

Population Variability
and its Ecological Significance
in
Arabidopsis thaliana (L.) Heynh.

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.....oOo.....

Introduction

During the 19th Century much work was done by botanists such as Darwin and Jordan on variation in wild and cultivated species. Attempts were made to relate this variation to natural selection but this was difficult with their current state of knowledge of mechanisms of inheritance. Mendel's work and that of other early geneticists clarified the laws of inheritance and started a second trend in the study of variation. At the beginning of the 20th Century these two lines were brought together especially by the work of Turesson (1922, 1925, 1930) who studied the genetical basis for the ecological variation within species.

Turesson noted that various species had different environmental tolerances and that the same species often displayed different morphological and physiological characters in different distinct habitats. His early work attempted to discover if this variation was due to the different expression of the same genotype in varying habitats or to the existence of different genotypes in each habitat.

This work on genecological differentiation was continued by Clausen, Keck and Hiesey (1940, 1945, 1948) who found that many widespread Californian species

(e.g., Potentilla glandulosa) could be separated into several local climatic races which showed distinct morphological and physiological characteristics when grown under uniform conditions.

By this time it had been well established that differences in the distribution of plant species were not caused by chance or historical accident but were due to differences in the tolerance of populations and species as a whole to various environmental factors. Climatic and edaphic factors could interact to provide special habitat complexes and the populations which occurred in these habitats often showed genetically based morphological and/or physiological characters which might have adaptive significance.

Meanwhile many advances had been made in the field of plant physiology and many of the mechanisms involved in controlling stages of plant development had been elucidated.

The work of Barton and Crocker among others had shed considerable light on mechanisms of seed dormancy and germination and the effect of environmental factors on these processes, (Barton and Crocker, 1948; Crocker and Barton, 1957). They found that seed dormancy could be caused by inappropriate environmental conditions and that it could occur despite the availability of all requirements for subsequent growth.

Similar advances were made in the understanding of flowering physiology. The flowering process was found to consist of several distinct stages - induction of the flowering state (Kleb's 'readiness to flower' (Maximov, 1934)), the changeover from vegetative to floral meristems, the morphological development of flowers and anthesis itself. Environmental factors were found to affect all of these processes independently and to help in synchronising the timing of these stages with appropriate climatic conditions.

One important effect, that of daylength on floral initiation, was first investigated fully by Garner and Allard (1920) who were able to distinguish plants that had a definite requirement for a particular photoperiod both in an obligate and a facultative manner. It was obvious that daylength requirements for flowering could play an important role in plant distribution.

Another important discovery was made by Russian workers on winter cereals. They found that these grains required a chilling pretreatment in the seed before they would flower. They called this process 'jarovisation' (vernalisation). Purvis (1934) working on rye found that vernalisation did not affect the formation of floral initials themselves but instead put the plant in a state 'readiness to flower'. It might be expected that variation in vernalisation requirements would affect the climatic tolerances of species.

Despite the advances made in plant physiology studies and the body of information which was accumulating on plant distribution and behaviour in the field, little attempt was made to correlate physiological characteristics observed in laboratory experiments with behaviour in the field.

In 1914 Shreve (1914) had emphasised "the viewpoint of the geographer, is too broad and general to give due respect to the actual physiological effects of temperature on plants; the point of view of the plant physiologist, on the other hand, is often too intensive to enable him to realize that the 'conditions' of his laboratory experiments are identical with the 'physical factors' of the environment of plants growing under a state of nature and he is therefore prone to neglect the bearing of his work on the problems of the field. There is no greater desideratum at the present time - with respect to the operation of all environmental factors - than to bring the intensive methods and exactness of logic which characterize physiological work to bear on the large and intricate problems of physiological plant geography".

However, until Went and his colleagues started working on the annuals of the Californian deserts in the 1930s little was known of how environmental factors and variation in physiology of species could interact to

influence the distribution of plants both spatially and temporally. Following the early work, Went became a pioneer of the use of growth chambers to simulate environmental conditions, Although the initial work failed to correlate directly the responses of plants under controlled conditions with responses in the field, (Lewis and Went, 1945), further investigation showed that the division of these species into summer and winter annuals and their abundance from year to year was directly correlated with field temperature after rainfall and that these species responded similarly in the field to temperature and moisture patterns simulated in his 'phytotron'. (Went, 1948, 1949; Went and Westergaard, 1949).

This and other work showed the value of controlled experiments in the laboratory to attempt to correlate specific environmental factors with physiological characteristics and so elucidate the importance of differences observed in the development and distribution of species.

In the 1950s the importance was realised of study of the 'ecological life cycles' of species if the critical stages of the life cycle for survival were to be discovered. This realization lead to the establishment of the 'Botanical Flora of the British Isles' instigated to a large degree by Salisbury (1928). Up to the

present day almost one hundred species have been studied in this way. In the U.S.A. Pelton (1953) and Stevens and Rock (1952) urged a similar approach and several studies have been made of either life cycles as a whole or critical stages of development such as establishment, vegetative growth and flowering especially in species growing under extreme conditions. Comparative studies of populations and species growing under different climatic conditions have also been made with either an ecophysiological slant e.g., Mooney and Billings, 1961; Mooney and Johnson, 1965; Ayodele Cole, 1967; or an ecogenetic bias e.g., Jain, 1969. Such comparative studies shed considerable light on the selective importance of specific factors in the habitat - environment complex.

Ratcliffe (1957, 1961, 1965) carried out work of this type on a group of eight winter annuals from the Derbyshire limestone. These included Arabidopsis thaliana, Saxifraga tridactylites, Arenaria serpyllifolia, Draba muralis and Hornungia petraea. They were found at well-defined times of year in open habitats where shallow soil and consequent summer drought excluded perennial vegetation. He found a common pattern for the life cycle in this taxonomically varied group. Flowering always occurs in the spring and seeds are usually shed by May. However, no germination was observed until late August even when the summer was relatively wet.

In the autumn these species produced compact rosettes or compact vegetative shoots^o which overwintered. Autumn flowering was rarely observed even if autumn conditions were particularly favourable. Flowering occurred relatively simultaneously in all individuals of the same species when warmer conditions and longer days arrived in the spring. These species are particularly suited to these habitats as they are able to complete their life cycle by the spring and so avoid the summer drought.

Ratcliffe found the inability of seeds to germinate in the summer was due to a post harvest dormancy or after-ripening requirement which was not complete until the early autumn. The failure to flower in the autumn even in warm weather was found to be due to the need for a cold treatment of the rosettes before floral initiation could take place. These two physiological characteristics were of primary importance in synchronisation of the life cycle with the most favourable time of year for survival of the species in each locality.

Several of these species have an extremely wide distribution range especially Arabidopsis thaliana and Arenaria serpyllifolia. Although the Derbyshire populations are well adapted to conditions pertaining in their localities it would be expected that in other parts of the wide distribution range different factors of the habitat-environment complex may be limited.

The aim of the present investigation was to extend Ratcliffe's work to populations of Arabidopsis thaliana from throughout its range to see if any correlation could be found between physiological variation observed under laboratory conditions and the climatic factors pertaining over the distribution range both on a widespread and local basis.

Arabidopsis thaliana is a very suitable species for an investigation of this nature. Its annual habit, small seed size (0.4 - 0.8 mm in length), large seed production per plant and small rosette size make it an ideal subject for experimentation and cultivation. It has long been used as an experimental organism in work on morphogenesis and mutagenesis and in the study of developmental physiology especially the effect of cold on flowering responses, (Laibach, 1965). More recently it has become widely employed in genetical studies and has been termed the 'botanical Drosophila', (Kribben, 1964).

This species was originally thought to be completely autogamous and indeed in greenhouse experiments A. thaliana is found to be usually self-fertilizing. However the recent work of Lawrence and his colleagues at the University of Birmingham has shown that a small but definite amount of outcrossing can occur if insect pollinators are allowed access to the plants, and there is evidence that outcrossing also occurs in the field,

(Snape and Lawrence, 1971; Jones, 1968, 1971 a,b,c; Westerman and Lawrence, 1970).

Laibach (1943) noted variation in several physiological factors including length of post harvest dormancy, the effect of light on germination and variation in vernalisation requirements. However, until the studies of Ratcliffe (1965) and later Cetl in Czechoslovakia (Ceřl, 1965; Cetl, Dobrovolna and Effmertova, 1965, 1967) little attempt had been made to establish the role physiological characteristics could play in determining the distribution of this species.

The purpose of this study was to investigate the effects of environmental factors at each stage of the life cycle from the seed to the flowering plant and to elucidate those physiological and environmental limitations which allow this plant to achieve its wide distribution and define its limits.

PART A Bio-geographical Information

A. 1. Geographical Distribution

Arabidopsis thaliana is common throughout Europe to 68° 30' North. Tutin, Heywood et al (1964) state that it is ubiquitous in Europe except for the Faroes, Iceland and Spitzbergen. It becomes more scattered eastwards in Europe but it reaches the Urals and Western Asia, where it occurs in the Caucasus, Dzungaria and the Altai Mountains ; (Schulz, 1957); the Pamir region (Benum, 1958) and in Central Asia (Ohwi, 1965); Hooker (1872) recorded it in the Himalayas from Bhotan to Kashmir, in Western Tibet and the Punjab; it occurs in Afganistan and in China in the Hupeh Province (Schulz, 1957). It is found in Korea and is widespread in Japan (Ohwi, 1965).

In the Middle East it is common in coastal regions in Turkey (Davis, 1965), the Balkans (Schulz, 1957) and in northern Persia, especially near the Caspian Sea (Rechinger, 1968). In North Africa it is reported from Morocco, Algeria, Tunisia, The Canaries and Madeira (Schulz, 1957). Herbarium specimen/s exist from Egypt and Libya (Herb. Kew.).

In East Africa A. thaliana is recorded in Eritrea, Somalia and Ethiopia, especially the Simien (Schulz, 1957); it is found further south on Mts. Elgon, Kenya and Kilimanjaro, in the Nivaisha area of Kenya, the Western Usambaras and Embagai crater, Tanzania (Herb. Kew.). Harvey (1838) did not record it from South Africa but its presence was noted in his 1868 edition. Now it is naturalised in scattered localities in Cape Province (Schulz, 1957).

There are no records from S.E. Asia, S. India, Indochina or the East Indies. Most Authors including Clapham, Tutin and Warburg (1952), Schulz (1957) and Hegi (1958) suggest that it is naturalised in Australia but Burbridge (1963) considers it occurs only as an introduced weed in the Canberra area. It is not recorded from New Zealand.

In the Western Hemisphere Pursch's 1814 flora and Nuttall (1818) describe A. thaliana as occurring in dry sandy fields and on old walls from New England to Virginia. At the present time it is common in the North East and Middle West of the U.S.A. (Fernald, 1950; Rickett, 1967; Seymour, 1969 etc.) and in North and East Texas (Correll and Johnston, 1970). In Missouri it occurs primarily along the river (Daniels, 1907; Steyermark, 1963). In the Western U.S.A. it is rare but has been reported from Washington, Idaho and Utah and in Oregon along the Willamette valley (Ryberg, 1932). It was first reported from California in 1892 and now is found in the coastal ranges and in the foothills of the Sierra Nevada (Howell, 1965) and as a weed in the Botanical Gardens of the University of California at Berkeley (Robbins, 1940).

Provancher's Flora Canadienne (1862) does not mention A. thaliana but Macoun (1883) recorded it at Point Abino, Lake Erie. Its distribution in North America; primarily in coastal regions spreading inland especially along river valleys, strongly suggests that it is an introduction and most authors have taken this view. However, Macoun (1883) commented "Abundant and, I think, indigenous". It is not reported from Central or South America.

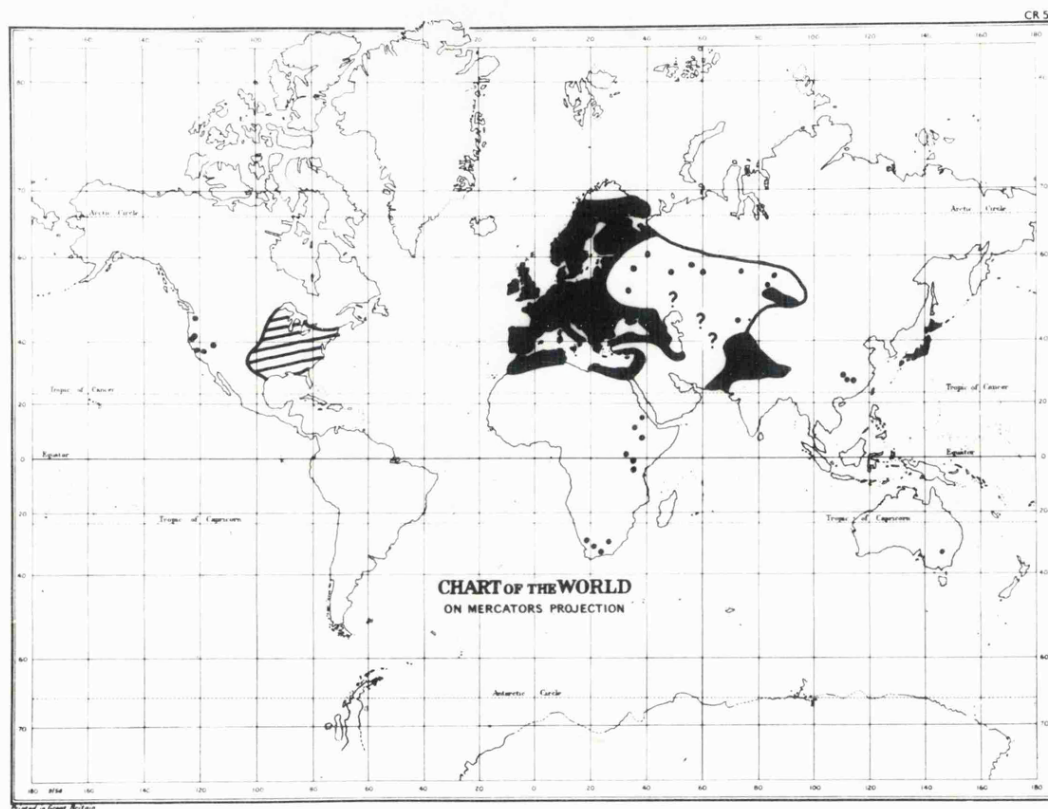


Figure A 1i. Geographical distribution.

The preceding information is summarised in figure A1 i.

Arabidopsis thaliana is widely distributed throughout the British Isles (figure A1 ii). It is more scattered in Ireland, Wales and parts of Scotland and Northern England though this may be due in part to inadequate recording. It is reported to be common or locally frequent in South and Central England, e.g. Bowen (1968), Clapham, (1969), Cadbury, Hawkes and Readett (1972).

Davey (1909) mentions it as being very local and rather rare in Cornwall while Riddelsdell (1948) reports it is locally common in Gloucestershire but appears to be missing from 'large areas of the Severn plain and the Cotswolds'. In Hertfordshire it is frequent on gravelly soils but rare on chalk and boulder clay (Davey, 1967) and in Northamptonshire it is locally abundant but rare on heavy clay soils (Druce, 1930).

Both the time of flowering and the altitudinal limits vary considerably throughout its range (Table A1 i).

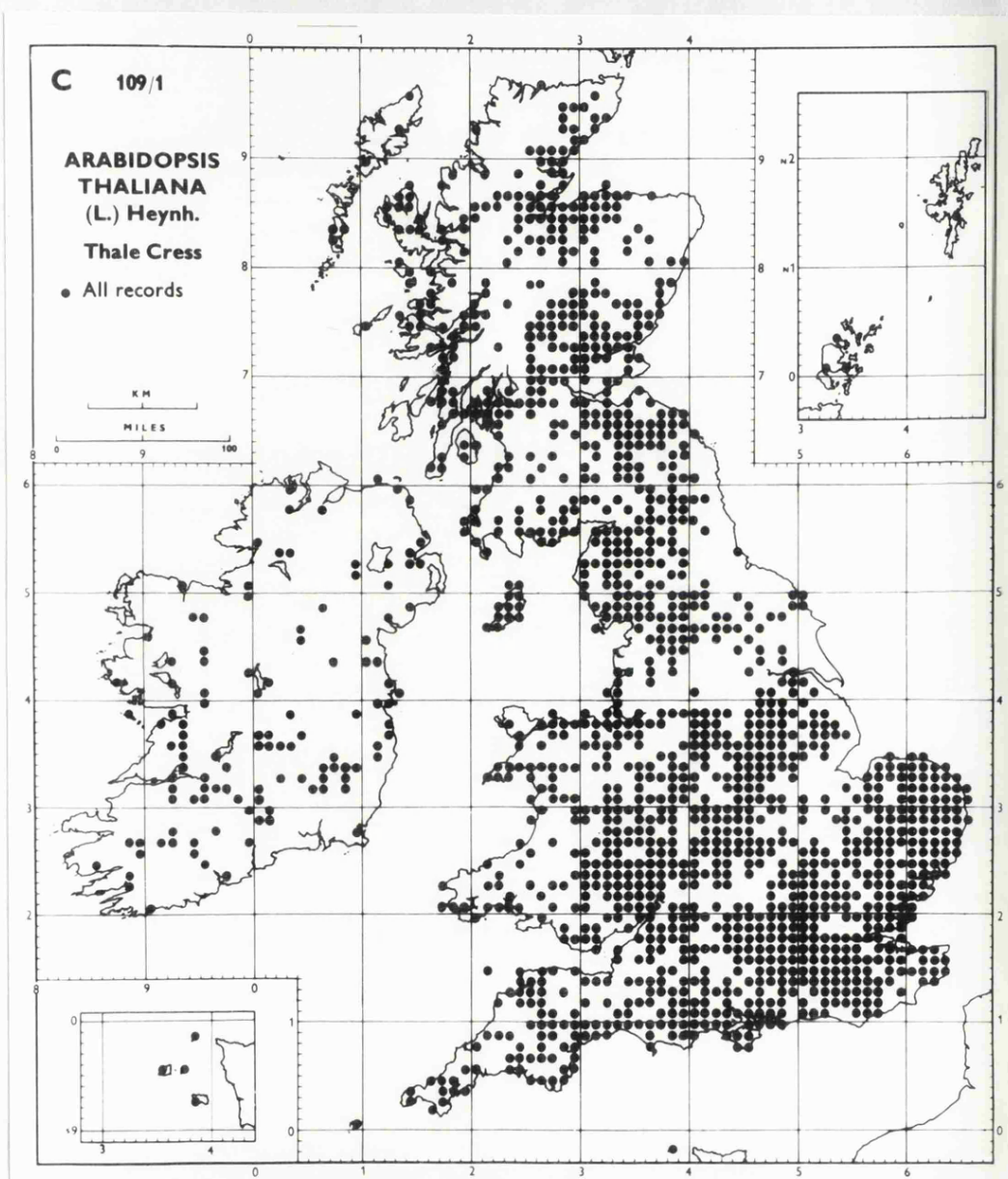


Figure A 1ii. Distribution in the British Isles.

TABLE A1 i

<u>Flowering time and upper altitudinal limits of Arabidopsis</u> <u>thaliana</u>		
<u>LOCALITY</u>	<u>FLOWERING TIME</u>	<u>ALTITUDINAL</u> <u>LIMIT</u>
British Isles	March - July (September)	800m
N. France, Belgium	March - July (September)	2000m
Switzerland, Germany	March - July (September)	2000m
Sweden, Norway	May - July	1150m
Denmark	April - June	?
Spain, N.Africa, Greece, Italy	February - May (July at high altitudes)	2400m
S. France, Canaries	December - March	2500m
Turkey	April - June	1800m
Iran	April - June	3700m
Himalayas	January - September	5000m
E. Africa	All year round	4500m
S. Africa	May - November	1800m
Japan	March - June	?
U.S.A.	March - June	?

From Clapham, Tutin and Warburg (1952), Fournier (1961), Lawalrée et al (1967), Hess, Landolt and Hirzel (1970), Lid (1963), Bornmuller (1925), Quizel and Santa (1962), Lid (1967), Davis (1965), Rechinger (1968), Ohwi (1965), and Rickett (1967).

In general there is a trend towards raising of altitudinal limits with decreasing latitude. There is also a strong tendency to later flowering with higher altitudes, at least in Europe and Asia. In Tajikistan Yunusov, Kosyanenko and Usmanov (1969) found that A. thaliana commenced flowering approximately one month later for each 600m increase in altitude from 700m, where it flowered from mid March, to 4000m where flowering began in mid July and continued until late September.

In Corsica specimens have been found ~~in~~ flowering at sea level on 25 January, at 600m on 19 April, 1600m on 28 June on a south facing slope and 18 ~~June~~^{July} on a north facing slope and at 2050 on 30 July (Briquet, 1913). Aspect is obviously important on a smaller scale; in Derbyshire on 24 May 1971 plants were in full flower or fruiting on a limestone scree of S.E. aspect in Dovedale, while in Lathkill Dale, five miles to the north east, plants on north west facing limestone ledges were only in bud.

The time of flowering will vary from year to year according to weather conditions; for example, in 1971 after a particularly mild winter, plants were reported in full flower on Blackford Hill, Edinburgh before the end of March (Grant Roger, 1971).

A.2. Ecological Distribution

Throughout its Eurasiatic and American distribution range this species is found in a wide range of habitats. These are usually early in seral situations, areas of disturbed ground, places where the soil is too shallow or unstable for perennial vegetation, or where the ground is trampled or grazed heavily.

It is often found on the early fixed stages of calcareous dunes (Salisbury, 1952) and Raunkiaer (1934) lists it as occurring with Salsola kali, Festuca rubra and Agropyrum junceum on a dune formation at Janø on the west coast of Jutland.

Frequently Arabidopsis thaliana occurs on unstable soils, for example, limestone screes of fine material in Derbyshire (figure A2 i); slate talus in Cumberland and Greece; on gravel in Ethiopia, Kenya and Cyprus; on sandy river banks in Siberia and Germany and on lava in Kenya and Cyprus. It is less common in grassland but may occur in more open vegetation of this sort, for example, in temporary spring pastures, the 'prados vernaes ephemeris' in Spain (de Bolos, 1950) and the 'poemnye luga' in Siberia (Krylova, 1931); in sub-alpine grassland (Braun Blanquet, 1961) and in sandy grass heath as in East Anglia. Mooney (Herb. Kew. specimen) describes it as occurring in 'very close grassy sward' on steep slopes at 11,300' at Gosmeda in Ethiopia;

the sward is grazed by Hyrax and the plants often flower at only $\frac{1}{2}$ " in height but occasionally reach 4" when protected by *Helichrysum* species. A. thaliana also occurs within closed grassland on 'islands' of disturbed soil, for example, on ant hills, in e.g., Lathkill Dale where it appears to spread from nearby rocky ledges.

In Mediterranean regions it is frequently found as a member of the therophytic spring communities in maquis, garigue and in open places in Quercus ilex woodland (figure A2 ii), it is also reported in beechwoods, in Alnus glutinosa woodlands in Holland and in open pine forests (*Pinus brutia*) in Turkey.

It is reported from saline places in Siberia (Krylova, 1931). It is found in a wide range of ruderal situations; ballast; rubbish tips; ash heaps; roadsides; footpaths; railway tracks (figure A2 iii) and old walls. It is a common weed of cultivated land including gardens, vineyards, fig orchards (in Turkey) and fields of clover, tobacco (Greece), potatoes and grain.

The substrates on which Arabidopsis thaliana is found are quite varied. It is common on siliceous soil but has been described as both a lime tolerant species (de Bolos, 1950) and a lime avoiding one (Hess, Landolt and Hirzel, 1970; Lawalrée et al, 1967). Perhaps the physical rather than the chemical features of the soil are the most important.



Figure A 2i. Limestone scree opposite Dove Holes, Derbys.



Figure A 2ii. *Quercus ilex* scrub near Monserrat, Spain.



Figure A2iii. Disused railway track, Friden Crossing
Derbyshire.

It appears to thrive on most light dry soils but is rare on chalk. It is infrequent on clay soil and when it does occur specimens are often depauperate.

A. thaliana is reported to prefer substrates rich in phosphates and nitrates (Hegi, 1958). Little is known of its tolerance to heavy metals but it has been collected from a serpentine soil on Holy Island, Ang^{er}sey, and in Penns~~X~~ylvania, it has been found growing on disintegrating sacks containing zinc oxide. Analysed plant material contained 9,000 ppm dry weight or 6% in the ash (Cannon, 1960).

It would appear that the habitats of A. thaliana, although superficially very diverse, have several features in common: they are often unstable or transient and may not support closed perennial vegetation but are characterised by a number of annual, perhaps pioneer, species.

Braun Blanquet (1961) describes it as an occasional species of dry grassland communities in the alpine valleys e.g., Stipeto - Poetum carniolⁱcae and Sileneto - Koelerietum vallesianae. In S.W. Germany Oberdorfer (1962) describes A. thaliana as typical of pioneer communities (field margins and paths which are warm but dry in summer and usually base rich), where there are a number of Aperetalia communities e.g. Papaveretum argenteae.

Hegi (1958) also says it is found in *Aperion spicae-venti* along with other pioneers e.g. Erophila verna, Holosteum umbellatum and Arenaria serpyllifolia. Tuxen (1950) places it in the *Agrostidion* (sic) *spicae-venti* section of his classification of the nitrophilous communities of Europe. In this section he describes a new community the 'Valerianello olitoriae - Arabidopsetum - thalianae' which has Valerianella olitoria, Arabidopsis thaliana, Veronica arvensis, Myosotis collina, M. Versicolor, M. micrantha and Gagea pratensis as faithful species. This association is found on the sloping terrasses of the Weser and Lahn valleys which are disturbed by grazing cattle in summer and ice movements in winter. He considers it to be a natural therophyte community which would be followed by an acidophilous dry grassland community with such species as Festuca ovina and Thymus angustifolius if the succession were undisturbed.

The striking feature ^{to} ~~in~~ common ~~with~~ all these communities is the high incidence of annuals with a similar distribution in Europe e.g., Saxifraga tridactylites, Erophila verna and Holosteum umbellatum. This suggests that A. thaliana is long established in other than ruderal habitats in Europe and that it cannot be considered as just a weed species (Ratcliffe, 1961).

These communities, however, have their counterparts in ruderal situations and on walls (Segal, 1969). 'Weedy' communities where it has been introduced are in all respects similar, for example, railway tracks in Southern Illinois (Voight and Mohlenbrook, 1959). In Southern Tennessee Baskin and Baskin (1972) reported it from therophytic communities in woodland clearings suggesting that despite its original introduction into N. America it has been able to find a niche in natural communities in at least one area.

The situation in East Africa is rather different. A. thaliana is found in dry tussock grassland at 4000m on Mt. Kenya. The dominant vegetation is Festuca^p~~Felgeri~~ with carpets of Alchemilla johnstonii; Cardamine obliqua, Myosotis keniensis and Galium glaciale also occur as occasional species. On Mt. Kilimanjaro at 3800m A. thaliana is found scattered with specimens of Cardamine obliqua and C. hirsuta among tussocks of Carex monostachya (Hedberg, 1963).

It is probable that in this considerably different climate A. thaliana has a different habit, life cycle and ecological distribution. This will be discussed in detail later.

A.3. Climatic factors and distribution

Arabis thaliana localities often have shallow, well drained soil and are subject to a high degree of insolation especially in the summer months which frequently leads to a cycle of rapid drying of the soil in early summer, drought and rewetting in the autumn. These characteristics are shared by A. thaliana habitats despite the wide distribution range covering many different climatic types; mediterranean, maritime, continental and montane including afro-alpine.

The Mediterranean regions have very warm, clear, dry summers with temperatures of 20 - 27°C (70 - 80°F) for two or more months except in exposed coastal stations. In summer diurnal fluctuations of temperature ~~at~~^{are} 17 - 20°C (30 - 35°F); in winter conditions are relatively equable with a range of 8 - 11°C (15 - 20°C). Summer drought is common owing to low rainfall. In September rain becomes more frequent and heavier; October is the wettest month with a second maximum in March. The annual growing season is 300 - 365 days. This climatic pattern is a feature of the whole Mediterranean region below 750 - 1200m and up to 150 kms inland. The dominant vegetation is characteristically broadleaved evergreens and sclerophyllous forms giving maquis or garigue with a high proportion of spring flowering therophytes.

The ~~temperature~~ maritime climate of the Atlantic seaboard from Portugal to Scandinavia gives way, eastwards, to a continental climate, which extends for approximately 5000 kms or more. The ~~temperature~~ maritime climate is characterised by mild winters, cool summers and abundant cloud and fog. The yearly range of temperature is rarely more than 11°C (20°F) at the coast, slightly more inland. Rainfall in places exposed to the sea is generally more than 100 cms (40"), over 50% falling between October and March. In Eastern Britain summer months may be the wettest owing to thunderstorms etc. In areas with a more continental climate winters are colder and the annual temperature range is larger e.g., Moscow 29°C (52°F), Warsaw 22°C (40°F).

However, the increased continentality of the climate eastwards is due less to an increase in summer temperatures than to a decrease in winter temperatures. Most precipitation occurs from April to September owing to the increased frequency of convective showers. The relative humidity is lower so there is less cloud or fog but there is more snow. Annual rainfall is 50 - 65 cms (20 - 25") on the North European plain, 65 - 75 cms (25 - 30") in S.E. Europe and over 100 cms (40") at high altitudes. The growing season varies from over 9 months on the French coast to 5 months east of the Baltic.

In the Mediterranean region therophytes form an important element of the vegetation. In northern Europe these annuals can only survive outside the forest in transient or unstable habitats. It is likely that the increasing rarity of A. thaliana in Eastern Europe and Asia is owing in some degree to the restriction of suitable habitats in the forest steppe and boreal forest.

It has been emphasised that the habitat types, the substrates and the associations where A. thaliana is found are rather similar despite their superficial variability. This is true to a certain extent of the climates also. When graphs of monthly temperature and rainfall of typical mediterranean, maritime and continental localities are superimposed, (figure A3 i), it can be seen that great similarity exists in the autumn and spring conditions. It is perhaps not chance alone that in annual species, the two most critical stages of the life cycle: Germination and flowering, occur at these times. The summer and winter climates show greater diversity. Continental regions are characterised by winter cold and high summer rainfall. Mediterranean and maritime areas have mild winters and maximum winter rainfall.

The annuals of Mediterranean and maritime regions are usually subjected to summer drought in the types of habitat where A. thaliana occurs and this unfavourable period is passed in the seed stage. Autumn germination and spring flowering follow.

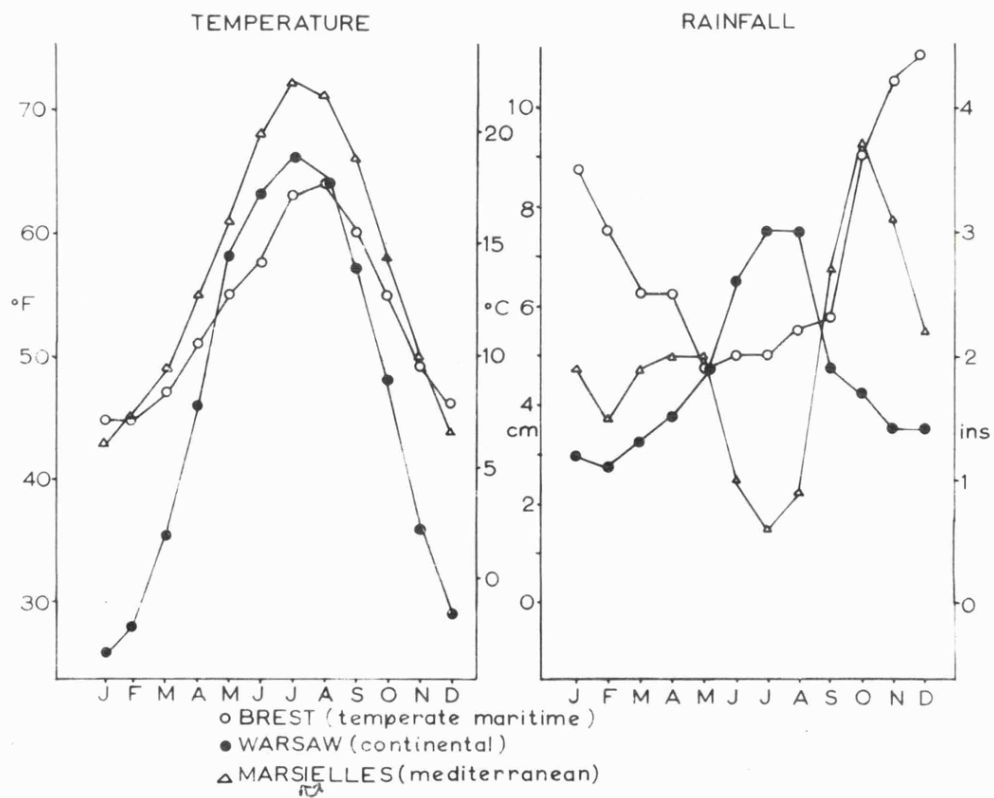


Figure A 3i. Climatic data.

In continental regions, the winter cold will obviously limit vegetative growth of any species and here some annuals may endure the winter as seeds to germinate in the spring and flower in the summer. On the other hand, where summer rainfall is unpredictable the winter annual habit may be more appropriate so long as the winter can be survived. It is obviously necessary to study the effects of environmental factors on various stages of the life cycle to elucidate the mechanisms by which A. thaliana has been able to overcome variation in micro-climate and occupy such a wide distribution range.

To be successful over this wide range considerable variation in physiological behaviour would be expected. However, as has been shown, it does not spread into the most extreme continental regions of Eurasia and North America. What is the reason for this limit ?

The first considerations of 'continentality' of climate were made by Amann (1929) who found he could explain the distribution of 'atlantic' mosses in Switzerland by considering the 'oceanité' of the climate using a formula involving precipitation, annual average temperature and the difference between the temperature of the hottest and coldest months. Poore and McVean (1957) used similar ideas in their studies of Scottish mountain vegetation. Important factors are the flattening of the annual temperature curve, the high numbers of days with temperatures between 0 - 10°C and the increase in the precipitation - evaporation ratio in the maritime climate. In addition, indirect factors

such as prolonged snow cover in continental regions and the poor melting of snow under warm, dry conditions as compared to cool, damp conditions, have to be considered in any estimation of length of growing season and the suitability of the seasons for plant development.

Following Böcher's work on the Greenland flora (Böcher, 1938), Meusel, Jäger and Weinert (1965) devised methods of expressing grades of oceanicity and continentality for Eurasia and discussed the distribution pattern of northern species in relation to these grades. It can be seen that the distribution of A. thaliana in Eurasia is very closely correlated with their oceanic grades and does not spread into truly continental regions, (figure A3 ii). This is probably due to the severe winter temperatures coupled with the irregularity of summer rainfall making an unsuitable climate for winter annuals and a precarious one for summer annuals.

The presence of several high altitude populations of A. thaliana shows that it can overcome to some extent the difficulties posed by the lack of a clear cut seasonal pattern and high diurnal temperature fluctuations found in these regions, (Rumney, 1968; Salt, 1954). Such populations might be expected to show physiological adaptation to montane conditions especially in the tropics. In temperate latitudes high altitude populations in southern regions might show similar patterns of variation to more northerly populations at lower altitudes.

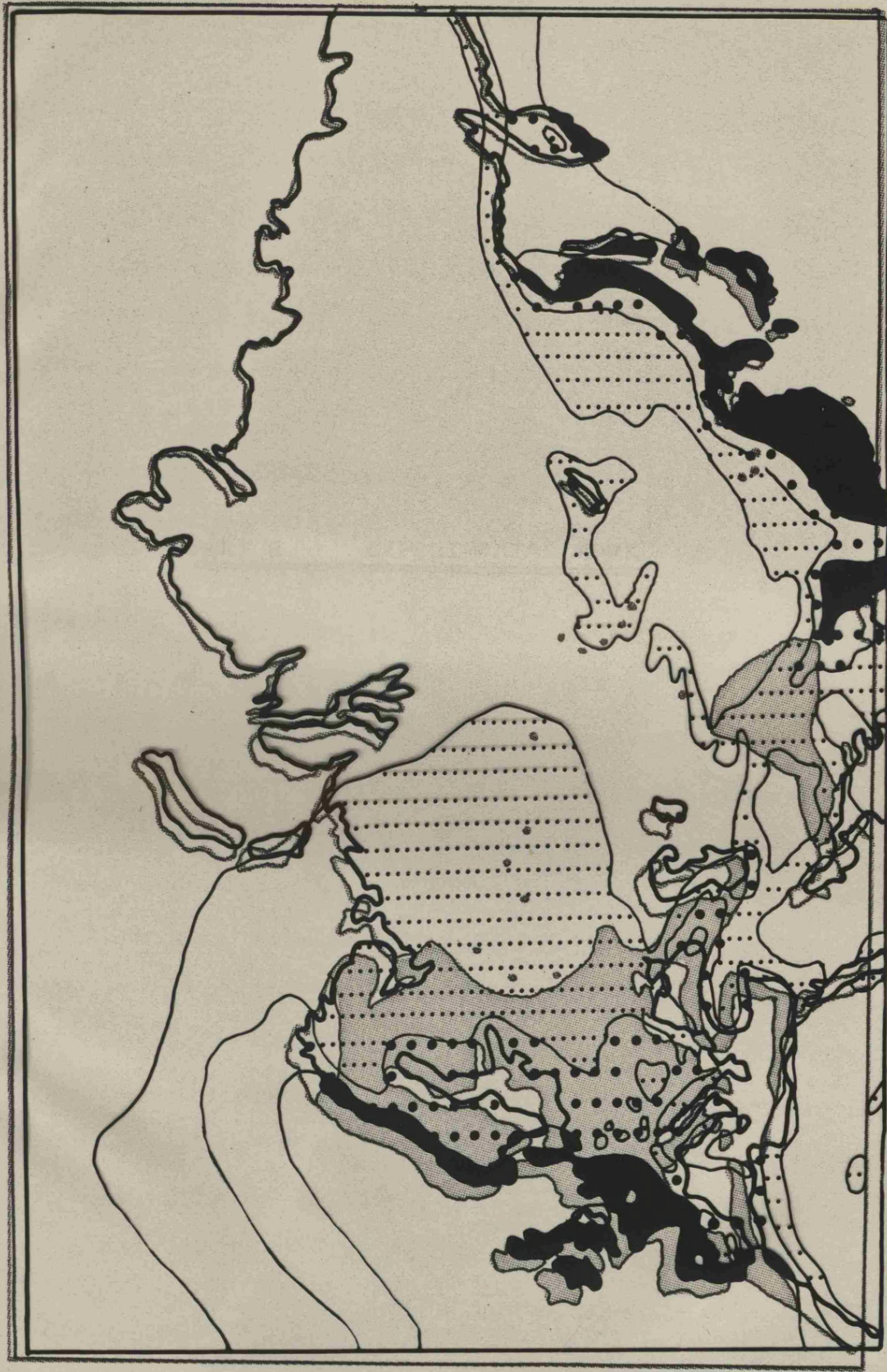
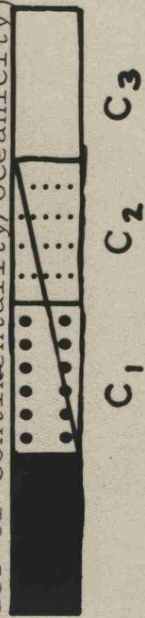


Figure A 3ii. Distribution in Eurasia. (Overlay: grades of continentality/oceanicity)



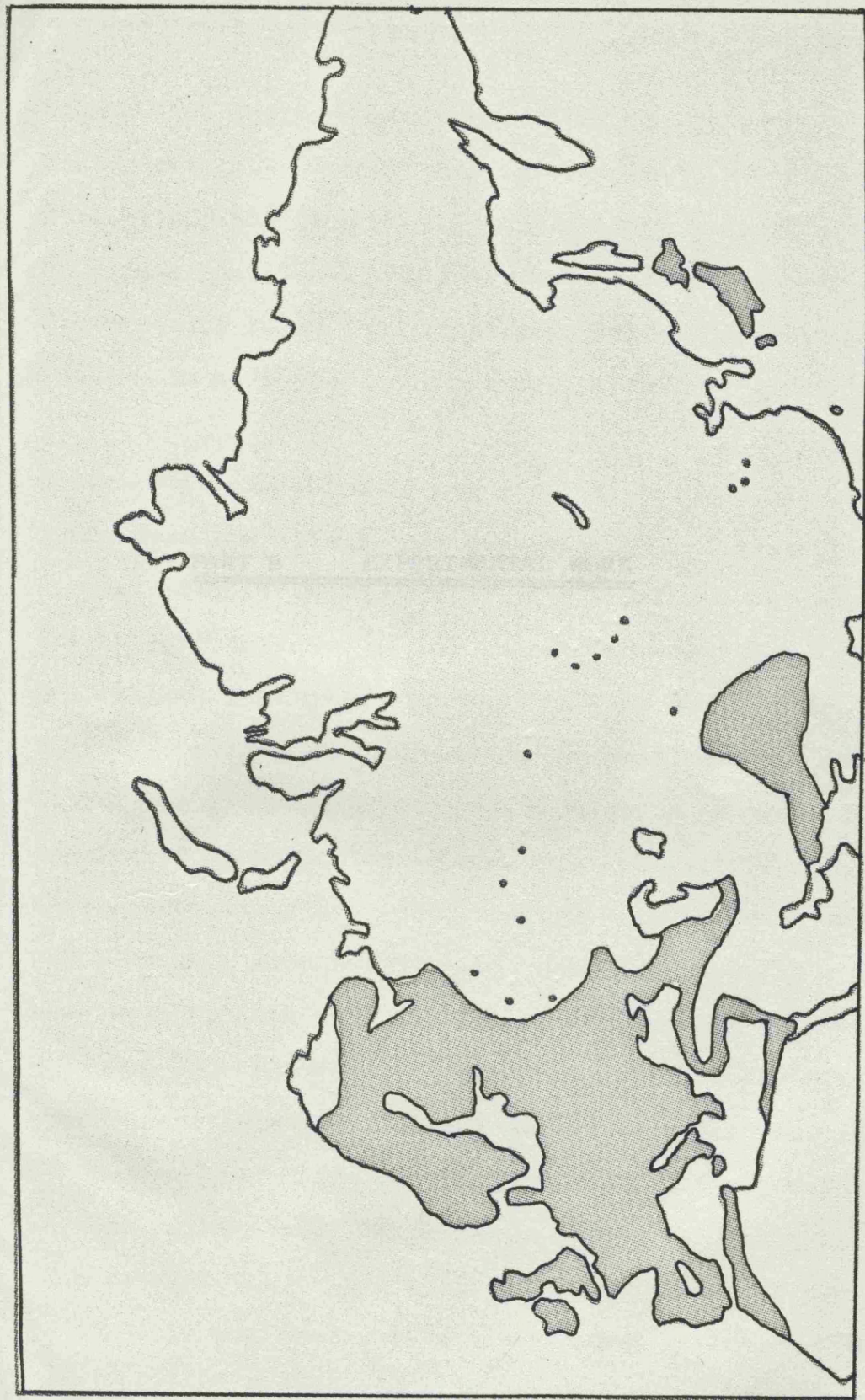


Figure A 3ii. Distribution in Eurasia. (Overlay: grades of continentality/oceanicity)

PART B EXPERIMENTAL WORK

B.1. Experimental Materials

Ninety seed samples from populations of Arabidopsis thaliana became available during this investigation. Even if all collections had been equally suitable, a reduction in numbers to a practical level would have been necessary. Several factors were taken into account in selecting those populations which were used in the experiments.

Firstly, it was hoped to use populations from diverse habitat types from the widest possible geographical range. Secondly, it was preferable to have as much knowledge as possible about the background of the population samples :- date of collection, storage conditions, habitat details and the number of plants represented in the seed sample. Finally, choice was restricted to those population samples which germinated in sufficient quantity at an early enough stage in the investigation. Some samples were too old, others had been sent from botanical gardens and herbaria and may have been treated with chemicals. Failure to germinate excluded some interesting localities among which were a few populations from the Scottish Highlands, some Mediterranean populations including one from Coimbra, a few populations from Eastern Europe e.g., Debrecen in Hungary and several populations from East Africa. Initially, as wide a range of populations as possible was used. This was reduced as the work proceeded and different combinations of populations were employed in different experiments.

TABLE B1 i

POPULATION DETAILS

SAMPLE	DATE OF COLLECTION	COLLECTOR*	HABITAT	ALTITUDE IN FEET	ASPECT	NUMBER OF PLANTS
PARSLEY HAY, DERBY.SK.1463	8/7/70	JAE	Disused railway track	1125	-	5 - 10
MOORSIDE, DERBY,SK.2956	8/7/70	JAE	"	700	-	10 - 20
DOVE HOLES, DERBY.SK.1453	9/7/70	JAE	Carboniferous limestone scree	550	SE	Over 20
DEEPDALE, DERBY.SK.1670	9/7/70	JAE	Carboniferous limestone ledges	750	NE	Over 20
FRIDEN CROSSING, DERBY.SK.1859	8/7/70	JAE	Disused railway track	1075	-	Over 20
CARSINGTON PASTURE, DERBY. SK.2554	8/7/70	JAE	"	1075	-	Over 20
JUG AND GLASS, DERBY.SK.1662	8/7/70	JAE	"	1100	-	Over 20
VIA GELLIA, DERBY.SK.2656	8/7/70	JAE	Limestone spoil heap	750	N	Over 20
LONGCLIFFE, DERBY.SK.2156	A. 8/7/70 B. 8/7/70	JAE JAE	Disused Railway track	1100 1100	- -	Over 20 10 - 20

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TABLE B1 i

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Table B1 i - Population Details Continued...

SAMPLE	DATE OF COLLECTION	COLLECTOR*	HABITAT	ALTITUDE IN FEET	ASPECT	NUMBER OF PLANTS
COBBLER'S NOOK, DERBY.SK.1958	A. 20/6/70 B. 8/7/70	DR JAE	Disused Railway track	1050 1050	- -	5 - 10 Over 20
LATHKILL DALE, DERBY.SK.1666	A. 7/7/70 B. c.1955 C. 7/71	JAE DR JAE	Limestone ledges Limestone quarry Limestone ledges	700 900 750	S S NE	Over 20 Over 20 10 - 20
HUSBAND'S BOSWORTH, LEICS	5-6/70	JAE	Disused railway track	375	-	Over 20
HEXHAM, NORTHUMBERLAND	6/66	DR	Ruins of Roman camp	250	-	2 - 5
HAILES, EAST LOTHIAN	4/70	JAE	Paths, castle ruins	500	-	5 - 10
DIRLETON, EAST LOTHIAN	5/64	DR	Gravel path	50	-	Under 5
GORT, BURREN	9/58	DR	Wall top	200	-	2 - 5
EDINBURGH	6/63	DR	Gravel soil	75	-	Over 10
BUSH, MIDLOTHIAN	6/63	DR	Gravel path in garden	500	-	Over 10
WEETING, NORFOLK	7/69	DR	Sandy grass heath	75	-	2 - 5

/Continued.....

Table B1 i - Population Details Continued...

SAMPLE	DATE OF COLLECTION	COLLECTOR*	HABITAT	ALTITUDE IN FEET	ASPECT	NUMBER OF PLANTS
WOEBLEY CASTLE, GLAM.	7/69	DR	Castle ruins	50	-	2 - 5
WHATTON, LEICS.	7/69	DR	Garden soil	200	W	Over 20
SORIA, SPAIN	4/61	DR	Sandy soil under <u>Quercus ilex</u>	3470	-	2 - 5
ENTRAYGUES, FRANCE	4/61	DR	Limestone ledges	500	S	5 - 10
OCHSENKOPF, AUSTRIA	8/70	DR	Rocky ledges	6000	S	Over 20
CREAG AN LOCHAN, ARGYLL	7/59	DR	Limestone ledges	3000	SE	5 - 10
BLACK MOUNT, ARGYLL	7/59	DR	Roadside	600	-	5-10
MICKLE FELL, N.W. YORKS	?	Dr. Derek Ratcliffe	Limestone rocky ledges	2600	W	5 - 10
BARRY SANDS, ANGUS	11/65	G. Smith	Sand dunes	Sea level	-	2 - 5
ST. CYRUS, KINCARDINE	I. 2/65 II. 11/64	G. Smith G. Smith	Turf over ruin. Cart track	100 Sea level	- -	2 - 5 2 - 5

/Continued.....

Table B1 i - Population Details continued.....

SAMPLE	DATE OF COLLECTION	COLLECTOR*	HABITAT	ALTITUDE IN FEET	ASPECT	NUMBER OF PLANTS
CATTERLINE, KINCARDINE	2/65	G. Smith	Edge of shingle beach	Sea level	-	2 - 5
ABERDEEN	2/65	G. Smith	Weed in ashy soil	100	-	2 - 5
BEN DHORAIN, SUTHERLAND	5/61	Dr. Derek Ratcliffe	Old red sand - stone crags	1500	?	2 - 5
CREAG NA EUN, LOCH NESS	5/61	Dr. Derek Ratcliffe	Calcareous crags of Moine Schist	1700	SE	2 - 5
LANARK	1961	A. McCosh	Railway track	?	-	10 - 20
ST. DOGMAEL'S ABBEY, CARDS.	5/70	A. Conolly	Old walls	50	-	10 - 20
KERRY	5/71	M. Long	Cliff top	?	-	5 - 10
HADLEIGH, IPSWICH	6/71	A.W.Punter	Gravel pit	?	-	5 - 10

* JAE - Author
DR - Dr. Denis Ratcliffe

Samples were also received from Botanical Gardens at Strasbourg, Dijon, Besançon, Lodz and Stockholm (3 samples).

The population samples collected by myself and Dr. Denis Ratcliffe formed the backbone of the collection as all background information was available. These were augmented by samples received from other botanists and botanical gardens. In some cases habitat information was sent with the seed samples, in others only the locality was given. Lack of information did not always exclude the use of such samples if they filled a gap in the geographical picture but results obtained from these populations need to be treated with greater caution.

Details of the populations used are given in Table B1 i. In some cases the date of collection does not give the age of the seed used as several generations have been raised in the greenhouse to ensure supplies of fresh seed in all cases where the original seed collection was made more than five years ago.

When material was grown in the greenhouse for use in germination experiments the seed from all available plants was collected so that no deliberate selection was made. When individual plants or groups of plants were selected this was always done deliberately.

Fresh seed samples were also obtained from plants of sixteen populations grown in the University Botanical Gardens at Leicester. Plants from each population were grown on both sides of a 3' fence erected in a line due east-west. The north side of the fence was cooler and damper than the south side which was exposed to direct sunlight so fresh seeds which had been matured in two different microclimates were available.

B.2. Factors affecting dormancy

Many factors both internal and external are involved in the induction and breaking of dormancy. As Mayber and Poljakoff - Mayber (1963) point out, when the time of shedding of seed does not coincide with the most favourable time for survival of the seedlings dormancy often occurs. The dormant state results in a reduced dependence on the environment and therefore will be of survival value when it spans unfavourable periods in the natural environment. It is likely that the onset and relaxation of dormancy will be under environmental control either directly or indirectly.

In many winter annual races of Arabidopsis thaliana there is an enforced dormancy period in the seed stage (Laibach, 1943; 1951; Ratcliffe, 1957). Even during a wet summer when sufficient soil moisture is available for germination the seeds do not germinate until the autumn. This 'after-ripening' requirement or post harvest dormancy is obviously important in determining the time of germination in the field. By delaying germination until the autumn, the after-ripening period ensures that the seeds do not germinate until there is likely to be sufficient soil moisture to allow establishment. Differences within and between populations should reflect variation in environmental conditions both from place to place and year to year.

The number of seeds germinating at any one time after dispersal will depend on several factors including age of seed, environmental factors such as temperature and light, the history of the seed and parent plant and on possible interactions between these.

Dry Storage and Dormancy

Many plant species have seeds which are dormant under normal favourable conditions when freshly harvested but lose this dormancy after periods of dry storage. This has been observed in many cereals e.g., barley, oats, rye and winter wheats (Larson, Harvey and Larson, 1936) and in wild grass such as Poa pratensis (Sprague, 1940) and Avena fatua (Toole and Coffman, 1940). Ratcliffe (1957) found post harvest dormancy in several winter annuals including A. thaliana, Erophila verna, Saxifraga tridactylites and Hornungia petraea.

Dormancy studies were initiated with several populations of A. thaliana. Usually the plants were collected (from the field, greenhouse, botanical gardens, etc.) when the siliquae had started to dry and turn yellow. The plants were stored in open polythene bags in an incubator at 25°C in the dark until completely dry. Then they were broken up and the seeds separated by passing through a 1mm mesh ^{ny} sieve. The seeds were stored in glass tubes with cork stoppers and kept in the dark until required at room temperature (20 - 25°C). Age of seed was calculated from date of harvest.

It was important to know if the conditions used for seed storage were suitable. Rehwaldt (1966) had found darkness and 55% relative humidity gave more rapid after-ripening in A. thaliana than light or storage over anhydrous calcium sulphate but he did not mention any temperature effects and presumably his experiments were carried out at room temperature.

In lettuce Borthwick and Robbins (1928) found low temperatures of storage to be no more effective in breaking post harvest dormancy than room temperatures. However, in barley and oats Larson, Harvey and Larson (1936) found low temperatures increased length of dormancy. On the other hand, high temperatures have been reported by several authors to shorten the after-ripening period : Toole (1940) found 30°C more effective than 20°C or 2°C in Oryzopsis hymenoides, Roberts (1965) found temperatures up to 57°C decreased the after-ripening period in rice and Atterberg (1907) showed that for barley 37°C was more effective.

Some incompletely after-ripened seeds of A. thaliana from twelve Derbyshire populations stored under the conditions described above, were given a period of two weeks at 37°C ± 2°C in an incubator in constant darkness. Germination of 100 seeds of each population previously stored under either continual room temperature or with a period at 37°C was compared at 20°C in an incubator in constant light. The seeds were set out on moist paper pulp in disposable petri dishes.

If, at the time of the 'test' after-ripening was almost complete (as measured by final percentage germination at 20°C) there was little difference between the two treatments. However, if on transfer to 20°C 90% germination was not achieved a greater proportion of seeds failed to germinate after the 37°C treatment. (Table B2 i).

TABLE B2 i

<u>Population</u>	<u>Final percentage germination</u>		
	<u>Age of seed in days</u>	<u>Without 37°C Pre-treatment</u>	<u>With 37°C Pre-treatment</u>
Carsington Pasture	73	100	100
Longcliffe B	73	100	100
Moorside	51	100	94
Dove Holes	51	100	90
Cobbler's Nook A	73	100	74
Longcliffe A	73	96	97
Cobbler's Nook B	51	95	82
Parsley Hay	51	91	74
Lathkill Dale	69	90	89
Friden Crossing	51	87	53
Deepdale	51	85	33
Jug and Glass	57	44	27

Justice (1941) reported that when Polygonum seeds were dried quickly at higher temperatures more were dormant than when they were dried slowly. Vegis (1964) suggested that high temperatures could induce dormancy in incompletely after-ripened seeds.

It appears that storage in the dark at room temperature and humidity does not substantially affect post harvest dormancy and if anything the results of dormancy experiments would err in the direction of shortening dormancy rather than the reverse. Most seed samples stored under these conditions for over $2\frac{1}{2}$ years show little loss of viability.

Inter-population variation

The initial seed collections used for investigation of length of after-ripening were obtained, by necessity, from plants grown in the greenhouse as the experiments were started in the autumn. Seeds were obtained from 1, 2 and 4 plants respectively of populations 'Lanark', 'Bush' and 'Besançon' and from 10 - 15 plants from populations 'Aberdeen', 'Dirleton' and 'Whatton' and stored under the 'standard' conditions described above. Subsamples of 50, 100 or 200 seeds, depending on the total number available in the bulk sample, were removed and set out at 20°C in constant light. The seeds were observed under a low power microscope to ensure only normal seeds were counted. Every 1 - 5 days the number of seeds germinated were counted and graphs of percentage germination against time from imbibition were drawn for each population, for example, see figures B2 i - ii~~x~~.

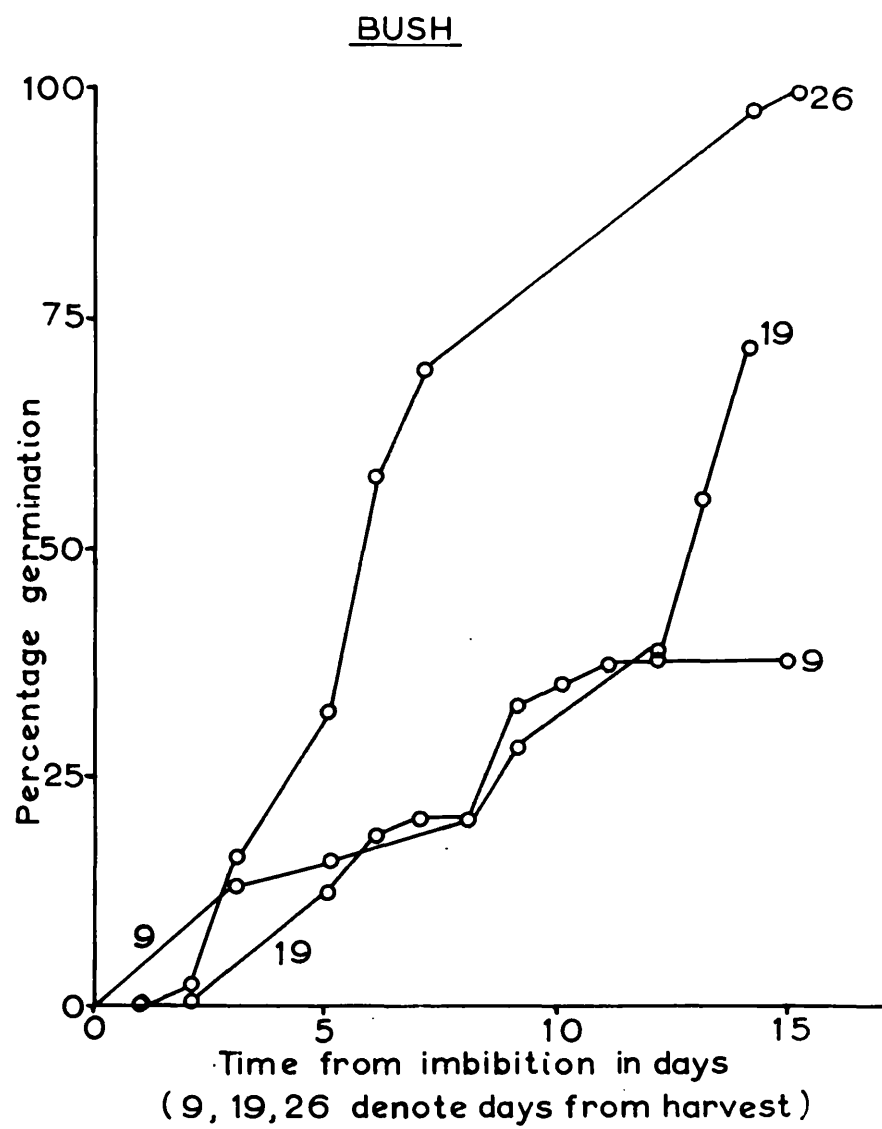


Figure B 2i. Post harvest dormancy in Bush.

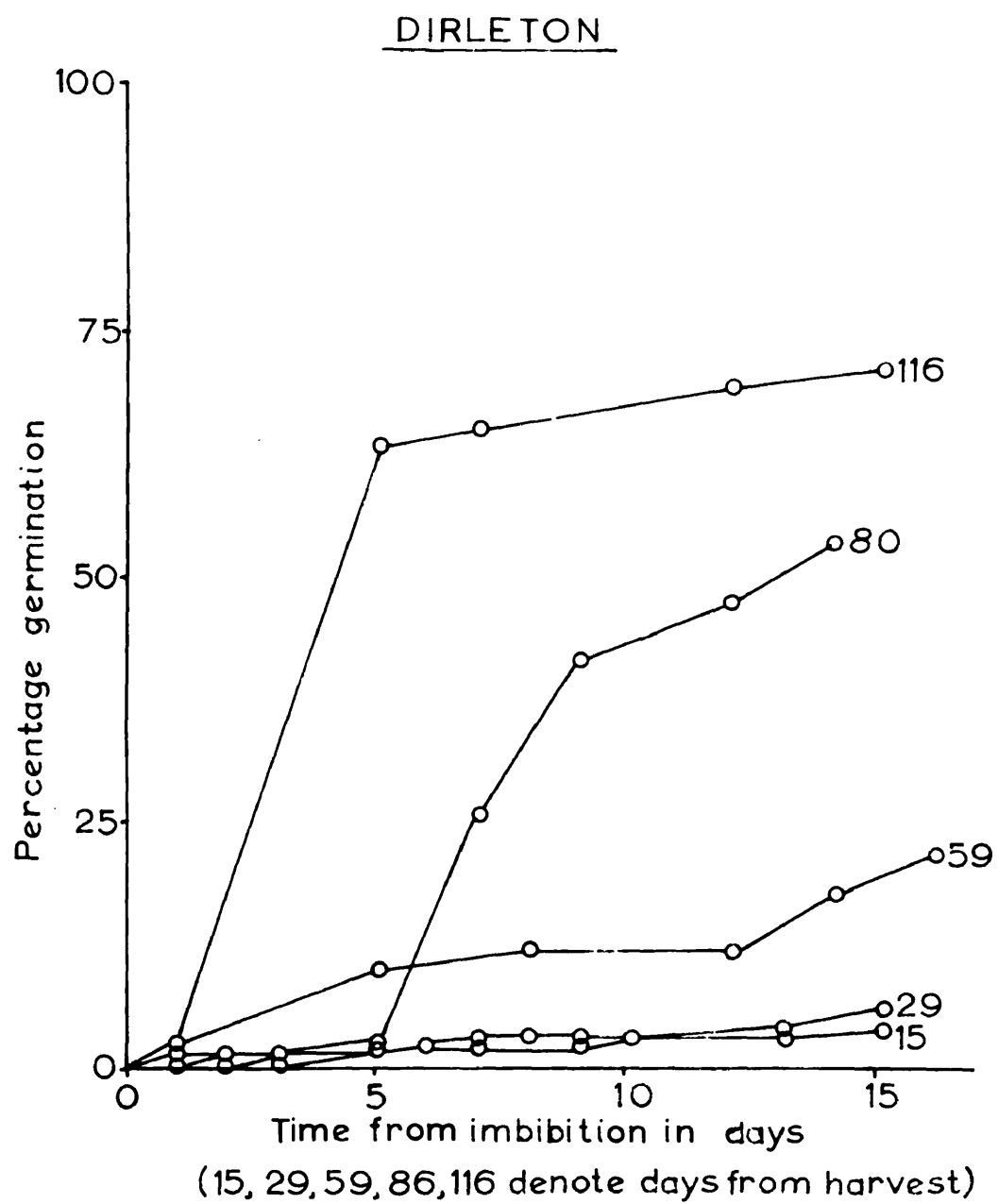


Figure B 2ii. Post harvest dormancy in Dirleton.

These initial results were confusing, in all populations there was a gradual increase in final germination percentage and rate of germination i.e., the slope of the germination curve, with length of dry storage. However, the time taken to reach maximum germination varied considerably. 'Lanark', 'Bush' and 'Besançon' had after-ripened completely within a month while 'Whatton' and 'Aberdeen' did not reach maximum germination until after 30 weeks dry storage and showed no germination at all until stored for 3 months. 'Dirleton' showed a gradual loss of dormancy from 15 days after harvest but again did not reach full germinability until kept dry for 30 weeks.

This large variation in after-ripening requirements made further investigation of dormancy imperative. In the early summer collections of large numbers of fruiting plants from twelve Derbyshire localities and from 'Husband's Bosworth', Leicestershire were made. At least twenty plants were collected for each bulk sample. At 'Husband's Bosworth' 22 individual plant collections were also made at random. These seeds were stored and tested as before, ^{for example see figure B2 iii.} Again there was a gradual loss of dormancy and increase in rate of germination with length of storage until a certain time after which the rate and final percentage germination remained constant. In some cases 100% germination was obtained after a short period of storage, but increases in rate continued until 100% germination could be obtained in 2 - 3 days at 20°C

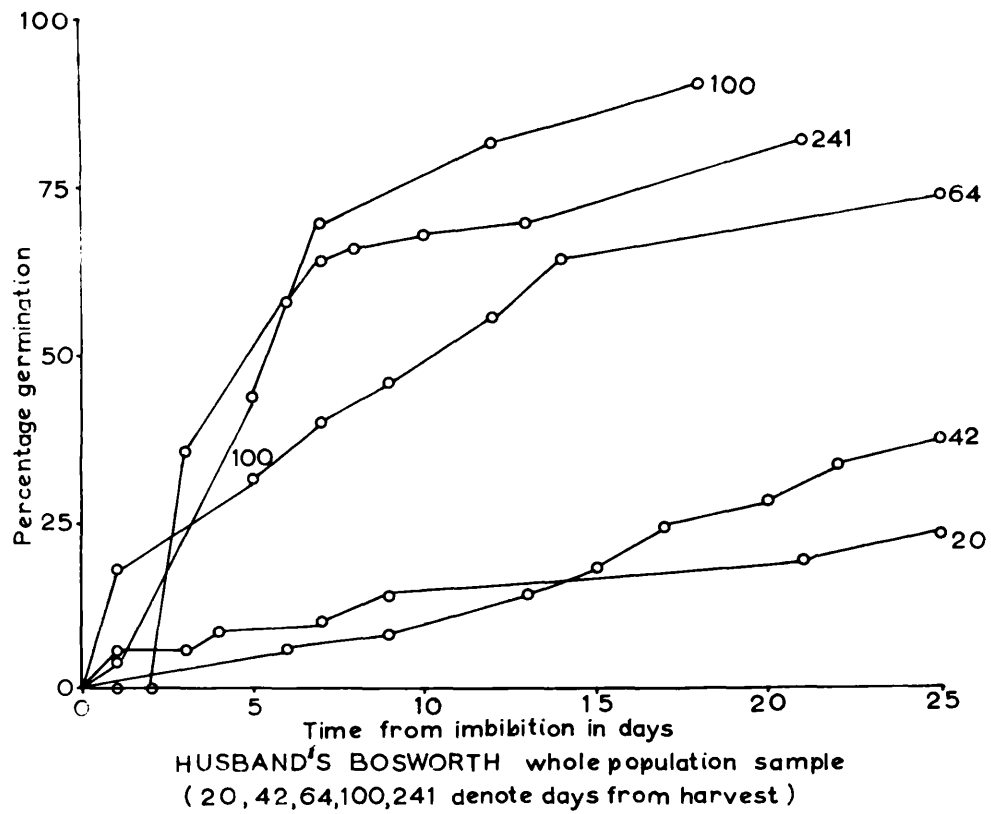


Figure B 2iii. Post harvest dormancy in Husband's Bosworth.

in constant light. Such seeds were considered to be fully after-ripened. All Derbyshire populations except 'Jug and Glass' achieved this condition. The pattern displayed by the Derbyshire populations were markedly similar to each other and different from 'Husband's Bosworth' or any of the earlier populations used, (figure B2 iv).

However, inter-population differences were evident. Populations from 'Carsington Pasture', 'Longcliffe', 'Cobbler's Nook', 'Moorside' and 'Dove Holes' were fully after-ripened, after 10 weeks, while those from 'Parsley Hay', 'Lathkill Dale', 'Jug and Glass', 'Deepdale' and 'Friden' took longer to lose dormancy completely. The populations which after-ripened quickly are mostly those from the South East of Derbyshire where the climate is generally warmer and drier than in the north and west (figure B2 v). It is probable that these populations flower earlier and ripen earlier.

The 'Husband's Bosworth' population has a similar pattern to the Derbyshire ones and indeed, its habitat, a disused railway track, is very like many of the Derbyshire localities. Its post harvest dormancy is somewhat longer; 4 months instead of 3 but this may be a response to the longer drier summers at this site as compared to the Derbyshire populations 50 miles further north and almost 600' higher.

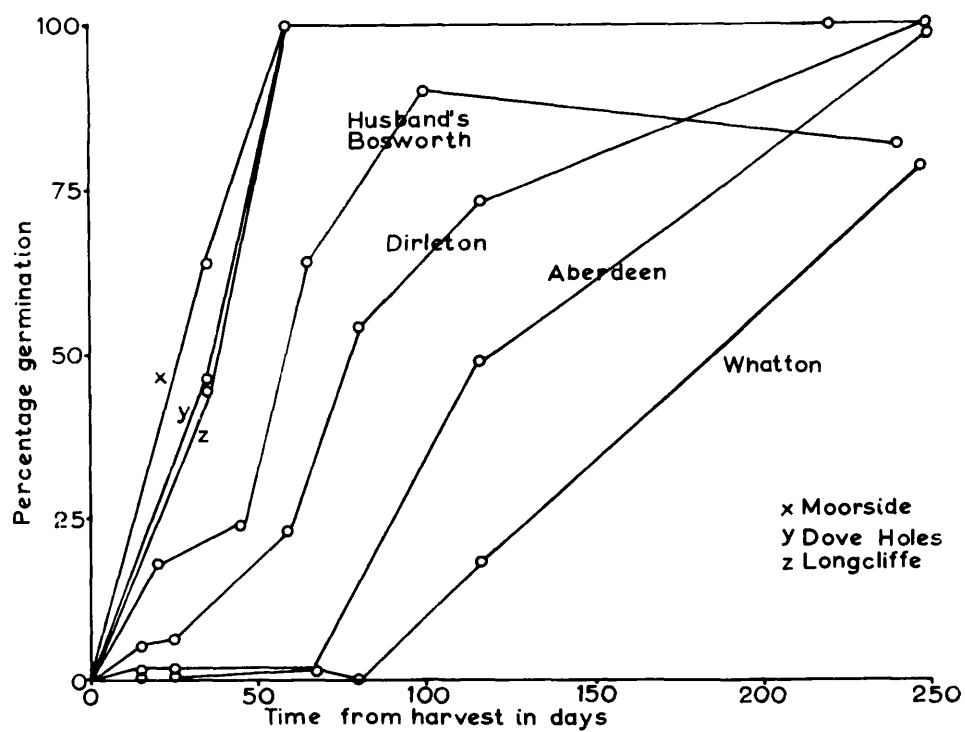


Figure B 2iv. Population variability in post harvest dormancy.

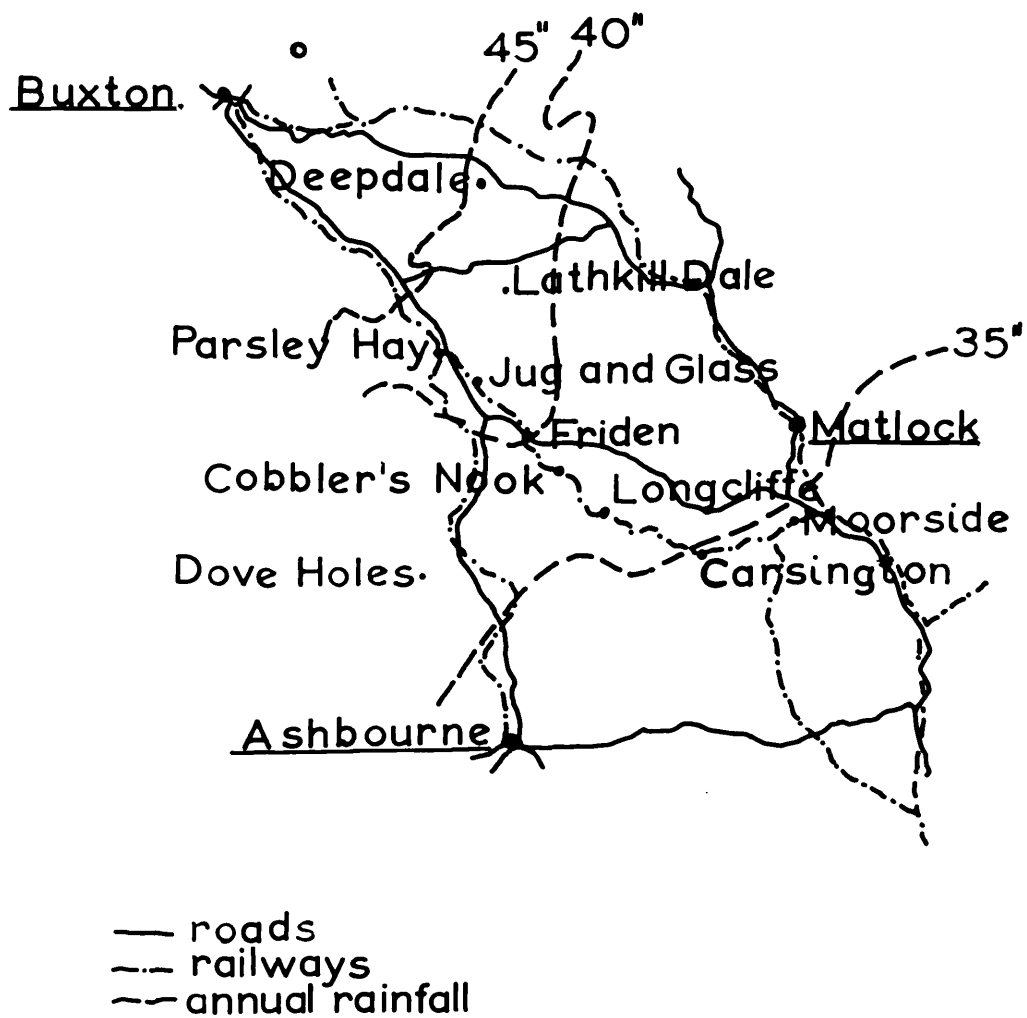


Figure B 2v. Location of Derbyshire populations.

The very long post harvest dormancy of the 'Aberdeen', 'Whatton' and 'Dirleton' populations may be related not so much to climatic factors as to their habitat : all were garden weeds and long post harvest dormancy could be advantageous in delaying germination until disturbance lessened in the late autumn i.e., selection by human interference.

The shorter post harvest dormancy in the 'Lanark', 'Bush' and 'Besançon' populations is more difficult to explain. 'Lanark' is a railway population similar to the Derbyshire ones, 'Bush' is a garden weed and though little is known of the actual habitat of the 'Besançon' population its more southerly and continental origin would suggest that a long dormancy period would be advantageous. All these populations, though showing the same pattern of after-ripening, were only dormant for six weeks.

Intra-population variation

It is possible that this pattern was not the result of any adaptation to habitat conditions but was due to the small numbers of plants used in the experiments. In the light of this it was considered necessary to study more closely within-population variability of after-ripening requirements. This was done using the 22 individual plant samples from 'Husband's Bosworth'. Subsamples of 25 or 50 seeds, depending on the total number available were removed from dry storage at intervals and set out at 20°C in constant light. There was considerable variation

between the individual plant samples in the numbers of seeds which germinated early. It was possible to place each sample into one of three categories with a short, intermediate or long post harvest dormancy, (Table B2 ii).

TABLE B2 ii

Mean percentage final germination of seeds of three categories of plants from 'Husband's Bosworth'. Figures in brackets are standard errors.

<u>Time from harvest in weeks</u>	<u>Length of Post Harvest Dormancy</u>		
	<u>Short</u>	<u>Intermediate</u>	<u>Long</u>
4	18.5 $\pm$ (6.5)	2.67 $\pm$ (0.74)	1.33 $\pm$ (0.66)
7	22.5 $\pm$ (9.1)	2.73 $\pm$ (0.58)	1.56 $\pm$ (0.65)
48	46.0 $\pm$ (14.2)	46.7 $\pm$ (6.8)	14.7 $\pm$ (5.6)
83	84.0 $\pm$ (7.0)	86.7 $\pm$ (5.1)	54.7 $\pm$ (6.5)

The rate of loss of dormancy was very similar in both the groups with short and long after-ripening requirements but the former group was characterised by a much higher percentage of non-dormant fresh seeds. The intermediate samples again had very few fresh seeds which germinated but initially they after-ripened rapidly and maintained this rate so that in the final test the percentage germination was very close to that of the 'early germinating' group, (figure B2 vi).

Examples of these three patterns of dormancy are shown in figure B2 vii. F and I are short dormancy, A and J long dormancy and N, E, C and R intermediate plant samples.

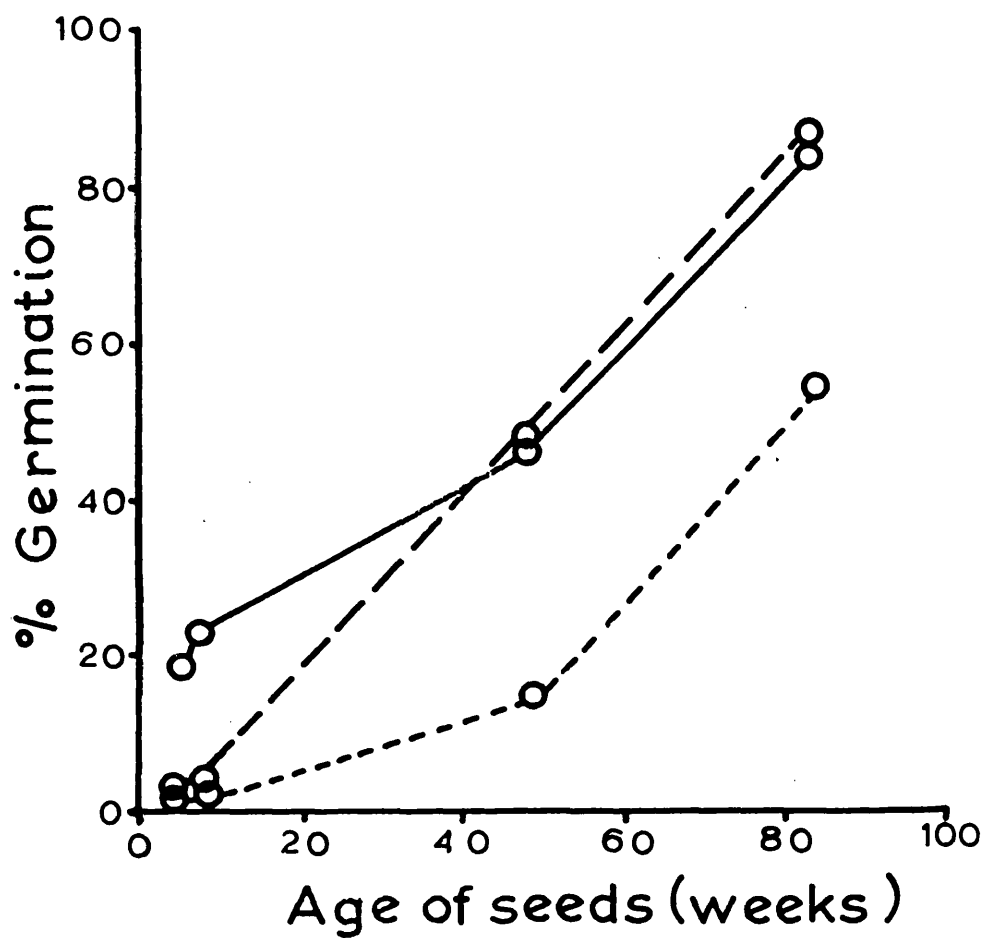


Figure B 2vi. Post harvest dormancy in Husband's Bosworth, mean values for:-

- 'early' germinating group,
- - - 'intermediate' germinating group,
- 'late' germinating group.

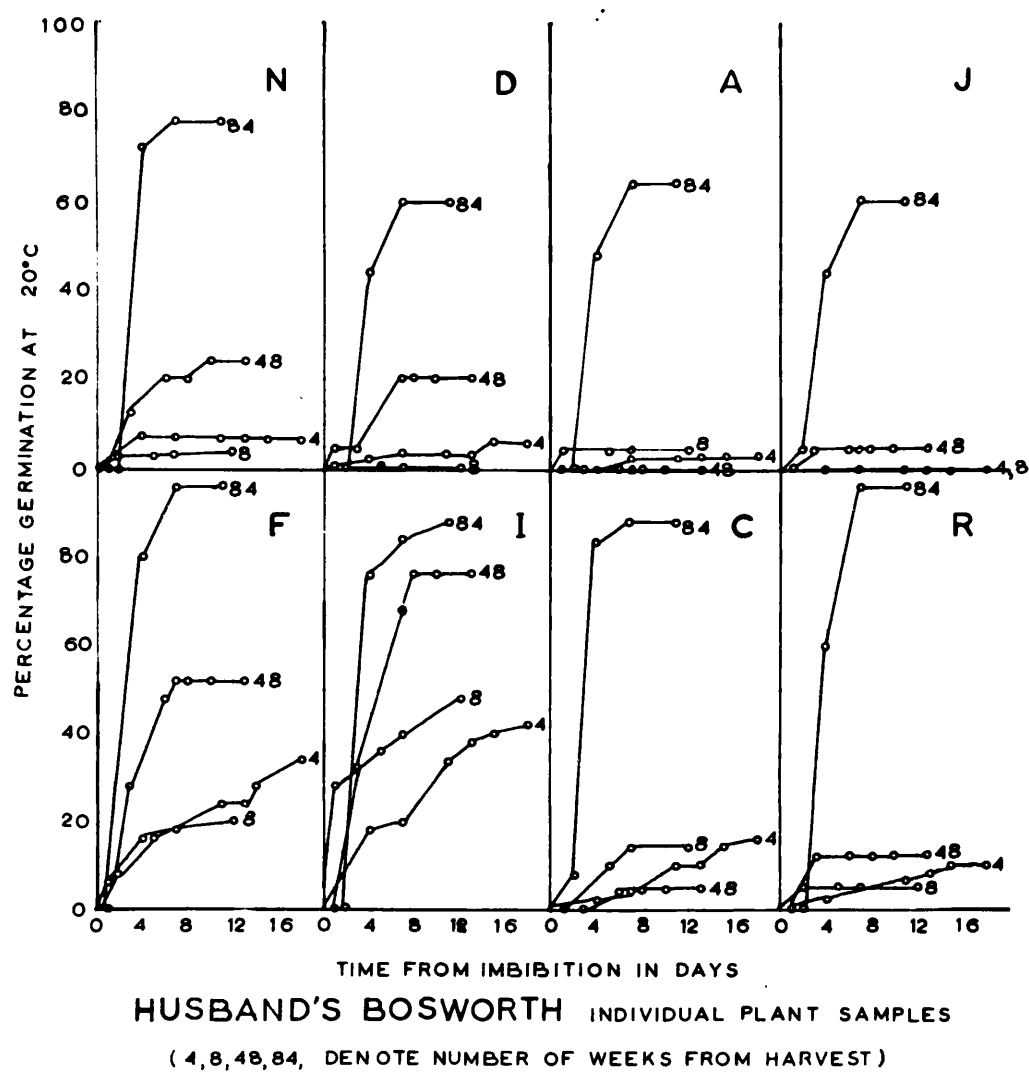


Figure B 2vii. Patterns of post harvest dormancy in individual plant seed samples from Husband's Bosworth.

This may represent genotypic variation. From histograms of the numbers of individual plant samples falling into classes of percentage germination at each seed age, there were indications of segregation, (figure B2 viii).

The shape of the individual germination curves suggests that there are differences even amongst the seeds obtained from a single plant. However, the numbers of seeds in the individual plant samples made it necessary to restrict the times the tests could be repeated so it would be unwise to compare these results directly with the 'Lanark', 'Bush' and 'Besançon' data. More information can be gained by comparing these with the results obtained from the bulk seed sample from 'Husband's Bosworth'.

Roberts (1961) working on rice seed found a normal distribution of 'dormancy' periods i.e., the time to germination among individual seeds of a 'pure line'. In the samples where exceptions to this pattern were noted the presence of more than one genotype was suspected. As different varieties were likely to differ in the length of their mean germination time a deviation from the normal distribution would be expected unless a large number of genotypes were mixed at random. With this in mind, the germination curves for 'Lanark', 'Bush' and 'Besançon' and 'Husband's Bosworth' were compared with calculated germination curves of normal distribution. Using tables of the normal probability integral oriented in terms of X/σ_x where X is the deviation from the mean and σ_x the standard

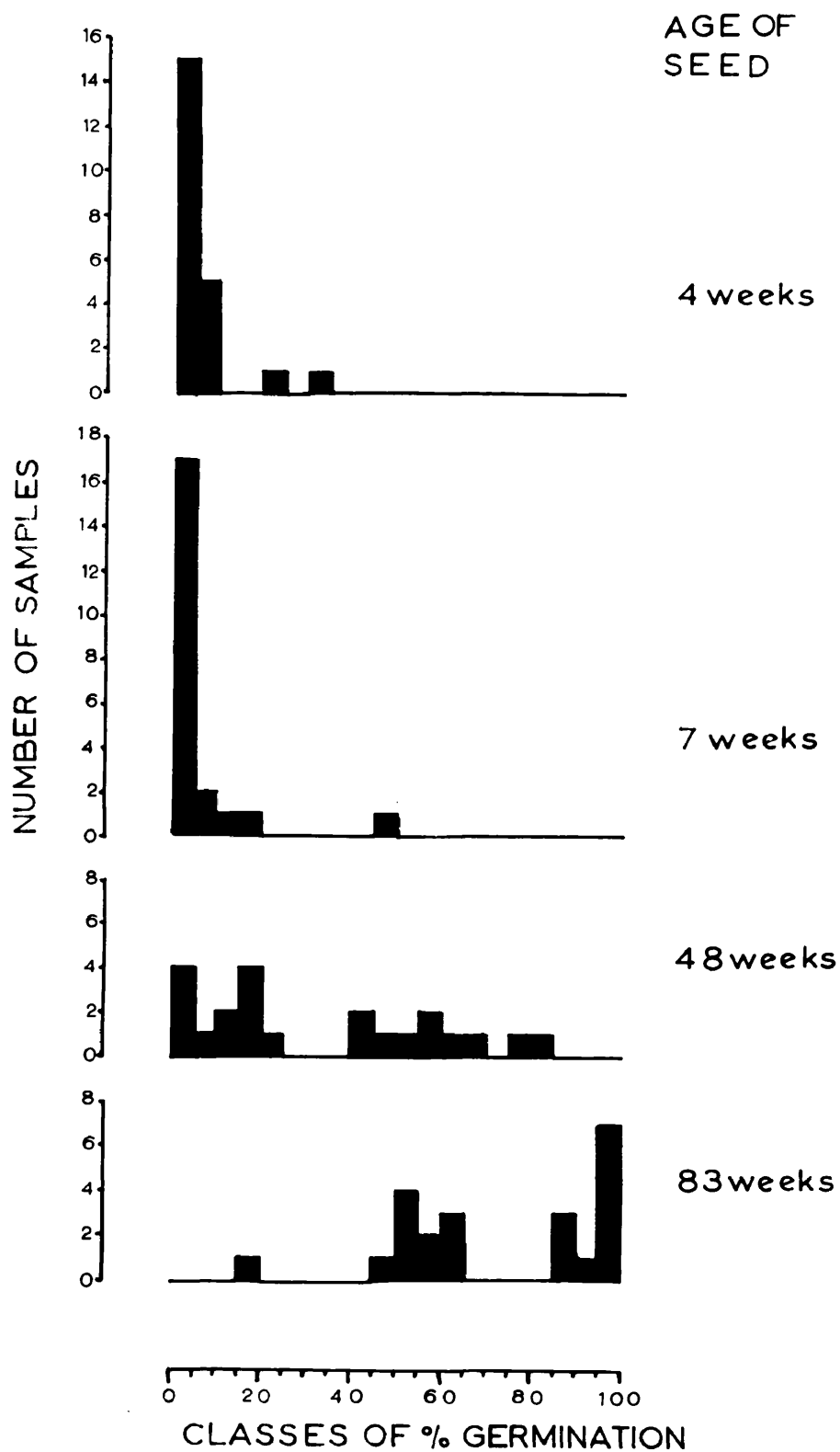


Figure B 2viii. Numbers of samples in various classes of percentage germination at different seed ages in Husband's Bosworth.

deviation ($1/6$ of the range), the expected daily increments for the percentage germination against time curve can be calculated and compared with the observed figures and subjected to a Chi-squared goodness of fit test. The results of this analysis are given in Table B2 iii.

<u>TABLE B2 iii</u>				
<u>CHI² Df</u>				
<u>Population</u>	<u>Age of seed in days</u>	<u>Chi²</u>	<u>df</u>	<u>P</u>
Bush	18	73.78	8	0.005 **
	25	11.67	6	0.1 - 0.05 N.S.
Besançon	21	157.21	10	0.005 **
	34	4.53	3	0.5 - 0.1 N.S.
Lanark	17	19.86	6	0.005 **
	21	20.17	9	0.025 - 0.01 *
	27	8.92	7	0.5 - 0.1 N.S.
	34	4.76	3	0.5 - 0.1 N.S.
Husband's Bosworth	64	198.04	5	0.005 **
	100	45.15	3	0.005 **
	241	18.63	4	0.005 **

In all the populations as after-ripening proceeded the distribution of germination periods became more normal. Each seed will differ to a certain extent in both its after-ripening requirement and the time it takes to germinate once after-ripened. Roberts (1961) suggests that a steep curve for percentage germination against time may

represent a small standard deviation of germination time within the population.

This is probably true for fully after-ripened seeds but in Arabidopsis thaliana the curve for these populations becomes steeper as the seeds after-ripen. When the seeds are fully ripened they can germinate quickly giving a steep curve within 4 - 5 days at their optimum temperature. Incompletely after-ripened seeds take longer or fail to germinate and so give flatter germination progress curves and/or the final percentage is lower. The probable reason for this lowering of final germination percentage and the fact that normal distribution of 'dormancy' periods is not found in 'younger' seeds is that some or all of the seeds acquire a secondary dormancy under these conditions. Once imbibed, seeds do not continue to after-ripen. This idea is born out by the fact that the failure to fit the normal distribution curves when the mid point of this is 50% is due to an abnormally high proportion of 'early germinators'. It would be interesting to subject the portion of the curve due to 'early germinators' to tests of goodness of fit to normal distribution curves with appropriate midpoints. It is possible that germination of 'early germinators' is itself normally distributed. Small seed numbers in these experiments did not allow this to be done with accuracy.

It is unlikely that the three seed samples from 'Lanark', 'Besançon' and 'Bush' contain many genotypes owing to the very small numbers of plants used. The most uniform results came from the seeds from the single plant derived from 'Lanark' seeds. It is possible that the plants whose seeds were used had produced 'early germinators' and that this led to the short dormancy observed. The possibility that an element with a long after-ripening requirement was lacking in the original collections can be discounted as later experiments show.

The 'Husband's Bosworth' population has very variable post harvest dormancy and the deviation from normal distributions (found even in fully after-ripened seeds) may be due to the occurrence in this seed sample of a few distinct genotypes indicating some degree of heterozygosity and reinforcing the evidence for genetic variability shown in the individual plant samples.

The value of such heterogeneity is obvious as it will ensure that the total number of seeds produced by a plant are not used up in a single 'attempt' at establishment. Some of the heterogeneity in dormancy patterns observed in any population will reflect genetic variation but, as Koller (1969) suggests, some quantitative variation in dormancy may be "a deliberate countermeasure" against the uncertainty factors in survival, i.e., disruptive environmental selection has operated. These populations from areas with marked annual fluctuations in local climate may show greater variation of this sort, while those from areas where the seasonal pattern does not change much from year to year e.g. The Scottish Highlands or the Mediterranean,

may be more uniform.

Temperature, light and dormancy

Germination in A. thaliana populations in the field does not appear to be a continuous process despite this heterogeneity, even during a wet summer. There is a characteristic burst of germination in the autumn especially in the Derbyshire populations when a period of warm dry weather in August or September breaks and the ground becomes continuously moist. Other factors apart from seed age are important in regulating the time of this burst of germination. When several populations were cultivated under uniform conditions in the University Botanical Gardens, it was possible to obtain large quantities of fresh seed from 5 - 25 plants from each population and so expand the dormancy experiments to investigate the effect of temperature and light on the germination of seeds through the after-ripening period.

Cold Treatment

One of the commonest means for breaking dormancy involves low temperature treatment of imbibed seeds, usually between 1 - 10°C. Seeds were harvested on 7 July 1971 from sixteen populations grown at the Botanical Gardens, Leicester (p.21). These were 'Barry Sands', 'St. Cyrus I', 'Catterline', 'Burren', 'Dirleton', 'Bush', 'Mickle Fell', 'Ben Dhorain', 'Besançon', 'Dijon', 'Woebley Castle',

'Whatton', 'Husband's Bosworth', 'Stockholm', 'St. Dogmael's Abbey' and 'Oschenkopf'.

They were tested and stored in the standard way. Sub-samples of 50 seeds from both N and S side seed samples were taken at intervals of 1, 3, 6 and 10 months from harvest and set up in incubators at 5, 10, 15, 20, 25 and 30°C. Duplicate sets were placed in continuous light and in black polythene bags from which the samples were only briefly removed for counting in dim light at appropriate intervals. The final count was made 28 days after imbibition; plates left longer showed no further germination. The results below refer to the south side samples except where otherwise stated.

A common pattern of germination was found in almost all the populations. After one month of dry storage percentage germination differed considerably with temperature with an optimum of 5°C. After three months storage germination at 5 and 10°C had usually declined slightly but was higher at higher temperatures. After six months the percentage germination was higher at all temperatures, after ten months some populations showed a further increase at high temperatures, others showed a decrease at high temperatures or at all temperatures, (figures B2 ix - xv).

When individual populations are studied in more detail they fall into several distinct patterns which appear to be closely correlated with environmental factors. 'Stockholm', 'Mickle Fell', 'Barry Sands', 'St. Cyrus I', 'Catterline' and 'Ben Dhorain' displayed the first pattern, (figures B2 ix - x). They are characterised by a relatively low proportion of dormant seeds at all temperatures below 30°C though 5°C is optimal. On the whole the germination at all temperatures has increased after three month's storage. After six months the seeds were fully after-ripened and germination usually 95 - 100% in light at all temperatures. This germinability was maintained after 10 months storage or longer (up to three years). Inter-population differences also were revealed. 'Stockholm' showed the lowest dormancy of all populations investigated. 'Barry Sands' and 'St. Cyrus I' had more dormant seeds at higher temperatures after one month storage but after-ripening was complete after three month's storage. 'Mickle Fell', 'Catterline' and 'Ben Dhorain' have not reached full germinability at any temperature after one month of storage, but at least 10% germination occurred at all temperatures except 30°C in light, increasing at all temperatures to over 35% after three months and 80% after 6 months.

The short dormancy at relatively high temperatures especially 15 and 20°C in these populations could be disadvantageous in any environment where the summer rains are unpredictable, but all these populations are from

Figures B 2ix-xv. Effect of seed age, temperature and light on post harvest dormancy in seven populations.

Open circles represent final % germination in continuous light; closed circles final % germination in continuous darkness.

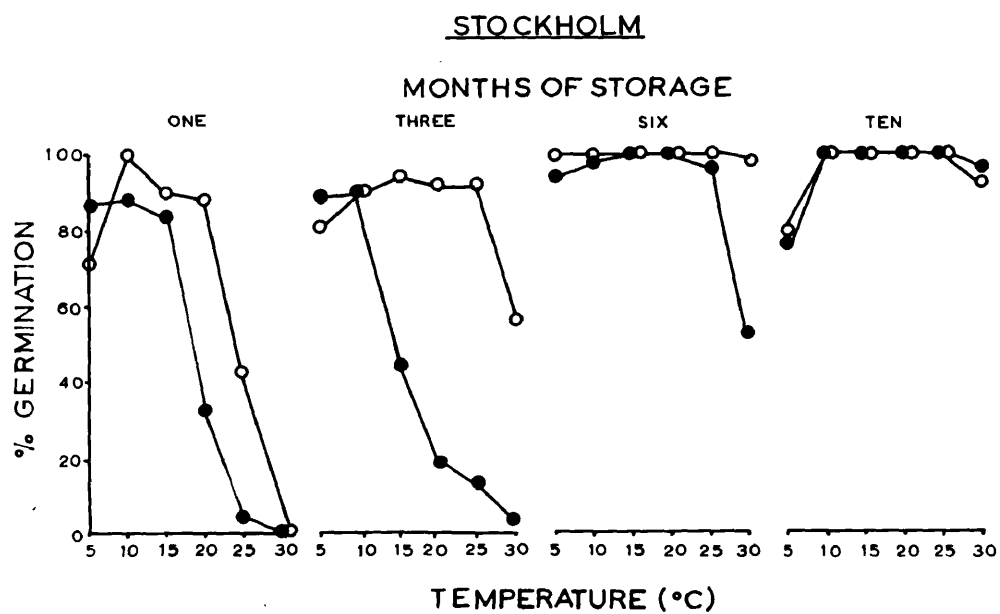


Figure B 2 ix.

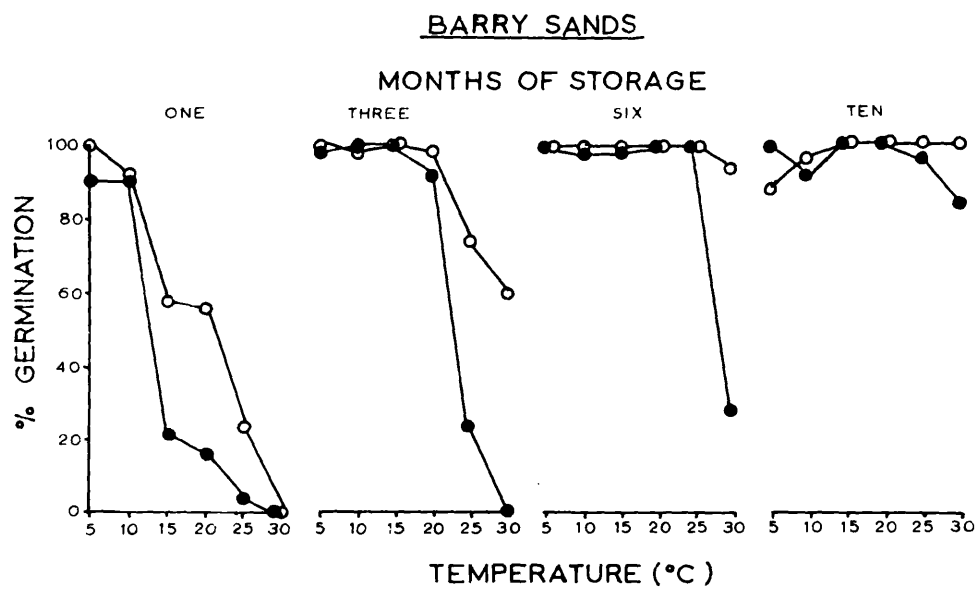


Figure B 2x.

northern latitudes and in habitats which may remain fairly wet and damp throughout the summer. These populations probably flower late in the summer - August or September; the winters are cold and long and the growing season is reduced. Early germination would allow the maximum vegetative growth before the very low winter temperatures.

The second pattern was shown by 'Woebley Castle' and 'St. Dogmael's Abbey'. These populations showed over 80% germination at 5°C after one month but there was a sharp decline at higher temperatures reaching zero at 15 or 20°C. After three months of storage there was an increase at 15 - 25°C to over 15% but with no germination at 30°C still. After six months storage germination was over 90% from 10 - 25°C (figure B2 xi).

These populations from the warm coasts of South and West Wales respectively would be unlikely to survive if they germinated in early summer. In fact they appear to be dormant at the likely ground temperatures after one month of storage. However, these areas are in the path of warm westerly winds which bring high rainfall, over 3" per month, from July onwards. By late August, the ground is likely to be damp enough to allow seedling establishment. Approximately 30% of seeds could germinate at the ambient ground temperatures, 15 - 20°C, three months after harvest.

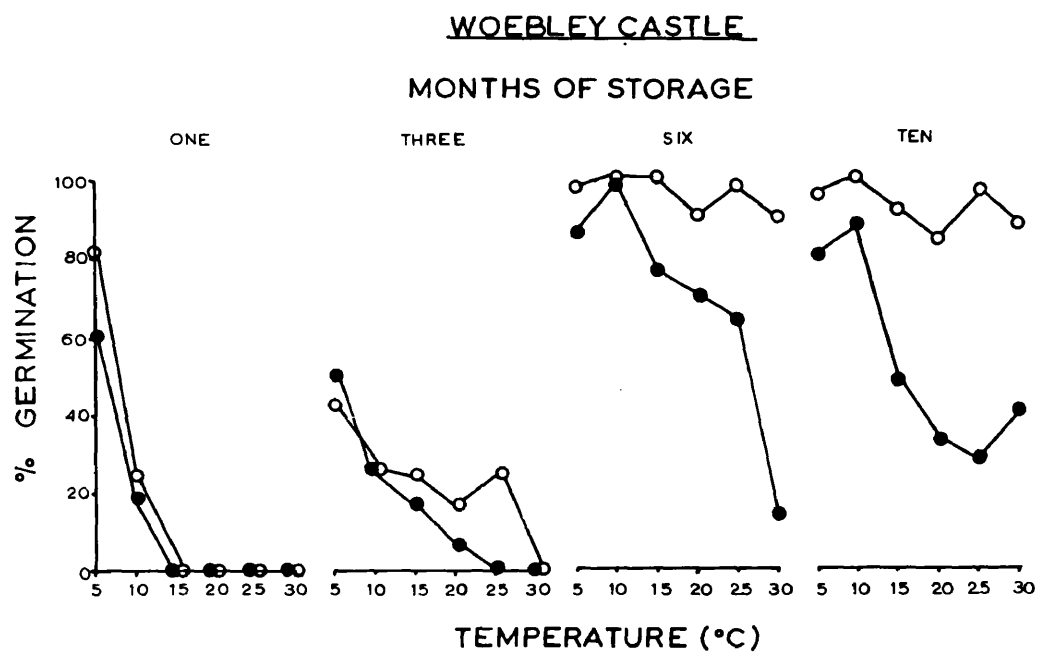


Figure B 2xi.

However, if there is a sudden drought in late summer 70% of the seed population would still remain dormant. After six months when the soil will be permanently damp germination would be high at all temperatures.

The two populations showing the third pattern, 'Husband's Bosworth' and 'Whatton House', are very similar in many respects to 'St. Dogmael's Abbey' and 'Woebley Castle'. After one month of storage there was again very high % germination at 5^oC with a sharp decrease at 10^oC and no germination at higher temperatures. A similar pattern was found after three months storage but in 'Whatton' the germination at 5^oC had decreased to 40^oC. After six months 98 - 100% germination was achieved at 5 - 15^oC falling off gradually to near zero at 30^oC (figure B2 xii). This pattern was maintained after 10 months and in fully after-ripened seeds. In these habitats the summer rains are very unpredictable and drought is common. Seeds germinating after summer showers are unlikely to survive to maturity and so it could be advantageous for these populations to have the prolonged dormancy they show at high temperatures.

'Bush' and 'Dirleton' show a fourth pattern. 'Dirleton' showed an optimum at 5^oC after one month of storage gradually decreasing to zero at 30^oC but the maximum germination was still less than 50%. After three months storage 10^oC was now optimal but the maximum was still only 45%. 'Bush' showed even lower maxima. 5^oC is

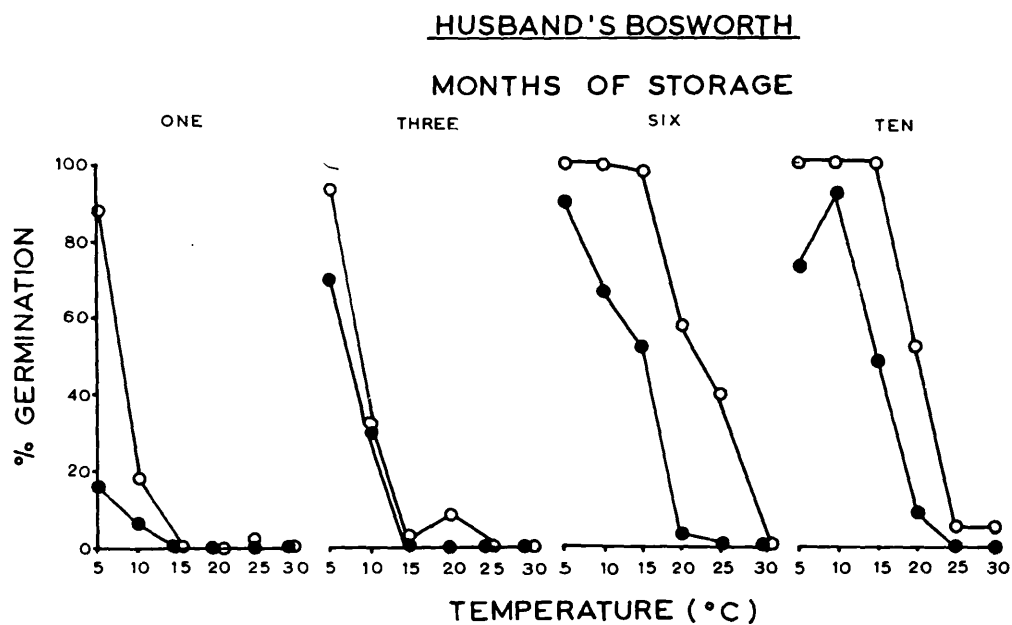


Figure B 2xii.

optimal : 14% and 24% were reached after 1 and 3 months respectively. There is a gradual decrease in germination to zero at 15°C after one month and 20°C after three months. After six months of storage over 90% germination is reached at 5 - 15°C in both populations and at 20°C in 'Dirleton' then there is a gradual decrease to 46% at 30°C. In 'Bush' the decline is more rapid : 60% at 20°C, 14% at 25°C and 4% at 30°C. After ten months percentage germination was lower at all temperatures in 'Dirleton' and at 15 - 30°C in 'Bush', (figure B2 xiii).

Both these are 'weedy' populations. Although summer rainfall is high in the Edinburgh region this rain is mostly in the form of convective showers separated by periods of dry weather so seedlings are unlikely to survive until the autumn. The prolonged dormancy at high temperatures shown by these populations may well be advantageous. The low but important fraction of seeds available capable of germinating at 15°C or more after three months of storage would provide a measure of opportunism, useful in such a ruderal situation.

The fifth pattern was shown by the European populations 'Dijon', 'Besançon' and 'Oschenkopf'. These populations had an optimum temperature for germination at 5°C with 75% or more after one month of storage but no germination at 15 - 20°C. After three months storage percentage germination was much lower at 5 and 10°C but approximately

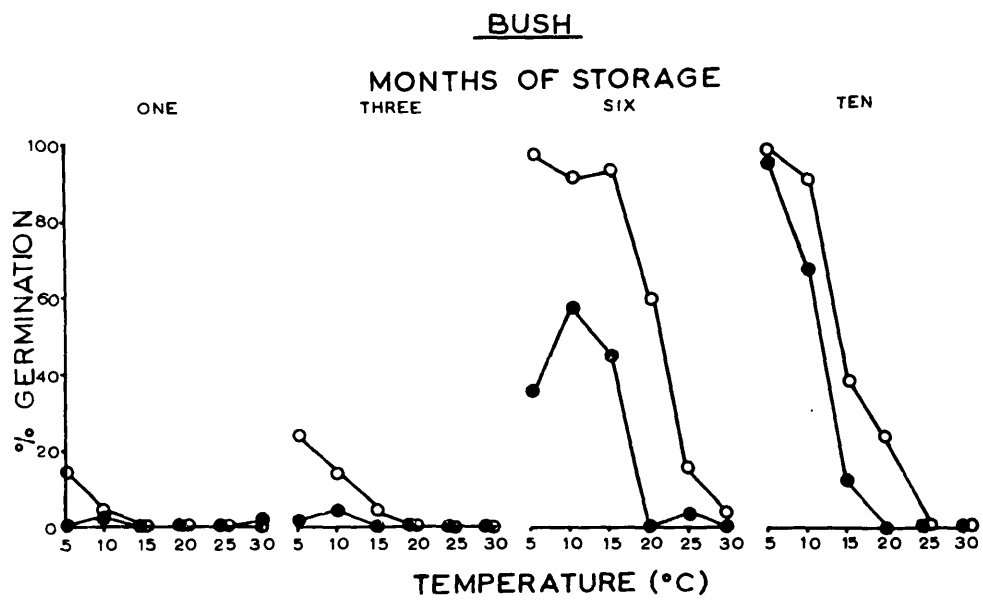


Figure B 2xiii.

15% at 15 and 20°C. in 'Besançon' and 'Oschenkopf'.

'Dijon' still showed no germination above 10°C.

After six months germination had increased to over 90% at 5 - 15°C in all three populations and at all temperatures in 'O^schenkopf'. IN 'Dijon' there was a rapid decline above 15°C to 2% at 30°C. In 'Besançon' there is only 25% germination at 20% but this rises to 52% at 30°C, (figure B2 xiv).

The low germination at high temperatures in these populations may again be a mechanism to avoid germination during the more continental summer when the rainfall, though high, is primarily in convective showers.

The reason for the sharp decline at 5 and 10°C after three months is not obvious. However, this was noted to a lesser extent in some of the other populations e.g., 'Woebley Castle' and 'Whatton'. The pattern of 'Dijon' and 'Besançon' after six months resembles that of 'Bush' and 'Dirleton' while the 'Ochsenkopf' pattern is very similar to that of the montane populations, 'Mickle Fell' and 'Ben Dhorain'.

Here it is worth noting that both the 'Besançon' and 'Lanark' populations show marked dormancy at 20°C after one month of dry storage, showing that the earlier results from these populations and already discussed (p.29) were not representative of the populations as a whole. It would appear from these experiments that an additional control of germination is achieved by the

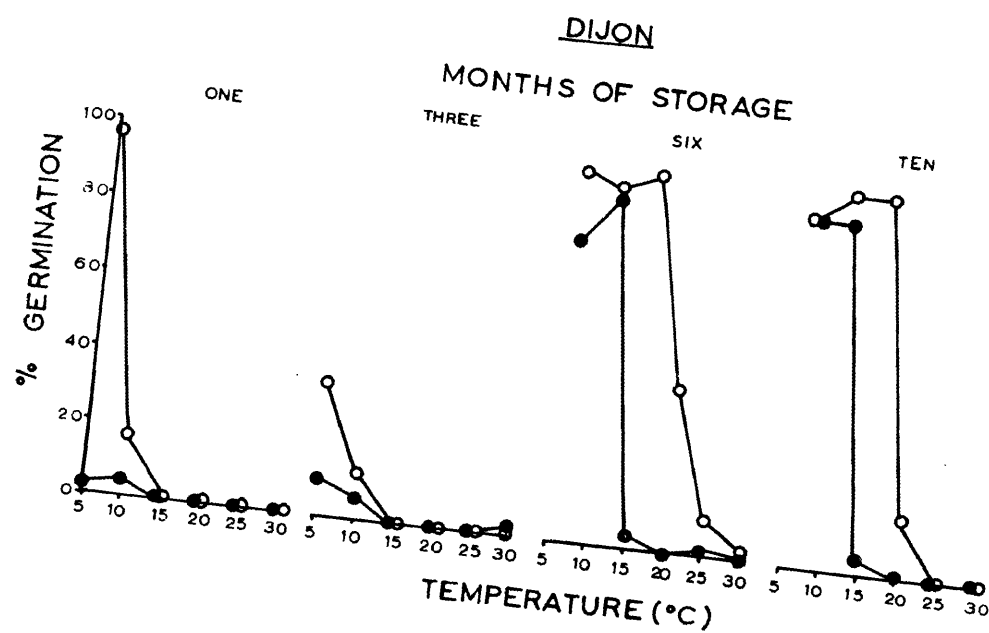


Figure B 2xiv.

variation in optimal germination temperature so that little germination will occur until the prevailing temperatures in the field coincide with the optimum temperature for germination in the after-ripening seeds.

The sixth pattern of behaviour is shown by the single population, 'Burren'. This population had a completely reversed temperature response, (figure B2 xv). Germination was high at high temperatures and declined rapidly with temperatures below 20°C at all stages of after-ripening. There is evidence that this population germinates in the spring. Flowering does not normally take place until August or even September yet the seeds would appear to be dormant at low temperatures for many months and so germination may be delayed until the Spring.

It is unfortunate that populations from extreme continental or extreme Mediterranean habitats could not be included in these experiments but very poor germination of the original samples gave insufficient parent plants.

Two possible patterns of germination could be suggested for Mediterranean populations. Either they may possess long post harvest dormancy making germination impossible until the autumn rains or they may have no dormancy as rainfall is so low that germination is unlikely. In the Continental populations there may be very long dormancy periods if germination occurs in the spring. The summer may be too dry in the types of habitat where A. thaliana occurs and the winters too cold to allow over-wintering.

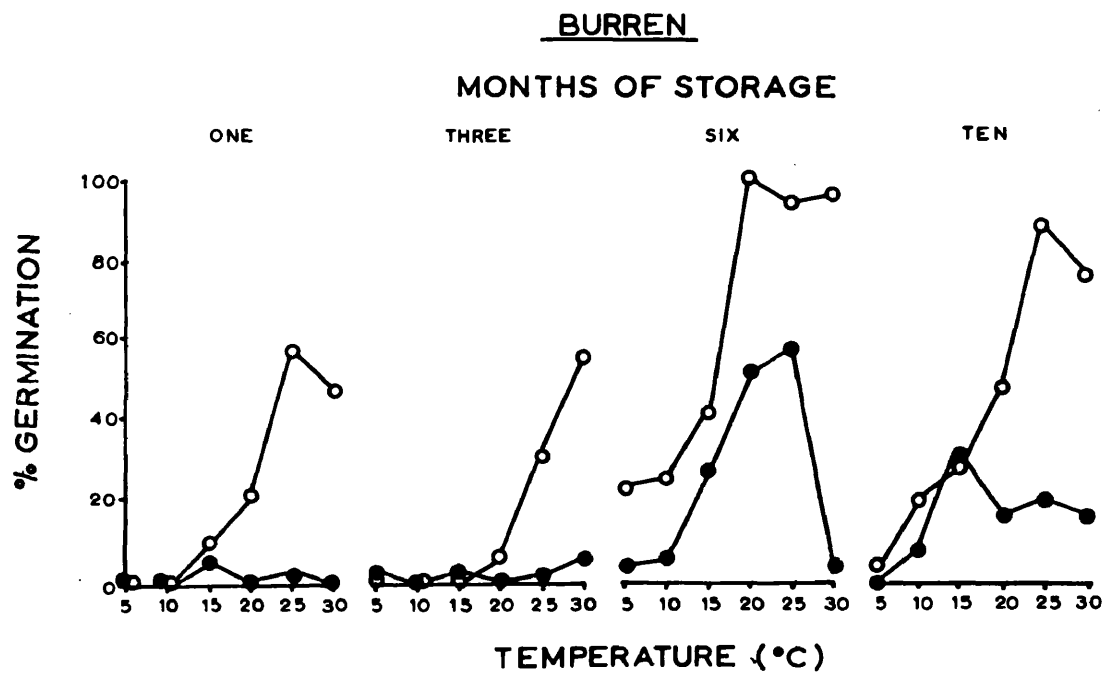


Figure B 2xv.

The life cycle may therefore be restricted to the spring and early summer. However no evidence is yet available as to the precise time of germination in the field in these regions. There is considerable controversy about this question between Central European workers. When fresh seeds of such populations become available it will be imperative to carry out similar experiments to complete the picture of dormancy patterns in the species as a whole.

Morley (1958) worked on *Trifolium* *T. subterraneum* from many sites in Europe. He found variation between populations in the inhibition of germination at temperatures above those encountered at the time of the autumn rains. Inhibitory temperatures were lower for populations from cooler, drier areas. This is very similar to the pattern observed in *A. thaliana*.

Variation of imbibition temperature

In any seed its time of germination will be determined by two factors, the length of its dormancy period and the time taken to germinate once dormancy is lost. At all seed ages in *A. thaliana* the time from imbibition to appearance of the radicle is inversely related to the temperature up to 25°C or above (e.g., Figure B2 xvi).

Although in fresh seeds germination percentage may be higher at 5°C than 25°C time to the appearance of the radicle is much longer. This would be a purely metab-

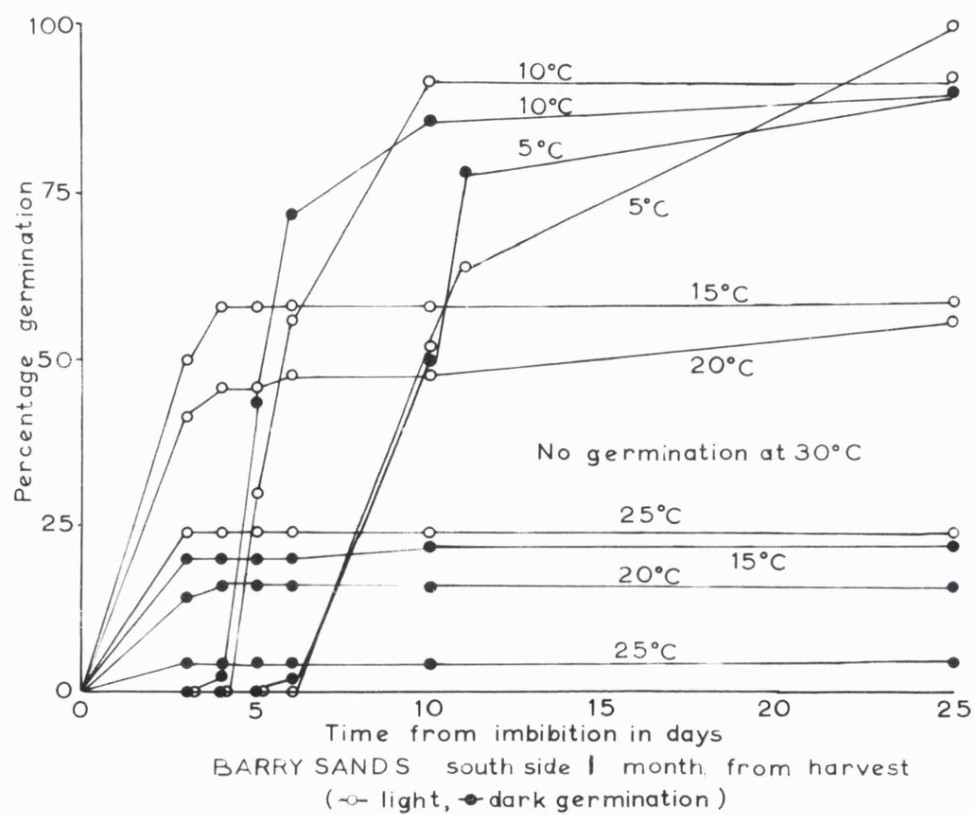


Figure B 2xvi. Percentage germination with time at different imbibition temperatures in seeds of Barry Sands.

-olic effect: once germination has been initiated the rate of growth will increase with temperature. It is possible therefore that temperature only controls the first stages of germination. In this case high temperatures would be expected to inhibit only these initial stages.

This was investigated in four populations after three months of dry storage. The populations chosen were 'Barry Sands' and 'St. Cyrus I' which have a high proportion of early germinating seeds and 'Bush' and 'Dirleton' which had many dormant seeds at 25°C. Five subsamples of 50 seeds of each population were placed at 10°C in continuous light, one sample of each population was transferred to 25°C in continuous light after 18, 45, 66 and 136 hours. The last sample was left at 10°C. A further subsample was kept at 25°C. Germinated seeds were counted on transfer to 25°C and at appropriate intervals afterwards. The results are shown in figure B2 xvii.

The 'early ripening' populations and 'Late ripening' populations showed different patterns but were very similar within each type. In all cases germination was lower in constant 25°C than in constant 10°C confirming the earlier results. In 'Barry Sands' and 'St. Cyrus I' a pretreatment of 18 hours at 10°C is sufficient to allow very rapid and complete germination on transfer to 25°C.

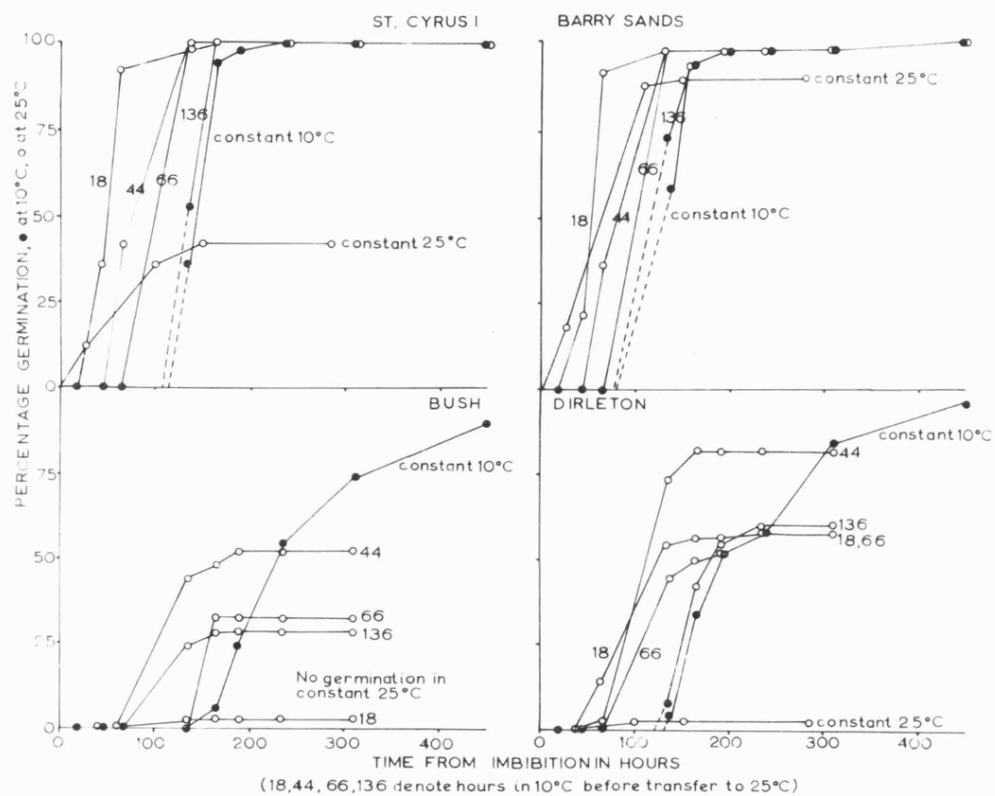


Figure B 2xvii. Effect of different lengths of pretreatment at 10°C on germination at 25°C in incompletely after-ripened seeds of four populations.

In 'Bush' and 'Dirleton' the 18 hours pretreatment brought about some increase in germination percentage at 25°C but was not as effective as the 44 hours pretreatment. Periods longer than 44 hours were inhibitory. No length of 10°C pretreatment used was sufficient to bring the percentage germination in 25°C to the level shown by the seeds in constant 10°C.

The 'Barry Sands' and 'St. Cyrus I' populations showed much lower dormancy than the others. Early germination could be advantageous in these habitats where flowering is late and the ground remains moist for much of the late summer. It would appear that one cold day at this time would be sufficient to allow germination in nearly all seeds even if high temperatures follow.

In 'Dirleton' and 'Bush' populations, which are early flowering from areas where the summer rains are unreliable, require two days at 10°C before they show greatly increased germination at 25°C. The higher final germination percentage reached after all lengths of pretreatment in 'Dirleton' appears to reflect the slightly lower dormancy shown by this population as compared to 'Bush'. These weed populations show some degree of opportunism in that a proportion of seeds can germinate at subsequent high temperatures after cold pretreatment. However, the lack of germination of a high proportion of seeds under this treatment might ensure that the population would not be wiped out by a summer drought.

The fate of those seeds which remain ungerminated at high temperatures may be important. They may still be capable of germination at low temperatures. In order to clarify this the subsamples of eight of the north side populations which had been tested (at 20, 25 and 30°C) after three months storage were placed at 10°C for a further three weeks and any increase in germination noted. The results are given in Table B2 iv.

<u>TABLE B2 iv</u>						
<u>Percentage increase in germination at 10°C</u>						
<u>Initial temperature treatment °C</u>						
Population	20		25		30	
	Dark	Light	Dark	Light	Dark	Light
Dijon	0	0	31.2	37.2	8.0	13.1
Besançon	0	0	81.2	0	8.0	0
Woebley Castle	2.3	0	74.2	11.6	10.0	0
Whatton	0	0	0	4.2	40.0	16.3
Stockholm	20.0	0	67.0	0	30.6	77.1
Husband's Bosworth	0	0	26.0	2.0	8.0	6.0
St.Dogmael's Abbey	0	0	30.6	36.3	8.0	23.4
Ochsenkopf	0	0	21.6	22.2	4.2	56.1
Mean	2.7	0	39.0	13.9	11.3	24.0

It would appear that few seeds ungerminated at 20°C were still capable of germinating at 10°C. Of the seeds ungerminated at 25 and 30°C some can germinate at 10°C suggesting they are not dormant but are unable to germinate at higher temperatures. In those seeds

which still remain ungerminated at 10°C some will have not after-ripened, in others secondary dormancy may have induced. As Vegis (1964) points out high temperatures may induce secondary dormancy in incompletely after-ripened seeds and it would appear that the proportion of secondary dormant seeds increases with increasing temperature.

Light effects

All seeds can be divided into light requiring, light inhibited or light indifferent (Meyer and Polkajoff-Mayber, 1963). No population of A. thaliana studied in this investigation had an absolute light requirement. Kugler (1951) reported that race Hm had an absolute light requirement from 7 - 38°C. It can be seen from the graphs (figures B2 ix - xv) of temperature against germination for differing seed ages, that there is considerable variation between populations in the ratio of light to dark germination. All races in this study showed some germination in darkness. The light sensitive germination of A. thaliana appears to be a typical red - far red response of a phytochrome system i.e., germination is promoted by red radiation between 5250 - 7000 Å and inhibited by far red 7000 - 8200 Å.

However, the phenomenon of light sensitivity does not appear to be independent of other factors. Black (1969) pointed out that light requirements can be circumvented by other factors. For examples, in Grand Rapids lettuce light is only required at temperatures above the optimum for germination. This phenomenon has been

recorded in many species. Shropshire, Klein and Elstad (1961) found this response in the race 'Estland' of A. thaliana. They considered it to be due to an accumulation of the far red absorbing form of the pigment or its active complex at lower temperatures leading to an increase of germination in the dark.

Working with Grand Rapids lettuce Hartmann (1966) considered that the far red absorbing form of the phytochrome pigment was destroyed under high energy, conditions and so the high energy part of the system, the blue far red reaction, should show characteristics of destruction of far red absorbing pigment. For example, reduction of temperature during irradiation should reduce the degree of inhibition of germination. This in fact proved to be so. He also suggested that one would expect a reduced sensitivity to red radiation after prolonged treatment with far red. This is important in relation to secondary dormancy which will be discussed in detail later. The above experiments could explain the light sensitivity observed in the A. thaliana population in this investigation. Most races studied show less inhibition of germination in the dark at low temperatures. The inhibition increases with increasing temperature and becomes severe or complete at 30°C. The level of inhibition at different

temperatures however, appears to vary both between populations and at different seed ages. At all ages of seed it appears that light is increasingly necessary for germination as the temperature for imbibition moves away from the optimum. However, it also appears that darkness becomes less inhibitory as the seeds after-ripen. Those populations which tend to after-ripen quickly e.g., 'Stockholm', 'Catterline', 'Barry Sands' tend to have little light requirement at low temperatures even in fresh seed. As the seed ages darkness is hardly inhibitory except at 30°C. In others 'dark' germination does not increase as much as light germination as the seed ages and after six months of storage there is still a distinct inhibition e.g., 'Husband's Bosworth' and 'Woebley Castle'. Other populations e.g., 'Burren', 'Bush' and 'Besançon' show little 'dark' germination when fresh even at low temperatures.

As darkness under experimental conditions is equivalent to buried seeds in the field, ratio of 'dark' to 'light' germination could be important, in enabling a population to survive by maintaining an insurance policy of buried seeds in the soil. In habitats with little year to year fluctuation in local climate especially where conditions are likely to be continuously favourable for establishment there would be little advantage in buried seeds remaining ungerminated e.g., 'Stockholm' and 'Ben Dhorain'; however, in weed

populations and those where summer conditions are liable to considerable fluctuation it may be advantageous to build up a reserve of seeds in the soil to counteract any decimation of the population after a very severe year e.g., 'Husband's Bosworth' or 'Dirleton'.

Other factors affecting dormancy

Other factors apart from light and temperature may affect seed dormancy. For example, Battle and Whittington (1969) found heavy rainfall delayed seed maturity and reduced germination in sugar beet. As the cultivation experiments at the gardens provided seeds of A. thaliana populations which had been subjected to different conditions during maturity it was possible to see if colder, damper ripening conditions affected dormancy.

In all populations loss of dormancy followed a very similar pattern in seeds from both the N and S sides of the fence. However, the process was delayed in the seeds from the N side which were never subjected to direct sunlight, (figures B2 xviii - ixx). It would appear that damper and cooler conditions during seed maturation delay after-ripening. The seeds probably dry out more slowly and the metabolic changes involved in after-ripening do not take place as quickly. It is known that imbibed seeds cannot continue to after-ripen (p.33) and may have to be dried out, stored and re-wetted before they can germinate.

Date of harvest may also effect dormancy. Marshall (1968) found in Corynephorus canescens that germination rate and the temperature range over which germination could occur, improved as the caryopses remained on the plants longer in the field. However, Roberts (1961) working on rice found premature harvesting reduced post harvest dormancy.

Figures B 2xviii-ixx. Effect of seed age and temperature
on post harvest dormancy in seeds
of two populations grown on the
north and south sides of a fence.
——— south side,
— — — — north side.

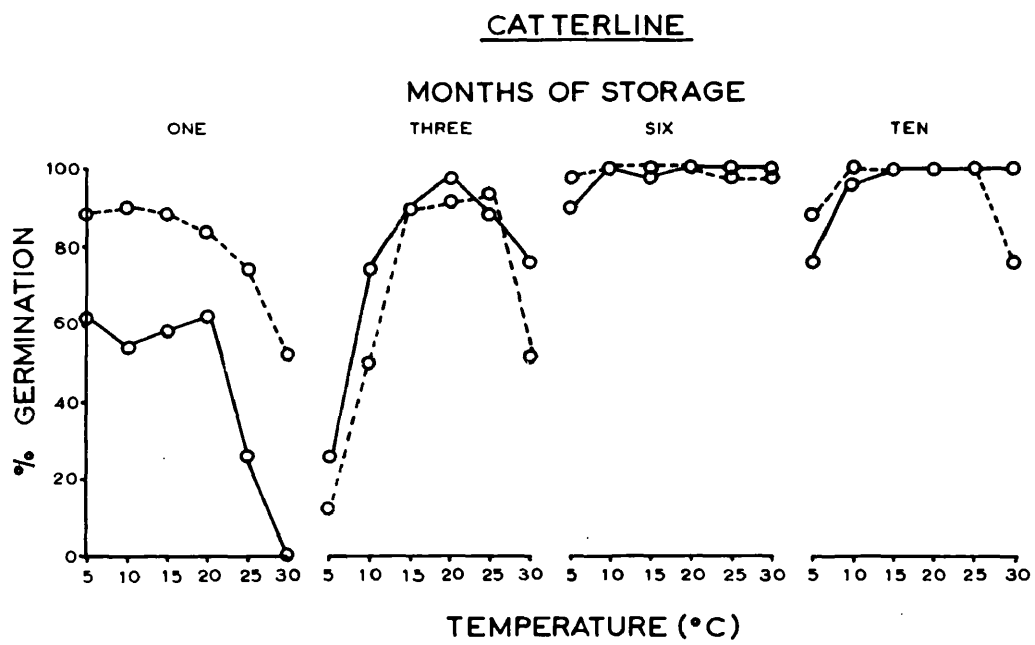


Figure B 2xviii.

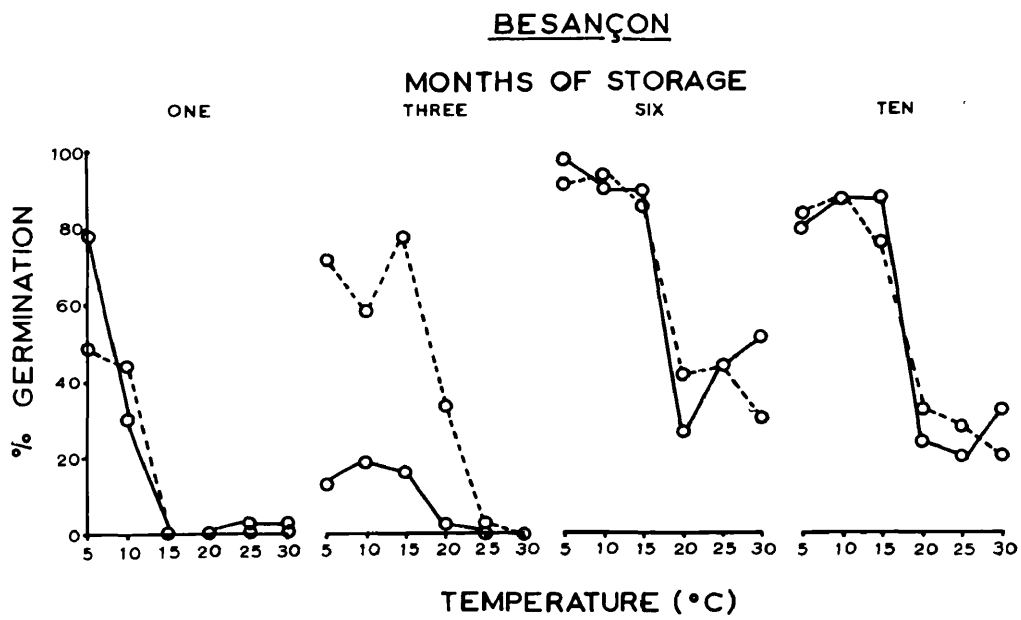


Figure B 2ixx.

In one of the Derbyshire localities 'Cobbler's Nook', collections were made on 20 June 1970 and 8 July 1970. Percentage germination at 20°C in constant light was higher in the collections harvested earlier, (Table B2 v).

TABLE B2 v

<u>Collection</u>	<u>Date of collection</u>	<u>Date of Imbibition</u>	% Germination	
			5 days <u>after imbibition</u>	12 days
Cobbler's Nook A	20/6/70	22/7/70	36	76
		28/8/70	100	100
		26/4/71	100	100
		7/7/71	100	100
Cobbler's Nook B	8/7/70	23/7/70	13	31.5
		28/8/70	93	95
		26/4/71	96	98
		7/7/71	100	100

It is probable that the seeds from the earlier collection stored in the laboratory dried and commenced after-ripening while those left on the plants dried more slowly. The alternate wetting and drying experienced in the field might also delay after-ripening and so give the germination pattern observed. If this was so, once after-ripening was complete, little difference in final germination percentage should be found : this was the case.

Earliest capability of germination would therefore be achieved in the absence of too rapid drying at high temperatures or too slow drying in damp cool conditions.

Selection experiments

The fact that seeds produced from populations grown under uniform conditions exhibited large differences in dormancy pattern certainly suggests that such differences are genetically controlled, as do the differences shown, by individual plants in a population. If this is so, selection of early and late ripening genotypes within the population should be possible and an attempt was made to do this. Using the populations from the garden experiment large seed samples were set out at 25°C after 3 months storage. Those seeds which germinated at this temperature were removed and planted in compost in the greenhouse and the rest of the seeds dried out. They were re-wetted after six weeks and a further selection of seeds which now germinated was made. Again the rest of the seed was dried out, re-wetted after a further six weeks and a final selection made. Unfortunately, at this time considerable problems were encountered in obtaining satisfactory compost and in controlling greenfly in the greenhouse. In only six populations a few plants from the first selection survived to flower and set seed. No plants from the second or third selection survived.

Subsamples of the second generation seeds were set out at 10°C and 25°C in the light. However, up to the present time about six months later, very little germination has been noted even at 10°C and it is possible that conditions in the greenhouse especially the high levels of insecticide used may have affected the viability of these seeds. It will be worthwhile to repeat these experiments when fresh seed becomes available from the garden.

Some seeds of the individual plant samples from the 'Husband's Bosworth' population were germinated, vernalised for six weeks, and allowed to flower and set seed; subsamples of this seed were set to germinate at 20°C in constant light. It was hoped that the progeny of early ripening strains would also have a high proportion of early ripening seed. So far this has not been shown. Few seeds have germinated at 20°C.

However, one interesting result was obtained from this experiment. The seed samples were all classified according to the date of anthesis of the parent and the experiments staggered so that the subsamples were set to germinate at the same time after anthesis of the parent plant. It was noted that those samples which had some seeds with short post harvest dormancy were those from late flowering parents regardless of whether these parents originated from plants classified previously as late, intermediate or early ripening, (P.30).

As the information was available on the flowering ~~side~~ date of each of these second generation plants it was hoped to select for early and late flowering strains. Some seeds from the earliest and the latest flowering progeny of eight of the original single plant samples (p.27) ^{were} ~~was~~ set to vernalise in the light at 5°C. After 28 days it was seen that 4 of these 16 subsamples showed a high germination percentage. All these were from late flowering plants. The experiment was repeated with other samples of the same seed and very similar results were obtained, (Table B2 vi).

<u>Table B2 vi</u>				
Original Sample	2nd Generation Plant	Days to anthesis	Final % germination of seeds	
			Imbibed 7/2/72	Imbibed 22/3/72
A	ii	68	0	0
	iii	79	100	100
C	i	58	0	0
	iii	77	96.88	100
D	iv	58	0	0
	i	77	0	0
F	ii	58	7.14	1.15
	iii	77	100	84.21
I	ii	73	0	0
	i	76	0	0
G	ii	65	0	0
	iii	77	95.65	100
N	i	63	0	0
	ii	73	0	0

It would appear that seeds from the early flowering plants have a lower dormancy period than those flowering 10 - 12 days later. Such a mechanism if it operated under field conditions would tend to synchronize the time at which germination became possible. It is possible that the expression of the genotype of the individual plant can be modified by environmental conditions. Obviously these experiments are very preliminary but offer a worthwhile line of investigation in the future.

It is possible that this is a feature which operates not only among the plants in an individual population but within the species as a whole. The earliest flowering populations e.g., 'Dirleton', 'Bush' and 'Besançon' all show a high degree of dormancy. Late flowering populations e.g., 'Mickle Fell', 'Stockholm' and 'Barry Sands' have short dormancy requirements and populations intermediate in flowering time e.g., 'Husband's Bosworth' are more intermediate in dormancy pattern.

It would appear that dormancy in A. thaliana is a very important factor in controlling the life cycle. Koller (1969) points out that the regulation of germination in a population must depend on selectivity, heterogeneity or both. Selectivity will allow response to specific environmental factors so that in some populations the whole seed production is used up and in others a proportion

is conserved. Heterogeneity will produce variation in the germination requirements among seeds from any individual plant ensuring that the seed supply is not exhausted in 'a single attempt at establishment'. Selectivity would appear to be operating in A. thaliana to produce the different patterns of dormancy observed allowing survival in the diverse climatic conditions experienced by the populations. The maintenance of heterogeneity especially in those habitats where conditions may be more extreme will ensure the continued existence of a population despite large year to year fluctuation in environmental factors.

B.3. Factors affecting germination and secondary dormancy.

Popay and Roberts (1970) point out that environmental factors influence germinationⁱⁿ two distinct ways :

- a) Loss of dormancy
- b) The rate and level of germination once dormancy has been lost.

For germination all seeds require adequate water, suitable temperature and a satisfactory gaseous environment. At very high and very low temperatures the germination of all seeds is prevented. A rise in temperature does not necessarily cause an increase in the rate or level of germination. Usually there is an optimal range above and below which germination is delayed. In some species an alternating temperature regime or exposure to some particular pretreatment is necessary before favourable conditions for germination are effective, (Meyer and Poljakoff - Mayber, 1963).

One group of plants whose germination has been studied in detail both by laboratory experiments and germination in the field ~~are~~^{is} the annuals of the South Californian deserts. Went and his colleagues found the abundance and distribution of annuals in any year ~~was~~^{were} determined by the number of seeds that germinate and the environmental conditions during attempts at establishment, (Went, 1948, 1949; 1955; Went and Westergaard, 1949, Juhren, Went and Phillips, 1956).

Constant Temperatures.

In order to ascertain if summer and winter annual races of A. thaliana had different germination requirements experiments were carried out with fully after-ripened seeds. Subsamples of fifty seeds of nineteen populations were set out on moist paper pulp in petri dishes at 5, 10, 15, 20, 25 and 30°C both in continuous light and in black polythene bags. The germinated seeds were counted at appropriate intervals at 1 - 5 days until no further germination was observed. Counting was carried out as quickly as possible in dim light at room temperature.

Considerable variation was observed in the final germination percentage between the populations but the results paralleled to a large extent the patterns seen in the incompletely after-ripened seeds. The populations could be divided into three groups, (for examples see figure B3 i).

- 1) Those with wide ranges of optimal temperatures -
'Barry Sands', 'Stockholm', 'St. Cyrus I and II',
'Catterline', 'Mickle Fell', 'Longcliffe A', 'Dove
Holes' and 'Lathkill Dale'.
- 2) Those with low optimal temperatures - 'Aberdeen',
'Bush', 'Hadleigh' and 'St. Dogmael's Abbey'.
- 3) Those with intermediate to high optimal temperatures
- 'Soria', 'Lodz', 'Burren', 'Ben Dhorain', and
'Besançon'.

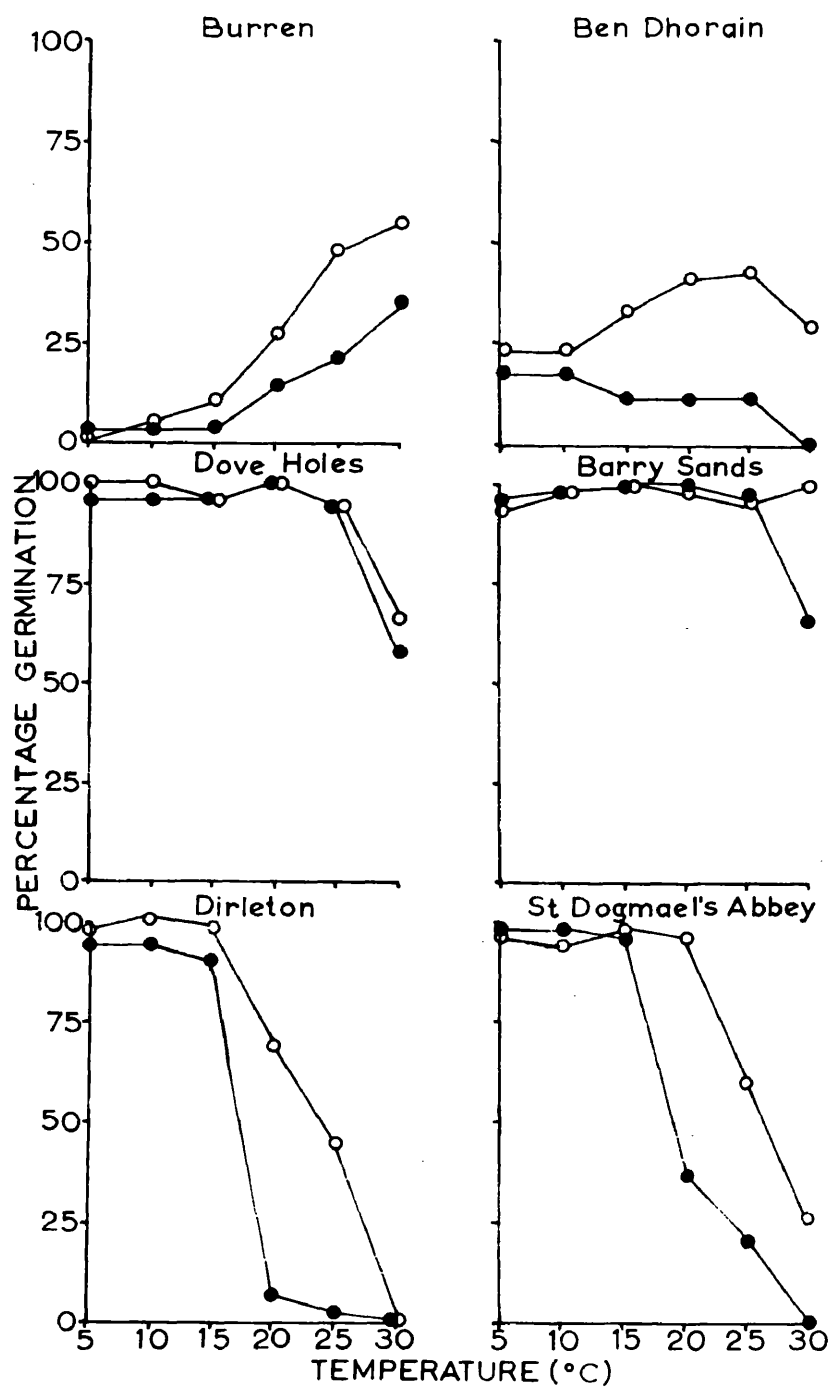


Figure B 3i. Effect of temperature and light on final % germination of fully after-ripened seeds of six populations.

The first group consists of many of the early ripening populations and of populations from similar areas with cool winters e.g., the Derbyshire populations. They show a wide range over which very high germination is achieved. The ratio of dark to light to germination is also high, reaching unity over 5 - 25°C in some cases. These populations would appear to possess great flexibility which may well be advantageous in areas with a cool damp climate, where germination can commence soon after flowering, e.g., 'Catterline' or 'Mickle Fell' or in weedy populations where a flexibility in relation to temperature could allow germination in new habitats e.g., 'Longcliffe A'. However, it is important to remember, as Koller (1969) points out that the optimum temperature range may be narrowed in the field by other stresses.

The second group with their low optimal temperatures show a very similar pattern in both incompletely and fully after-ripened seeds. It appears that these populations are never able to germinate well at high temperatures. This correlates well with their long post harvest dormancy and the high probability of summer drought in their place of origin.

The dormancy patterns where known, of the third group of populations are very different from each other. It is possible if high temperatures^{ve} optima are of any adaptive significance in these populations, that different factors in the environment had^{ve} led to selection of this similar response.

Morley (1968) states that, in general, seeds of species which normally germinate in the autumn and grow through the winter have low temperature optima as shown by Went in the desert annuals.

This would appear to be true for the second group of populations discussed here. However, the third group probably includes both summer and winter annuals. The high temperature optima for summer annuals e.g., 'Lodz' and 'Burren' is understandable as these probably germinate in warmer spring weather. Populations from the Mediterranean such as 'Soria' need to be capable of germination at high temperatures as temperatures below 10°C are rarely experienced until mid winter. The 'Dijon' and 'Besançon' populations present a problem. They have short vernalisation requirements and will flower, in time, without any cold treatment. However, they have a long dormancy. It is possible that these populations may be spring germinators. Little is known of their original localities but the climate must be more continental than that of Britain or the Mediterranean. Taking the time of flowering and the length of dormancy into account these populations should be capable of germinating in the autumn at low temperatures or in the spring in warmer conditions.

Billings and Mooney (1968) found that the optimum temperature for germination measured by both speed and completeness of germination ~~were~~ ^{was} high for most tundra and high altitude species. Values of 20 - 30°C were common. Little germination was found at constant 5°C.

They concluded that germination must occur early enough in the year for good growth before the return of freezing temperatures. If the temperature thresholds were too low, germination would occur in a season which was too cold for adequate photosynthesis and the plant would be too small to overwinter. This could be the case in the 'Ben Dhorain' population from 1500 ft. in the north of Scotland which has an optimum of 25°C.

The populations from more extreme environments whether this is winter cold or summer drought show much reduced germination in darkness. In these habitats not all buried seeds will germinate and a reserve of seeds may be built up in the soil.

Ayodele Cole (1967) studied germination responses in several populations of the winter annuals, Erigeron cinereum and E. fasciculatum in California. Populations from the coastal beach habitats similar to Mediterranean localities had narrow ranges of optimal germination: 15 - 25°C. Those from the less extreme coastal lowlands, coastal mountains and inland valley populations had much wider ranges : 5 - 35°C. In A. thaliana there seems to be an analogous situation.

In populations of A. thaliana where the temperature range is wide, low temperatures delay germination. However, the pattern of germination is very similar at all temperatures. On each of two bowls of compost

approximately 30 x 40 cms, 1000 seeds of 'St. Cyrus II' were scattered. One bowl was left in the warm greenhouse at $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$. Daylight was supplemented by mercury vapour lamps to give a 24 hour photoperiod. The other bowl was placed outside the greenhouse where it was subject to normal diurnal fluctuations of temperature and photoperiod. The seeds which germinated each day were counted and removed until no further germination occurred.

Using thermistors attached to a Grant ⁱminiature temperature recorder the temperature of the soil surface in the two bowls was recorded every hour; from the records of temperature the area under the temperature curves was calculated using the formula :

$$\int_a^b = \frac{1}{3} \left[y_0 + 2 \sum y_{\text{odd}} + 4 \sum y_{\text{even}} + y_i \right]$$

Where y_0 is the temperature recorded at 0 hrs; $\sum y_{\text{odd}}$, the sum of the temperatures at the odd hours (1, 3, 5 etc); $\sum y_{\text{even}}$, the sum of the temperatures at the even hours (2, 4, 6 etc) and y_i , the temperature at 23 hours. (Simpson's rule).

This calculation gave the integrated temperature in degree hours for each day i.e., the total 'heat sum' received at the soil surface. This was corrected to give the integrated temperature above 5°C which was considered to be the likely lower limit for active growth.

The experiment was carried out in late November - December. It can be seen from figure B3 ii that the 'heat sum' in the greenhouse was between 280 - 440⁰hrs per day while outside it rarely reached 50⁰hrs and frequently was negligible. Indoors, germination commenced four days after imbibition, outside no germination occurred until the 19th day. However, once germination started the rate for the first three - four days was very similar to that recorded in the greenhouse after which germination was almost 100% indoors. 'Outside' germination continued at a much lower rate for another 8 - 10 days and final germination was approximately 20% lower outside indicating that in the fluctuating conditions occurring in the field, the very high germination % observed in laboratory experiments is not necessarily achieved.

It would seem that high temperatures do not affect the rate of germination. In a uniform completely after-ripened seed sample of high viability the same characteristic burst of germination is obtained under widely differing temperature conditions. However, the time taken to reach a stage when the seeds are capable of germinating is considerably increased by lower temperatures due no doubt in part to low level respiration and slow mobilisation of food reserves.

In some species germination will not proceed at high temperatures until a period at low temperatures has been experienced. This is similar to the germination of

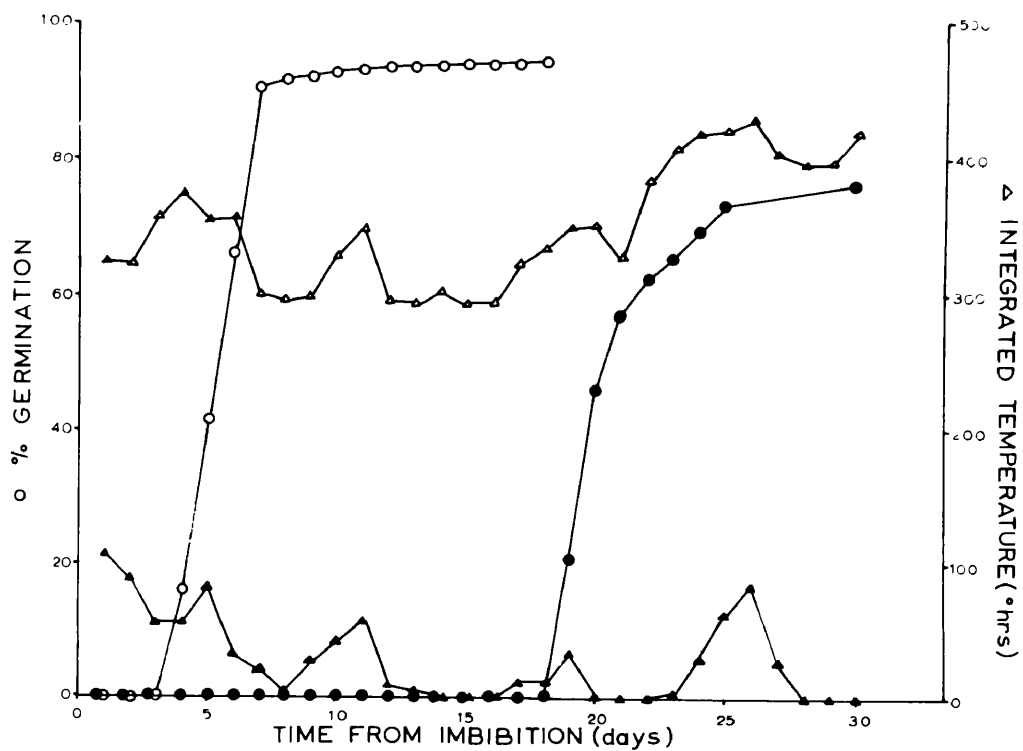


Figure B 3ii. Percentage germination of seeds of St. Cyrus II and temperature integrals out of doors in November-December (closed symbols) and in a warm greenhouse (open symbols).

incompletely after-ripened seeds of most A. thaliana populations. Cooper (1963) found that many species from areas with cold winters had a freezing requirement which delayed germination until the spring.

Two subsamples of fifty seeds were taken from each of twenty populations. One sample was set out at 25°C in continuous light. The other sample was given a three week pretreatment at 5°C in light before being placed in 25°C. The final percentages ~~received~~^{achieved} are given in Table B3 i.

TABLE B3 i

<u>Population</u>	<u>Final Percentage Germination at</u> <u>25°C</u>	
	<u>With 5°</u> <u>Pretreatment</u>	<u>Without 5°</u> <u>Pretreatment</u>
Stockholm I	96	98
Stockholm II	98	98
Longcliffe B	100	100
Mickle Fell	78	72
St. Cyrus I	100	100
Husband's Bosworth	78	26
St. Dogmael's Abbey	94	64
Friden Crossing	90	78
Ochsenkopf	94	60
Besançon	26	12
Woebley Castle	90	50
Ben Dhorain	50	32
Whatton	22	4
Palermo	2	6
Lodz	14	22
Strasbourg	16	24
Weeting Heath	4	20
Creag an Lochan	2	6
Loch Ness	0	8
Burren	18	54

Populations which have wide optimal ranges for germination e.g., 'Stockholm', 'Longcliffe B', 'Mickle Fell' and 'St. Cyrus I' all showed very similar germination

both with and without a cold pretreatment.

Germination in 'St. Dogmael's Abbey', 'Friden Crossing', 'Ochsenkopf', 'Besançon', 'Woebley Castle' and 'Whatton' was increased by a cold pretreatment. All these populations come from areas with warm summers and a high incidence of summer drought and have a long post harvest dormancy. Most of these populations have low optima for germination. None has an absolute requirements for cold pretreatment and it is unlikely that germination in any of these populations is delayed until the spring.

Germination of 'Ben Dhorain' was also increased by the cold pretreatment. The final germination of 50% achieved was greater than that observed previously in constant 5°C and possibly in this population germination of some seeds could be delayed until spring especially if the autumn temperatures were suboptimal.

In the other populations percentage germination was reduced by the cold pretreatment. The summer annuals 'Lodz', 'Strasbourg' and 'Burren' have high temperature optima. The 'Burren' population is known to have a long dormancy at low temperatures and germination is likely to be prevented until the spring. It is possible that a similar mechanism is operating in 'Lodz' and 'Strasbourg'.

Palermo is likely to have a high temperature optimum for germination as low temperatures are unlikely to occur often in this locality. In the 'Weeting Heath' population germination probably occurs in the autumn but it would be advantageous to restrict germination to the early autumn in this more continental climate. Late germinating plants may not grow sufficiently to survive the cold winter. This is probably also true for 'Creag an Lochan' and 'Loch Ness'. The dormancy patterns of these three populations are not yet known.

Alternating temperatures.

The experiments so far discussed were carried out at constant temperatures. However, this obviously does not occur in the field. In many species germination is enhanced by alternating temperatures. The effect of four alternations on the germination of twenty populations of A. thaliana were studied using a controlled environment Saxcil cabinet. In each case the higher temperature was maintained for 16 hours in continuous light, the lower for 8 hours darkness. Regimes of 27/20, 27/14, 27/7 and 20/14°C were used. Fifty seeds of each population were used in each regime. The final germination percentage was measured after three weeks.

Several different patterns of behaviour were observed. (Table B3 ii).

TABLE B3 ii

<u>Population</u>	<u>Final Percentage Germination</u>			
	Temperature regime			
	(Day temp/night temp °C)			
	27/20	27/14	27/7	20/14
Dove Holes	98	100	100	100
Longcliffe A	98	98	100	100
Mickle Fell	92	94	90	96
Barry Sands	98	100	92	100
Stockholm I	94	94	96	96
St. Cyrus I	100	100	100	100
St. Cyrus II	96	100	90	98
Catterline	84	94	94	96
St. Dogmael's Abbey	94	100	96	100
Aberdeen	2	62	66	70
Dirleton	32	100	94	84
Bush	20	54	52	32
Lathkill Dale	76	96	94	98
Dijon	26	2	4	14
Lodz	10	2	0	16
Ben Dhorain	50	56	18	50
Besançon	16	52	26	44
Parsley Hay	84	96	58	98
Woebley Castle	40	52	12	28
Burren	26	38	36	54

In the case of 'Dove Holes', 'Mickle Fell', 'Barry Sands', 'Longcliffe A', 'Stockholm I', 'St. Cyrus I & II', 'Catterline' and 'St. Dogmael's Abbey' very similar germination was found in all regimes. With the exception of 'St. Dogmael's Abbey' these populations have a wide range of optimal temperatures. 'St. Dogmael's Abbey' had poor ~~growth~~ germination in constant light at 25 or 30°C or in constant dark at 20°C. It would appear that low night temperatures were sufficient to give optimal germination at 27/14 and 27/7. The optimal germination at 27/20 suggests that the alternation of temperature had increased germination.

In several populations, 'Aberdeen', 'Dirleton', 'Bush', and Lathkill Dale', germination was much poorer in 27/20 than with cooler night temperatures suggesting that, as Kugler (1951) found in other races of A. thaliana, night temperatures may be most effective in controlling final germination. This would appear to be especially marked in populations with low temperature optima. Populations such as 'Dijon' and 'Lodz' germinated less well when cool nights were accompanied by high day temperatures. Went (1949) found in desert species that alternations of warm days and cool nights gave little germination. These conditions will occasionally occur in early or late summer when there are clear skies and little rain and establishment is unlikely. Other populations showed a similar reduction only at 27/7 e.g., 'Ben Dhorain', 'Besançon', 'Parsley Hay' and 'Woebley Castle'. The last three populations have low temperature optima for germination and also showed a reduction at 27/20. 'Burren' showed similar germination in high day temperatures regardless of night temperatures, but germination was highest in the 20/14 regime. This is surprising considering this population has an optimum of germination of 30°C both in light and dark.

In the field seeds are not subject to single alternations of temperature and light but to a continually changing pattern throughout the year. Towards the end of this study a refrigerated incubator became available. It was possible to use this to study germination under a wider variety of temperature alternations. Using the

values of mean monthly maximum and minimum temperature for Buxton (the nearest place with adequate records to the Derbyshire localities) (Clapham, 1969) and the daylength for this latitude, (Table B3 iii), the incubator could be set to simulate to some extent the Buxton climate.

TABLE B3 iii

Buxton Climatic Data

Mean Monthly Temperature °C

	<u>Min</u> ^m	<u>Max</u> ^m	<u>Daylength</u>
January	0	4.5	7h 34m
February	0	4.7	8h 50m
March	0.7	7.2	10h 48m
April	2.7	9.8	12h 53m
May	5.0	13.4	14h 54m
June	8.2	16.4	16h 29m
July	10.5	18.0	16h 53m
August	10.1	17.5	15h 49m
September	8.2	15.0	13h 59m
October	5.3	11.1	11h 56m
November	2.6	7.3	9h 51m
December	0.8	5.1	8h 00m

The light period temperature was set at the average maximum temperature and the night temperature at the average minimum for each month. Unfortunately, each set of conditions could be maintained for only 10 days in order to complete the series in a reasonable time. Subsamples of fifty seeds of nineteen populations were placed on petri dishes filled with moist compost. One subsample of each population was placed in the incubator

at the beginning of each regime. The percentage germination at the end of that regime and the final germination achieved by each population was recorded. Examples of the final germination responses are shown in figures B3 iii - iv. In addition duplicate dishes wrapped in aluminium foil to simulate burial were placed in the August - December regimes. These were uncovered at the end of the experiment and the final germination counted.

Many populations showed high germination in all regimes. These include 'Longcliffe B', 'Ben Dhorain', 'Barry Sands', 'Woebley Castle', 'Dove Holes', 'Whatton', 'St. Cyrus I', 'Catterline', 'Ochsenkopf', 'Husband's Bosworth' and 'St. Dogmael's'. Those populations have high germination in constant temperatures below 18°C, the maximum in these tests.

It is obvious that fully after-ripened seeds of these populations can germinate in all temperature alternations used, even when the night temperature drops to 0°C. This emphasises the importance of dormancy mechanisms in restricting germination to favourable periods for establishment. In several populations a reduction in germination is noted in cooler regimes. This is pronounced in the 'Stockholm' and 'Mickle Fell' populations where late germination is unlikely to result in overwintering plants.

Figure B 3iii-iv. Final % germination in simulated
Buxton temperatures and photoperiod.
Open circles represent germination in
alternating light and darkness, closed
circles represent germination in
continuous darkness.

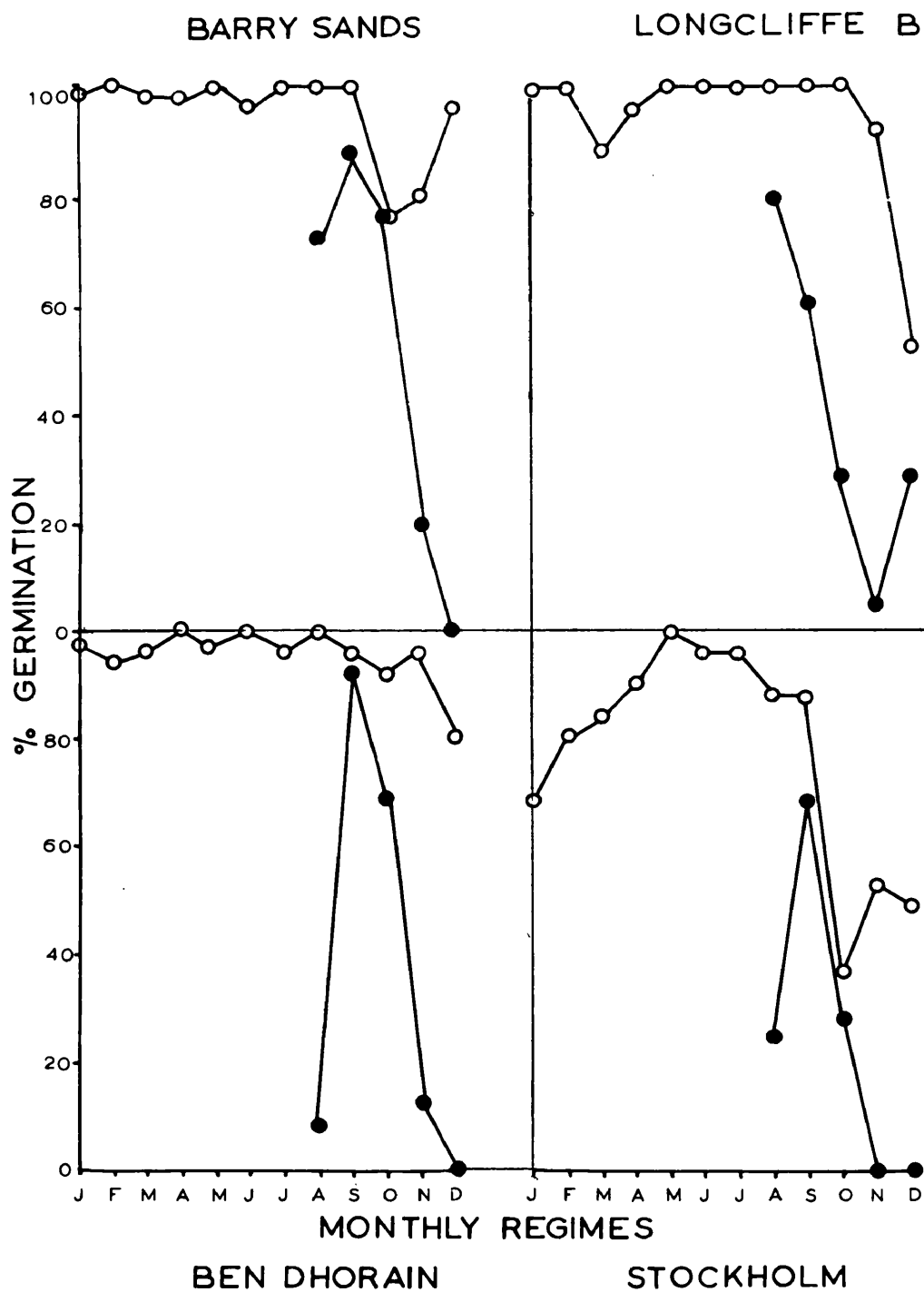


Figure B 3iii.

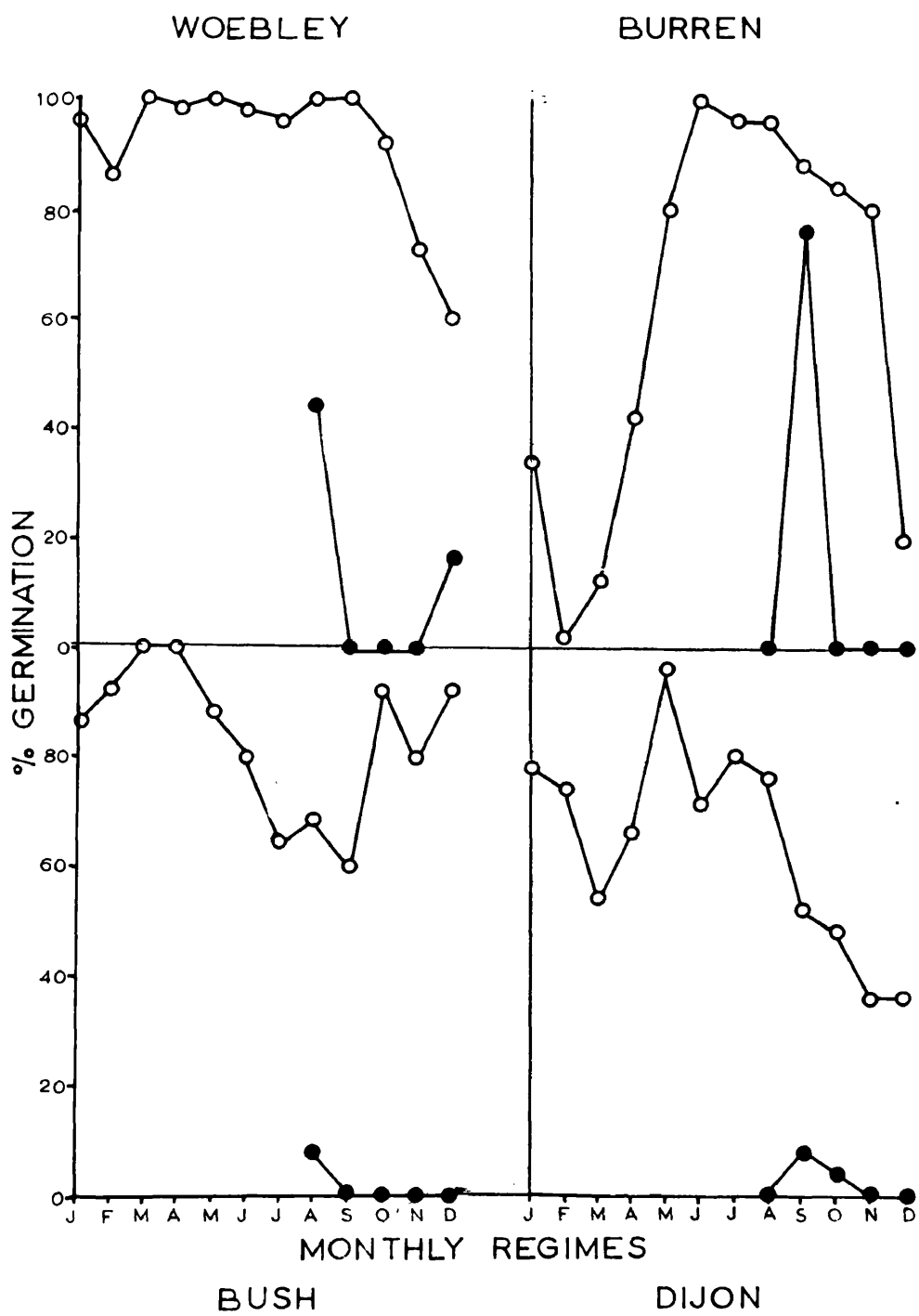


Figure B 3iv.

Populations from areas where summer drought is common e.g., 'Bush' and 'Dirleton' show optimal germination only in spring and autumn conditions indicating that in these populations lack of summer germination may also be due to an inhibition of germination in high temperature regimes. The 'Hadleigh' population is similar. 'Burren', 'Dijon' and 'Besançon' germinate best under Buxton summer conditions. These populations may be spring germinating or they may be unable to germinate at the very low Buxton winter temperatures which are below their optima. Most populations have an optimum of 'dark' germination in the September regime. At temperature alternations above and below this, germination may be very reduced. This would suggest that buried seeds are much more sensitive to temperature alternations than those on the soil surface and that burial of seeds may be important in the preservation of ungerminated seeds in the soil. In some populations the optimum dark germination occurs in the August regime but then there is a rapid decrease.

These experiments were of necessity carried out with fully after-ripened seeds. It would be valuable to repeat them with fresh seeds. Baskin and Baskin (1972) studied one population of A. thaliana from Tennessee. Seeds were collected after the plants had flowered in May and were then subjected at monthly intervals to a series of alternations corresponding with the mean monthly temperature ranges from May - October in Tennessee.

They found that fresh seeds only germinated in the September or October regime. Not until September did the seed become capable of germinating well in the alternations prevailing in the summer months in the field.

Secondary dormancy

Obviously in many populations optimal germination is not achieved in all conditions. Too low or too high a temperature, especially in conjunction with darkness, appears to induce secondary dormancy even in fully after-ripened seeds. This is a common phenomenon in many species and such induction of dormancy may be associated with particular light, temperature or gaseous conditions (Mayer and Poljakoff - Mayber, 1963; Roberts 1969; Harper, 1957). Such secondary dormancy could be valuable in ensuring the survival of a population if it brought about the build up of a reserve of seeds in the soil; both by safeguarding the population against unfavourable conditions and by increasing the heterogeneity of the population, as seeds of several generations may germinate in the same year.

In most of the previous experiments high temperatures in darkness often reduce percentage germination. It was necessary to ascertain if seeds which did not germinate under such conditions did germinate when returned to favourable conditions or if secondary dormancy had in fact been induced.

TABLE B3 iv

TABLE B3 iv

<u>Temperature of Dark Incubation</u>	<u>Temperature of Subsequent Light Incubation</u>	<u>Final Percentage Germination</u>				
		<u>Longcliffe B</u>	<u>Mickle Fell</u>	<u>Besançon</u>	<u>Dirleton</u>	
5	5	100	72	16	96	
	10	100	100	36	100	
	25	100	100	20	96	
	30	100	96	16	100	
10	5	100	96	52	100	
	10	100	100	56	100	
	25	100	100	60	100	
	30	100	100	32	96	
25	5	100	92	28	100	
	10	96	100	28	96	
	25	100	100	16	8	
	30	100	100	8	4	

Temperature and light effects

Initial experiments were carried out using 'Longcliffe B', 'Mickle Fell', 'Besançon' and 'Dirleton'. Sub-samples of twenty-five seeds from each population were placed on moist filter paper in petri dishes and put in black polythene bags. Four dishes of each population were placed in incubators at 5, 10 and 25°C. After one week the dishes were exposed to the light, the germinated seeds counted and one dish of each population from each incubator subsequently placed in an incubator at 5, 10, 25 and 30°C. The final germination achieved was recorded after 3 weeks, (Table B3 iv).

It would appear that germination in 'Longcliffe II' was not inhibited by any of the treatments. 'Mickle Fell' germinated slightly less well when maintained throughout at low temperatures but high temperatures throughout had no inhibitory effect. In 'Besançon' germination was also reduced at continuous 5°C which is sub optimal for this population. After a dark period at 10°C germination is reduced by exposure to 30°C. After dark imbibition at 25°C however, a further reduction in light germination at all temperatures but especially at 25 and 30°C is found. The germination of 'Dirleton' is also reduced drastically by a dark incubation at 25°C if this is followed by light incubation at 25°C or 30°C.

Dark incubation would then seem to have little effect on subsequent germination at any temperature in 'Longcliffe B' and 'Mickle Fell'. These are both stable populations from relatively high altitudes in the Pennines. Dormancy mechanisms prevent germination in 'Longcliffe B' until the autumn rains. In 'Mickle Fell' germination is possible soon after flowering as the ground is then moist and little dormancy is shown at the prevailing temperatures. 'Besançon' has^a much more continental climate and 'Dirleton' also comes from an area where summer rain is unpredictable. In these populations it could be advantageous to have a reserve of seeds in the soil. It would appear that burial at high temperatures could induce a secondary dormancy that subsequent light exposure could not break unless accompanied by a reduction in temperature. This would help to prevent the germination of early ripening seeds buried during the summer but would not prevent their germinating if uncovered during the autumn and so would not ensure carry over of seeds until the next year.

This mechanism of induced dormancy seemed worthy of further investigation and further experiments were carried out using 'Longcliffe B' and 'Dirleton'. Firstly the temperature range was altered. Secondary dormancy could be induced in 'Dirleton' by 25°C but not by 10°C so the experiments were repeated using 10, 15, 20 and 25°C. In 'Longcliffe B' 25°C had not proved effective so the experiments were repeated at 10, 25 and 30°C. Secondly, the effect of the length

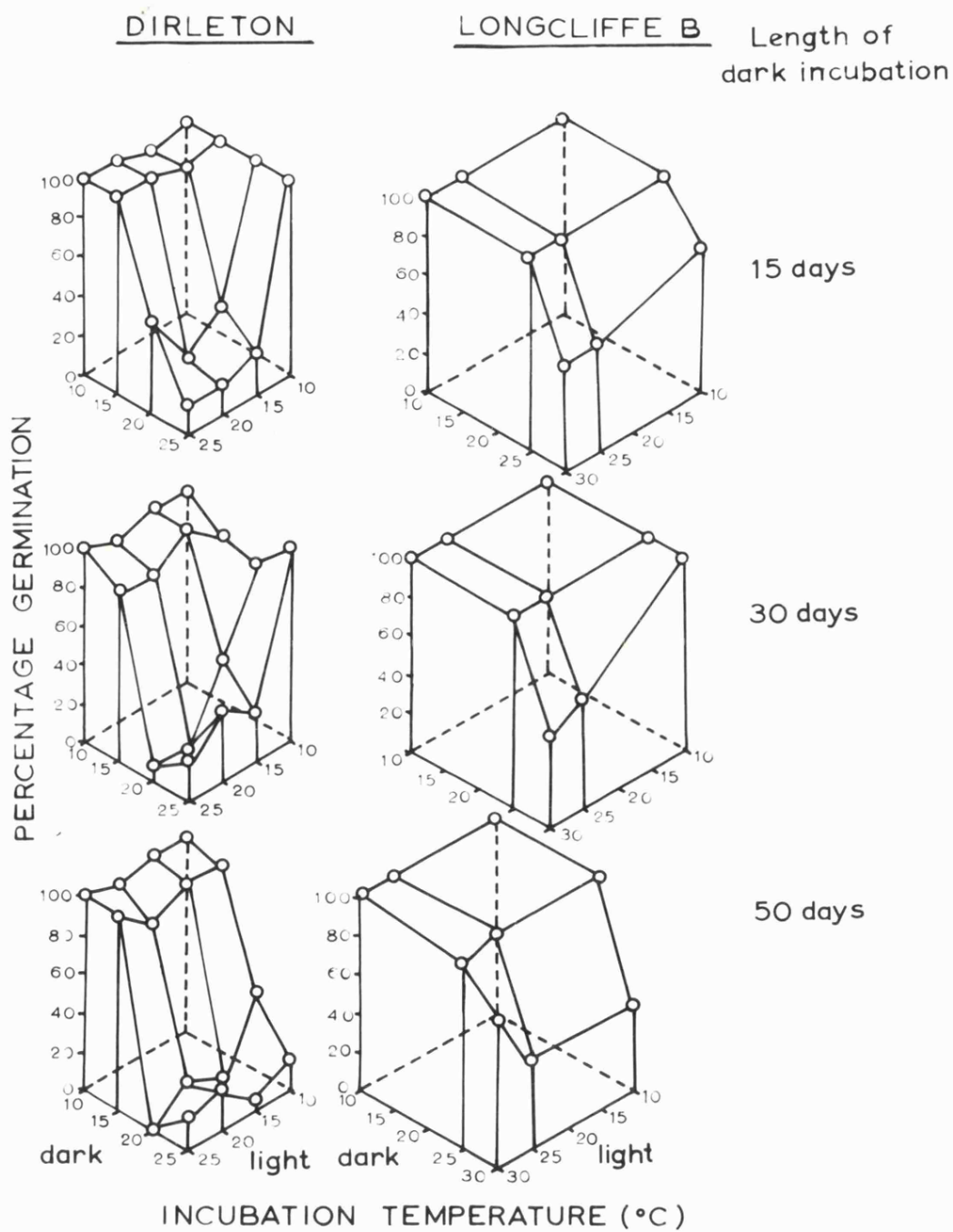


Figure B 3v. Final % germination in light after various lengths of dark incubation for seeds of two populations.

of the dark incubation period was investigated in order to see if the number of dormant seeds was increased by a longer dark pretreatment. The experiments were carried out with dark incubation periods of 15, 30 and 50 days, (figure 3v).

It can be seen that in 'Dirleton' only temperatures above 15°C were effective in inducing secondary dormancy and that 20°C though less effective than 25°C still produced a high degree of secondary dormancy. After 15 and 30 days dark incubation, subsequent exposure to 10°C in the light was sufficient to break this dormancy. However, after 50 days germination was also much reduced at 10°C suggesting that prolonged burial at relatively high temperatures was capable of inducing dormancy in over half the seeds which was not broken by subsequent exposure to light or low temperatures. This would be effective in allowing carry over of seeds through winter. Drying out the seeds and a second after-ripening could allow germination in the following autumn if conditions were favourable.

In 'Longcliffe B' a similar mechanism operates but does not come into effect at temperatures of 25°C or below. Dark incubation at 30°C induced secondary dormancy in over 25% of the seeds which was not broken by subsequent light exposure at 25 or 30°C. After 50 days dark incubation, 10°C in light was similarly ineffective.

Returning all dishes to 10°C produced a little fresh germination when the dark incubation had been 15 or 30 days but a high proportion of seeds remained ungerminated in almost all dishes. No increases were noted in the 50 day dishes. Clearly, burial at sufficiently high temperatures for a suitable length of time is enough to induce secondary dormancy in a significant proportion of seeds in these populations that subsequent exposure to light at any temperature will not break. With seeds as small as these (0.4 - 0.9 mm in length) burial must be of common occurrence. The fact that 'Dirleton' seeds go dormant at lower temperatures and with shorter periods of dark incubation compared to 'Longcliffe II' is in keeping with the greater fluctuations of temperature and rainfall in its habitat from year to year.

It is well known from the work of Brenchley and Warrington (1930) and others that many weed species have large reserves of seeds in the soil and that disturbance by cultivation for example, can allow many of these seeds to germinate.

Sauer and Struik (1964) considered that the germination of some of these seeds might be due to a 'light flash' and that the ability to respond to this depended on the history of the seed as regards imbibition and temperatures experienced during burial. The effect of a light flash on germination of 'buried' seeds was investigated in the 'Dirleton' population and at the same time the effect of lowering the temperature of the dark incubation

for a short while was examined. Subsamples of 100 seeds were placed on moist paper pulp in petri dishes and kept in the dark at 20°C for 6, 20 and 30 days. After each time period the seeds that had germinated were counted and a subsample was placed in each of three conditions : 10°C in light, 10°C in dark and 20°C in light. The seeds destined for the 10°C dark treatment were counted under a Bright Green spectrum filter which was considered unlikely to affect germination. After 48 hours all seeds were returned to 20°C in the dark and left for a further 10 days when final germination counts were made. A control of 100 seeds kept at 20°C in the dark continuously gave only 2% germination. The increase in germination found after the 48 hour treatment was calculated for each subsample, (Table B3 v).

<u>TABLE B3 v.</u>			
<u>Increase in % Germination</u>			
Length of incubation before 48hr pretreatment (days)			
48 hour treatment	6	20	30
10°C dark	7	2	3
10°C light	95	4	1
20°C light	26	1	0

After 6 days dark incubation, a light period of 48 hours was very effective in increasing germination at 10°C and to a lesser extent at 20°C. However, after 20 and 30 days dark incubation the light exposure had very little effect. The drop of temperature in the dark had a small effect after 6 days incubation at 20°C and even less after 20 and 30 days incubation. It would therefore

seem likely that seeds buried at high temperatures retain an ability to germinate for a short while only. Longer periods of dark incubation induce a secondary dormancy which cannot be broken easily by light exposure or a lowering of temperature.

Burial and the effects of gaseous conditions.

These experiments suggest that a complex mechanism of secondary dormancy exists in A. thaliana and that it may be more easily induced in populations where the environmental stresses are greater. However, the demonstration of such a mechanism only is proof that it can operate under experimental conditions, not that it operates in the field. To ascertain if carry over of seeds could take place under field conditions, seeds were artificially buried in the 'Via Gellia' population in Derbyshire. In late April 1970 before the seeds had been dropped an area of ground, 50 x 50 inches, was cleared of plants, stones etc. and a piece of black polythene laid over the soil and firmly weighted down with stones, (figure B3 vi). In April 1972 before seed set occurred the polythene was removed and all the surface soil to a depth of $\frac{1}{2}$ cm was removed and brought back to the laboratory where it was dried and sieved through a 1mm mesh sieve. A total of 430.22 gms was obtained. A 25 gm sample was sprinkled thinly on a sheet of moist paper pulp in a polythene tray approximately 40 x 20 cms and the tray covered with plastic sheeting to reduce evaporation. The tray was placed in the refrigerated incubator set to the Buxton September regime for 10 days



Figure B 3vi. Site of carry over experiment at
Via Gellia, Derbyshire.

and then for 10 days in the October regime. After 20 days, 38 seeds of A. thaliana had germinated. This would give a rough estimate of 650 seeds capable of germination in the total soil sample. As this represents the seeds from only $\frac{1}{4}$ sq. metre this would indicate a considerable reserve of seeds in the soil in this population. This seed could only have come from plants flowering in 1969 or earlier and so have remained ungerminated for at least three years. In the field disturbance of the soil coupled with a suitable light and temperature regime and adequate moisture may be sufficient to cause germination of at least some of this buried seed reserve.

It is unlikely that temperature and light effects are the only factors causing secondary dormancy. Emergence of seedlings from different depths of soil is often variable. Cole (1957) found in Chenopodium album that the number of seedlings to emerge usually decreases with increasing depth of burial. Subsamples of 100 seeds of the 'Barry Sands' population of A. thaliana were buried at depths of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 5 cms in pots of compost and kept in a warm greenhouse at $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in continuous light. A control of 100 seeds was scattered on the surface of a further pot of compost. The seedlings that emerged in each pot were counted and removed until no further germination was observed, (Figure B3 vii). Burial even at 0.5 cm is effective in reducing emergence considerably. From below 1.5 cms

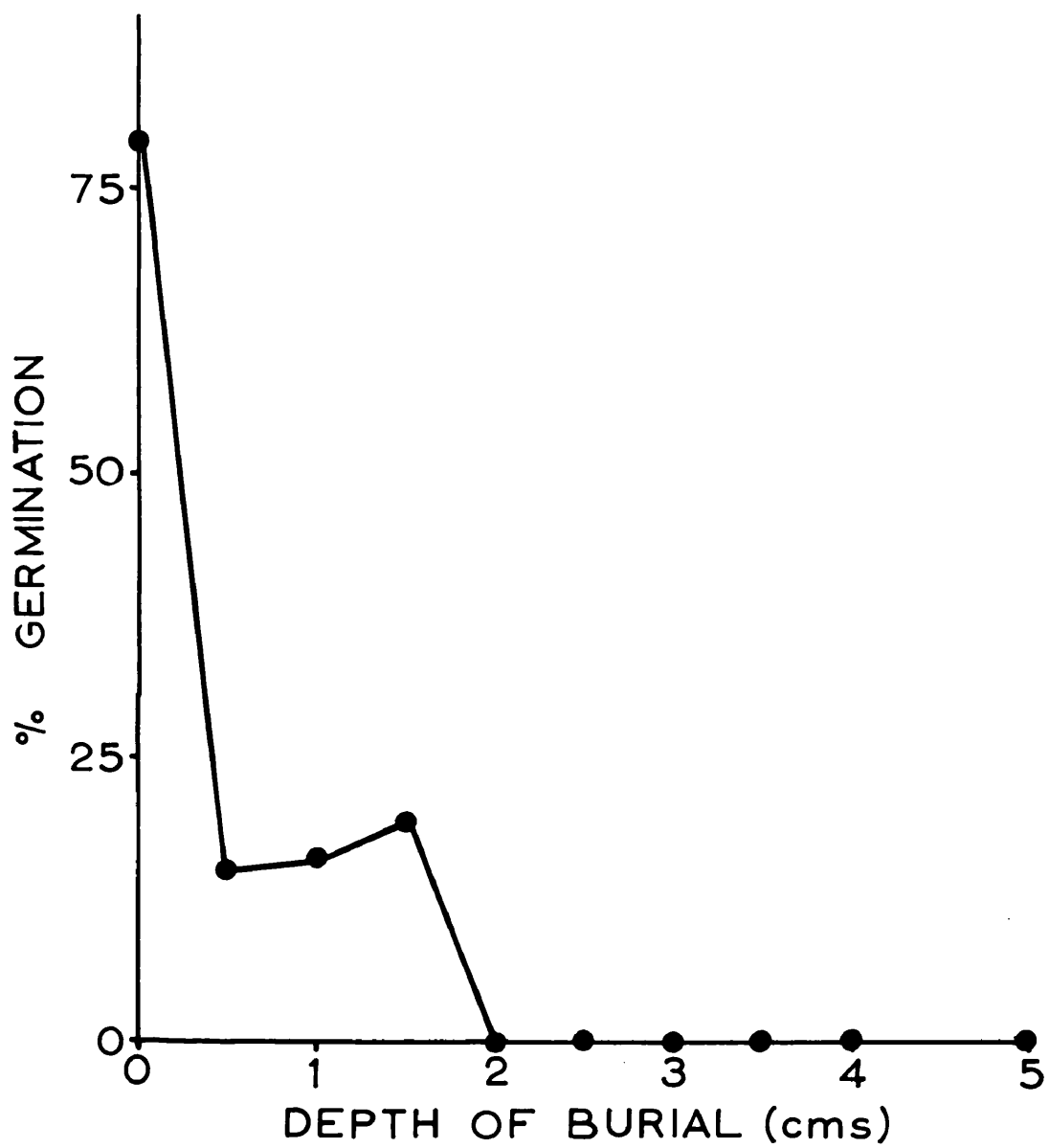


Figure B 3vii. Emergence of seedlings of
Barry Sands from different
depths of soil.

no emergence occurs. Subsequent examination showed that the failure of seedlings to emerge was due to failure to germinate rather than to starvation of the seedlings before they reached the surface. It is unlikely that the variation in light or temperature at the different depths is sufficient ^{to account} for this discrepancy.

It is possible however that the gaseous environment within the soil could affect germination. An earlier experiment had indicated that high carbon dioxide levels affected germination. Four subsamples of 100 seeds of 'St. Cyrus II' were placed on moist paper pulp in petri dishes. Two open dishes were placed in each of two dessicators. These were evacuated and filled with pure CO₂.

One was placed in an incubator at 10°C in continuous light, the other in an incubator at 25°C in continuous light. After one week the dishes were removed and exposed to the air. One dish from each dessicator was placed at 25°C and one at 10°C in the light. The final germination after a further two weeks was noted, (Table B3 vi).

<u>TABLE B3 vi</u>			
Temperature of Incubation at in CO ₂	Temperature of Subsequent incubation in air.	Final Percentage Germination	
		CO ₂ Pretreatment	
		<u>With</u>	<u>Without</u>
10	10	84	87
	25	98	100
25	10	10	87
	25	6	100

Exposure to CO₂ at the higher temperature was effective in reducing germination regardless of the subsequent temperature in air. It is possible that the increase in carbon dioxide with increasing depth of soil may affect germination at differing depths of burial. However inhibition^{bi} of germination may not be caused by high CO₂ levels but low oxygen levels.

Further experiments were carried out using the populations 'Barry Sands', 'Dirleton' and 'Husband's Bosworth'. Subsamples of 100 seeds of each population were buried at 1 and 5 cms in pots of compost or sprinkled on soil surface. The pots were placed on the window sill in a laboratory at 20 ± 3°C and the seedlings counted and removed as they appeared. When no further emergence had occurred for two weeks the soil containing the seeds buried at 1 and 5 cms was dug up and stirred before being placed in petri dishes and left on the window sill. Further emergence in these dishes was observed and the seedlings counted and removed. Controls of 100 seeds of each population were placed on moist paper pulp in petri dishes and kept in constant dark and in the diurnal light fluctuations experienced by the pots (Table B3 vii).

<u>TABLE B3 vii</u>					
Final Percentage Germination					
<u>COMPOST</u>				<u>PETRI DISH</u>	
Population	Soil Surface	Below Surface		Darkness	D/L
		1 cm	5 cms		
Barry Sands	90	7	0	97	96
Dirleton	78	0	0	84	94
Husband's Bosworth	92	0	0	31	83

Emergence was high for the samples on the soil surface in all populations. Only in 'Barry Sands' did any seedlings emerge from 1 cm. No seedlings emerged from 5 cms in any population. When these subsamples were exposed to the light some germination occurred. However, this did not reach the level of germination of the surface subsamples in any population suggesting that secondary dormancy had been induced in some seeds. The numbers of seeds apparently showing this secondary dormancy increased with depth of burial. Four subsamples of 100 seeds of these populations were also placed on sterilised moist paper pulp in Thunberg tubes. The tubes were evacuated and divided into two sets each containing 2 tubes from each populations. The first set was filled with pure CO₂ ; the others remaining evacuated giving a very low partial pressure of oxygen. In each set one tube from each population was placed in a light proof aluminium box. The other tube was placed in an incubator at 20°C in continuous light and opened after one week. The germinated seeds were counted and the seeds left exposed to the air. The other tubes were removed from the box

and placed in the light and the germinated seeds were counted. After one week the tubes were opened and the germinated seeds counted then and after a week's exposure to the air, (Table B3 viii).

<u>TABLE B3 viii</u> <u>Final Percentage Germination</u>						
Low partial pressure ϕO_2				CO_2		
Light $\longrightarrow$ Air				Light $\longrightarrow$ Air		
Barry Sands	92		100	0		12
Dirleton	36		76	0		8
Husband's Bosworth	0		76	0		36
Dark $\longrightarrow$ Light $\longrightarrow$ Air				Dark $\longrightarrow$ Light $\longrightarrow$ Air		
Barry Sands	52	52	72	0	0	0
Dirleton	0	0	12	0	0	0
Husbands Bosworth	0	0	40	0	0	36

It would appear that under conditions of a near vacuum in the light only 'Barry Sands' retains a high ability to germinate. However, subsequent exposure to air allows high germination in 'Dirleton' and 'Husband's Bosworth' also suggesting that the seeds have not become dormant but that there is just too little oxygen for germination. The ability of 'Barry Sands' to germinate with little oxygen may be related to its sand dune habitat where seeds will be frequently buried by drifting sand. In the dark 'Barry Sands' achieves over 50% germination, the other populations fail to germinate. No further germination is found when the seeds are exposed to

light suggesting that the inability to germinate is not lack of light but a combination of dark and low oxygen. Subsequent exposure to air increases germination in all populations but not to the levels achieved in the light indicating some degree of secondary dormancy has been induced by a combination of dark and low oxygen levels.

In neither light nor dark do seeds exposed to high CO₂ levels germinate. When the seeds incubated in light are exposed to the air some increase in germination occurs but the final germination is far below that found in the evacuated tubes suggesting high CO₂ levels may be more effective than low oxygen levels in inducing dormancy. The seeds incubated in the dark show no germination on subsequent exposure to light, again suggesting that the inability to germinate is not due to lack of a light stimulus. Subsequent exposure to air is only effective to a slight degree in 'Husband's Bosworth'.

There is some indication therefore that high CO₂ and to a lesser extent low oxygen levels can induce secondary dormancy in buried seeds and that this may explain differences in the numbers of seeds emerging from different depths of soil. These experiments are only preliminary but it would appear that light, temperature and soil atmosphere all interact to effectively induce secondary dormancy in a proportion of seeds in A. thaliana and so

bring about the carry over of seeds from year to year.

A further influence is suggested by MacDonough (1968). He found seeds of Phacelia tanacetifolia and Lactuca sativa underwent a loss of photosensitivity when imbibed in the dark for long periods especially at high temperatures. However, the extent of this was influenced by the substrate hydration. Low substrate hydration during the inhibitory dark period reduced the subsequent ability of the seeds to respond to conditions favourable for germination.

If such a mechanism exists in A. thaliana it is possible that burial during a hot dry summer would increase the level of secondary dormancy induced. This aspect would be worthy^{of} further investigation in the future.

B.4. Factors affecting vegetative growth

Establishment

Variation in seed size and weight have been reported in several species. For example, MacWilliams, Landers and Mahlstedt (1968) found a cline of decreasing seed weight from S. Central Canada to S. Illinois in Amaranthus retroflexus and a similar, though less pronounced, trend in European populations. They considered that a large seed weight would allow more rapid germination and establishment, owing to larger food reserves, thus being advantageous in more northern populations where the growing seasons were shorter. In the Antarctic species Chionochoa rigida, Mark (1965) found mean seed weight decreased with altitude.

Inspection^{of} seeds of various A. thaliana populations shows that seed size varies considerably from population to population. To quantify this, the length and breadth of ten seeds from eight populations; 'Barry Sands', 'St. Cyrus I' and 'II', 'Catterline', 'Soria', 'Edinburgh', 'Black Mount' and 'Creag an Lochan' and from two generations of 'Dirleton' seeds, were measured and the results subjected to an analysis of variance. The 'within population' variability was insignificant while between populations variability was highly significant, (Table B4 i).

TABLE B4 i

Analysis of VarianceSeed Breadth

Source of variation	df	SS	MS	F	P
Between populations	9	1273.9	141.54	6.25	0.001 ***
Within populations	9	127.3	14.14	0.62	N.S.
Remainder	81	1834.8	22.65		
Total	99	3236.0			

Seed length

Source of variation	df	SS	MS	F	P
Between populations	9	4475.9	497.3	31.24	0.001 ***
Within populations	9	313.4	34.8	2.18	0.5 - 0.1
Remainder	81	1289.9	15.92		
Total	99	6079.2			

Similar measurements were taken and analysed from the individual plant seed samples of 'Husband's Bosworth'. Neither within population or within sample variability was significant.

The two generations of 'Dirleton' are very similar despite one being obtained from the original locality and one being derived from plants grown in a greenhouse. This character would appear to be stable within populations and is probably genetically fixed.

Seed weight measurements were also made on three samples, each of 100 seeds, from sixteen populations, (Table B4 ii).

TABLE B4 ii

<u>Population</u>	Mean weight of 100 seeds (mgms)
Lodz	8.00
Creag an Lochan	27.67
Husband's Bosworth	7.33
Barry Sands	26.33
St. Cyrus I	39.67
Aberdeen	22.00
Dove Holes	12.00
Ochsenkopf	20.00
Dijon	23.00
Besançon	20.67
Burren	38.00
Stockholm I	26.33
Ben Dhorain	30.00
Mickle Fell	34.00
Soria	48.00
Parsley Hay	18.33

Within population differences were small but there was considerable variation between populations. However, there were no apparent correlations with any obvious environmental factor.

Although no apparent significance can be attributed to differences in seed size and weight it is obvious that these differences do exist. A possible relationship is with radicle growth over the first few days after germination.

Seeds from 23 populations were set out at 20°C in the standard way. Approximately 65 hours after imbibition

TABLE B4 iv

TABLE B4 iv

Population	Mean rate of radicle growth (mgms/hr x 10 ⁻²)		
	0 - 65	65 - 90	90 - 115 hrs
Barry Sands	2.18	4.59	5.70
Mickle Fell	0.70	5.82	6.35
Dirleton	1.29	3.91	4.47
Dove Holes	1.84	2.71	4.15
Lathkill Dale	2.00	2.33	3.60
Burren	1.19	4.47	4.70
Ochsenkopf	1.77	1.57	3.91
Longcliffe A	2.60	2.30	3.62
St. Cyrus I	1.76	5.58	5.58
Cobbler's Nook A	1.58	3.23	5.87
Stockholm I	2.59	5.32	6.37
Jug and Glass	1.84	3.21	3.59
St. Dogmael's Abbey	1.79	2.50	3.63
Deepdale	1.96	4.21	5.54
Catterline	1.96	6.42	7.50
Friden Crossing	2.31	4.12	5.03
Woebley Castle	1.82	3.18	5.71
Moorside	1.82	1.90	4.31
Besançon	1.77	1.76	3.53
Carsington Pasture	2.13	2.77	3.58
Aberdeen	0.83	3.41	4.18

the length of the radicles of 10 seeds which had germinated and which appeared to be healthy were measured beneath the microscope. This was repeated at approximately 90 and 115 hours after imbibition. The populations were measured in the same order each time so differences in time between measurements for each population were minimised. The absolute values obtained after 90 hours for those 23 populations were subjected to analysis of variance. Between population variance was significant at the 0.001 level, (Table B4 iii).

TABLE B4 iii
Analysis of Variance

Source of variation	df	SS	MS	F	P
Between populations	22	15.31	0.6959	4.4580	0.001
Within populations	124	19.36	0.1561		
Total	146	34.67			

The rates of growth from 0 - 65, 65 - 90 and 90 - 115 hours were calculated with the exception of 'Ben Dhorain' and 'Dijon', (Table B4 iv).

There was considerable variation in rate of radicle growth. Most populations from areas with microclimates dry in summer such as the Derbyshire populations; 'Besançon' and 'St. Dogmael's Abbey' had high initial rates of growth. The growth rates over the next two periods however, were not as high as those of populations from areas with cool damp summers and cold winters. Some of these populations had a low initial growth rate ('Mickle Fell' and 'Aberdeen'). Others^{had} a high initial rate

('Barry Sands' and 'Stockholm'). In the field two factors are likely to influence the growth rate of radicles, rainfall and temperature. A regression analysis of radicle growth rate between 65 - 115 hours after imbibition against mean October temperature and rainfall of each locality (where this could be obtained with accuracy) was made, (October was taken as the month when germination should be possible in all these localities with the possible exception of 'Burren'), (figure B4 i).

Relationships between greater rate of radicle growth ~~with~~ ^{and} higher rainfall and lower temperature are apparent. These regressions were subjected to an analysis of variance, (Table B4 v).

TABLE B4 v					
<u>Analysis of Variance</u>					
<u>Temperature</u>					
Source of variation	df	SS	MS	F	P
Explained by regression line	1	13.60	13.60	11.60	.005 - .001
Unexplained	17	20.12	1.18		**
Total	18	33.72			
<u>Rainfall</u>					
Source of variation	df	SS	MS	F	P
Explained by regression line	1	451.17	451.17	1.96	.25 - .10 NS
Unexplained	17	3881.99	228.35		
Total	18	4333.16			

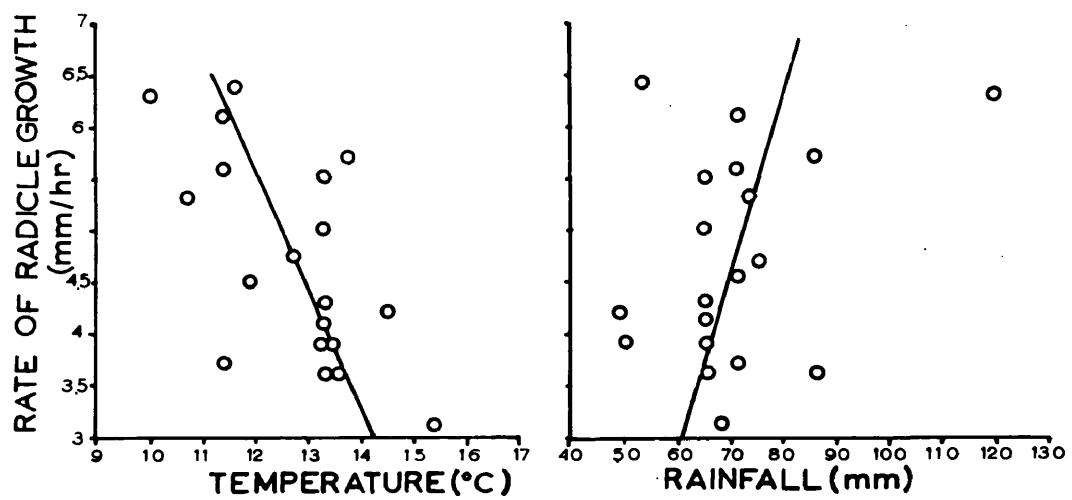


Figure B 4i. Regression of mean October temperature and rainfall on rate of radicle growth.

A significant relationship between mean October temperature and rate of radicle growth was shown. This kind of relationship must be treated with caution. However, it is entirely possible that each population is adapted to the particular conditions in its habitat at the time of germination even though dormancy mechanisms restrict germination to times when sufficient rainfall is available for establishment so that precipitation is unlikely to exert a strong selective pressure. However, rapid growth and establishment in areas where germination is soon followed by low temperatures would be advantageous, especially if flowering is late; in populations from areas where the active growing season extends into late autumn or early winter, high rates of radicle growth may not be of any great advantage.

Vegetative Growth

Experiments were carried out to see if the populations from areas with short growing seasons maintain higher growth rates at a later stage of vegetative development. Rate of leaf production was studied in six populations, 'Catterline', 'Lanark', 'Burren', 'Mickle Fell', 'Lodz' and 'Whatton'. Some seeds of each race were vernalised for six weeks at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in darkness. Then both vernalised and unvernalsed seed was scattered onto pots of compost. For each population three pots of unvernalsed and three of vernalised seed were prepared. The pots were

arranged in a Latin square (figure B4 ii) and placed in a Saxcil controlled environment cabinet at 19°C (Dew point 17°C), both with a photoperiod of 8 hours and in continuous light. The light intensity was similar to that for a bright winter's day. Once germination was complete the plants were thinned out in each pot to give a suitable number. The number of rosette leaves on each plant was counted at intervals of approximately five days and the mean leaf number per pot and per population calculated for unvernalsed and vernalised seed.

An analysis of variance carried out on the results of the first experiment showed no significant differences between the replicate rows of the Latin square (Table B4 vi).

TABLE B4 vi

Analysis of Variance

Source of variation	df	SS	MS	F	P	
Between rows AEC	2	0.7233	0.3617	0.0917	NS	
Between rows BDF	2	2.7902	1.3951	0.3539	NS	F. P.
Between groups unvernalsed	4	10.6675	2.6669	0.6764	NS	) 3.5337 NS
Between groups vernalised	5	47.1168	9.4234	2.3902	10.05	
Remainder	19	74.9078	3.9425			
Total	32	136.2056				

It was therefore unnecessary to move the pots around in the chamber. The 'Lanark' unvernalsed block was removed from the analysis as only one seed germinated.

Figure B 4ii.

Latin square.

Arrangement of pots in the Saxcil cabinet.

A	B	C	D	E	F
Cu	Bu	MFu	Lou	Wu	Lau
Bv	MFv	Lov	Wv	Lav	Cv
MFu	Lou	Wu	Lau	Cu	Bu
Lov	Wv	Lav	Cv	Bv	MFv
Wu	Lau	Cu	Bu	MFu	Lou
Lav	Cv	Bv	MFv	Lov	Wv

(C=Catterline, B=Burren, MF=Mickle Fell, Lo=Lodz,
W=Whatton, La=Lanark, u=unvernalised, v=vernalised.)

TABLE B4 vii

1 2 3 4 5 6 7 8 9 10 11 12

1 2 3 4 5 6 7 8 9 10 11 12

1 2 3 4 5 6 7 8 9 10 11 12

1 2 3 4 5 6 7 8 9 10 11 12

TABLE B4 vii

Population	Vernalisation	Mean Leaf Number			
		24 hours		8 hours	
		Flowering No.pts	Non-flowering No.pts	Non-Flowering	No.pts
Catterline	-	-	15.93	11.12	25
	+	16.00	17.50	9.50	2
Burren	-	-	18.19	10.60	20
	+	-	20.50	11.25	4
Lanark	-	-	19.00	5.00	1
	+	10.20	18.40	9.38	8
Mickle Fell	-	-	19.55	11.45	22
	+	12.00	20.33	9.00	4
Lodz	-	10.70	20.00	10.21	19
	+	12.50	-	9.00	8
Whetton	-	-	15.75	8.54	13
	+	13.27	15.67	10.82	11

Where some plants had flowered under a 24 hour photoperiod the numbers of leaves on vegetative and germinative plants were calculated separately, (Table B4 vii).

Under an 8 hours photoperiod (close to that experienced by winter annual races during most of their vegetative growth), the larger mean leaf numbers are shown by the populations from the areas likely to have the shortest growing seasons - 'Mickle Fell' and 'Catterline', indicating they maintain the higher growth rates suggested by the study of radicle growth. The 'Whatton' population, from a much milder area, and the probable summer annuals, 'Lodz' and 'Burren' have lower mean leaf numbers. It is possible that winter annual populations which experience shorter daylengths during their growth period are able to produce more leaves under short days than summer annual ones.

Under continuous light the winter annual populations 'Catterline' and 'Whatton' show small leaf numbers compared to the probable ^{Summer} annual populations. 'Mickle Fell' is the one exception and resembles 'Lodz' and 'Burren'. Perhaps in this population, where flowering probably does not occur until late summer, vegetative growth continues throughout the long days of spring and early summer and the population has a high growth rate under all daylengths.

Leaf number is only one measure of growth and may bear little relationship to leaf area or dry matter production so relative growth rate experiments were carried out with eleven populations to ascertain if differences in rates of dry matter production existed. Fifteen seedlings of each population were transplanted to individual pots of compost. The 165 pots were placed in a randomised block in a Saxcil cabinet at 20°C (Dew point 18°C) in continuous light. Three pots of each race were removed after 1, 2, 4, 5 and 6 weeks. The plants were dried at 25°C $\pm$ 2°C to constant weight. Complete results were obtained from only six races ('Oschenkopf', 'Besançon', 'Dove Holes', 'Mickle Fell', 'Barry Sands' and 'Stockholm') as some plants died. The relative growth rates between harvests for the six populations for which the results were complete were calculated according to the formula :

$$RGR = \frac{\log_e W_t - \log_e W_o}{t_1 - t_o}$$

Where W_t is the dry weight at time t_1 , and W_o the dry weight at time t_o . The results are given in Table B4 viii.

TABLE B4 viii				
Population	Relative growth rate (mgms dry weight/day)			
	1 - 2	2 - 4	4 - 5	5 - 6 weeks
Ochsenkopf	2.2465	1.0784	0.3524	0.4909
Besançon	2.8995	1.3428	0.0264	0.3029
Dove Holes	1.9249	1.2561	0.3976	0.7869
Mickle Fell	1.9505	1.5784	0.2199	0.2772
Barry Sands	1.8772	1.2848	0.4185	0.7938
Stockholm I	2.0397	1.5552	0.6672	0.0593

TABLE B4 ix

TABLE B4 ix

Population	Relative Growth Rate (mgms dry weight/day)	Leaf Area Ratio (mgms/cm ²)	Net Assimilation Rate (mgms/cm ² /day)
Ochsenkopf	2.2465	.4460	4.0276
Besançon	2.8995	.4010	4.7884
Dove Holes	1.9249	.3940	4.3354
Parsley Hay	2.1559	.5263	3.6797
Mickle Fell	1.9505	.4505	4.0629
Barry Sands	1.8772	.4142	4.1964
Creag an Lochan	2.9216	.4282	4.4191
Stockholm I	2.0397	.4485	3.9861
Soria	2.3884	.4025	4.5255
Lodz	6.4106	.5365	3.6134

The leaf area of plants harvested after 2 weeks was also measured. Leaf area ratios and net assimilation rates were calculated according to the formula :

LAR = Leaf area / dry weight,

$$\text{NAR} = \frac{W_2 - W_1}{t_2 - t_1} \bigg/ \frac{L_1 + L_2}{2}$$

Where W_2 and W_1 are dry weights, L_2 and L_1 leaf areas at time t_2 and t_1 respectively. In this case the leaf area at t_1 was negligible ^{ad}comparable to that at time t_2 and so was taken as zero, (Table B4 ix).

It can be seen that the differences observed in RGR were primarily due to variation in NAR and that LAR was similar between the populations.

An analysis of variance was made on the results at each harvest. At no harvest was there significant within population variance but differences between populations were significant, (Table B4 x).

Analysis of Variance

Week 1						
Source of variation	df	SS	MS	F	P	
Between populations	9	275.07	30.56	5.1335	.005 -	.001 **
Within populations	2	0.0167	0.0084	0.0014	NS	
Remainder	18	107.1533	5.953			
Total	29	382.24				
Week 2						
Between populations	7	303.037	43.291	4.7053	.01 -	.005
Within populations	2	4.1953	2.0976	0.2280	NS	
Remainder	14	128.8052	9.2004			
Total	23	436.0375				
Week 4						
Between populations	7	31820.5	4545.785	2.4245	.05 -	.02 *
Within populations	2	13136.4	6568.2	3.5031	NS	
Remainder	14	26249.4	1874.957			
Total	23	71206.3				
Week 5						
Between populations	7	40054.25	5722.04	3.5057	.025 -	.01 *
Within populations	2	8867.99	4434.00	2.7166	.25 -	.10 NS
Remainder	14	22850.71	1632.19			
Total	23	71772.95				
Week 6						
Between populations	9	282389.23	31376.58	5.8338	.001 ***	
Within populations	2	6309.44	3154.72	0.5865	NS	
Remainder	18	96812.06	5378.45			
Total	29	385510.73				

TABLE B4 x

The relative growth rate was high initially but tailed off to a low value between the 3rd and 4th harvest (4th and 5th harvest for 'Stockholm'). Results from the 6th harvest were not reliable due to the constantly recurring cultivation difficulties. The results are not clear cut but there is some indication that populations from areas with severe winters are adapted to shorter growing seasons by having high rates of assimilation. Populations from milder areas have slightly lower initial growth rates but maintain these rates for a longer time so that actual dry matter production is similar after the six week experimental period. The exception to this initial relative growth rate is 'Besançon' which had a high initial growth rate which fell very rapidly when the plant flowered. It is probable that populations which have no vernalisation requirement and which may complete the cycle from seedlings to flowering plants in a few weeks have high initial growth rates but produce much less dry matter before flowering.

Further experiments were designed to compare vegetative growth under differing light and temperature regimes. Eight populations were germinated at 20°C in continuous light. After ten days ten plants of each population were planted out in pots of compost and placed in the greenhouse at approximately 25°C $\pm$ 5°C, in daylight supplemented with mercury vapour lamps to give a 24 hour photoperiod. After four weeks the plants were transferred to the Saxcil cabinet which had a 16 hour photoperiod, a photo-

TABLE B4 xi

TABLE B4 xi

	Mickle Fell			Barry Sands		St. Dogmael's Abbey	
Temperature regime	27/20	27/4	20/14	27/20	27/14	27/20	27/14
Fresh weight (mgms)	254.01	157.64	31.7	216.45	81.14	187.50	80.47
Dry weight (mgms)	34.16	21.51	14.0	25.74	12.98	24.20	10.13
Rosette diameter (mm)	56.29	44.44	14.0	52.25	28.7	46.0	33.83
Leaf number	22.0	19.44	16.33	19.33	17.2	15.0	18.33
Leaf length (mm)	15.5	12.17	5.17	15.38	9.0	12.5	9.17
Leaf breadth (mm)	8.93	8.72	3.33	9.19	6.0	8.5	6.0
Leaf length/breadth	1.70	1.40	1.55	1.67	1.46	1.50	1.43
Total leaf area (cm ²)	12.68	7.88	1.20	10.92	3.54	8.68	3.37
Petrole length (mm)	12.21	11.17	3.17	10.5	6.6	9.5	7.33
Dry weight/fresh weight	0.13	0.14	0.40	0.12	0.16	0.13	0.12

period temperature of 27°C (Dew point 25°C) and a dark period temperature of 20°C (D.P. 18°C). Two weeks later measurements were made of fresh weight, dry weight, rosette diameter, leaf number, length, breadth, petiole length of longest leaf and total leaf area. The experiment was repeated with alternations of 27°C (25°C D.P.) with 14°C (D.P. 12°C) and 20°C (18°C D.P.) with 14°C (D.P. 12°C).

Unfortunately, as with the relative growth rate and leaf number experiments, many plants died. This was due in part to insect damage especially greenfly and to deficiencies in the compost which could not be sterilised or mixed efficiently at this time. Only one population 'Mickle Fell', survived in reasonable numbers (3 - 8 plants) in all three regimes. Several plants of 'Barry Sands' and 'St. Dogmael's Abbey' survived in the 27/20 and 20/14 regimes. The results are given in Table B4 xi.

All measurements were greater in the 27/20 regimes than in 27/14 indicating that the reduction of the night temperature had a distinct effect on vegetative growth. All measurements except leaf breadth were greatest in 'Mickle Fell' and showed least reduction with night temperature in this population. This high altitude population may have a high growth rate over a wide range of night temperatures. The 'Barry Sands' populations had higher measurements than 'St. Dogmael's Abbey' but these were much closer in the 27/14 regimes. The 'St.

Dogmael's' population had a greater leaf number in 27/14 than in 27/20. This was due to a greater number of smaller leaves. The weight and rosette diameter decreased.

In the 'Mickle Fell' population a drastic reduction in all measurements was achieved when the light temperature was dropped to 20°C. Perhaps reduction in night temperature will reduce vegetative growth by reducing translocation during the dark periods. Reduction of photoperiod temperature to a similar extent will severely cut down the assimilation rate and cause a large decrease in vegetative growth.

All upland or high latitude plants presumably need to have an adequate level of metabolic activity at lower temperatures in order that their dry matter production will not be severely restricted during a shorter growing season. In many species higher rates of photosynthesis and respiration have been recorded for northern and upland races. For example, Mooney and Billings (1961) found in Oxyria digyna that northern populations in N. America had higher respiration rates at all temperatures and higher photosynthetic rates at lower temperatures than southern populations. Bjorkmann, Florell and Holmgren (1960) found alpine populations of Solidago vigaurea had lower temperature optima for apparent photosynthesis than inland or maritime races.

It would appear that in *A. thaliana* winter annual populations from areas with cold winters have greater capacity for vegetative growth under all experimental conditions tested than populations from milder areas. Summer annual populations with much shorter life cycles may also have high growth rates. The fact that these differences are found despite acclimatisation for four weeks in constant conditions suggests that genetic variability is involved, though plasticity in vegetative growth is also likely. Further experiments with plants acclimatised under differing temperature regimes would be valuable. Measurements of absolute rates of respiration and photosynthesis would help to confirm these indications of differing metabolic rates.

Loginov and Usmanov (1972) have made preliminary studies on photosynthesis of *Arabidopsis* in Tajikistan. They found species and ecotypes from different vertical zones had very different assimilative capacities. The four populations of *A. thaliana* studied showed the greatest photosynthetic rate measured in mgms CO₂/g/hr at the experimental station closest in altitude to their original habitat. Optimal temperatures for photosynthesis at the intermediate altitude station were initially 22 - 26°C for the race from 1100m and 16 - 18°C for that from 3600m. After five generations the difference in optima had been reduced to 3 - 4°C. This suggests that differences in photosynthesis ability exist between *A. thaliana* populations but that they can adapt quickly to similar climatic conditions presumably by selection of favourable genotypes.

B.5. Factors affecting Flowering

In Britain Arabidopsis thaliana populations rarely flower in the autumn even in favourable conditions. It is possible that the growing season is too short to allow sufficient vegetative growth before flowering. However, if plants are grown in a warm greenhouse where conditions are very favourable for vegetative growth plants of most populations produce many leaves and large rosettes before showing bud development and some show no signs of flowering even after six months. It is unlikely that the shorter growing season experienced by winter annual populations is the main reason for their failure to flower in the autumn.

Populations from continental regions of Europe e.g., 'Strasbourg' and 'Lodz' may germinate in the field in the spring and flower in the summer. Plants from these populations flower readily within a month in a warm greenhouse in continuous light.

Autumn flowering in most winter annual populations would be disadvantageous as in most years early winter temperatures are too low for successful seed set. Autumn flowering would also upset the timing of the life cycle. It is unlikely that seed dormancy mechanisms within a population would be suitable for restricting germination in both winter and summer. In the putative summer annual populations from continental areas a short life cycle would be necessary in order for it to be completed before

the spring thaw when germination can occur and the advent of high summer temperatures and the likelihood of drought.

Many factors will affect time to flowering including time of germination and length of the vegetative growth period. Photoperiod and low temperature pretreatments are also known to directly influence flowering responses.

Photoperiodic effects

Gregory and Hussey (1953) found that a race of A. thaliana from near Cambridge flowered most rapidly in continuous light but flowering in short days was considerably delayed so this race at least could be considered a facultative long day plant. In their experiments plants given 8 hours photoperiod then 16 hours dark period remained vegetative for 60 days before buds became visible. When light of low intensity replaced the 16 hours dark period the time to bud formation decreased as the light intensity increased to 50 f.c. above which there was no further acceleration of flowering. Buds were then formed in 25 days. Assuming the photoperiod reaction of this race is representative of the species as a whole, the time to flowering will be relatively short even under short days; daylight is therefore unlikely to be directly involved in limiting its distribution. There may well be quantitative differences between populations but photoperiod is not likely to be a critical factor and so has not been given any attention.

Cold Treatment

As autumn germinating plants inevitably overwinter some degree of adaptation to the widely differing winter conditions over the range of the species is to be expected. In fact, Arabidopsis thaliana displays all degrees of low temperature requirement from none to absolute. Laibach (1940) considered that true winter annual populations (those with an absolute vernalisation requirement), were rare. He divided the populations into 'early', 'medium' and 'late' summer annuals, and winter annuals. The earliest races reached 'ripeness to flower' five days after germination; three days later the first initials could be seen. 'Later' races, even under the most favourable conditions produced more leaves before flowering.

Napp Zinn (1959, 1965) clarified the situation by classifying populations into :

- 1) Early summer annuals flowering in less than 40 days
(at 20°C and in continuous light of 500 f.c.)
- 2) Medium summer annuals flowering in 40 - 55 days.
- 3) Late summer annuals flowering in 55 - 92 days.
- 4) Winter annuals only flowering in more than 92 days.

Whether these types are winter annual or summer annual in the ecological sense would depend on their time of germination. Napp Zinn considered that the time to flowering in unvernalsed plants was correlated with their low temperature requirement.

One cannot consider separately the effect of temperature treatments on flowering in A. thaliana and photoperiod effects. Laibach (1951) found clear physiological differences between 'summer' and 'winter' annual types. He found that 'early' races could flower in a 4 hour photoperiod. 'Late' races could not. However, when a temperature of 0°C was imposed during the 20 hour dark period the 'late' races also flowered. In a 5 hour photoperiod the 'late' races flowered in a constant 23°C regime earlier than with nightly cooling, and also produced more leaves. With winter annuals there were few flowering plants even in constant light. The favourable factors for flowering were temperature dependant. Promoting factors could be replaced by cold treatments in winter annuals or with a shorter daylight at cooler than normal temperatures in 'late' summer annuals.

It would appear that autumn germinating populations, whether they are winter or summer annuals in the physiological sense are likely to flower most rapidly under long day conditions but that this effect may be, to a large extent, replaced by a low temperature pretreatment. Initiation of flowers of these autumn germinators must, in the field, take place in relatively short days. Long days may accelerate bud break and subsequent elongation of the flowering stem but it might be expected that more southerly populations would be less daylight sensitive than that more northerly ones i.e., be able to flower more rapidly in short days. This has not been studied experimentally.

Cetl and his colleagues studied a series of populations in Western Moravia. Plants were subjected to $25 \pm 3^{\circ}\text{C}$ in continuous light of 1250 lux, conditions which would induce flowering in the 'summer annual' types but inhibit it in the 'winter annuals'. After the plants had been growing for 42 days the percentage of generative plants was scored in each population. These were assumed to be summer annual types. Seventy-eight percent of the populations consisted wholly of 'summer' or 'winter' types, the remainder had a mixture of both. The boundary between the summer and winter types corresponded to the limit of the range of 'xerotherm' vegetation. The summer annuals were found in the 'cool, wet highlands' while the winter annuals were found in the 'warm, dry lowlands'. The mixed populations occurred at the boundaries between those climates. There was a significant positive correlation between percentage generative plants and height above sea level and a significant negative correlation between percentage generative plants and mean temperatures, especially with the monthly means for September and February - March. He considered that lack of summer annuals in the lowland region was probably connected with the danger of premature flowering in the autumn owing to lack of a low temperature requirement and the consequent loss of overwintering ability. There would be great selective pressure against summer annuals in the lowland region where autumn temperatures are high, (Cetl, 1965; Cetl, Dobrovolna and Effmertova, 1965, 1967).

The lack of autumn flowering in the British populations even when conditions ^awere particularly favourable, is very likely due to too short a period at low temperatures to bring about 'ripeness to flower' rather than to restriction of the growing season by lowering temperatures. This is certainly the case in Derbyshire where Ratcliffe (1957) has shown that flower initials are not produced until December. However, low temperatures would cause cessation or at least reduction of growth retarding flowering until warmer conditions and longer days arrived in the spring.

Effect of length of cold pretreatment

The variation of flowering times observed in the field could be due to varying cold requirements. Populations which do not flower until late summer, for example, the Scottish Highland populations, and that from 'Burren' could have very long cold requirements or they could be summer annual. Obviously detailed studies of low temperature requirements could shed light on the factors governing the differences in flowering time in the field. This low temperature requirement although usually experienced in the rosette stage in the field can be replaced by a similar treatment immediately after germination, (Napp Zinn, 1959, 1965).

Seeds of several populations of *A. thaliana* were subjected to varying lengths of cold treatment. They were set out under the standard conditions and left at room temperature for approximately 24 hours to allow

imbibition and the initiation of the respiratory processes necessary for vernalisation. The dishes were then transferred to a dark cold room maintained at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After vernalisation they were placed in a warm greenhouse or incubator at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and as the seedlings developed they were transplanted onto trays of compost and kept in the warm greenhouse at approximately 25°C in continuous light.

Table B5 i lists the number of days from transplanting to anthesis in several populations after 33 days vernalisation. It can be seen that flowering was earliest in the populations from areas with mild winters, 'Burren' being the only exception. The populations from areas which may be presumed to have very cold winters did not flower after 33 days vernalisation, except for 'Lodz' which flowered after 43 days. Only 'Mickle Fell' subsequently flowered, but only after 130 days.

TABLE B5 i

Days to anthesis after 33 days vernalisation	
Population	(figures in brackets are standard errors)
Lodz	43.14 ($\pm$ 0.59)
Lanark	53.00 ($\pm$ 1.54)
Woebley Castle	41.20 ($\pm$ 3.00)
Aberdeen	43.54 ($\pm$ 1.90)
Besançon	46.59 ($\pm$ 1.58)
Weeting Heath	52.00 ($\pm$ 1.62)
Whatton	43.33 ($\pm$ 1.34)
Burren	57.33 ($\pm$ 2.34)
Mickle Fell	130.67 ($\pm$ 2.33)
Hexham	60.00 ($\pm$ 2.00)

No flowering in six months in 'Creag an Lochan' or 'Ben Dhorain'.

Only a few races flowered within 40 days with no cold treatment. In 'Lodz' flowering was 22 - 23 days later after 33 days vernalisation than without a cold treatment indicating that vernalisation retarded flowering.

Again this population is very probably summer annual and spring germinating. 'Strasbourg' also flowers without vernalisation and may be another summer annual. 'Pontivy' and 'Lanark' are probably autumn germinating but may have a very short vernalisation requirement which is not absolute. Some plants of these populations flower without vernalisation.

Plants from 'Burren' and 'Whatton' did not survive in the greenhouse but some seedlings which had been left in the petri dishes also flowered without vernalisation. Due to the nutrient deficiency they must have been experiencing only the cotyledons and one pair of leaves appeared, before flowering occurred and only one silique with a few seeds was produced on each depauperate plant. The fact that 'Burren' can flower without vernalisation but took 57 days to anthesis after 33 days cold treatment suggests that it is another summer annual and so reinforces the evidence for this behaviour shown by the dormancy experiments.

Some British populations appear to be capable of flowering after short periods of vernalisation. These requirements could be satisfied early in the winter but low temperatures would delay bud break and anthesis until spring. Possibly in a year when the autumn was cold but followed by a period of warm weather flowering could occur in these populations. The rare occurrence of such conditions probably accounts for the rarity of autumn flowering.

Clark (1969) found in two other winter annuals, Erophila verna and Saxifraga tridactylites, growing in very similar habitats and often associated with A.thaliana that their vernalisation requirements were satisfied by mid November in the habitats studied. However, high temperatures were necessary for bud break and this prevented winter flowering.

Taking populations 'Aberdeen', 'Besançon' and 'St. Dogmael's Abbey' it can be seen that there was a decrease in number of days to anthesis with increasing length of low temperatures up to a point beyond which little further acceleration of flowering was observed (figure B5 i). This number of days to anthesis under optimal vernalisation conditions was very similar in all these races suggesting that a certain minimum time is required to complete the flowering process. This optimum length of cold treatment varied among the three populations from 2 - 4 weeks in 'St. Dogmael's' to 6 weeks or more in the other two populations where winter conditions are probably much more severe.

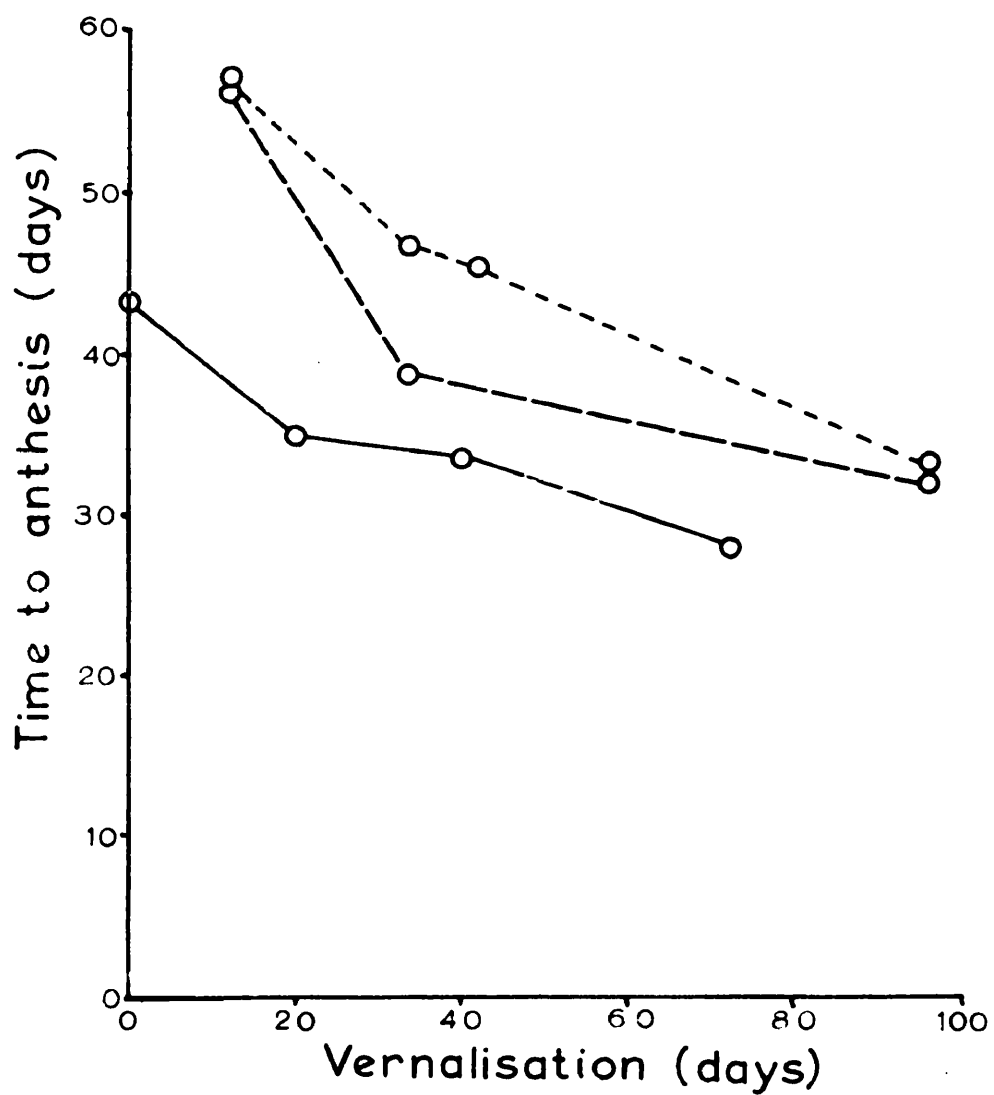


Figure B 5i. Effect of various lengths of vernalisation on time to anthesis in three populations.

— St. Dogmael's Abbey,
 - - - Aberdeen,
 - - - - Besançon.

Effectiveness of vernalisation temperature.

In the field in many localities mean winter temperatures as low as 2°C are relatively rare. It was considered necessary, to ascertain whether a higher temperature was as effective in inducing flowering. Seeds from seven populations were vernalised for four weeks at both 2°C and 5°C, grown in the warm greenhouse and days to anthesis measured, (Table B5 ii).

TABLE B5 ii

Days to anthesis after 28 days
vernalisation at

Population	2°C	5°C
St. Dogmael's Abbey	29.08	31.74
Stockholm III	43.67	37.14
Whatton	39.50	31.67
St. Cyrus II	52.80	45.50
Husband's Bosworth	42.50	37.50
Parsley Hay	46.00	41.09
Mickle Fell	65.00	72.00

In all but the 'Mickle Fell' population the differences between the 2 and 5°C treatments were very small e.g., 'St. Dogmael's Abbey' or flowering was more rapid at 5°C, perhaps due to faster development of the seedling once sufficient cold had been received. 'Mickle Fell' showed a 7 day reduction in time to anthesis at 2°C, perhaps because the four week vernalisation period at the higher temperature was too short for an optimal response.

It is probable, that given sufficient length of cold treatment, any temperature below 5°C is effective for vernalisation.

These experimental results clearly suggest that the length of the low temperature treatment required by A. thaliana populations is related to the local climatic conditions. Similar results have been found in other plants. Ketellapper (1960) found that the cold requirement of Phalaris tuberosa was closely related to the conditions in the place of origin of each population. He found that there was a significant negative correlation between the cold requirement needed to give 100% generative plants in his samples and the mean temperature of the coldest month in the original locality. The vernalisation requirement varied from almost none in an Algerian sample from 108m to 8 weeks or more for a Greek population from 400m. This is similar to the pattern in A. thaliana.

Ratcliffe (1965) considered the populations could be divided into four groups :

- 1) Mediterranean types - winter annuals with negligible or short cold requirements.
- 2) W. European types - winter annuals with a distinct cold requirement and post harvest dormancy.
- 3) Central European types - summer annuals, no cold requirement, varying post harvest dormancy depending on local conditions.

- 4) N. West European types - very long low temperature requirement, summer or late flowering winter annuals.

This classification tallies with Napp Zinn's division, into medium, late and early summer annuals and winter annuals.

This variation in vernalisation requirements coupled with the time of occurrence of suitable temperatures for bud break would produce the differences in flowering time observed among the autumn germinators.

In A. thaliana there appears to be a strong correlation between the 'oceanicity' of the climate and flowering date, (figure B5 ii). Details of flowering date and location were taken from herbaria specimens and the oceanicity of the climate for each record calculated according to the formula :

$$\text{Index of oceanicity} = \frac{N \text{ dt}}{100\Delta}$$

(Poore and Mackean, 1957)

Where N = precip. in mm
dt = no. of days with mean temp. bet. 0 - 10°C.

Δ = diff. bet. mean temp. of warmest and coldest months

Also it has been possible using the details of days to anthesis for all of Laibach's series of races (Robbelen, 1965) to divide these into early, medium and late summer annuals, and winter annuals and plot the occurrence of each flowering type geographically (figure B5 iii).

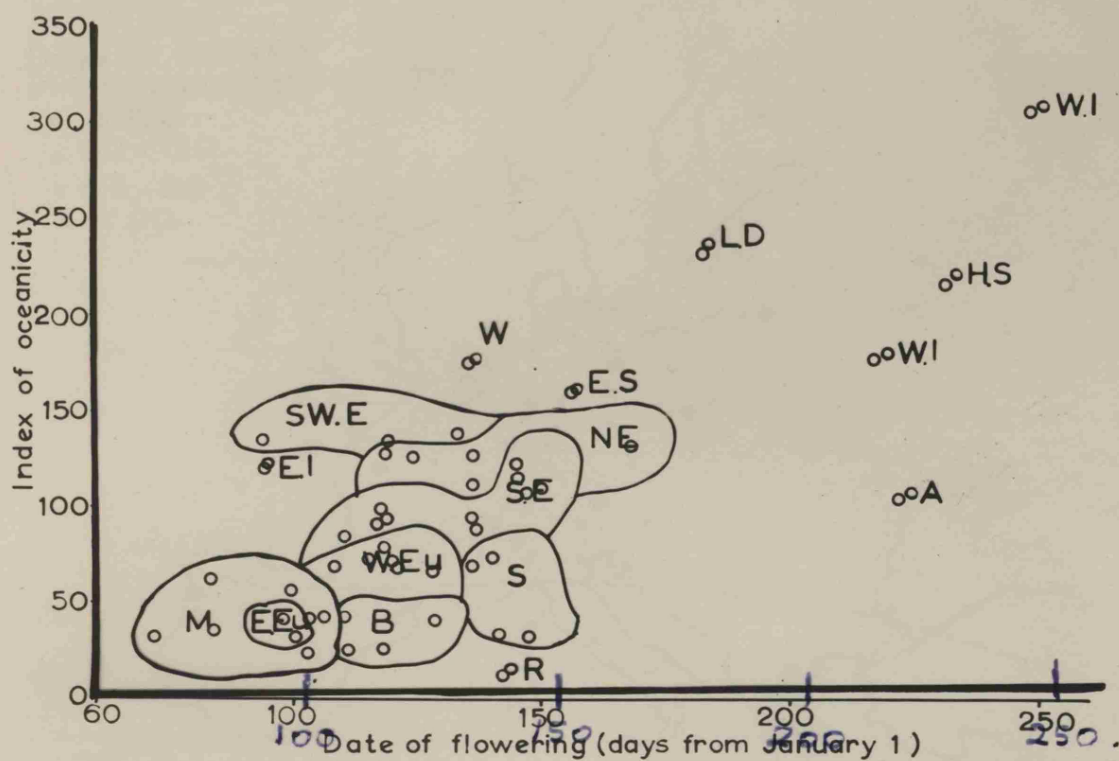


Figure B 5ii. Relationship between oceanicity and flowering time.

Overlay:- places of origin of sample groups,

M=Mediterranean, S=Scandinavia,
 E.Eu=Eastern Europe, B=Balkans,
 W.Eu=Western Europe, R=Russia,
 S.E.=Southern England, W=Wales,
 N.E.=Northern England, A=Austria,
 SW.E.=South West England,
 E.S.=Eastern Scotland,
 H.S.=Highland Scotland,
 L.D.=Lake District, E.I.=Eastern
 Ireland, W.I.=Western Ireland.

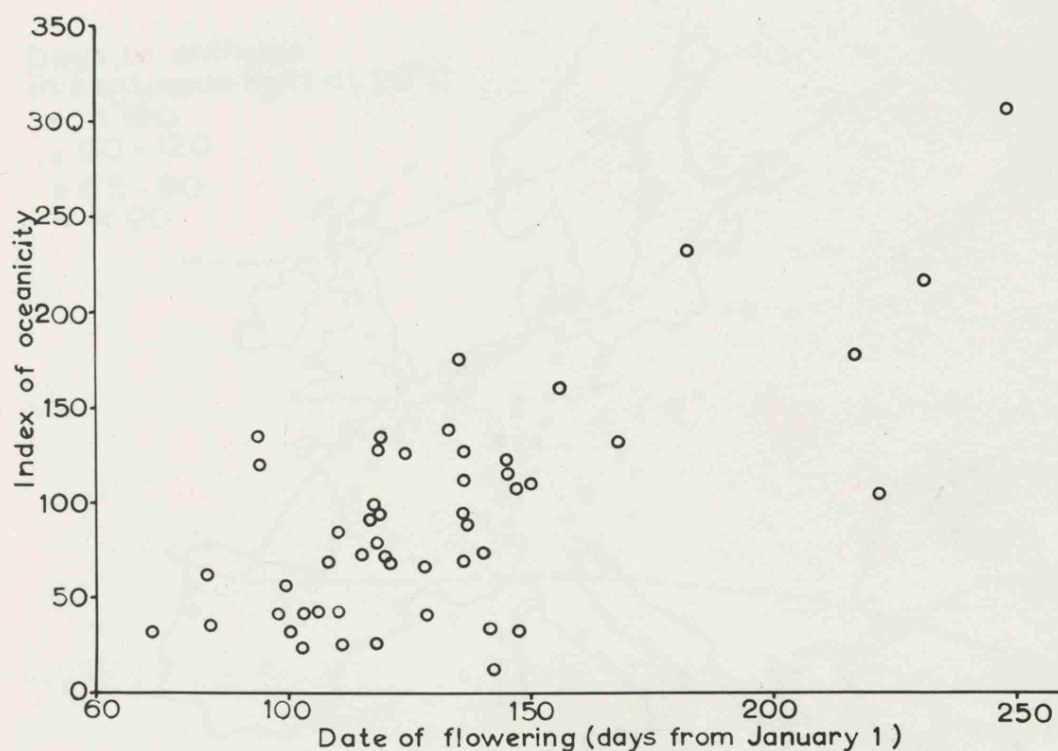


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 E.S.=Eastern Scotland,
 H.S.=Highland Scotland,
 L.D.=Lake District, E.I.=Eastern
 Ireland, W.I.=Western Ireland.

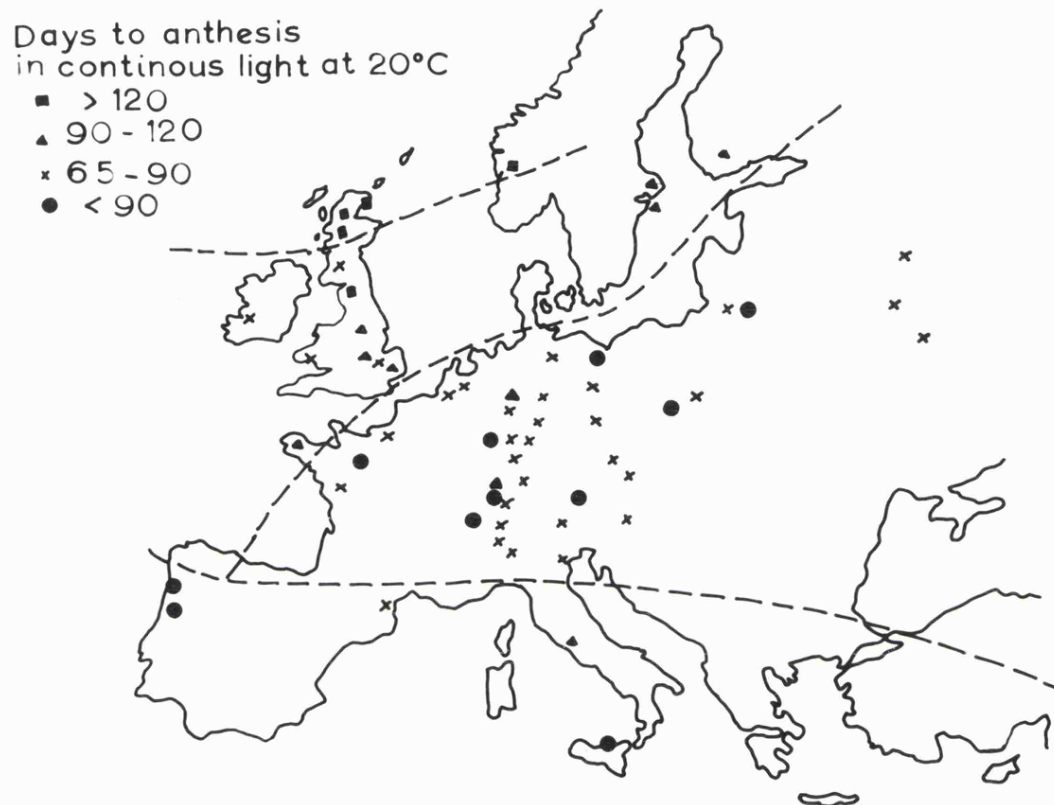


Figure B 5iii. Occurrence of 'early', 'medium' and 'late' summer annuals, and winter annuals in Europe.

The early summer annuals are in fact restricted to continental regions of Central Europe while the medium summer annuals occur around the Mediterranean. Late summer races occur in western Europe or at high altitudes in more southerly regions and the winter annual races are restricted to Scandinavia and northern Britain.

An analagous pattern was found in Holcus lanatus by Bocher and Larsen (1958). This species has a wide distribution range in West, Central and Southern Europe. Populations from the north are true ^{perenn}~~perenn~~ials. Populations from Southern Europe are usually short lived and are found in open or dry habitats. In Copenhagen they usually flowered in the first summer after germination. Populations in which a few plants flowered late in the first summer originated south-of a line from the English Channel to Yugoslavia. Populations from north east of this line flowered in their second year only.

In Derbyshire, Ratcliffe (1957) reported that most individuals within a population flowered almost simultaneously. In the vernalisation experiments little within-population variation was observed as is indicated by the standard errors of the means. Unlike germination the simultaneous flowering of all individuals in a population as soon as conditions become suitable would not be disadvantageous. While the flowering period is normally extended by the later production of axillary flowering stems, under unfavourable conditions these

secondary inflorescences will not develop until conditions improve. Unlike germination of an individual seed no all or nothing gamble is involved.

In general terms therefore it would appear that in most west and north west populations a low temperature pretreatment is a prerequisite for rapid flowering so that in combination with mechanisms which ensure autumn germination they are best fitted to a relatively mild winter in which rainfall is maximal. The Mediterranean populations are similar but with a suitably reduced low temperature requirement. Conversely, central European races may well be spring germinating types better fitted to the more severe winters and summer rainfall maximum prevalent there. These populations have no cold requirement for flowering and anthesis occurs after much less vegetative growth.

More information is necessary on actual time of germination in the field and possible dormancy mechanisms in the seed before the probable situation in Central Europe can be clearly expressed. The rarity of spring germinators in Britain where there are relatively warm mild autumn conditions may be due to selection against these types, as Cetyl found in Western Moravia^{vi.}.

This pattern in *A. thaliana* is paralleled in several other species. For example, in several therophytic Scrophulariaceae and Gentianaceae studied by Wettstein (1900), the early flowering spring germinating forms

showed reduced vegetative development and more restricted geographical and ecological distribution compared to the later autumnal types. He considered that the vernal forms were probably derived from the autumnal ones due to a loss of vernalisation requirements. Their development tended towards acceleration of differentiation of flowers at the expense of vegetative growth as a means of completing the life cycle in the shortest possible time. It is very likely that an analogous situation exists in Arabidopsis thaliana.

PART C Cultivation Experiments and Field Observations

C.1. Cultivation of populations under uniform conditions

Laboratory experiments provided clear indications of the mechanisms by which synchronisation of the life cycle of individual populations with local microclimate could be achieved. Nevertheless the demonstration, under experimental conditions, of possible mechanisms is not evidence of their operation in the field. In order to ascertain whether the laboratory findings were in fact reflected in field behaviour, **S**ixteen populations were grown in the open at the University Botanical gardens, both on the south and north side of an east-west orientated fence whose height was such that the plants on the north side never received direct sunlight at any time during the growing season, (figure C1 i).

The populations used were 'Barry Sands', 'St. Cyrus I', 'Burren', 'Dirleton', 'Bush', 'Mickle Fell', 'Ben Dhorain', 'Dijon', 'Besançon', 'Woebley Castle', 'Whatton', 'Husband's Bosworth', 'St. Dogmael's Abbey', 'Ochsenkopf' and 'Stockholm I'. The seeds were set out at 20°C in continuous light in an incubator and twenty - thirty seedlings of each population were transplanted into bowls (40 x 30 cms) of compost. The bowls were placed out of doors for seven weeks until the seedlings were well established and on 30 April 1971 transferred to the fence site where the bowls were buried so that the soil surfaces inside and outside were level.

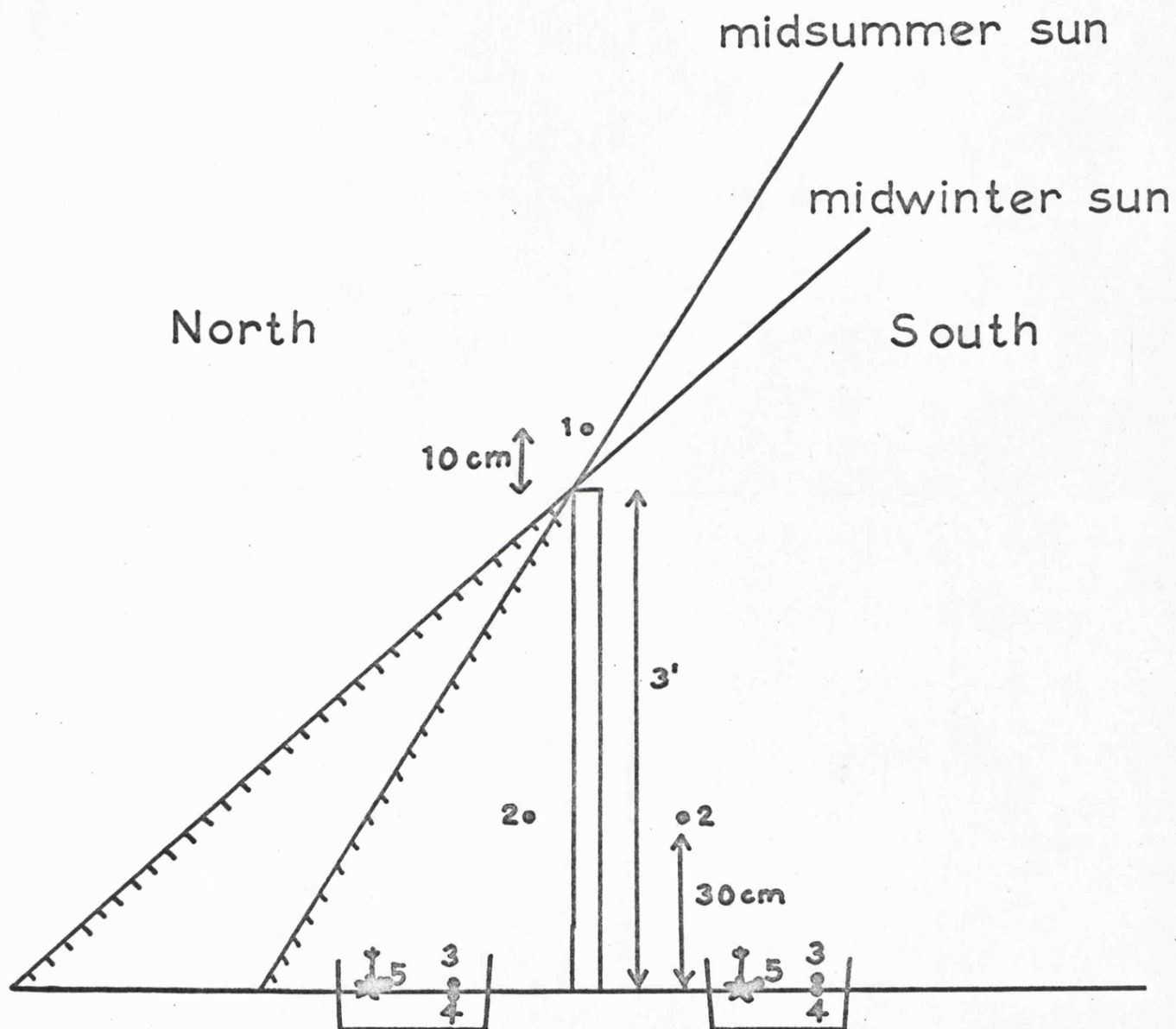


Figure C 1i. Diagram to show position of bowls,
fence and thermistors in garden
experiment.

TABLE C1 i

TABLE C1 i

Population	Mean Diameter *		Mean Flowering Stage*	
	North	South	North	South
Barry Sands	33.06	29.06	-4.41	-5.00
St. Cyrus I	31.79	17.69	-4.50	-5.00
Catterline	13.67	15.56	-5.00	-5.00
Burren	23.75	14.29	-5.00	-5.00
Dirleton	15.00	16.25	-5.00	-5.00
Bush	23.30	21.80	+3.17	+2.86
Mickle Fell	22.78	24.33	-5.00	-5.00
Ben Dhorain	21.00	23.50	-5.00	-5.00
Dijon	20.00	15.00	-3.12	-5.00
Besançon	21.20	23.33	+0.33	-0.06
Woebley Castle	18.82	19.29	+0.22	+0.43
Whetton	21.00	16.67	-0.10	-2.83
Stockholm I	18.57	24.17	-3.29	-5.00
Husband's Bosworth	20.00	25.00	-5.00	-3.24
St. Dogmael's Abbey	26.32	23.82	-2.39	-3.88
Ochsenkopf	24.67	23.24	-3.33	-5.00

- * Measured to the nearest 5mm
- ** according to Turesson's scale (1930)
- 5 no buds
- 4 unnoticeable buds
- 3 small buds
- 2 buds of medium size
- 1 large and swelling buds
- 0 anthesis
- +1 sparse flowering
- +2 ample flowering
- +3 abundant flowering
- +4 fading flowering
- +5 very much faded

Initial flowering behaviour

The diameter and flowering stage of each rosette was recorded when the bowls were placed in the garden, (Table C1 i).

At least five plants and usually more than fifteen survived in all bowls. The variation in diameter within and between populations and between the duplicate bowls was subjected to analysis of variance. Only between population variation was significant, (Table C1 ii).

TABLE C1 ii

Analysis of variance

Source of variation	df	SS	MS	F	P
Between populations	10	3398.64	339.86	8.69	0.001 **
Within populations	9	418.86	46.54	1.19	N.S.
Between north & south	1	28.37	28.37	0.73	N.S.
Remainder	200	7824.22	39.12		
Total	219	11670.09			

Many of the plants had already started to flower and anthesis had occurred in several individuals from the 'Bush', 'Besançon' and 'Woebley Castle' populations. These populations had been shown to have low vernalisation requirements in the greenhouse experiments. Many individuals from 'Dijon', 'Whatton', 'St. Dogmael's Abbey', 'Ochsenkopf' and 'Stockholm I' which had been shown to require 4 - 6 weeks cold treatment, were in bud but had not flowered. Almost all plants from 'Barry Sands', 'St. Cyrus I', 'Catterline', 'Mickle Fell', 'Ben Dhorain' and 'Husband's Bosworth' showed no sign

TABLE C1 iii

Population	Mean % increase in diameter				Mean flowering stage			
	North		South		North		South	
	7 May	28 May	7 May	28 May	7 May	28 May	7 May	28 May
Barry Sands	195.36	1.20	210.35	4.10	-3.18	-0.76	-1.94	+4.00
St. Cyrus I	134.88	8.48	328.81	33.15	+2.87	+4.00	+1.54	+4.00
Catterline	346.23	96.72	371.29	82.58	-3.4	+1.60	-2.67	+3.67
Burren	213.68	43.62	288.88	20.82	-0.25	+3.75	-0.29	+4.00
Dirleton	326.67	77.73	325.64	57.83	-2.00	+3.25	-1.67	+3.83
Bush	46.64	22.93	36.47	0.84	+4.00	+4.00	+4.00	+4.00
Mickle Fell	279.59	71.40	346.64	44.94	+4.77	+2.20	-3.29	+2.80
Ben Dhorain	177.06	38.57	295.74	30.11	-5.00	-5.00	-5.00	-4.56
Dijon	211.50	42.22	128.00	49.12	+3.00	+4.00	+4.00	+4.00
Besançon	141.83	20.29	110.54	26.33	+4.00	+4.00	+4.00	+4.00
Woebley Castle	108.55	29.94	62.00	-8.00	+4.00	+4.00	+4.00	+4.00
Whatton	80.00	9.91	78.46	5.04	+4.00	+4.00	+4.12	+5.00
Stockholm I	164.25	18.34	145.06	9.74	+3.93	+4.00	+4.00	+4.00
Husband's Bosworth	293.94	80.86	272.63	29.89	-2.63	-2.00	-0.94	+4.00
St. Dogmael's Abbey	242.94	20.76	248.45	6.63	+4.00	+4.00	+4.00	+4.00
Ochsenkopf	222.41	19.37	225.41	16.36	+3.85	+4.00	+3.94	+4.00

of flowering: their long ^{vernalisa}vegetation requirements had presumably not been satisfied during the initial seven week period out of doors. 'Burren' and 'Dirleton' have a very low cold requirement but did not show signs of flowering. These populations may require higher temperatures for floral development.

On 17 May and 28 May 1971 (19 and 30 days respectively after the start of the experiment) diameter and flowering stage were again recorded and the percentage increase in diameter calculated, (Table C1 iii).

Initially all populations on the south side grew more rapidly than those on the north, no doubt due to warmer conditions. Subsequently, the responses were more complex but there were three major trends.

- a) 'Early' populations (as their behaviour at the start of the experiments indicated) e.g., 'Bush', 'Woebley Castle', 'Besançon' and 'Dijon' showed small increases in vegetative growth : these plants flowered when the rosettes were relatively small. There was more vegetative growth and flowering was later on the north side.
- b) In 'Late' populations e.g., 'Barry Sands', 'St. Cyrus I', 'Mickle Fell' and 'Ben Dhorain' little flowering had been achieved (on either side) by 17 May and rosettes were larger on the south side.
- c) The 'Intermediate' populations e.g., 'Whetton', 'Ochsenkopf' and 'St. Dogmael's Abbey' showed little difference in percentage increase between sides, probably the increased rate of flowering on the

S. side balanced the reduction in growth due to cooler conditions on the north side.

This behaviour is exactly what would be expected from the differing low temperature requirements under experimental conditions (page 112). On 17 May all populations except 'Ben Dhorain' showed signs of flowering. 'Burren' and 'Dirleton' were in full flower indicating that their 'lateness' was due more to the low temperatures during March and April, than to inadequate cold treatment. On 28 May flowering was complete on the south side in all but the two latest populations, 'Mickle Fell' and 'Ben Dhorain'. On the north side several other late populations e.g., 'Husband's Bosworth', 'Barry Sands' and 'Catterline' were also delayed. 'Burren' and 'Dirleton' were also later on this side.

Obviously seven weeks in the open under Leicester conditions in March and April was sufficient to induce rapid flowering in the populations with low or intermediate low temperature requirements. However, the remaining populations (excluding 'Ben Dhorain') managed to achieve flowering by the end of May in this locality; their later flowering ~~much~~^{must} either be due to the need for longer periods at low temperature or for higher temperatures for floral development. In their original localities flowering may be delayed still further by the prevailing cooler summer temperatures resulting in the reported July and August flowering in the field.

TABLE C1 iv

TABLE C1 iv

Population	North		South	
	6 July	29 July	6 July	29 July
Barry Sands	0	0	0	0
St, Cyrus I	0	0	0	0
Catterline	0	0	0	0
Burren	0	1	0	1
Dirleton	0	0	0	0
Bush	4	5	5	5
Mickle Fell	0	1	0	1
Ben Dhorain	0	0	0	0
Dijon	0	0	0	0
Besançon	1	2	3	5
Woebley Castle	0	0	3	5
Whatton	4	5	4	5
Stockholm I	1	5	3	5
Husband's Bosworth	0	0	0	0
St. Dogmael's Abbey	0	0	0	0
Ochsenkopf	0	2	2	4

- 0 - No germination
1 - scattered, small rosettes
2 - several small - medium rosettes
3 - several medium - large rosettes
4 - many medium - large rosettes
5 - very abundant, mostly very large rosettes.

The 'Burren' seeds were originally collected in September and so had flowered very late. The lack of a long low-temperature requirement shown earlier, and the need for higher temperatures for 'bud break' makes it unlikely that flowering would be delayed for as long as this in the mild climate of Western Ireland if the population germinated in the autumn. Late flowering in this locality may well be due to spring germination.

Subsequent summer behaviour : Germination

Seed collections were made from each bowl on the 6 July 1971. Ripening had been delayed by the ^{un}usually cold and wet June in 1971. Sufficient siliquae were left on the plants to ensure an adequate seed supply to produce the next generation. As early as 6 July some germination was observed, the extent of this in each bowl was recorded on 6 July and 29 July, (Table C1 iv).

Although in the dormancy experiments a very low percentage of seeds ~~were~~ ^{was} capable of germinating after 1 month of storage several bowls had large numbers of seedlings. In those experiments low temperatures had been shown to be optimal for early germination and it is possible therefore that the low June temperatures were responsible for the germination in July, but germination was poorer on the north side. However, this latter behaviour may have been due to delayed after-ripening by the moist conditions.

Some of these populations which did not achieve germination even by 29 July had been shown to be early germinators. Nevertheless these populations were late flowering and so their lack of germination at this time is not necessarily anomalous.

Summer behaviour : flowering

In several of the early populations :- 'Bush', 'Whatton', 'Besançon' and 'Woebley Castle' this new generation had flowered and set seed by 29 July. Obviously these populations are much more flexible than would be supposed from laboratory experiments, for in a damp summer a second generation of plants may develop and go through their life cycle in 8 - 10 weeks.

Further progress

The further progress of these garden populations was carefully followed and at the same time temperature conditions were monitored at hourly intervals using a Grant 9 channel temperature recorder. Records were started on 30 September 1971. Type A thermistors, shielded from direct radiation where appropriate, were placed as indicated in figure C1 i. Temperature integrals in $^{\circ}\text{hrs}$ were calculated by the same method as has been described in Chapter B3. These measurements were continued until the end of May 1972. Unfortunately malfunctioning of the recorder caused several gaps in the records. The data are summarised in figures C1 ii - iv.

Figures C 1ii-iv. Climatic data recorded at the
University Botanic Gardens;
open circles represent S side
probes, closed circles N side
probes.

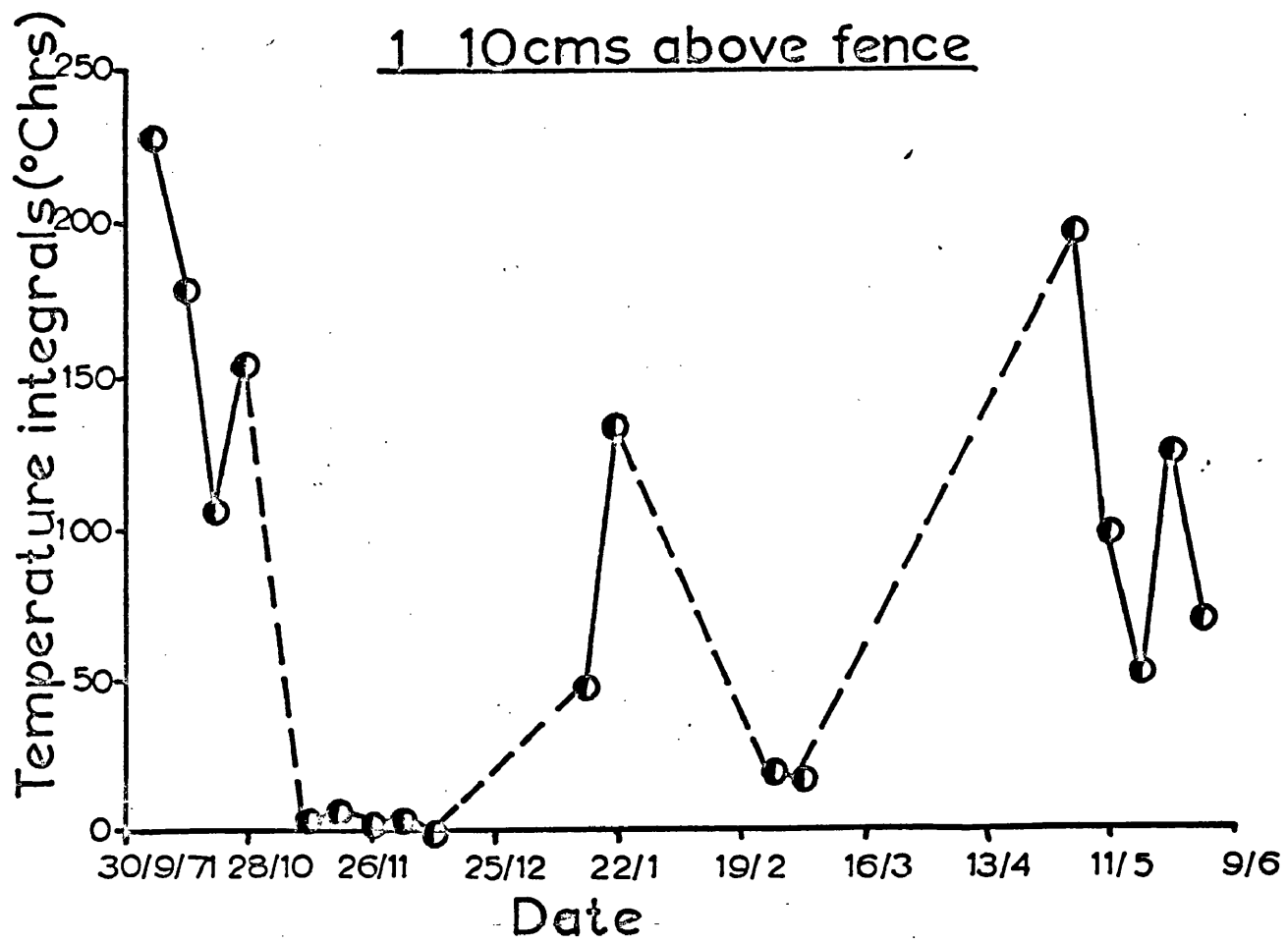


Figure C 1ii.

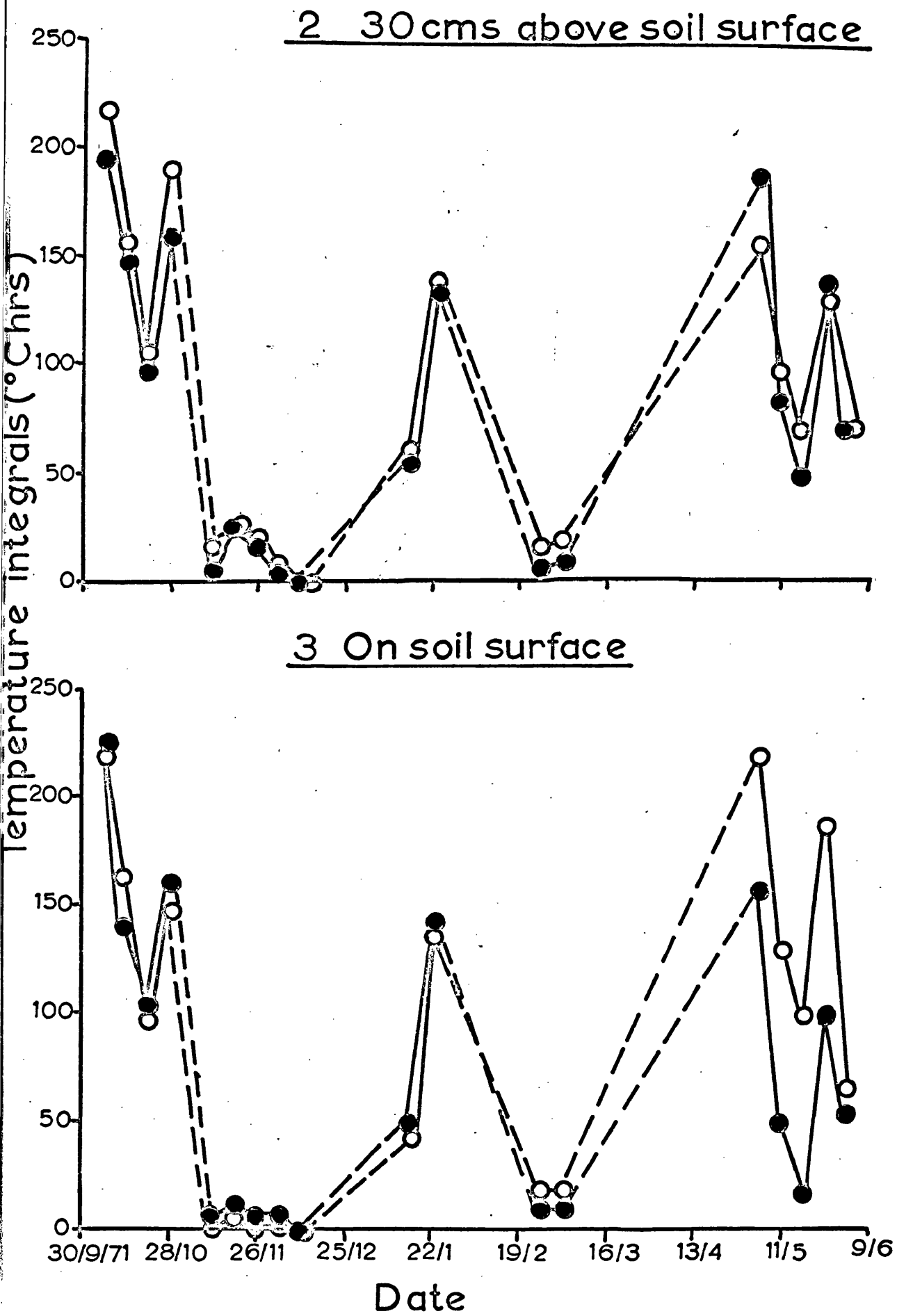


Figure C 1iii.

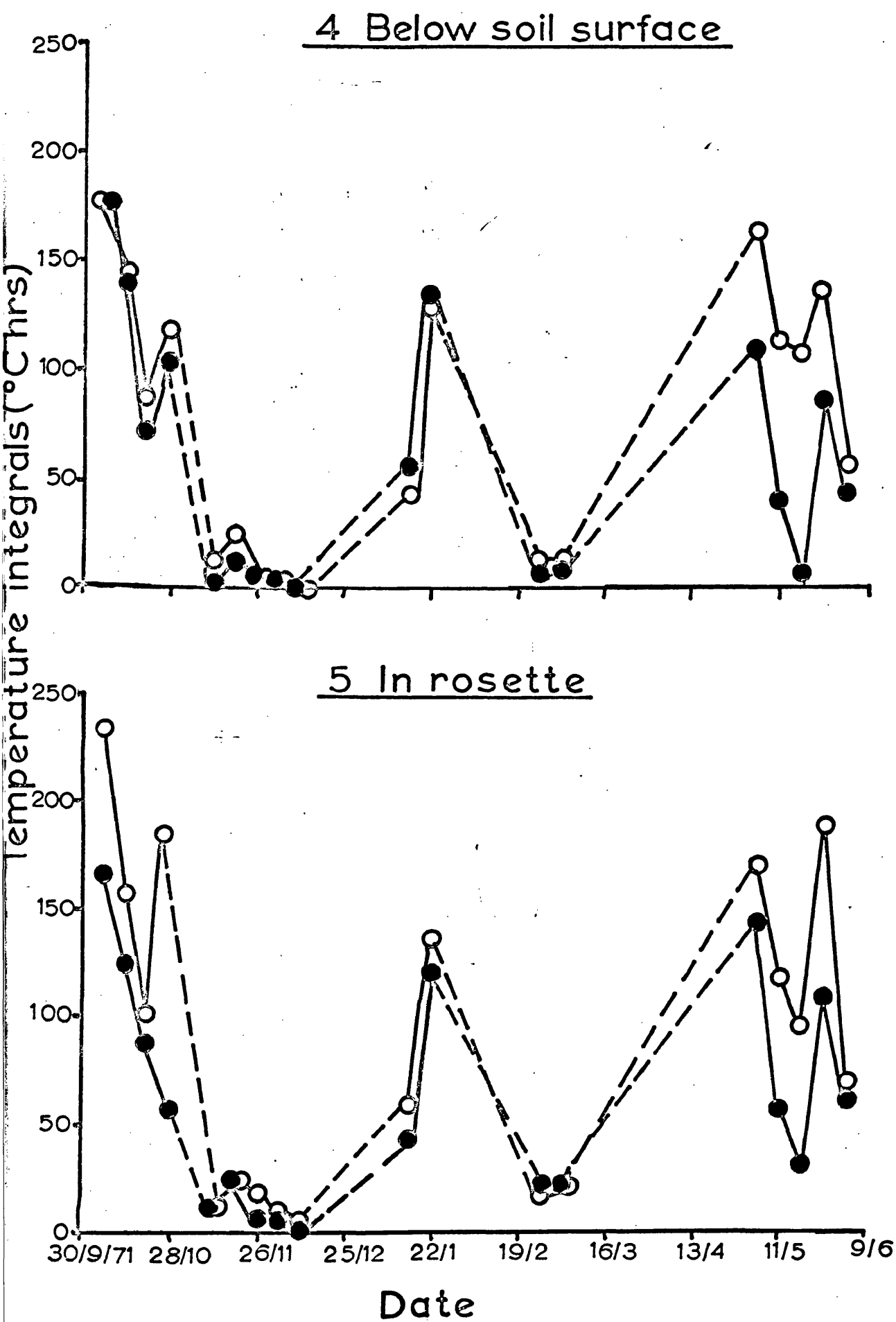


TABLE C1 v

Population	North	South
Barry Sands	Continuous germination. Abundant small - large rosettes	Occasional small - medium rosettes and fresh germination
St. Cyrus I	Occasional medium rosettes	Several medium - large rosettes and seedlings
Catterline	Very abundant small - medium rosettes	Continuous germination. Seedlings - very large rosettes
Burren	Numerous small - medium rosettes. All flowering	Seedlings - medium rosettes. All flowering
Dirleton	Medium - very large rosettes. Continuous germination, largest plants flowering	Seedlings - very large rosettes. Continuous germination, largest flowering
Bush	Seedlings - medium rosettes. No further flowering	Few medium rosettes. Abundant seedlings
Mickle Fell	Abundant medium - very large rosettes	Continuous germination. Seedlings - very large rosettes
Ben Dhorain	Seedlings recently germinated	Seedlings - very large rosettes - Continuous germination
Dijon	Fresh - very large rosettes. Continuous germination. Occasional flowering	Continuous germination. Numerous medium - large rosettes. Few seedlings. Occasional flowering.
Besançon	Seedlings - medium rosettes, occasional flowering	Seedlings - medium rosettes, occasional flowering
Woebley Castle	Medium - large rosettes and seedling Discontinuous germination	Medium - large rosettes and seedlings

/Continued...

Population	North	South
Whetton	Seedlings - very large rosettes - all but seedlings flowered	Seedlings - large rosettes Occasional flowering
Stockholm I	Occasional large rosettes and seedlings. Discontinuous germination	Occasional flowering. Seedlings - large rosettes - Continuous germination
Husband's Bosworth	Occasional small - medium rosettes and seedlings. Discontinuous germination	Continuous germination. Seedlings recently germinated
St. Dogmael's Abbey	Numerous small - medium rosettes	Occasional small - medium rosettes and seedlings
Ochsenkopf	Numerous small - large rosettes - very occasional flowering	Seedlings - very large rosettes continuous germination

TABLE C1 v (Continued)

Germination

The pattern of development in each population was noted on 30 September, (Table C1 v).

A burst of germination had occurred within the previous two weeks in many bowls. In others germination had been continuous throughout September. Some bowls showed evidence of discontinuous germination : Some large rosettes had obviously come from seeds germinating in early summer.

Flowering

Several populations were already flowering at this time. Almost all 'Burren' plants on both sides of the fence were in flower, indicating that spring germination and autumn flowering may well be the normal pattern in this population. A large proportion of the plants of 'Dirleton' and 'Dijon' populations were also flowering but in their original localities ~~would~~ cool conditions may delay bud break until the spring.

A large proportion of plants of 'Whatton', 'Besançon' and 'Woebly Castle' also flowered. Some of the larger plants had probably germinated in July but as many very small plants flowered a third generation of plants may have been produced. Under favourable conditions these populations may resemble some of the summer annual types described by Laibach (1943): the most extreme of these had a life cycle of 30 days and could produce 10 - 12 generations a year. Under field conditions dormancy

regulating mechanisms will be more critical in determining the yearly cycle of behaviour.

However, these results indicate that the very high dormancy found in laboratory experiments may not represent the total picture under field conditions. With the very large numbers of seeds produced by A. thaliana plants only a small percentage need be capable of early after-ripening to allow some summer germination. The considerable early germination observed was no doubt due in part to the cool damp weather experienced in 1971 in combination with poorly drained soil conditions. In most years the habitats where A. thaliana usually occurs would be too dry to allow germination. When it did occur plants would be killed by summer drought. However, the existence of a small proportion of 'opportunistic' seeds in some populations would suggest that summer germination may occur if conditions are favourable.

Winter behaviour

On 24 November ten plants from each bowl were brought back to the greenhouse and transplanted into trays of compost and kept at $25 \pm 5^{\circ}\text{C}$ in continuous light. Unfortunately many plants died but the days from transplanting to anthesis were recorded for the surviving plants (Table C1 vi).

TABLE C1 vi

Population	Mean time to anthesis (Days)	No. Plants
Whatton (South)	15.33	6
Dirleton (North)	24.83	6
Bush (South)	16.67	3
Stockholm (South)	28.60	5
Catterline (North)	39.00	2
(South)	32.00	1
Barry Sands (North)	36.00	2
St. Dogmael's Abbey (South)	29.00	1
Dijon (South)	21.00	4
St. Cyrus I (North)	36.00	2
Ochsenkopf (North)	30.00	1

The populations with short low temperature requirements, which had flowered early in the garden experiment, flowered within 22 days in the greenhouse. These included 'Whatton', 'Bush', and 'Besançon'. In these populations the cold temperature requirement can be considered to have been fully satisfied by this time. These were followed by populations intermediate in both cold requirement and time to flowering in the garden e.g., 'Dirleton', 'Stockholm I', 'St. Dogmael's' and 'Ochsenkopf'. The late flowering plants needing a long cold treatment e.g. 'Catterline', 'St. Cyrus I' and 'Barry Sands' took over 32 days to flower and presumably had not yet received sufficient cold. However, it would appear that floral initiation had taken place in all populations by this time.

As can be seen from the temperature integrals the weather was still relatively mild at this time. Flowering was still proceeding in 'Whatton', 'Dirleton', 'Besançon' and 'Burren'. Soon after this time temperatures dropped well below 5°C and flowering ceased.

During the last week in February, 1972 the bowls were brought back to the laboratory and photographed before being returned to the gardens. Germination had already occurred in some bowls (Table C1 vii).

TABLE C1 vii

Population	Germination *	24 February
	North	South
Barry Sands	0	0
St. Cyrus I	0	0
Catterline	0	0
Burren	1	1
Dirleton	0	0
Bush	5	2
Mickle Fell	0	0
Ben Dhorain	1	0
Dijon	0	0
Besançon	3	2
Woebly Castle	3	2
Whatton	0	1
Stockholm I	0	0
Husband's Bosworth	1	2
St. Dogmael's Abbey	0	1
Ochsenkopf	2	1

* See Table C1 iv

This was most evident in early flowering types e.g., 'Bush', 'Besançon' and 'Woebly Castle' again indicating that these may produce more than one generation each year. 'Burren' also showed germination, which was to be expected if this was a spring germinator. However, conditions were still cool at this time and seedlings

were few in number. Several of the late flowering populations showed some germination e.g., 'Ochsenkopf', 'Ben Dhorain' and 'Husband's Bosworth' suggesting some degree of seasonal dichotomy in germination within these populations.

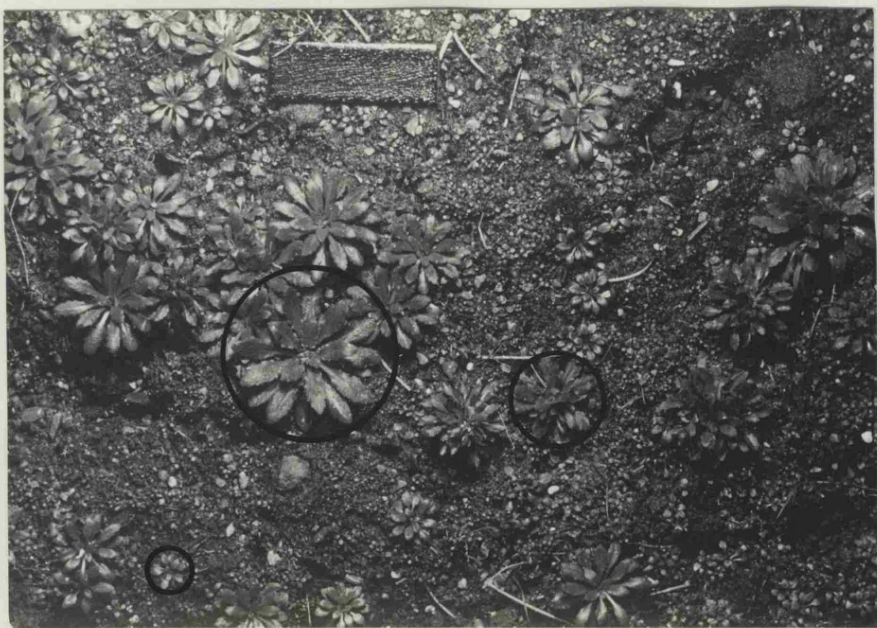
From the photographs it is obvious that large differences exist between the populations despite their being grown for a year in uniform conditions. This indicates that differences in behaviour are indeed genetically determined and heritable though no doubt some plasticity in expression of the life cycle exists.

Several populations show variation between the north and south sides (figures C1-vi). There was variation in numbers of plants ('St. Cyrus I', 'Dirleton' and 'Bush'); both numbers and size, ('Mickle Fell', 'Ben Dhorain', 'Woebley Castle', 'Whatton' and 'St. Dogmael's Abbey'). Little difference was seen between 'Burren', 'Catterline', 'Besançon', 'Stockholm I', 'Husband's Bosworth' and 'Ochsenkopf'. When differences in size were found, plants on the S. side were usually larger.

Large differences between populations are also evident, though populations from adjacent areas or those with similar microclimate are more alike, than those from different areas (figures C1^{vii}-viii). In general there is a gradual increase in size from the early flowering populations e.g., 'Bush', 'Woebley Castle' and 'Besançon' through to the late flowering 'Ben Dhorain' and 'Mickle Fell'. The causes of the variation in size and numbers

Figures C Iv-vi. Variation between plants of the same
population grown on the north and south
sides of the fence.

(Wooden markers in figures C Iv-viii
are 5cm long.)

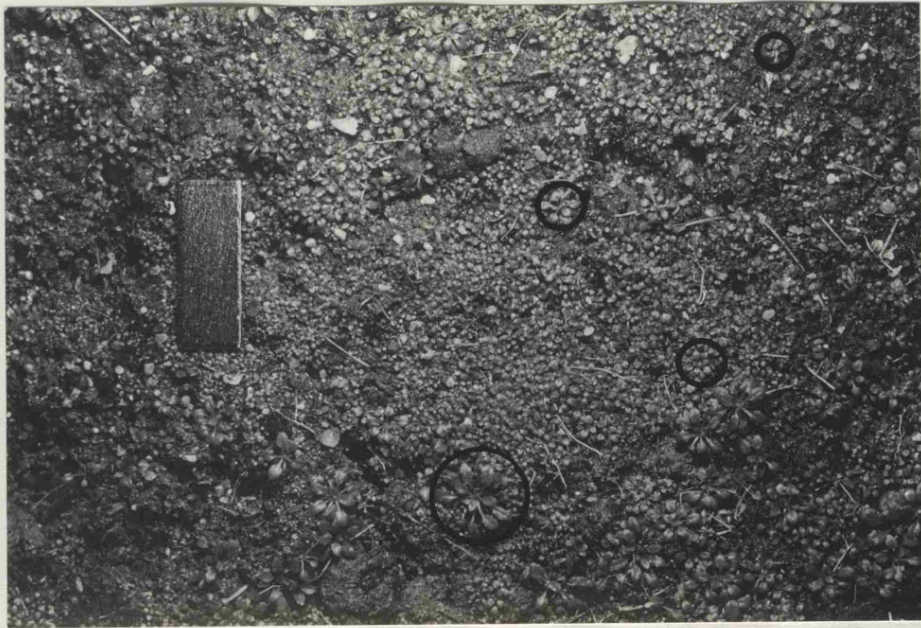


St. Cyrus South.



St. Cyrus North.

Figure C Iv. Variation in size of plants.

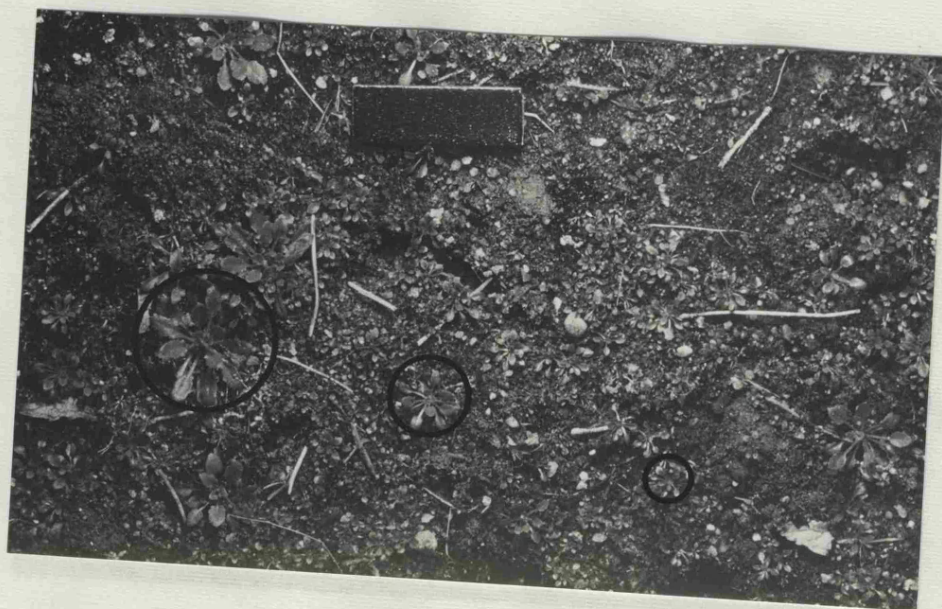


Woebley Castle South.



Woebley Castle North.

Figure C Ivi. Variation in size and number of plants.

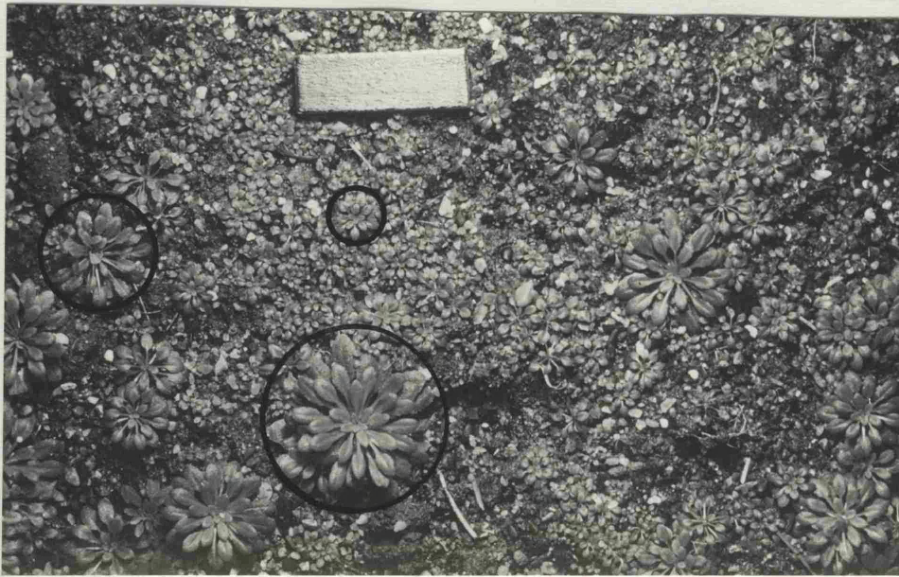


Besançon North.



Ochsenkopf North.

Figure C Ivii.



Mickle Fell South.



Catterline South.

Figure C Iviii.

of plants are probably complex and interrelated. Variation in germination time will probably be important in addition to rate of growth under low temperature conditions and vernalisation requirements.

On 20 March, after a week of above average temperatures many of the early populations had flowered e.g., 'Bush', 'Dijon', 'Besançon', 'Woebley Castle', 'Whatton', 'Dirleton' and 'St. Dogmael's Abbey'. Some plants of 'St. Cyrus I' had also flowered but these plants were dwarfed and branched from the base resembling plants grown in the greenhouse with insufficient cold. This was also true of several plants of 'Catterline' which had not yet reached anthesis. Flowering was delayed on the N. Side (Table C1 viii).

TABLE C1 viii

Populations	Range of flowering stages ** 20 March			
	North		South	
Barry Sands	-5	-4	-5	
St. Cyrus I	-5	-4	-3	+3
Catterline	-5	-4	-5	-3
Burren	-5	-4	-2	
Dirleton	-5	-3	+3	
Bush	-4	+1	-5	+2
Mickle Fell	-5	-4	-4	
Ben Dhorain	-5		-5	
Dijon	-2	+3	+0	+3
Besançon	-5	+1	-3	+2
Woebley Castle	-5	-3	+3	
Whatton	-4	+10	+0	+2
Stockholm I	-4	-1	-4	-3
Husband's Bosworth	-5		-5	
St. Dogmael's Abbey	-5	-4	-3	+3
Ochsenkopf	-5	-4	-5	-4

** See Table C1 i

By 2 May almost all the overwintered plants on the S side had flowered. On the N side flowering was less advanced especially in the later flowering types. The ability of the later flowering populations like 'Mickle Fell' and 'Ben Dhorain' to flower at this time would suggest that their delay of flowering until midsummer reported in the field is not entirely due to the long low temperature requirement as this was satisfied during the much milder Leicester winter, but also to the delaying effect of low spring temperatures in their original localities.

The cultivation experiments with A. thaliana have shown that the extrapolation of laboratory results to field conditions is not a clear cut process. To a large extent the populations maintained the patterns of variation they had displayed in laboratory experiments. However, several populations especially 'Bush', 'Whatton', 'Woebly Castle', 'Dijon' and 'Besançon' displayed the ability to respond to favourable conditions by germinating in spring, summer and autumn and by flowering in spring and again in autumn.

The first three populations are known to be weed populations. It would be informative to know the habitats types of the two continental ones. Similar polymorphic (sic) populations have been described by Jones (1971 b.c.) from railway tracks in Warwickshire.

These results emphasis the necessity of outdoor cultivation experiments for comparison with laboratory results if satisfactory explanations of field behaviour patterns are to be put forward. Moreover, it is imperative wherever possible to supplement this by field observations.

C.2. Field Observations

Although this was not primarily a field investigation, regular visits were made to observe the pattern of behaviour in Derbyshire populations, (the first II populations in Table B1 i). Altogether four natural populations from limestone screes and rock ledges and seven ruderal populations were observed. The ruderal populations occurred on a disused railway track along which altitude and annual rainfall gradually decreased from NW to SE. The localities of the populations varied therefore in habitat, aspect and microclimate, (see figure B2 v).

The first year of observation (1969 - 1970) was characterised in this area by a mild winter followed by a warm dry spring. In all localities seeds were being shed when the first collections were made in late June - early July, 1970.

Ratcliffe (1957) reported that during his observation of some of these sites in 1952 - 56, germination did not occur until the early autumn. However, in 1970, some germination was observed when the dry weather ended in mid July and rosettes of more than 20mm diameter were found in September. The early flowering and perhaps associated early after-ripening allowed by the dry weather, were probably responsible for this.

The cultivation experiments clearly showed only a small proportion of early ripening seeds need exist in a population to allow this 'exploitation' of favourable summer conditions. Ratcliffe had concentrated on the natural screes and rock ledges on limestone e.g., Dove Holes and Lathkill Dale. In 1970 these remained dry well into August and no germination occurred at these sites before late August. In fact, early germination was only observed in the ruderal populations on the ballast of the railway track where the fine cinder substrate remained moist following heavy rainfall in July.

Even where early germination occurred no autumn flowering was observed. This was to be expected as these populations have long low temperature requirements for flowering. On 24 April 1971 the height and flowering stage of at least 20 plants chosen at random were measured in seven Derbyshire populations, (Table C2 i).

TABLE C2 i

Population	Mean height (cm)	Mean Flowering stage **
Via Gellia	5.8 ($\pm$ 0.41)	+1.85 ($\pm$ 0.13)
Dove Holes	16.9 ($\pm$ 0.98)	+3.95 ($\pm$ 0.05)
Cobbler's Nook	4.2 ($\pm$ 0.47)	+3.00 ($\pm$ 0.21)
Moorside	7.1 ($\pm$ 0.74)	+2.73 ($\pm$ 0.36)
Friden Crossing	4.3 ($\pm$ 0.40)	+2.65 ($\pm$ 0.18)
Jug and Glass	4.6 ($\pm$ 0.43)	+3.00 ($\pm$ 0.10)
Lathkill Dale 'c'	5.1 ($\pm$ 0.92)	-1.20 ($\pm$ 0.46)

** see Table C1 i

Figures in brackets are standard errors of the mean

In both flowering time and plant height within population variation was insignificant while between population differences were highly significant (Table C2 ii).

<u>TABLE C2 ii</u>						
<u>Analysis of Variance</u>						
Source of variance	df	SS	MS	F	P	
<u>Plant height</u>						
Between populations	6	1414.55	235.76	13.23	0.001	**
Within populations	19	127.80	6.73	0.38	N.S	
Remainder	114	2030.75	17.81			
Total	139	3573.10				
<u>Flowering Stage</u>						
Between populations	6	279.60	46.60	26.03	0.001	**
Within populations	9	14.60	0.77	0.43	N.S	
Remainder	114	204.40	1.79			
Total	139	498.60				

The earliest population was on a sheltered and well insolated south east facing slope near Dove Holes. At this time flowering in the railway ballast populations was also well advanced but the 'Via Gellia' and 'Lathkill Dale' populations with north and north east aspects respectively, were still at an early stage of flowering. Within populations there was a tendency for the taller plants to reach anthesis earlier. However, between populations there was no such correlation. The height of individual plants probably depends on a combination of flowering physiology and microclimate.

In late June further collections of seeds were made. There had been no distinct period of drought following the mild winter and the month preceding collection had been much cooler and wetter than average. At this time the seeds were barely ripe and no germination was noted. The wet weather had probably delayed both flowering and after-ripening and, as far as is known, germination was more or less confined to the early autumn as no unusually large rosettes were present in December.

Further observations were made on March 30 1972. The warm weather which brought about early flowering of the populations in the cultivation experiments either did not occur or evidently did not have the same effect on the Derbyshire populations as little flowering was apparent. In a few plants buds could be seen e.g., at 'Carsington Pasture' (figure C2 i), but generally the plants were still vegetative. By mid June 1972 plants in 'Lathkill Dale' had flowered and set seed but the fruits were still very green, due no doubt to the cool damp June weather.

Germination usually takes place in the autumn but in ruderal populations on damper substrates some germination may occur earlier in a damp summer. Plants overwinter as rosettes and flower in spring. Plants within a population flower relatively simultaneously but the timing of this burst is affected by yearly climatic fluctuations and is delayed by cool damp conditions. These observations agree to a large extent with those of Ratcliffe (1957) especially with regard to natural populations.



Figure C 2i. Plant in bud at Carsington Pasture on 30 March 1971.



Figure C 2ii. Large numbers of very small plants at Friden Crossing on 30 March 1971.

The number of plants in a population may also vary considerably from year to year. In 1969 - 1970, the 'Friden Crossing' population consisted of 500 - 1000 plants but in spring 1971 less than 100 depauperate specimens could be found. These obviously represented a negligible proportion of the seed production from 1970. In 1972 the population consisted of many thousands of plants. Many were overcrowded and remained very small, (figure C2 ii). These plants were far too numerous to have arisen solely from the seed dropped in 1971. This is further evidence that carry over of seeds takes place in the field and that, if conditions are unfavourable in any year, a large number of seeds remains ungerminated. Not only does this mean that the population will not easily be wiped out but also that the selection pressure on seeds of widely differing germination physiology can be very great.

PART D Discussion

D. Discussion

This investigation is an attempt to clarify the relationship between a species and its environment by studying the probable adaptive characteristics of the plant and attempting to correlate these with the environment. This essentially, means a study of variation, predominantly between populations, of those physical characteristics which can be presumed, beyond reasonable doubt, to be of adaptive value.

The studies of Went and his colleagues on annuals of the Californian deserts (Went, 1948, 1949; Lewis and Went, 1945; Went and Westergaard, 1949) showed that the varied seasonal occurrence of these species was correlated with their optimum germination temperatures under experimental conditions and with field temperatures after rainfall in spring and autumn. This was surely a clear cut example of adaptive response. Ratcliffe (1957, 1961) showed that eight winter annual species including Arabidopsis thaliana, Saxifraga tridactylites and Erophila verna grew in the same type of habitat over a wide geographical range in Europe and had several physiological characteristics in common. These included the after-ripening requirements of the seeds, their optimum temperature for germination, the reaction of germination to light and dark, small size of seeds, and time of germination in the field. Time of vegetative growth in the field, vernalization requirements for floral initiation and the time of flowering were also found to be comparable. The occurrence of such similar patterns of development in species from different taxonomic groups certainly indicates that these major plant characteristics are adaptive.

Baskin and Baskin (1970, 1972), working in the U.S.A., have confirmed Ratcliffe's conclusions for some of his species including A. thaliana and E. verna and have shown additional adaptive features; they have demonstrated that the relationship between decreasing field temperatures in the autumn and the changes under experimental conditions in optimum germination temperature with increasing age of seed gives an additional control of germination time.

The works of Napp Zinn (1959, 1965), Cetl and his co-workers (Cetl, 1965 a,b) and more recently Jones (1968, 1971 a, b, c) have provided much information on the genetics of Arabidopsis thaliana.

It is apparent that A. thaliana is reaching the northern limit of its range in Britain and it might be expected that climatic differences within this country would have led to local differentiation of populations. The availability of seed samples from populations from a wide range of climates in Britain with some additional European material provided the opportunity to study the situation.

The most prominent seasonal pattern in A. thaliana is autumn germination, floral initiation in winter, spring flowering, seed set in early summer followed by post-harvest dormancy. However, deviations from this pattern do occur. Initially it will be convenient to consider different stages of the life cycle separately.

1) Time of flowering

The time of flowering and maturation of the seed directly affects the time of germination of the seed and so will be considered first.

On the whole, Ratcliffe's and Baskin and Baskin's picture of spring flowering and autumn germination for Arabidopsis thaliana has been confirmed in most of the populations investigated. However, there is some evidence for deviation from this pattern and this may be listed as follows:

(i) Some of the montane populations do not flower until late summer. This, coupled with relatively high optimum temperatures for germination, could indicate spring germination in at least some of the seeds.

(ii) The absence of a vernalization requirement in some of the continental populations, e.g., Strasbourg and Lodz, could indicate a pattern of spring germination and summer flowering. Additional work on germination and dormancy combined with field observations are necessary before the situation can be clarified in these populations.

(iii) The Burren population also appears to be spring germinating. This population is also very late flowering but unlike the montane populations requires very little or no vernalization. However, there is a prolonged post-harvest dormancy at low temperatures which would seem to preclude autumn or early winter germination. The very oceanic climate experienced by this population would make summer drought

extremely unlikely and vegetative growth throughout the summer would be possible. However as the seed sample from this population was small, further investigation would be preferable before any final conclusions are reached. Nevertheless there is other supporting evidence, as follows:

It was also found that cold pretreatment of imbibed seeds delayed flowering in the Burren and Lodz populations (p. 114). This was the reverse of the situation in the populations requiring vernalization, especially the montane ones. This is consistent with the probability of spring germination in the Burren and Lodz populations.

Cultivation of plants in the botanical gardens helped to confirm laboratory results as the variation in flowering time between the populations was as predicted from experimental data (Table Cliii, p. 127). The coastal and lowland populations tended to flower first, e.g., Dirleton and Bush, while the montane populations, e.g., Mickle Fell and Ben Dhorain, were late flowering. Despite an unusually damp summer the main burst of germination was still autumnal in most populations though in some of the lowland populations this occurred in late July (Table Cliv, p. 128). The planting of rosettes of the Burren race in spring no doubt led to the phenomenon of early flowering in this race. However the subsequent germination under moist warm conditions and second flowering in the autumn observed in this race would be in keeping with the experimental data on dormancy and flowering responses.

Transfer of plants from the gardens to the greenhouse in November also confirmed the laboratory results. Early flowering populations with short cold temperature requirements flowered rapidly indicating that their vernalization requirements were fully satisfied by this time. Populations intermediate in flowering on the basis of both the laboratory and garden experiments flowered next followed by the late flowering populations requiring long cold treatments. Possibly the late flowering populations had not received sufficient cold treatment for rapid flowering by late November, although floral initiation had obviously taken place in all these plants (Table Clvi, p. 135). The fact that the relatively mild Leicester winter satisfied the low temperature requirement of even the most montane populations - Mickle Fell and Ben Dhorain - is perhaps to be expected, as they must be able to flower even after the mildest winter (for autumn germinating plants) or the spring and summer conditions must be sufficiently cool to satisfy their vernalization requirements (after spring germination).

Thus, with the exception of the Burren population, in Britain winter annual populations are found in the lowlands and coastal areas where high autumn temperatures could lead to premature flowering if vernalization was not necessary. Montane populations require very long periods of cold pre-treatment. They may be spring germinating, however, and so the pattern of summer living populations in the uplands and overwintering populations in the lowlands may well be the same in both Britain and Western Moravia as described by Cetl (Cetl, 1965; Cetl, Dobrovolna and Effmertova, 1965,

1967, p. 111). From Briquet's data (Briquet, 1913) on flowering times in Corsica a similar situation may well exist there. At sea level plants were found flowering on 25 January indicating that these plants were overwintering, while at 2050m flowering did not take place until 30 July, which might indicate the existence of summer annual populations. Whether or not these populations require cold pretreatment for flowering would naturally have to be ascertained by experimental methods.

Insufficient data are available from Central European populations to allow any conclusions to be reached on their flowering behaviour though similar patterns of variation may exist.

2) Vegetative growth

Similar regional variation was apparent when vegetative growth was investigated.

(i) Rates of radicle growth were greater in upland populations from areas with cool damp summers and cold winters than in lowland populations (Table B4iv, p. 97). Studies of leaf production also indicated higher rates in upland populations (Table B4vii, p. 97).

(ii) Investigations of relative growth rates indicated that montane populations have higher initial growth rates than more lowland populations but maintain these for a shorter time period. Actual dry matter production was similar after a six week experimental period (Table B4ix, p. 102).

(iii) In another experiment when vegetative growth was compared under three alternating temperature regimes the individuals from montane populations showed greater vegetative growth in all temperature regimes (Table B4xi, p. 104).

All these results indicate that montane populations which experience short growing seasons and cold winters are adapted to these conditions by having high rates of vegetative growth. Lower growth rates were observed in lowland and coastal populations where the winters are milder and the growing season extends at least until early winter. Hypothetically the highest rates of vegetative growth would be expected in winter annual populations from more continental areas where the changeover from summer drought conditions to winter cold is rapid. Further investigation is necessary here.

No measurements of metabolic activity were made in this investigation. However, Loginov and Usmanov (1972) showed that differences in photosynthetic ability existed in A. thaliana populations from Tajikistan where more lowland populations had much higher optimum temperatures for photosynthesis than montane populations.

The overall picture of vegetative growth and flowering in Arabidopsis thaliana clearly indicates adaptive variation in relation to climatic factors, especially winter conditions. Flowering is earliest in the eastern and southern parts of the range and gets progressively later towards the west and north. Similarly, populations requiring little or no

vernalization are found in the continental and mediterranean areas while the vernalization requirements of other populations increase towards the more maritime and montane areas. Superimposed on this pattern is altitudinal variation with summer living populations being found at higher altitudes. These populations may (Britain) or may not (Western Moravia) require cold pretreatment for flowering and this again may be an adaptive response to the differing climates in different montane areas.

3) Germination and Dormancy

Investigation of dormancy and germination responses indicate a much more complex situation than exists in the flowering process.

One major problem involves the collection of seed samples for such experiments. Seed collections are intended to represent the natural seed rain and should include all variation of genotype and phenotype even if in different proportions from the total seed production. The usual occasion for seed ripening in the field is summer drought. When this occurs all mature seeds on the plant ripen rapidly and further flowering ceases. In laboratory experiments plants grown for seed were well watered until they had flowered profusely and produced fruits. When watering ceased ripening of the pods was rapid and almost all seeds from a particular plant could be harvested. This technique was felt to approximate as closely as possible to seed ripening in the field. Using the seed from field and laboratory collections dormancy and germination experiments

were carried out and variation in quantifiable characteristics was studied at three levels: geographical, interpopulational and intrapopulational.

(i) Post-harvest dormancy

When lengths of post-harvest dormancy were studied it was found that populations from the Derbyshire railway tracks and limestone ledges had faster rates of after-ripening, and shorter post-harvest dormancy, than either ruderal populations of the Scottish coasts or Midland ruderal populations, both of which may well be more likely to suffer summer drought due to lower summer rainfall (Figure B2iv, pp. 27-29).

Within the Derbyshire populations variation in length of post-harvest dormancy also exists: populations from the cooler, wetter, north-western sites remained dormant longer than the south-eastern populations. This could be due to later flowering and seed maturation in the north-western populations as these experiments were carried out on field seed collections. Dormancy was short in all Derbyshire populations and very few dormant seeds were found after two months. In the natural Derbyshire habitats, however, no early germination was ever observed even in wet summers. Probably the shallow soil, good drainage and high insolation in these sites themselves preclude germination until heavy rains occur in the autumn. In the more poorly drained railway sites wider variation in germination time was observed.

Investigation of the seed samples in the Husbands Bosworth populations gave evidence of intrapopulation variability,

as differences in dormancy were observed even amongst the seeds obtained from a single plant. However, taking the plant samples together three patterns of dormancy were observed in this population and these may well represent genotypic variation (Figures B2vi-viii, between pp. 30-31).

Differences in the germination patterns of newly harvested seeds and seeds a few months old may also indicate some genetic variability.

Further evidence of variability is provided by the spread of time of germination from first imbibition which usually becomes more normal (statistically speaking) as after-ripening proceeds. However, in the bulk sample from the Husbands Bosworth population a normal distribution curve for germination was never achieved even in fully after-ripened seeds. This undoubtedly indicates some genetic heterozygosity and reinforces the evidence for genetic variability indicated in the individual plant samples from this population.

Variation of this nature could obviously be important in ensuring that not all seeds are 'used up' in one attempt of establishment.

The normal situation is that where there is prolonged rain at the end of summer, the shallow soil of most *A. thaliana* sites becomes moist and a characteristic burst of germination takes place. However, where this autumn change in soil moisture level is not so clear-cut the presence of early

germinating and late germinating seeds may be of prime importance: The early germinators allow for opportunism in a favourable year, the late germinators ensure that 'all eggs are not in one basket' in a variable climate.

(ii) Optimum germination temperature

The period of absolute dormancy is quite short in most populations. After this has been completed the optimum germination temperature changes with seed age. As the seeds after-ripen the optimum germination temperature increases. In the field, however, the soil temperature would be falling, until in late summer or early autumn it would coincide with optimum germination temperature of the seeds. Thus an additional control of germination time exists. From the experimental evidence it is obvious that geographical variation exists in this controlling mechanism.

The populations studied can be divided into six groups:

a, A northern group, which has very short dormancy at all temperatures. This correlates well with their late summer flowering. Their climate is one of high rainfall and germination soon after flowering would be both possible and advantageous in allowing sufficient vegetative growth before the cold winter and to gain maximum time for the vernalization of the rosette (Figures B2ix-x, following p. 37).

b, is a western group whose seeds can germinate only at high temperatures after three months ensuring little summer germination even under moist conditions. There are

many non-germinating seeds after three months, however, and this may be a response to the uncertain nature of the climate (Figure B2ix).

c, and d, are weedy Midland populations and north-east coastal populations respectively. Both are from very variable habitats. In the former this is due to cultivation and in the latter to the surface of dunes or shallow soil reacting quickly to changes in climate and therefore experiencing wide variation in soil moisture. Both these groups show poor high temperature performance even after six months after-ripening. This ensures that germination is prevented until damp conditions prevail in autumn and allows for a considerable store of non-germinating seeds to be built up in reserve (Figures B2xii-iii).

e, the European populations investigated have high low temperature germination after one month's after-ripening, but this falls off after three months. As these populations are late flowering this may prevent germination late in the autumn when temperatures are too low for sufficient overwintering growth to be made (Figure B2xiv).

f, This group consists of the single population, Burren. This shows the opposite pattern to all other populations studied. Non-after-ripened seeds have optimum germination at high temperatures. As the population is very late flowering this may prevent germination until the spring or, at least, restrict autumn germination.

g, Insufficient data are available for central European

Key to Table D i.

A=autumn, S=spring, Sum=summer.

G=time of germination, F=time of flowering.

M=Matthews' classification (Matthews, 1955)

OS=oceanic southern

ON=oceanic northern

M =mediterranean

CS =continental southern

H=Hultén's classification (Hultén, 1950)

18=European sea shore plants

20=Subatlantic plants

26=West European-middle Siberian plants

27=West European-south Siberian plants

46=Plants very strongly spread by culture

a. European-west Siberian

c. circumpolar.

Table D i.

Species	Distribution	M	H	G	Dormancy	F	Habitat
<i>Tuberaria guttata</i>	S,W, Europe	OS		A	Testa impermeable	Sum	Rocky heaths nr. sea
<i>Phleum arenarium</i>	"	OS	18	A	None	Sum	Dunes, sandy fields
<i>Hordeum murinum</i>	Holarctic	OS		A		Sum	Ruderal hab. esp. nr. sea
<i>Cochlearia danica</i>	W, Europe	ON	18	A		S	Sandy or rocky shores
<i>Atriplex glabriuscula</i>	"	ON	18	A		Sum	Sand or gravel shores
<i>Anthemis cotula</i>	Supraholarctic	M	46a	A&S	Over 4 months	Sum&A	Arable weed
<i>Draba muralis</i>	S,W,Cen.Europe		20	A	2-3 months	S&Sum	S and W-facing sunny slopes
<i>Veronica hederifolia</i>	Eurasian		20	A		S	Arable weed
<i>Saxifraga tridactylites</i>	Europe		20	A	2-3 months	S	Dry open habitats, walls
<i>Hornungia petraea</i>	S,W, Europe	CS	20	A	2-3 months	S	Dry open habitats, dunes
<i>Plantago coronopus</i>	S,W,Cen.Europe	CS	20	A	None	Sum	Cliffs, salt marshes
<i>Papaver argemone</i>	"	CS	20	A&S	Fresh seed dormant	S	Cornfields, ruderal habs.
<i>Corrigiola littoralis</i>	S,W, Europe	CS	20	S		Sum	Shale, shingle beaches
<i>Torilis arvensis</i>	Supraholarctic	CS		A&S		Sum	Arable weed
<i>Valerianella carinata</i>	S,Cen.Europe	CS		A		S	Arable weed
<i>Fumaria parviflora</i>	S,W, Europe	CS		A&S		Sum	Arable weed
<i>Chenopodium ficifolium</i>	S,W,Cen.Europe	CS		S		Sum	Arable weed
<i>Sagina apetala</i>	Supraholarctic		26	A		S	Ruderal habitats, dunes
<i>Scleranthus annuus</i>	Eurasian		26	A&S		Sum	Sandy habitats, arable weed
<i>Arenaria serpyllifolia</i>	Holarctic		27	A	2-3 months	Sum	Dry turf, walls
<i>Veronica arvensis</i>	Eurasian		27	A,occ		S&Sum	Arable weed
<i>Papaver rhoeas</i>	"		26	A&S	Fresh seed dormant	Sum	Arable weed
<i>Aster tripolium</i>	"		26	S		Sum&A	Shoals, mud flats
<i>Impatiens parviflora</i>	"		27	S	Over 6 months	Sum	Moist shady habitats
<i>Sinapis arvensis</i>	Cosmopolitan		27	S	Several months	Sum	Arable weed
<i>Cerastium glomeratum</i>	Cosmopolitan		46c	A		Sum	Ruderal habs., walls, dunes
<i>Juncus bufonius</i>	"		46c	Sum.		Sum	Moist habitats
<i>Chenopodium rubrum</i>	Supraholarctic		46c	S,occ		Sum&A	Ruderal habitats, marshes
<i>Polygonum persicaria</i>	Eurasian		46c	S	(Reduced by sub-	Sum	Arable weed
<i>Polygonum hydropiper</i>	Holarctic		46c	S	mergence at 2-4°C	Sum&A	Moist habs., inundated in winter
<i>Spergula arvensis</i>	Cosmopolitan		46c	S		Sum	Sandy soils
<i>Arabidopsis thaliana</i>	Eurasian		27	A&S	2-6 months	S&Sum	Dry open habs., walls, dunes

or Mediterranean populations to allow any hypotheses to be promulgated about their behaviour. However, Morley found that in Trifolium subterraneum dormancy was important in areas where summer rains were unreliable (Morley, 1958). In contrast, however, little dormancy was found in warm, dry Mediterranean areas like Morocco or Algeria, where summer rains were very rare, presumably because dry weather made germination impossible and dormancy unnecessary. A similar situation may well exist in A. thaliana.

(iii) Light and darkness

Another important factor observed in germination experiments was the variation between light and dark germination. In some populations germination is poorer in darkness, especially at high temperatures. This is very marked in certain populations, e.g., Burren, Bush and Besancon. This would appear to strengthen the idea that non-germinating, i.e. dormant, seeds may well be a feature of selective advantage (see later).

(iv) Alternating temperatures

Experiments on ripe seed in alternating temperatures are of particular relevance to the field situation. From the experiments in the simulated Buxton temperature regimes it was apparent that in all populations 'buried' seeds were prevented from germinating except under autumn conditions and even then some remain dormant. This would appear to be another mechanism which helps restrict the possible germination time. There is also a fall off in light germination after the autumn regimes in several populations (Figures

B3iii-iv, p.71), and this could provide a source of non-germinating seeds. These results tie in well with those of Baskin and Baskin (1972) on fresh seeds of A. thaliana and it would be worth while repeating the Buxton simulation on fresh seeds of the populations investigated here.

(v) Secondary dormancy

The germination and dormancy experiments have confirmed that 100% germination is not achieved under all conditions. Excessively high or low temperatures, especially in darkness, induce secondary dormancy even in fully ripe seeds. A combination of high temperature with darkness was the most effective condition and if the high temperature treatment in darkness is long enough, subsequent light treatment is ineffective in breaking dormancy in a large proportion of the seeds so that in the field churning or cultivation of the soil would be ineffective after three weeks or more (Table B3v, p. 79).

In general, secondary dormancy was induced by relatively lower temperatures in populations from those sites where summer drought was most likely, compared with the need for relatively high temperatures in populations from areas with more reliable summer rainfall (Figure B3v, following p. 76).

It is probable therefore that in a warm wet summer in Britain a combination of high temperatures and a moist soil will induce secondary dormancy, and since no treatment other than a further period of after-ripening will bring about subsequent germination of most of these seeds, they

are thus added to the soil 'fund' until at least the following year.

The field experiment described on page 80 showed that for one site as many as 650 germinable seeds persisted in an area of 0.25m^2 for as long as three years and demonstrated conclusively the importance of secondary dormancy in building up a soil reserve. The importance of the seed reserve cannot be underestimated.

The overall picture of the life cycle is presented in Figure Di. There are obviously several sources for dormant seeds at each stage in the life cycle. The seed rain in A. thaliana is so large that a wide range of physiological variation must be present. Even after unfavourable circumstances, e.g., if early high percentage germination is followed by death of a large proportion of seedlings by drought, there will still be a large number of seeds left for the autumn burst of germination. These can be added to by the fund of seeds in the soil which also represent a wide range of variation. Both the population itself and much of its physiological variability will therefore be maintained. Naturally the seeds in the soil do not carry only characteristics of germination variability but also variation in the vegetative growth and flowering physiology.

All this variability in the seed 'bank' and in the 'new'

seeds in any one year will be 'sieved' by environmental conditions. However, place to place, month to month and year to year variation in the environment exists but the variability of the seed fund can presumably provide individuals whose germination characteristics, growth and/or flowering physiology fits them for almost all eventualities. Nevertheless the production of very large numbers of seeds by the population and the build up of reserve in the soil may in itself be adaptive and may be related to the relatively low rate of generation of genetic variability in a species with such a low proportion of cross fertilization (Snape and Lawrence, 1971; Jones, 1968, 1971 a,b,c).

All populations studied have different proportions of genotypes with various characteristics - long or short dormancy, high or low potential for inducement of secondary dormancy, greater or lesser ratio of light to dark germination, long or short cold requirement for flowering. This was apparent in variation in flowering physiology in Cetl's work in Western Moravia (Cetl, 1965; Cetl, Dobrovolna and Effmertova, 1965, 1967) as referred to on page 111 and is also apparent in dormancy mechanisms in the present investigation.

Obviously it is not possible for all populations to be fit in all environments at the same time. The optimum 'strategy' of each species must be to allocate its resources so that different combinations of characters produce fit populations in different environments. The frequency of individual population types will depend on the relative frequency of the different environments (Levins, 1969).

This in turn must reflect the 'graininess' of the environment. As far as A. thaliana is concerned, the environment must be considered coarse-grained. Each individual plant must pass all or most of its life in a single environment. The graininess of the climate varies from season to season and year to year. There will be regular yearly climatic cycles but superimposed on this will be a short term graininess involving wet or dry, hot or cold periods. This short term variation will differ in different environments. The mediterranean climate is not very grainy. Summers are warm and rainfree; soil remains dry all summer; winters are mild. However, in maritime climates, there is little predictability in the day to day weather. The montane and continental climates are again less grainy and more predictable. It would appear that population variability in A. thaliana reflects this pattern. It is possible that the genetics of the population is also grainy and that step-wise 'adaptations' of physiological systems involving simple or at most a few genes occur rather than gradual selection of polygenic systems, especially in maritime populations. The combination of grainy climate and grainy habitat - both in a special sense, i.e. from shallow, unstable soil to soil suitable for perennial colonization, and in a temporal sense due to instability from cultivation, shifting sand, etc. - may well make the selection of genotypic variability as opposed to stability imperative.

The ability to colonize a wide range of habitats or climatic types must be dependent on the possession of a great store of variability either obvious or concealed (Stebbins, 1942).

As Baker (1953) suggests it is theoretically easier for an outbreeding species to enter new niches than an inbreeder because it can produce a greater number of adaptive re-combinations. Species with many biotypes will be widespread and common but those with only a few biotypes will be rare unless each biotype has a wide range of tolerance or unless the conditions to which it is adapted are common. However, successful colonizers are frequently self fertilizing and this obviously allows the rapid establishment of a large population of well adapted individuals from the chance introduction of perhaps only one fit individual (Stebbins, 1957).

It might be thought that the most effective mechanism for successful colonization of all sites would be an all purpose genotype which conferred a wide tolerance and plasticity on the plant bearing it. This would be the multi- or general-purpose genotype described by Baker (1965). On the whole, limitations of biological systems probably make it impossible to achieve such a high degree of adaptation by plasticity alone, so although plasticity may be important, some genetic differentiation is required.

The outbreeding species, while able to produce a high number of recombinants and so achieve greater genetic variability and tolerance, is not suited to the rapid perpetration of well adapted genotypes. On the other hand, the predominantly selfing species will consist of numbers of local populations, each distinguished from other populations by morphological and physiological characteristics.

Each population can be finely adjusted to its local environment by continual selection and maintenance of the best adapted individual genotypes (Mather, 1953).

The evidence reviewed for Arabidopsis thaliana suggests that most natural populations do not consist of general purpose genotypes. In laboratory experiments many characters including dormancy, germination and flowering responses show distinct patterns which distinguish the populations. In cultivation under uniform conditions most populations maintain their patterns of variability. However, certain ruderal populations e.g., Bush, Whatton House and Woebley Castle, show much greater plasticity under cultivation than they would indicate from experiments made under laboratory conditions. This may indicate the occurrence of some general purpose genotypes within the species.

Baker (1965) considered that the necessity of self fertilization for the rapid establishment of weedy populations would limit the rate of production of recombinants and thus the colonizing ability of such populations. They might therefore be expected to rely more on multi-purpose genotypes. Nevertheless populations long established in ruderal situations and populations in natural habitats might be expected to show finer adjustment to their environments due to the continual segregation of well adapted types and the depletion of less fit biotypes.

On the other hand it could be argued that production of a large number of seeds in combination with a small degree

of outbreeding could increase the probability of occurrence of a sufficient number of well-adapted individuals to the level comparable to that of a habitually outbreeding species so that either a new site could be colonized and the population subsequently maintained or a population could be maintained successfully in an extreme and/or variable environment. Genetic studies are proceeding which have already shed much light on the breeding system in this species and evidence of outcrossing is now strong (Snape and Lawrence, 1971; Jones, 1968, a,b,c).

It is likely that such an environment forces the development of the particular genetic system on the plant, and the resulting 'strategy' is very much one of defence. However, the opportunities provided by such a genetic system confer an added advantage on the plants and this has allowed Arabidopsis thaliana to be successful over such a wide geographical and climatic range.

It is suggested that this investigation has demonstrated that (a) variability, often somewhat disjunct, is present in a wide range of physiologically important characters, (b) the seed reserve in the soil is extremely important in maintaining the variability, and (c) the variability in various characteristics is correlated with the broad features of the environment in such a way that a great deal of it must be presumed beyond reasonable doubt to have adaptive value.

Obviously there are large gaps where much further work is required. The present investigation has made possible the recognition of several important times in the life cycle of A. thaliana where physiological variation is probably adaptive and where selection could take place. Selection experiments along the lines of that described on page 53 would be one direction worth following in future studies of germination and flowering time. The unexpected, apparently genetic, correlation between late flowering and the production of non-dormant or short dormancy seeds and vice versa regardless of the original dormancy pattern of the parent plants tempts one to see a clear adaptive effect but perhaps it is more important that disjunct differences like this occur at all. Clearly selection experiments concerned with this and other stages in the life cycle where selection might occur in the field would be very rewarding, though difficult to carry out.

One variable feature with no obvious adaptive significance is that of the rather extreme variation in seed size. In general, species occupying open habitats and those low in seral successions in temperate regions tend to produce small seeds (Salisbury, 1942). Arabidopsis thaliana is no exception. The largest mean seed weight in any population measured in this investigation was only 0.48 mgm. However, considerable variation from 0.07 to 0.48 mgm was found between the populations in this character. The significance of these differences is not immediately apparent. Variation in mean seed weight from 0.09 to 0.30 mgm was reported by Baker (1972)

in Potentilla glandulosa from California. However, in this species seed weight was found to decrease with increasing altitude. No such pattern of variation is apparent in A. thaliana. If anything, high altitude populations, in Britain at least, possess larger seeds than those from more low lying areas.

Baker also found that in Californian species as a whole there was a tendency for seed weight to increase with the likelihood of the seedling's being exposed to drought. Again no such tendency can be seen in A. thaliana populations. However, Baker did find that desert plants did not fit this general pattern and frequently had small seeds. He presumed that their precise temperature and moisture requirements for germination made the need for large seeds and consequent rapid growth less pressing as germination could only take place when conditions were suitable for seedling survival. Clearly further work on the importance of seed size in A. thaliana is necessary.

In addition actual measurements of seed production under various environmental conditions still need to be made. Also the 'death' rate of seeds in the soil must be investigated. According to Harper and White (1970) the decay rate of seeds in the soil is about 25% per year for other annual species such as Capsella bursapastoris and Chenopodium album.

Finally it should now be possible to select critical

environmental factors on which to make more precise measurements. For example, the precise soil surface and sub surface temperatures and moisture levels necessary for germination could be recorded with advantage if appropriate techniques could be developed.

In conclusion the words of Amen (1966) are particularly appropriate: 'The more diverse mechanisms a plant possesses that preserve order, insure growth, or time developmental phases appropriately the greater are its chances of ecological and reproductive success.'

Appendix 1.

Experimental results.

Chapter B 2. Dormancy experiments.

Population	Age of seed (days)	% germination after 15 days imbibition
Besançon	17	18
	21	27
	27	78
	34	100
Bush	9	37
	19	72
	26	100
Lanark	17	100
	21	100
	27	100
	34	100
Husband's	20	16
Bosworth	42	18
	64	64
	100	84
	241	76
	331	24
	403	36
Aberdeen	15	1
	29	0
	59	2
	80	0
	116	48
	256	98
	346	56
	418	52
Whatton	15	0
	29	0
	59	4
	80	2

Appendix 1/2.

Population	Age of seed (days)	% germination after 15 days imbib.
Whatton	116	18
	256	78
	346	8
	418	20
Dirleton	15	4
	29	6
	59	22
	80	64
	116	74
	256	95
	346	76
	418	96
Lathkill Dale	30	64
	61	96
	220	94
	302	92
	374	96
Carsington	30	68
Pasture	61	100
	220	100
	302	100
	374	100
Longcliffe A	30	58
	61	97
	220	98
	302	96
	374	100
Longcliffe B	35	44
	61	100
	220	100
	302	100
	374	100

Appendix 1/3

Population	Age of seed (days)	% germination after 15 days imbib.
Deepdale	35	13
	61	85
	220	100
	302	98
	374	92
Dove Holes	36	46
	61	100
	220	100
	302	100
	374	100
Moorside	35	66
	61	100
	220	98
	302	96
	374	100
Jug and Glass	35	20
	61	44
	220	78
	302	60
	374	78
Cobbler's Nook A	35	86
	61	100
	220	100
	302	100
	374	100
Cobbler's Nook B	36	64
	61	95
	220	100
	302	98
	374	100

Appendix 1/4

Population	Age of seed (days)	% germination after 15 days imbib.
Friden Crossing	36	19
	61	87
	220	100
	302	98
	374	100
Parsley Hay	36	61
	61	91
	220	96
	302	98
	374	96

Appendix 1/5		Final % germination											
One month of storage		Imbibition temperature											
Population		5D	1L	10D	L	15D	L	20D	L	25D	L	30D	L
Barry Sands	S	94	100	90	92	22	60	16	56	4	28	0	0
	N	90	94	80	96	56	54	14	48	10	20	4	4
St. Cyrus I	S	30	82	0	22	0	0	2	2	0	0	0	0
	N	56	76	62	56	14	16	2	2	4	0	2	0
Catterline	S	30	62	46	54	10	58	8	62	4	26	0	0
	N	84	88	96	90	86	88	64	84	68	76	54	52
Burren	S	0	0	0	0	6	10	0	24	2	58	0	50
	N	0	2	2	2	0	2	0	4	0	32	0	60
Dirleton	S	0	42	6	26	2	8	0	6	2	4	0	0
	N	10	22	2	22	0	4	0	2	0	2	0	0
Bush	S	0	14	2	4	0	0	0	0	0	0	0	0
	N	0	8	0	0	0	0	0	0	0	0	0	0
Mickle Fell	S	8	30	14	54	4	8	4	14	0	14	0	4
	N	84	94	58	96	24	86	12	82	6	78	10	36
Ben Dharain	S	38	88	20	78	8	26	8	58	2	38	0	2
	N	60	90	90	70	40	70	40	90	70	30	10	10
Dijon	S	2	96	4	16	0	0	0	0	0	0	0	0
	N	0	36	8	32	2	18	0	0	0	0	2	2
Besançon	S	20	78	24	30	0	0	0	0	0	2	0	2
	N	34	48	22	44	0	0	0	0	0	0	2	0
Woebley	S	62	82	22	24	0	2	0	0	0	0	0	0
Castle	N	44	76	8	34	0	0	0	0	0	0	0	0
Whatton	S	8	96	0	62	0	2	0	0	0	0	0	0
	N	0	26	0	16	2	2	0	0	0	0	0	0
Stockholm I	S	88	94	88	100	84	90	32	88	4	44	0	4
	N	84	80	82	92	66	84	8	30	6	24	0	10
Husband's	S	16	94	6	18	0	0	0	2	0	2	0	0
Bosworth	N	4	60	2	12	0	0	0	0	0	0	0	0
St. Dogmael's	S	84	82	48	54	16	16	0	8	0	2	0	0
	N	12	52	20	30	0	2	0	0	0	2	0	0
Ochsenkopf	S	98	100	58	80	2	16	0	2	2	0	0	0
	N	76	98	59	90	0	34	0	0	0	0	2	0

Appendix 1/6

Final % germination

three months of storage

Imbibition temperature

Population		5D	L	10D	L	15D	L	20D	L	25D	L	30D	L
Barry Sands	S	98	100	100	98	100	100	92	98	24	74	2	60
	N	60	68	92	96	74	94	42	82	44	66	2	4
St. Cyrus	S	92	100	100	100	94	98	34	66	12	18	2	6
	N	0	8	14	38	28	80	10	42	12	4	0	0
Catterline	S	24	26	84	74	60	90	44	98	10	88	2	74
	N	6	12	40	50	76	90	72	92	76	94	32	52
Burren	S	2	0	0	0	0	2	0	6	2	32	6	56
	N	2	0	0	0	0	4	0	8	0	12	0	10
Dirleton	S	22	26	16	46	4	20	4	22	6	2	0	4
	N	0	22	0	20	0	4	2	10	0	6	0	0
Bush	S	2	24	4	14	0	4	0	0	0	0	0	0
	N	0	0	0	8	4	4	0	0	0	0	0	0
Mickle Fell	S	22	58	78	84	64	90	20	88	2	62	2	38
	N	30	62	28	50	28	96	16	94	34	82	2	72
Ben Dhorain	S	16	70	32	76	14	38	32	52	12	54	4	54
	N	50	30	20	20	50	10	40	50	30	40	20	20
Dijon	S	10	34	6	12	0	0	0	0	0	0	2	0
	N	18	76	6	60	14	76	10	76	4	48	0	8
Besançon	S	2	28	0	18	2	16	0	2	0	0	0	0
	N	60	72	64	58	52	78	20	34	2	2	0	0
Woebley	S	42	42	26	26	16	24	6	16	0	24	0	0
Castle	N	88	100	84	100	64	94	12	76	2	14	0	0
Stockholm I	S	84	76	90	90	44	94	18	92	12	92	2	56
	N	86	94	90	88	92	92	90	96	70	88	2	12
Husband's	S	64	94	30	32	0	2	0	8	0	0	0	0
Bosworth	N	72	96	8	90	4	52	0	12	0	0	0	0
St. Dogmael's	S	50	34	34	30	18	40	8	56	4	22	0	0
Abbey	N	46	76	42	64	44	78	22	74	2	42	0	6
Ochsenkopf	S	2	4	0	14	4	6	0	16	0	2	2	0
	N	66	70	52	66	50	88	52	84	26	82	4	16
Whatton	S	18	42	6	12	0	2	0	0	0	0	0	0
	N	38	78	14	34	6	46	0	35	0	4	0	2

Appendix 1/7 Final percentage germination
6 months of storage Imbibition temperature

Population	D	L	5	10	15	20	25	30	L
Barry Sands	S 100	100	98	100	98	100	100	28	94
	N 90	98	100	100	96	100	96	8	94
St. Cyrus I	S 98	100	100	100	100	98	100	28	100
	N 94	100	100	100	96	98	92	34	98
Catterline	S 64	90	92	100	96	100	96	12	100
	N 80	98	96	100	98	100	100	24	98
Burren	S 4	24	6	26	28	52	58	4	96
	N 4	30	18	26	18	8	10	0	78
Dirleton	S 82	98	78	100	74	84	8	0	46
	N 60	98	78	96	36	20	2	0	4
Bush	S 72	98	58	92	46	0	4	0	4
	N 0	100	12	96	12	0	0	4	0
Mickle Fell	S 72	92	94	100	96	50	56	2	88
	N 98	96	96	100	100	82	70	6	92
Ben Dhorain	S 90	100	88	100	88	75	76	14	98
	N 30	30	20	20	33	0	20	0	10
Dijon	S 80	100	94	96	40	0	2	0	2
	N 50	86	38	76	12	2	2	2	34
Besançon	S 42	98	70	90	42	14	14	0	52
	N 72	92	66	94	66	0	12	0	30
Woebly	S 86	98	98	100	76	70	64	14	90
Castle	N 100	100	96	100	92	14	20	0	66
Whatton	S 42	100	72	100	88	0	0	0	4
	N 66	98	54	100	68	0	0	0	0

Appendix 1/7 Continued.....

Population		5	10	15	20	25	30
Stockholm	S	D 94	L 100	D 98	L 100	D 96	L 100
	N	86	96	94	98	80	100
Husband's	S	90	100	66	100	0	40
Bosworth	N	92	100	58	100	0	14
St. Dogmael's	S	90	78	80	100	54	92
Abbey	N	72	74	64	84	14	58
Ochsenkopf	S	60	98	68	96	24	96
	N	58	90	70	100	18	98

Final percentage germination

		10 months of storage						Imbibition temperature												
		5			10			15			20			25			30			
		S	D	L	S	D	L	S	D	L	S	D	L	S	D	L	S	D	L	
Population																				
Barry Sands	S	100	96	100	96	100	100	100	100	100	100	100	100	96	84	100	84	100	100	
	N	96	96	100	96	100	100	100	100	100	100	100	100	89	56	92	56	100	100	
St. Cyrus I	S	100	100	100	100	100	100	100	100	100	100	100	100	100	52	100	52	80	80	
	N	84	92	100	92	100	96	96	96	96	96	96	96	88	56	100	56	80	80	
Catterline	S	80	76	100	100	96	96	96	96	96	96	96	96	80	16	100	16	100	100	
	N	92	88	92	100	100	100	100	100	88	88	88	88	88	68	100	68	76	76	
Burren	S	0	8	8	8	20	32	28	16	48	20	88	20	8	20	88	20	76	76	
	N	8	12	8	16	4	36	36	0	20	4	40	4	8	40	40	8	32	32	
Dirleton	S	100	92	100	92	84	76	76	16	64	0	44	0	4	44	4	40	40	40	
	N	100	96	92	92	84	48	48	0	34	0	8	0	4	8	4	4	4	4	
Bush	S	96	100	68	92	100	12	40	0	24	0	0	0	0	0	0	0	0	0	
	N	88	96	28	100	0	44	44	0	0	0	0	0	0	0	0	0	0	0	
Mickle Fell	S	100	96	96	96	96	72	88	20	72	16	84	12	12	72	84	12	72	72	
	N	96	96	88	96	88	96	100	32	100	60	100	12	12	88	100	12	88	88	
Ben Dhorain	S	96	100	100	100	92	84	68	92	88	56	92	36	36	68	92	36	68	68	
	S	92	92	92	92	100	4	100	0	16	0	0	0	0	0	0	0	0	0	
Dijon	N	28	72	44	64	20	98	98	0	60	4	24	0	0	20	24	0	20	20	
	S	60	80	68	88	48	88	88	16	24	4	20	12	12	32	20	12	32	32	
Besançon	N	60	84	96	88	44	76	76	4	32	4	28	0	0	20	28	0	20	20	
	S	80	96	88	100	48	92	92	32	84	28	96	40	40	88	96	40	88	88	
Woebley	N	52	100	84	96	40	92	92	24	76	8	44	8	8	56	44	8	56	56	
	S	100	84	96	100	32	92	92	4	16	0	0	0	0	0	0	0	0	0	
Castle	S	100	84	92	100	24	100	100	0	12	0	0	0	0	0	0	0	0	0	
	N	84	92	84	92	100	24	100	0	12	0	0	0	0	0	0	0	0	0	

Appendix 1/8 Continued.....

Population	5		10		15		20		25		30	
	D	L	D	L	D	L	D	L	D	L	D	L
Stockholm S	80	76	100	100	100	100	100	100	100	100	92	96
N	88	72	80	84	100	96	76	84	44	80	8	88
Husband's S	72	100	92	100	98	100	8	52	0	4	0	4
N	96	100	84	100	20	96	4	24	4	16	0	16
St.Dogmaels S	88	84	96	88	100	84	52	68	48	76	48	68
N	60	68	52	92	68	60	8	32	12	24	4	28
Abbey S	56	80	88	92	52	80	60	92	32	96	36	100
N	52	64	72	100	20	52	68	80	24	80	20	56

Appendix 1/9

Chapter B 3.

Constant temperatures

Final % germination

Population

Imbibition temperature

	5D	L	10D	L	15D	L	20D	L	25D	L	30D	L
Soria	2	4	2	4	6	13	18	22	12	22	-	-
Burren	4	0	4	10	14	28	22	48	36	56	-	-
Lodz	0	0	2	0	0	2	8	6	6	8	0	2
Aberdeen	76	90	68	94	2	62	2	40	0	14	0	0
Dirleton	94	98	94	100	90	98	6	68	2	44	0	0
Bush	28	72	24	66	20	44	4	32	0	4	0	4
Ben Dhorain	18	24	18	24	12	34	12	42	12	44	0	30
Dijon	2	0	6	4	6	12	6	14	0	0	4	0
Besançon	34	30	36	40	22	52	14	36	14	16	2	0
Longcliffe A	76	76	96	100	100	100	100	100	88	94	60	96
Dove Holes	96	100	96	100	96	96	100	100	94	94	58	66
Lathkill Dale	68	88	78	98	30	98	30	88	16	66	2	32
Barry Sands	96	94	98	98	100	100	100	98	98	96	66	100
Mickle Fell	88	78	86	90	86	92	82	92	72	92	10	76
Stockholm II	80	68	96	88	96	100	44	98	36	100	8	68
Whatton	0	4	10	14	16	18	4	16	0	6	0	2
Hadleigh	67	84	66	76	57	71	1	12	0	0	0	0
St. Dogmael's Abbey	98	96	98	94	98	98	84	94	36	60	20	26

Appendix 1/10

Population	Alternating temperatures				Refrigerated incubator													
					Final Percentage Germination													
					Monthly regime													
J	F	M	A	M	J	J	A	L	S	D	L	O	D	L	N	D	D	
Barry Sands	98	100	98	98	100	96	100	100	72	100	88	76	76	80	20	96	0	
St. Cyrus I	94	100	100	100	100	96	100	100	76	100	100	96	76	100	36	100	0	
Catterline	94	56	100	100	96	96	100	100	0	96	96	84	44	100	0	88	0	
Burren	34	2	12	42	80	100	96	96	0	88	76	84	0	80	0	20	0	
Dirleton	68	94	80	86	100	100	88	76	0	88	12	68	0	88	0	76	0	
Bush	86	92	100	100	88	80	64	68	8	60	0	92	0	80	0	92	0	
Mickle Fell	86	68	86	96	100	100	92	72	72	80	60	72	40	76	8	84	0	
Ben Dhorain	98	94	96	100	96	100	96	100	8	96	92	92	68	96	12	80	0	
Dijon	78	74	54	66	96	96	80	76	0	52	8	48	4	36	0	36	0	
Besançon	78	90	80	90	72	88	52	64	8	44	60	56	0	44	4	32	0	
Woebley Castle	96	86	100	98	100	98	96	100	44	100	0	92	0	72	0	60	16	
Wharton	60	76	100	80	76	68	48	80	68	40	0	48	0	52	0	28	0	
Stockholm I	68	80	84	90	100	96	96	88	24	88	68	36	28	56	0	48	0	
Husband's Bosworth	90	82	94	100	92	100	96	96	72	68	8	88	0	84	0	64	0	
St.Dogmael'sabbey	94	92	100	100	100	100	100	100	36	88	56	80	32	88	4	20	0	
Ochsenkopf	78	90	84	100	100	100	100	60	0	96	44	100	24	76	0	0	0	
Dove Holes	52	97	88	89	100	88	92	64	32	96	80	96	88	68	0	4	8	
Longcliffe B	100	100	88	96	100	100	100	100	80	100	60	100	28	92	4	52	28	
Hadleigh	52	60	64	94	62	62	44	68	0	28	0	20	0	44	0	0	0	

Appendix 2

Compost

Considerable difficulties in obtaining satisfactory compost were found throughout this investigation. The three chief problems were the inability to sterilise loam easily, water logging and the difficulty found in reducing insect infestation in the greenhouse. These problems led to the loss of many experimental plants throughout the vegetative growth and flowering experiments and led to a greater emphasis being placed on the germination and dormancy work which did not rely on compost.

Initially a mixture of four parts sterilised sieved loam from the University Botanical Gardens, Leicester; two parts coarse sand and two parts fine sands were used. $1\frac{1}{2}$ oz. John Innes Base and $\frac{3}{4}$ oz. calcium carbonate were added to each eight gallons of compost. The plants failed to thrive on this mixture. There was a tendency for the soil to become waterlogged if overwatered; if watered occasionally the soil surface formed a hard crust. The addition of 4 lbs ground limestone to each eight gallons improved the consistency of the soil somewhat and plant growth was improved. However the percentage survival of seedlings was still low and logging remained a serious problem.

This compost was then mixed with an equal quantity of Levington potting compost. With this mixture waterlogging seldom occurred and seedling survival rose to over 90%. However, plants growing on this compost were very susceptible to insect infestation especially black fly and greenfly which got under the rosette leaves and proved very difficult

to control.

Plants were sprayed with solutions of Abol X (with Disystol added as a wetting agent) but this failed to solve the problem. Fumigation of the greenhouse and the use of Vapona strips were similarly ineffective.

Appendix 3.

Programme to compute mean integrated temperature per day and mean hourly temperature over a one week period.

```
& JOB;
& ALGOL;
"BEGIN" "REAL" SUM 1, SUM 2, TOTAL;
"INTEGER" M, N, I, J, Q, X;
"READ" M, N;
"BEGIN" "REAL" "ARRAY" A$:M, 0:N!, B$:0:23!;
"FOR" I:=1 "STEP" 1 "UNTIL" M "DO"
"FOR" J:=0 "STEP" 1 "UNTIL" N "DO"
"READ" A$(I, J!);
"FOR" J:=0 "STEP" 1 "UNTIL" N "DO"
"BEGIN" B$(J!):=0.0;
"FOR" I:=01 "STEP" 1 "UNTIL" M "DO"
B$(J!):=B$(J!)+A$(I, J!);
"PRINT" B$(J!)/7;
"END";
SUM 1:=0.0;
"FOR" J:=0, 1 "STEP" 2 "UNTIL" 23 "DO"
SUM 1:= SUM 1 + B$(J!)*2;
SUM 2:=0.0;
"FOR" J:=2 "STEP" 2 "UNTIL" 22 "DO"
SUM 2 := SUM 2 + B$(J!)*4;
"READ" Q;
TOTAL := 0.0;
TOTAL := (TOTAL + SUM 1 + SUM 2)/Q;
"READ" X;
"PRINT" " L, X=, , SAMELINE, X, "L, TOTAL =, , SAMELINE, TOTAL;
"END"
"END";
```

Read in data: 7(M), 23(N); temperature recorded at 00hrs, 01hrs, 02hrs ---23hrs on day 1, 00hrs---23hrs on day 2, -----00hrs---23hrs on day 7; 3; code for operation (X).

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