequently developed into plants and flowered, and in experiments 17 and 18, directly cooling the growing point only, resulted in normal flowering whereas in experiment 19 heating the growing point only during a low temperature treatment prevented flowering. Leaf primordia and young leaves up to about 1 cm in length were present in the growing point but flower induction of swedes can occur in darkness and so it is not photoperiodic in nature. The primordial and young leaves may or may

not be involved in vernalisation but it is likely that the apical meristem is the chief site of induction, as in vernalisation of cereals (Purvis 1940, 1961).

Within the growing point and in the axils of expanded leaves there are many axillary meristems and if these are growing actively, for instance when apical and upper meristems are removed, releasing lower meristems from apical dominance, these lower axillary meristems can be induced to flower, even meristems in the axils of the cotyledons (see experiment 23). The growth activity of these axillary buds appears to affect the efficiency of vernalisation. In experiment 22 the lowest four or five buds on the plant released from apical dominance before a six-week low temperature treatment did not subsequently flower, although some control plants did, probably because the growth of the lower buds was too slow during low temperature treatment.

Experiment 24 demonstrated that some axillary buds can be vernalised even when the apical meristem is present during the low temperature treatment and the growth of the axillary buds suppressed by apical dominance. These axillary buds may be induced by a substance translocated from the apical meristem during the low temperature treatment, or some buds may be induced because they developed from an apex that was itself already induced but as axillary buds can be induced in the absence of the apical meristem and as some axillary buds (see experiment 26) were already present at the beginning of low temperature treatment and later flowered, it is most probable that the axillary buds are induced independently in the same way as the apical meristem is or develop from an induced apex, rather than being induced by a translocatable substance from the apex. As axillary

buds released from apical dominance after low temperature treatment in experiment 22 did not flower whereas some of the apical buds on the control plants did, it is probable that the vernalisation process is slightly slower in axillary buds under the influence of apical dominance than in the more actively growing apical bud.

The main site of vernalisation in the swede is without doubt the apical bud and probably chiefly the apical meristem, although axillary buds can also be vernalised. In experiment 26 the lowest buds (cotyledon and first and second leaf buds) flowered much less than higher buds when released from apical dominance at the time the main axis flowered. This may have been because their growth was too slow during low temperature treatment for induction or because these lower buds were induced and then devernalised after the low temperature treatment in the same way as basal buds on the chrysanthemum and devernalised unless the rest of the plant is removed immediately after low temperature treatment (Schwabe 1954b). The chrysanthemum is a perennial however, and requires a mechanism to prevent all buds flowering in one year whereas the swede is a biennial and does not persist beyond one flowering season, and so the flowering mechanisms of the two species may be very different.

3.15 Possible translocation of a flowering stimulus

Unlike the photoperiodic response there is very little evidence that induction by low temperature results in the production of a translocatable flowering substance. No *Brassica* species has provided any evidence of the existence of a mobile substance although there is some evidence from beet (*Beta vulgaris*) (Curtis *et al* 1964),

crested dog's tail (*Cynosurus cristatus*) (Purvis 1961) and henbane (*Hyoscyamus niger*) (Salisbury 1963) that such a substance exists in these species. In this study there was no indication that a mobile substance was produced by the swede, although no conclusive evidence that there was no such substance.

Vegetative young swede plants grafted onto flowering swedes and swedes on the point of flowering grew and formed close unions with the stock, but remained vegetative. This does not prove the non-existence of a mobile substance as it might be produced for only a short time, possibly long before flowering, or before a full graft union bud formed.

It has been suggested by Margara (1964) that axillary buds of rape (*Brassica napus*) and beet (*Beta vulgaris*) are induced by a substance translocated from the apex. This does not seem likely in the swede, as removal of apical meristem or the apical bud immediately after low temperature treatment did not prevent the highest remaining axillary bud from flowering normally and promptly (see experiment 24). Experiment 23 has also shown that all buds are capable of being induced independently of the apical meristem and so there is no obvious need for a mobile flowering stimulus in the plant. In experiment 26 the removal of various proportions of the shoot at flowering showed that all but the very lowest buds were induced. As upper axillary buds may be induced by the end of low temperature treatment it seems more likely that axillary buds are induced directly by the low temperature treatment and not by a substance translocated from the apical bud during low temperature treatment. Axillary buds near the apical meristem at flowering probably flower because they developed from the induced apex during or after low temperature treatment, whereas

lower buds already present at the beginning of low temperature treatment are probably induced independently of the apical meristem.

3.16 The mechanism of vernalisation

The nature of the vernalisation process in any species is not clear, but in the swede it is certain that the process occurs in the growing point or actively growing buds and that light is not necessary for the process. Induction in the swede occurs at 11° and below and is reversed at higher temperatures (15° and above) which fits in with the theoretical model of vernalisation (see 3.3), but it is not known whether the eventual result of vernalisation is the accumulation of a substance or the gradual destruction of an inhibitor, as in the release of buds from dormancy, by chilling.

In cauliflower (Thomas *et al* 1971), radish (Suge 1970) and iris scales (Pereira 1964), higher levels of gibberellin-like substances were produced after low temperature treatment and in the swede the effects of exogenous gibberellic acid on stem growth are similar to the effects of a period of low temperature on stem growth but gibberellic acid does not cause flowering although it may promote it. It is possible that flowering is caused by the accumulation of a gibberellin but either this is not the same substance that causes stem extension, or some other additional substance is involved in flowering, as stem extension and flowering are not affected in the same way by gibberellic acid or by devernalisation.

Vernalisation in honesty (*Lunaria biennis*) (Wellensiek 1962b) has been demonstrated to occur only in actively dividing tissue and this may also be the case in the swede. Whether dividing cells are

necessary for vernalisation because they are the only cells with a suitable level of metabolic activity at the temperatures at which vernalisation occurs, or whether cell division itself is essential for the process is not clear.

The rate of flower development varies with the duration of low temperature treatment, which suggests that the process is to some extent quantitative, although part of the process is clearly qualitative in nature, as a plant either becomes reproductive, or remains vegetative with no real gradation between these states. The quantitative factor, however, governs the rate of change from vegetative to reproductive (see experiment 1) and the rate of stem extension.

The visible change from vegetative to reproductive does not usually occur immediately after a period of low temperature, and vegetative leaves may be produced by apices which later flower and so there must be a gradual accumulation of some flower promoting substance or depletion of a flower inhibiting substance after low temperature treatment has ended. One explanation is that substance D, the flowering substance in the theoretical model, is an enzyme, or a co-factor increasing the activity of an enzyme already present. The more D is present at the end of the low temperature period the more enzyme will be available for the production of a substance that, at a certain concentration, induces the apex to change from vegetative to reproductive, which would explain the earlier flowering after longer durations of low temperature treatment. Some other substance, possibly gibberellin-like, that causes stem extension and whose production is not affected so much by high temperature must be produced during low temperature treatment and this substance may not only cause stem extension but

possibly also promote flower bud growth and development. At least two substances must be produced but there may be many more involved.

Carbohydrate level affects flower induction in cauliflower and broccoli but defoliation or the absence of light did not greatly affect vernalisation in the swede. A high level of carbohydrate is not therefore essential for the process but low levels probably do reduce the efficiency of the process, possibly simply by reducing cell activity and division.

Little is known of the substances or reactions involved in vernalisation despite over 30 years of research on the subject. Unlike photoperiodic induction, there is no clear evidence of a translocatable substance involved in the process and therefore much less chance of easily isolating a substance involved in the process.

One approach to the subject is to examine the levels of certain substances during and after low temperature treatment. Such studies have revealed the accumulation of gibberellin-like substances and the depletion of auxins in some species, and an increase in starch and sugar levels. One of the disadvantages of this method is that the various treatments used, low temperature and auxiliary treatments, such as defoliation, the absence of light or applications of SADH, may affect both flowering and the levels of the substances studied but provide no evidence of a causal relationship.

Another approach is to look for differences between varieties, cultivars or lines within a species that differ in resistance to flowering. Differences in the available sulphydryl (-SH) and di-sulphide sulphur (-S-) content between resistant and susceptible sugar beet have been demonstrated (Lexander 1974).

There is a difference of only one gene between annual and biennial forms of rye and henbane (Purvis 1948), and a comparison of the accumulation of substances at low temperature within annual and biennial forms might provide some information on the substances involved in vernalisation, particularly if lines could be bred which were as similar as possible in all characteristics apart from this one gene. In the theoretical model this difference might be the absence of reaction II, so that all substance B produced is converted to D and no substance C is produced.

CONCLUSIONS

1. At temperatures of 11° and below, swedes can be vernalised. The swede has an obligate requirement for vernalisation and if grown at temperatures above 13° to 14° will not flower. The optimum temperature for vernalisation is around 5°, 5° to 6° being more suitable for Wilhelmsburger and 3° to 6° for Doon Major.

2. High temperatures immediately after low temperature devernalise and reduce the proportion of plants flowering and this reversal of vernalisation may continue for three weeks after low temperature treatment. Interruptions of low temperature with short periods of high temperature also devernalise and reduce flowering and in both cases the higher the temperature the greater the devernalisation.

3. Swedes have a juvenile stage during which they cannot be induced to flower, but it is very short, below 4 days at temperatures around 15° and even at vernalising temperatures plants grow out of the juvenile stage in one to two weeks. Some cultivars have a slightly shorter juvenile stage, for instance Harrietfield or Doon Major, in which it is less than or around 2 days. Once seedlings have emerged they have passed out of the juvenile stage. In Wilhelmsburger it was calculated that plants at the changeover from juvenile to adult had 0.8 of a leaf primordium. A long juvenile stage in a cultivar confers useful bolting resistance during early growth but would be difficult to select.

4. Stem extension in the swede is caused by a period of low temperature, like flowering, but its response to gibberellin and temperature is slightly different. Exogenous gibberellin readily

causes stem extension but not flowering. Stem extension is less affected by devernalisation than flowering is, stems sometimes extending after low temperature treatment when apices are devernalised and vegetative. When devernalisation and gibberellin are used in combination devernalised plants extend much more than unvernalised plants, in response to gibberellin. There must therefore be more than one reaction involved in the induction of flowering and its associated stem extension.

5. The higher the light intensity during low temperature treatment the greater the proportion of plants flowering, but swedes can be vernalised in the dark and so light is not essential for flower induction.

6. High levels of nitrogen promote earlier flowering although all plants flowered in the treatment with very low nitrogen.

7. Cultivars vary considerably in susceptibility to flower induction, and there was 9 to 12 days difference between the number of calculated days required to cause 50 per cent flowering of the most susceptible and most resistant cultivars in two experiments. The flowering behaviour of some cultivars is more variable than that of other cultivars and early and late flowering lines selected from Wilhelmsburger, a variable cultivar, differed much more from the parent cultivar than lines selected from Doon Major, a uniform cultivar.

8. Vernalisation affects swedes qualitatively, causing some plants to flower, and quantitatively, the longer the duration of low temperature treatment the earlier plants flower. Plants can take from 40 to 200 days to flower depending on the duration of low temperature but in a population in which at least 50 per cent of plants are flowering the time is usually 70 to 90 days. After longer durations of low temperature, over 40 days, the response in terms of reduction in time to flower as duration of low temperature increases declines. Different cultivars respond at slightly different rates to increasing duration of low temperature but in general resistant cultivars flower more slowly than more susceptible cultivars.

9. There was a relationship between rate of development as measured by the rate of leaf production during low temperature treatment and susceptibility to flower induction. Cultivars with higher leaf production rates during low temperature and nitrogen and light intensity treatments that increased leaf production had higher proportions of plants flowering.

10. To select resistant or susceptible individuals from a population of swedes, plants over 4 days old should be given a low temperature treatment at about 5° for 20 to 30 days depending on the degree of selection required followed by at least 14 days at 12° to 14°. If the duration of low temperature selected does not give the required proportion of plants flowering, late flowering plants can be included with non-flowering plants.

11. To reduce the reproductive cycle to a minimum, swedes just over 4 days should be given 50 days low temperature treatment at around 5^{o} followed by 14 days at 12^{o} to 14^{o} before transfer to a higher temperature. Light intensity and nitrogen nutrition should be good during and after low temperature treatment. Flowering should occur within 130 to 150 days of sowing.

12. The normal site of vernalisation and devernalisation is the growing point although axillary buds can be vernalised in the presence and absence of the growing point and axillary buds that develop from a vernalised apex during or after low temperature treatment may be induced. There is no evidence of a translocatable flowering stimulus in the swede.

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APPENDICES

APPENDIX A

EXPERIMENTAL METHODS

Sources of seed

The Pentland Harvester (1974) seed was obtained from the breeders, the Scottish Plant Breeding Station. In experiments 2, 3, 6, 7, 9, 10, 11, 12, 16, 17, 18, 19, 21, 22 and 25, Wilhelmsburger (1973) was from Garton's seed merchants and Doon Major (1974) from Sinclair and McGill. In all other experiments the source of seed was as listed below:

Cultivar	Supplier of seed
Wilhelmsburger (1976)	Sharpes
Doon Major (1976)	Barclay, Ross and Hutchinson
Ruta Otofte (1976)	Danish Plant Breeders
Harrietfield (1975)	Sinclair and McGill
Seefelder (1975)	Petersen (Germany)
Marian (1977)	National Seed Development Organisation
Della (1977)	National Seed Development Organisation

Growing conditions

Unless stated otherwise, seeds were initially sown in 7.5 cm diameter plastic pots and repotted later as necessary. John Innes No. 2 compost was used in all the experiments in which Wilhelmsburger seed was from Garton's (listed above) and also in experiments 1, 8 and 13. Levington potting compost was used in all other experiments. Two or three weeks after sowing or repotting, plants were given twice weekly applications of a fertiliser solution, 'Solufeed' (23 per cent N, 19.5 per cent soluble P_2O, 16 per cent K_2O, at a concentration of 150 g per litre) either watered on or, when the leaf canopy was very dense, 30 ml applied direct to the soil surface.

In many experiments the swedes were transplanted from pots to a field plot which in all but experiment 10 had been previously fertilised at the rate 625 kg/ha, the fertiliser containing 15 per cent N, 14.55 per cent water soluble P, 0.45 per cent insoluble P and 21 per cent K.

Plants in experiments 1 and 8 were raised in a glasshouse with daylight supplemented to 16 hours by warm white fluorescent light tubes.

In all other experiments plants were raised before and after treatment in a glasshouse with daylight supplemented to 16 hours (experiment 2 and 10) or 18 hours by sodium vapour lamps giving a light intensity of around 20,000 to 30,000 lux in winter at plant level.

Temperature measurement and low temperature treatment

Most temperature records were based on weekly charts from thermohydrograph recorders, but in a few experiments, 1, 8, 11, 17 and 18, daily maximum minimum mercury thermometer readings were used over at least part of the experiment. In the three growth cabinets temperature records were taken from the two hourly readings of thermistor temperature detectors. Daily mean temperatures were calculated from the mean of 12 two hourly readings for each day, and the mean of the daily mean temperature calculated over the appropriate period, for thermohydrograph and thermistor records.

Glasshouse temperatures often varied daily by as much as $\pm 5^{o}$ from the mean temperature but as plant growth and devernalisation increase with increasing temperature a fluctuating temperature will have similar effects to a steady temperature with the same mean.

Low temperature treatment was out of doors, utilising natural low temperature, in an unheated glasshouse with no supplementary light

or in controlled conditions. Experiments 3 and 12 were carried out in a growth chamber with good temperature control and warm white fluorescent light tubes supplying 10,000 to 12,000 lux at plant level for 16 hours (experiment 3) or 18 hours (experiment 12) a day. Plants were also given low temperature treatments in a growth chamber with less good temperature control, illuminated with warm white fluorescent light tubes on for 16 hours a day (experiments 2 and 10) or 18 hours (experiments 9, 13 and 14).

In experiments 4, 5 and 15 plants were given temperature treatments in three identical growth cabinets with generally good temperature control and cool white fluorescent light tubes for 18 hours each day supplying between 5500 lux at the extreme corners of the cabinet to 11,000 lux in the central area. Low temperature treatment in experiment 11 was carried out in a refrigerator with good temperature control.

Daily variation in uncontrolled low temperature environments was not usually greater than $\pm 3^{\circ}$ about the daily mean but over a long period of treatment the range between highest and lowest daily mean temperature might be 7° or 8°.

Temperatures in the growth chamber with good temperature control, and generally in the growth cabinets fluctuated by $\pm 0.5^{\circ}$ at the most, and daily mean temperature was constant. In the growth chamber with less good control, temperatures fluctuated about $\pm 1.5^{\circ}$ and the range in daily mean temperatures was sometimes as much as 5° or 6° over a treatment period but temperatures were always less variable than temperatures out of doors.

Immediately after low temperature treatment plants were usually moved to an environment of moderate temperature (11° to 15°) to limit

devernalisation which might be caused by an immediate return to a higher temperature. In experiments 2, 3 and 10 plants were kept in growth chambers with good temperature control, and 16 hours daylight, in experiment 13 in the chamber with less good temperature control and 18 hours daylength, and plants treated in the growth cabinets remained in them after low temperature treatment, the temperature being raised. In most other experiments plants were moved to a glasshouse compartment maintained at a lower temperature than the main glasshouse area, and supplied in all cases except experiment 22 with mercury vapour lamps supplementing daylight to 18 hours.

Replication

In a large number of experiments more than one duration of low temperature treatment was used. In most of these experiments all plants were exposed to the same post low temperature treatment conditions but plants were raised from sowing to low temperature treatment at different times. The effect of a low temperature treatment cannot therefore be separated from the effect of pre-treatment conditions and so duration of low temperature treatments can only be properly replicated over time, for instance by repeating the experiment.

As replicates of duration of low temperature treatments within an experiment were not strictly valid and the inclusion of more replicates restricts the number of treatments and therefore the number of points on a response curve, in many experiments duration of low temperature treatments were not replicated. Plants were mainly grown in controlled or glasshouse environment and variation within an experiment would be much less than in field experiments.

Pest and disease control

The only important fungal disease affecting the plants in the glasshouse and in the field was powdery mildew (*Erysiphe polygoni* DC) and it was controlled initially with ethirimol ('Milgo E' Plant Protection) applied at 0.6 per cent concentration (of product, not of active ingredient) and later more effectively by 'Persulon' (Bayer) in 0.2 per cent concentration. A number of plants in the glasshouse were infected with turnip mosaic virus in the summer of 1976 and the glasshouse was cleared of brassicas for a month to provide a disease break.

Aphids, chiefly *Myzus persicae* but also *Brevicoryne brassicae* were controlled by pirimicarb ('Pirimor' Plant Protection) at 0.5 g/ litre concentration in the glasshouse and in the field. Pollen or blossom beetle (*Meligethes aeneus*) on flowering plants in the field plots were controlled with azinphos-methyl ('Gusathion' Plant Protection) in a 0.625 per cent concentration applied at the rate of 340 litres/ha. The beetles eat into flower buds to lay their eggs and feed on the pollen and can cause premature opening of flowers. Chlorfenvinphos granules ('Sapecron' Ciba Geigy) were sprinkled round plants at 10.5 kg/ha shortly after planting out to protect against cabbage root fly attack.

Fenitrothion ('Ciba-Geigy Fenitrothion 50EC' Ciba Geigy) at 0.1 per cent concentration was applied to the centre of the plant rosettes to protect against swede midge (*Contarinia nasturtii* Kieff.) attack in a late flowering experiment (4).

Measurements

Plant size was measured at the beginning and end of low temperature treatment in most experiments. In seedlings, hypocotyl height,

from soil level to cotyledons, and cotyledon width (the longest dimension of the cotyledon) were measured as they both increase as the seedling emerges. The length and, where possible, the width of the first, or largest, true leaf was measured and the number of leaves over 1 cm long counted. The hypocotyl, cotyledon and leaf size measurements were used to compare plants in different treatments and experiments but were of little use in comparing plants of different ages, or over a long period of time, as hypocotyls and cotyledons stopped growing after about 25 and 16 days respectively, and leaves after about 2 to 4 weeks from their first appearance.

With older plants, the number of visible leaves was the most convenient non-destructive measurement of plant development. Leaves 1 cm long and over were usually easily seen in the rosette and so 1 cm was taken as the minimum leaf size. All leaf scars except cotyledon scars were included in the visible leaf number.

In some experiments, to obtain the most accurate measurement of plant development, sample plants were dissected and the total number of true leaves - scars, visible leaves and all leaf primordia - were counted. This is the only quantitative measurement of plant development in the swede which can be applied to all plant ages from the seed upwards.

The aim of this project is to study the bolting and flowering of swedes. Bolting plants are in the process of extending and flowering. The most easily measured point in the reproductive cycle is the opening of the first flower and pollen shedding from the anthers (anthesis). Anthesis usually occurs shortly after the opening of the petals or at least within a day of the first signs of petal opening. Anthesis was selected as the main criterion of whether a

plant is reproductive or not as it is the least affected by subjective judgement. The word 'flowering' in this study is taken to mean anthesis. As this is a purely qualitative measurement a more quantitative measurement, the number of days from the end of low temperature treatment to the time anthesis occurs, was recorded in most experiments.

APPENDIX B

STATISTICAL ANALYSES USING A COMPUTER

Analysis of proportion of plants flowering

The most important data in most experiments was the number of plants flowering in each treatment. As a plant has either flowered or not flowered by the end of an experiment, the data is binomial in distribution. In some experiments a few plants died and so the flowering data is expressed as the number of flowering plants as a proportion of the number of plants remaining in each treatment.

In some simple comparisons, especially when only two treatments were involved, for instance between the flowering of juvenile and adult plants, as in experiment 2, between the flowering of Wilhelmsburger and Doon Major cultivars, and when numbers of flowering plants are small, a straightforward χ^2 analysis is appropriate. In larger experiments with several treatment factors χ^2 is not so suitable and a more complex analysis is required.

The GLIM (General Linear Interactive Modelling) program used on EMAS enables binomial data to be transformed to a logit, probit or complementary log log scale and be fitted iteratively to a model of treatment factors and interactions of treatment factors. When treatment levels are evenly spaced the linear or linear and quadratic effects of the treatment factor can be used in the model instead of all effects of the treatment factor.

An example of a GLIM analysis of binomial data (the proportion of plants flowering in experiment 4) is shown below (pages 269-272) and the lines of the analysis have been numbered for descriptive convenience.

The logit scale is used in default if no '£LINK' setting is declared after '£ERROR B' (declares binomial distribution) (see line 21) and as the logit scale tends to straighten out S-shaped curves (see Figure 2.11, experiment 15, for an example of an S-shaped curve of proportion of plants flowering) it was suggested as an appropriate scale by Michael Franklin, ARC Unit of Statistics. A logit is $\log_e (\frac{p}{1-p})$ where p is the probability of an event happening, in this case the probability of a plant flowering.

Lines 1 to 18 put the data into the program in the correct order, and instruct that duration and temperature may be examined on a linear scale (lines 17 and 18). '£C' at the beginning of a line introduces a non-operative comment. Line 19 declares the terms that may be included in a fitted model, line 20 declares the y- variate and at line 22 the fitting process begins. In the absence of a model term after '£FIT' the grand mean is fitted and the deviance of the data from the mean is calculated.

Deviance is equivalent to variance, and the 'DEVIANCE' calculated in the program (for example, line 37) is a measure of the variation of the individual values from the calculated means for a given model. In line 25, the deviance is a measure of the variation of individual points from the grand mean. Model terms can be fitted and the importance of any term in the model assessed by examining the decrease in deviance produced by fitting that term. When a term is fitted some interactions are included in that term and so the reduction in deviance attributable to that term must be adjusted for these interactions. Instead of fitting D (effect of duration of low temperature) and subtracting the deviance for fitted D from the deviance for the fitted grand mean to find the importance of D, all main

factors are fitted, in this example D, T and H (see line 26) and then the factors that are not being examined, that is, T and H, are fitted (line 30). The difference in deviance between these two fittings is solely attributable to the effect of D (adjusted D) as any interactions with T and H have been removed from both larger and smaller deviances in both models by the fitting of T and H, as T and H include these interactions.

In this example the adjusted effect of D (duration of low temperature) is:

FIT T+H	(line 30)	Deviance	113.3	DF 32	(degrees of freedom)
FIT D+T+H	(line 26)	Deviance	-26.48	DF 30	
adjusted D		is	86.82	DF 2	

As there are 2 degrees of freedom for duration the mean deviance is 86.82 ÷ 2, that is 43.21. This is compared directly with χ^2 with the same number of degrees of freedom, that is 2, and as it is greater than χ^2 at the $p = 0.001$ level, the effect of duration is significant at this level.

The linear effect of duration (DL) can be examined in the same way:

FIT T+H	(line 30)	Deviance	113.3	DF 32
FIT DL+T+H	(line 42)	Deviance	-28.78	DF 31
adjusted DL		is	84.52	DF 1

This leaves only 86.82 - 84.52 = 2.3 deviance for the effect of the rest of D which is not significant and so the only significant effect of D is its linear effect.

Other terms including interactions are examined in a similar way and any terms which by their addition to a model significantly reduce the deviance can be included in a final model (line 69). A print-out

of this model can be obtained (lines 73 to 119) and the 'LIN.PRED' column gives the fitted values of the model on a logit scale and can be used for the preparation of graphs of the fitted model.

As the adjusted deviance of each factor is compared directly with χ^2 there is no great advantage in the analysis of having replicates.

```
Line
   1    £C WILDGOOSE EXPERIMENT 4
        £UNITS 36
        £FACTOR B 2 D 3 T 2 H 3
        £C B= REPS D=DURATION T=CULTIVARS H= TEMPERATURES
   5    £DATA 36 R N
        £READ
        0 7 0 7 0 7 0 7 0 7 0 7
        1 7 0 7 0 7 0 7 0 7 0 7
        2 7 3 7 4 7 2 7 0 7 0 7
  10    1 7 4 7 4 7 1 7 0 7 0 7
        7 7 5 7 5 7 6 7 0 7 0 7
        7 7 4 7 7 7 3 7 1 7 0 7
        £CAL B=%GL(2,6)
        £CAL D=%GL(3,12)
  15    £CAL T=%GL(2,1)
        £CAL H=%GL(3,2)
        £CAL DL=D
        £CAL HL=H
        £TERMS R+D+T+H+DL+HL+D.T+D.H+T.H+DL.T+DL.H+HL.D+HL.T
  20    £YVAR R
        £ERROR B
        £FIT £
           35 DF
        DEVIANCE CYCLE
  25       172.3   3
        £FIT D+T+H £
           30 DF
        DEVIANCE CYCLE
           26.48   5
  30    £FIT T+H £
           32 DF
        DEVIANCE CYCLE
           113.3   5
        £FIT D+H £
  35       31 DF
        DEVIANCE CYCLE
           31.52   5
```

```
Line
      £FIT D+T £
         32 DF
 40    DEVIANCE CYCLE
         98.53   5
      £FIT DL+T+H £
         31 DF
      DEVIANCE CYCLE
 45      28.78   5
      £FIT D+T+HL £
         31 DF
      DEVIANCE CYCLE
         49.55   5
 50   £FIT D+T+H+D.T £
         28 DF
      DEVIANCE CYCLE
         21.43   9
      NO CONVERGENCE BY CYCLE 10
 55   £FIT -D.T+D.H £
         26 DF
      DEVIANCE CYCLE
         24.43   9
      NO CONVERGENCE BY CYCLE 10
 60   £FIT -D.H+T.H £
         28 DF
      DEVIANCE CYCLE
         24.19   9
      NO CONVERGENCE BY CYCLE 10
 65   £FIT D+T+H+DL+DL.T £
         29 DF
      DEVIANCE CYCLE
         24.29   5
      £FIT DL+T+H £
 70      31 DF
      DEVIANCE CYCLE
         28.78   5
      £DISPLAY D E R
      ERROR BINOMIAL LINK LOGIT
 75   Y-VARIATE R
      DEVIANCE =  28.78 DF = 31
```

Line		ESTIMATE	S.E.	PARAMETER
	1	-1.105E 01	1.50E 00	GM.
	2	9.523E -01	4.31E -01	T 1
80	3	5.149E 00	1.09E 00	H 1
	4	4.969E 00	1.09E 00	H 2
	5	2.357E 00	3.41E -01	DL

	UNIT	OBS	N	FITTED	RESIDUAL	WEIGHT	LIN.PRED
	1	0	7	0.49	-0.72	4.53E -01	-2.59E 00
85	2	0	7	0.20	-0.45	1.91E -01	-3.54E 00
	3	0	7	0.41	-0.66	3.87E -01	-2.77E 00
	4	0	7	0.16	-0.41	1.61E -01	-3.73E 00
	5	0	7	0.00	-0.06	3.04E -03	-7.74E 00
	6	0	7	0.00	-0.03	1.17E -03	-8.69E 00
90	7	1	7	0.49	0.76	4.53E -01	-2.59E 00
	8	0	7	0.20	-0.45	1.91E -01	-3.54E 00
	9	0	7	0.41	-0.66	3.87E -01	-2.77E 00
	10	0	7	0.16	-0.41	1.61E -01	-3.73E 00
	11	0	7	0.00	-0.06	3.04E -03	-7.74E 00
95	12	0	7	0.00	-0.03	1.17E -03	-8.69E 00
	13	2	7	3.09	-0.83	1.73E 00	-2.36E -01
	14	3	7	1.64	1.22	1.25E 00	-1.19E 00
	15	4	7	2.78	0.94	1.68E 00	-4.16E -01
	16	2	7	1.42	0.55	1.13E 00	-1.37E 00
100	17	0	7	0.03	-0.18	3.18E -02	-5.39E 00
	18	0	7	0.01	-0.11	1.23E -02	-6.34E 00
	19	1	7	3.09	-1.59	1.73E 00	-2.36E -01
	20	4	7	1.64	2.11	1.25E 00	-1.19E 00
	21	4	7	2.78	0.94	1.68E 00	-4.16E -01
105	22	1	7	1.42	-0.39	1.13E 00	-1.37E 00
	23	0	7	0.03	-0.18	3.18E -02	-5.39E 00
	24	0	7	0.01	-0.11	1.23E -02	-6.34E 000
	25	7	7	6.25	0.92	6.69E -01	2.12E 00
	26	5	7	5.34	-0.30	1.27E 00	1.17E 00
110	27	5	7	6.12	-1.28	7.69E -01	1.94E 00
	28	6	7	5.10	0.76	1.38E 00	9.88E -01
	29	0	7	0.32	-0.58	3.08E -01	-3.03E 00
	30	0	7	0.13	-0.36	1.26E -01	-3.98E 00
	31	7	7	6.25	0.92	6.69E -01	2.12E 00

Line	UNIT	OBS	N	FITTED	RESIDUAL	WEIGHT	LIN.PRED
115	32	4	7	5.34	-1.19	1.27E 00	1.17E 00
	33	7	7	6.12	1.00	7.69E -01	1.94E 00
	34	3	7	5.10	-1.79	1.38E 00	9.88E -01
	35	1	7	0.32	1.22	3.08E -01	-3.03E 00
	36	0	7	0.13	-0.36	1.26E -01	-3.98E 00

Analysis of days to flowering

The date of flowering was recorded for each plant and so the number of days from the end of low temperature treatment to flowering could be calculated. This data was only recorded from flowering plants and so the number of plants providing data for each treatment depended on the number of plants flowering in the treatment. This unbalanced data could not be analysed in a standard analysis of variance and instead was analysed using the GLIM (General Linear Interactive Modelling) program. There is a facility in this program for analysing quantitative data from variable numbers of individuals in each treatment, or, if the data is presented as a mean for each treatment, weighting that mean by the number of plants contributing to the mean. Both methods are, in effect, an analysis of the data for each individual although in the latter case within treatment error is not present.

The presentation of the data is similar to the preceding analysis (pages 269 to 272) but the fitting is non-iterative and a '£ERROR' directive is not necessary as the distribution of the data is normal. The example shown on pages 276 to 279 is of the days to flowering data of Wilhelmsburger lines only in experiment 12. In line 4, EFW,W and LFW refer to early flowering Wilhelmsburger lines, the Wilhelmsburger parent and late flowering Wilhelmsburger lines and the order in which the three groups are presented in the data (lines 8 to 15). TL (line 22) refers to the linear effect of lines so that if the effect of lines increases or decreases regularly from early flowering through the parent to late flowering lines this effect can be examined in the analysis.

The deviance attributable to each term is derived in the same way as in the previous example, for instance the deviance attributable to the effect of duration of low temperature treatment (4, 5 or 6 weeks) is:

FIT T	(line 34)	Deviance	16420	DF 36
FIT D+T	(line 30)	Deviance	-3594	DF 34
adjusted D		is	12826	DF 2

The mean deviance for the effect of duration is 12826 ÷ 2 which is 6413.

This mean deviance is not compared with χ^2 as in the previous analysis but with F in a variance ratio test. The error term used is that for the appropriate model, that is the deviance left after fitting the model, in this case D+T, deviance 3594, with 34 degrees of freedom (lines 30 to 33). This gives a mean deviance for error of 3594 ÷ 34 = 105.71 and the adjusted deviance for D is compared with this in a variance ratio 6413 / 105.71 with 2 / 34 degrees of freedom, that is 60.67, compared with F from tables 8.77 (2 / 30 DF p=0.001) and so the effect of duration is significant at the $p<0.001$ level and should be included in the final model.

In this example the linear effect of duration (deviance 12816 DF 1, line 37 minus line 45) accounted for most of the reduction in deviance attributable to fitting duration to the model and similarly with the effect of line (T) the linear component being by far the greater part (line 41 minus line 49). The model which fits the data best is the linear effect of duration and the linear effect of line (DL+TL) (line 62) as no interactions were significant. When this model is printed out, the column 'FITTED' (lines 74 to 119) show the fitted values in the model, which can be used if required in the preparation of graphs.

In this experiment there were replicates and each deviance included some within treatment error but in several other experiments there were no replicates and no within treatment error. The error deviance used in these experiments is the deviance left after subtracting the main effects only, that is the deviance attributable to second and third order interactions. In these unreplicated experiments second order interactions were examined and compared with this error deviance but third order interactions which could not be examined were not expected to occur nor considered to be of any interest.

```
Line
  1    £C EXPERIMENT 16
       £UNITS 45
       £FACTOR B 5 D 3 T 3
       £C B=REPLICATES D=DURATION T=LINES,EFW,W,LFW
  5    £DATA 45 V1 V2
       £C V1=DAYS TO FLOWER V2=NO. OF PLANTS
       £READ
       93 15 80 17 77 16 0 0 90 4 72 3
       0 0 99 2 92 8 94 10 88 16 73 19
 10    100 1 82 2 73 4 0 0 94 1 85 4
       88 17 80 20 72 18 0 0 82 4 77 4
       0 0 94 4 83 7 90 18 82 20 75 17
       91 1 94 4 76 4 0 0 97 2 83 11
       88 15 76 20 76 20 92 3 84 4 73 4
 15    94 1 98 2 89 7
       £WEIGHT V2
       £CAL B=%GL(5,9)
       £CAL D=%GL(3,1)
       £CAL T=%GL(3,3)
 20    £CAL DL=D
       £CAL DQ=DL*DL
       £CAL TL=T
       £CAL TQ=TL*TL
       £TERMS V1+D+T+DL+TL+D.T+DL.T+TL.D
 25    £YVAR V1
       £FIT £
          38 DF
       DEVIANCE CYCLE
          0.1859E 05  0
 30    £FIT D+T £
          34 DF
       DEVIANCE CYCLE
          3594.       0
       £FIT T £
 35       36 DF
       DEVIANCE CYCLE
          0.1642E 05  0
```

```
Line
      £FIT D £
         36 DF
40    DEVIANCE CYCLE
         9660.    0
      £FIT DL+T £
         35 DF
      DEVIANCE CYCLE
45       3604.    0
      £FIT D+TL £
         35 DF
      DEVIANCE CYCLE
         3967.    0
50    £FIT D+T+D.T £
         30 DF
      DEVIANCE CYCLE
         3133.    0
      £FIT D+T+DL+DL.T £
55       32 DF
      DEVIANCE CYCLE
         3444.    0
      £FIT D+T+TL+TL.D £
         32 DF
60    DEVIANCE CYCLE
         3356.    0
      £FIT DL+TL £
         36 DF
      DEVIANCE CYCLE
65       3990.    0
      £DISPLAY D E R
      ERROR NORMAL  LINK IDENTITY
      Y-VARIATE V1
      DEVIANCE =  3990. DF = 36
```

Line

		ESTIMATE	S.E.	PARAMETER
70	1	9.194E 01	1.79E 00	GM.
	2	-8.071E 00	7.54E -01	DL
	3	5.882E 00	8.19E -01	TL

	UNIT	OBSERVED	FITTED	RESIDUAL
75	1	9.30E 01	8.97E 01	1.26E 01
	2	8.00E 01	8.17E 01	-6.92E 00
	3	7.70E 01	7.36E 01	1.36E 01
	4	0.00E 00	9.56E 01	0.00E 00
	5	9.00E 01	8.76E 01	4.88E 00
80	6	7.20E 01	7.95E 01	-1.30E 01
	7	0.00E 00	1.02E 02	0.00E 00
	8	9.90E 01	9.34E 01	7.86E 00
	9	9.20E 01	8.54E 01	1.87E 01
	10	9.40E 01	8.97E 01	1.34E 01
85	11	8.80E 01	8.17E 01	2.53E 01
	12	7.30E 01	7.36E 01	-2.65E 00
	13	1.00E 02	9.56E 01	4.37E 00
	14	8.20E 01	8.76E 01	-7.87E 00
	15	7.30E 01	7.95E 01	-1.30E 01
90	16	0.00E 00	1.02E 02	0.00E 00
	17	9.40E 01	9.34E 01	5.56E -01
	18	8.50E 01	8.54E 01	-7.46E -01
	19	8.80E 01	8.97E 01	-7.21E 00
	20	8.00E 01	8.17E 01	-7.51E 00
95	21	7.20E 01	7.36E 01	-6.83E 00
	22	0.00E 00	9.56E 01	0.00E 00
	23	8.20E 01	8.76E 01	-1.11E 01
	24	7.70E 01	7.95E 01	-4.98E 00
	25	0.00E 00	1.02E 02	0.00E 00
100	26	9.40E 01	9.34E 01	1.11E 00
	27	8.30E 01	8.54E 01	-6.28E 00
	28	9.00E 01	8.97E 01	1.06E 00
	29	8.20E 01	8.17E 01	1.43E 00
	30	7.50E 01	7.36E 01	5.74E 00

Line	UNIT	OBSERVED	FITTED	RESIDUAL
105	31	9.10E 01	9.56E 01	-4.63E 00
	32	9.40E 01	8.76E 01	1.29E 01
	33	7.60E 01	7.95E 01	-6.98E 00
	34	0.00E 00	1.02E 02	0.00E 00
	35	9.70E 01	9.34E 01	5.03E 00
110	36	8.30E 01	8.54E 01	-7.87E 00
	37	8.80E 01	8.97E 01	-6.78E 00
	38	7.60E 01	8.17E 01	-2.54E 01
	39	7.60E 01	7.36E 01	1.07E 01
	40	9.20E 01	9.56E 01	-6.29E 00
115	41	8.60E 01	8.76E 01	-3.12E 00
	42	7.30E 01	7.95E 01	-1.30E 01
	43	9.40E 01	1.02E 02	-7.51E 00
	44	9.80E 01	9.34E 01	6.44E 00
	45	8.90E 01	8.54E 01	9.60E 00