THE EFFECTS OF INORGANIC NITRATE SOLUTIONS ON DORMANT SEEDS.

By

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FOR DEBORAH

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ABSTRACT

The role of nitrate solutions in stimulating the germination of seeds in soil was investigated with a series of laboratory and field experiments. Comparisons were made between ruderal species from disturbed sites, calcicole species from calcareous grassland and calcifuge species from acidic heathland.

Germination tests showed that maximum germination of four ruderal species (*Artemisia vulgaris*, *Cardamine hirsuta*, *Senecio vulgaris* and *Stellaria media*) occurred in 10^{-2}M to 10^{-3}M potassium nitrate. Twenty-two ruderal species were incubated on a thermobar, with and without 10^{-2}M nitrate, in light and darkness in eleven temperature regimes with different diurnal temperature ranges. The greatest additional germination with nitrate in many of these species occurred at the larger diurnal temperature ranges which were already stimulatory. The calcicole and calcifuge species generally failed to respond to nitrate solutions but were stimulated by alternating temperatures.

Seeds of the four ruderal species were buried outdoors for varying periods and then incubated in controlled conditions. Their response to nitrate was lost and their responses to light and alternating temperatures were reduced. Naturally buried seedbanks of soil from the three habitats, when incubated in controlled conditions, also failed to show any stimulation by nitrate.

Field experiments to test the effects of added nitrate on seedling emergence from natural seedbanks were performed in 1981 and 1982. In 1981 a nitrification inhibitor was used to control natural nitrate levels in the soil but its apparent stimulation of emergence made interpretation difficult. The only significant response to nitrate was in *Poa* spp., with 20% higher germination in nitrate than

in water treatments. In 1982 no responses to nitrate could be isolated.

These results were discussed in terms of the possible role of nitrate in the detection of safe sites for germination and the adaptive nature of any response to nitrate by ruderal species.

CONTENTS

CHAPTER ONE

Introduction and Literature Review.

1. Introduction and Literature Review.

1.1 General Introduction : The importance of dormancy.

Dormancy may be defined as the lack of germination of viable seeds, even though the minimum requirements for the regrowth of the embryo (water, oxygen and reasonable temperatures) are available (Villiers 1973). The ability of seeds to remain dormant is an important characteristic of many plant species and is responsible for the accumulation of large seedbanks in both cultivated and natural soils. Many estimates of the numbers of seeds present in a wide variety of seedbanks have been made (Roberts and Stokes 1966, Roberts 1970, Thompson and Grime 1979, Roberts H.A. 1981), although the majority of work has involved arable soils (reviewed by Murdoch 1982). Numbers of arable weed seeds ranging from 3×10^6 ha^{-1} up to 860×10^6 ha^{-1} were cited by Chancellor (1981) in his review on weed behaviour.

Chancellor (1982) has reviewed the ways in which types of dormancy have been classified. A widely used system was a division between primary and secondary dormancy. Primary dormancy is present when the seed is shed. However, if conditions are unfavourable for germination when primary dormancy is lost, a secondary dormancy can be induced. Harper (1957) suggested a classification into three types of dormancy; innate, induced and enforced. Innate dormancy corresponds to primary dormancy, whilst induced dormancy results from conditions inclement for germination but remains even when favourable conditions return. Enforced dormancy is that which occurs when environmental conditions are unfavourable for germination, but does not persist once the conditions have changed. A third classification by Vegis (1964) divides seeds into those which are absolutely dormant

and will not germinate under any conditions, and those which are conditionally dormant and require specific environmental conditions for germination.

Henceforth, in discussing these experiments, the last of these classifications, employing the concepts of conditional and absolute dormancy, will be used. Greater germination under particular environmental conditions and not others is thus taken as evidence of conditional dormancy. Evidence from the study of Avena fatua seeds suggest that the factors capable of breaking primary and secondary dormancy are of the same type (Chancellor 1982) and such a division is consequently considered unnecessary in this context.

The importance of seed dormancy has been widely discussed and reviewed (Thurston 1959, Barton 1962, Vegis 1964, Roberts E.H. 1972, Chancellor 1982) with emphasis on ruderal (*sensu* Grime 1979) species. Seed dormancy is one strategy that can enable plants to avoid conditions unfavourable for growth or survival, such as periods of extreme climate or disturbance. In this way some species are able to survive extreme heat and drought or the cold of winter as well as being able to recolonise areas cleared by cultivation or natural disturbance such as fire. This ability to remain dormant is responsible for many serious agricultural weed infestations despite physical and chemical methods of weed control (Murdoch and Roberts 1982).

A random loss of dormancy would serve to spread germination over a wide period but since it could still frequently occur in conditions unfavourable to seedling survival the advantage gained would be limited. It would be more beneficial if germination occurred at a time and place where seedlings had a higher chance of survival. The concept of "safe sites", situations in which germination can occur, was originally introduced by Harper, Williams and Sagar (1965), and

in a later article by Cook (1980) the term was extended to describe sites in which seedling survival following germination was also favoured. If dormancy is assumed to be adaptive it is to be expected that germination sites will provide a relatively high likelihood of establishment for the seedlings emerging in them. Cook's extended usage of the term "safe site" is therefore used here.

The occurrence of safe sites varies both temporally and spatially. Examples of the former are where seedlings would benefit from avoiding the hot and dry conditions of summer or the cold of winter when mortality due to dessication or frost damage could be high. Spatial variation of two kinds can be identified; firstly related to the depth of seed burial and secondly associated with open conditions and gaps in vegetation. The energy reserves of seeds buried deep in the soil may be exhausted before the seedling reaches the surface, resulting in death. Germination at a shallower depth therefore constitutes a form of safe site although clearly the actual depth would depend to a large extent on seed size (Black 1956). Open conditions can also provide safe sites for germination in that competition for light, water and nutrients, which might otherwise result in seedling mortality can be avoided. Thus, the chance of seedling survival immediately after germination is greater in each of these types of safe site.

If seeds are to benefit from these opportunities for safe germination, they must be able to respond to the environmental characteristics of the safe sites they encounter. It has been shown that the following factors can be involved in breaking dormancy and hence may help to identify safe sites.

1. Light (e.g. Evenari 1965, Wesson and Wareing 1967, 1969, Toole 1973).
2. Alternating temperatures; large diurnal temperature ranges (e.g. Thompson 1977, Totterdell and Roberts 1980, Roberts and Totterdell 1981).
3. Chemical treatments; in particular inorganic nitrates and ethylene (reviews by Roberts E.H. 1973, Chancellor 1981).
4. Chilling (stratification) (e.g. Taylorson and Hendricks 1969, Vincent and Roberts 1977, Roberts and Benjamin 1979).
5. Oxygen and carbon dioxide levels (e.g. Popay and Roberts 1970a).

In addition, moisture stress may be an overriding factor in limiting the germination of non-dormant seeds in the soil (Roberts and Potter 1980).

A recent literature review by Murdoch (1982) discussed these factors in detail and additional references were also presented above. Further discussion is left to appropriate points later in the text. It should, however, be noted that a single dormancy breaking factor may not be sufficient to identify a safe site or indeed be efficient in doing so. Interactions between these dormancy breaking factors, particularly light, alternating temperatures and nitrate, have been widely observed and have important influences on the germination patterns of many species (Roberts E.H. 1973, Vincent and Roberts 1977, 1979).

Depth of dormancy and the resulting response to particular dormancy breaking factors are changing characteristics of seeds. Some species exhibit an annual cycle of dormancy changes (Karssen 1980/81b, Baskin and Baskin 1981, Froud-Williams *et al.* 1984). An induction of dormancy by prevailing environmental conditions may occur which alters the response to the dormancy breaking factors

involved. Other environmental factors later in the year may then reduce the depth of dormancy returning the seed to, or near to, its original state of dormancy. Depth of seed dormancy has also been shown to vary with seed age, both in the laboratory and in the soil (Roberts and Lockett 1975, 1978b). Vegis (1964) described a widening of requirements for germination as a result of after-ripening. Thus the state of dormancy may vary not only seasonally but also with seed age.

Despite the wide agricultural use of inorganic nitrates and the use of nitrates to stimulate germination in the laboratory their effect still remains poorly understood in comparison to the other dormancy breaking factors. Few efforts have been made to assess their importance in the field and recognise their involvement, if any, in the identification of safe sites for germination. As well as comparing the responses to inorganic nitrate solutions of a variety of species (22 ruderals, 6 calcicoles and 4 calcifuges) in the laboratory, this study therefore attempts to assess the importance of nitrates in field conditions.

1.2 Nitrate literature review : Laboratory experimentation.

The earliest record of stimulation of germination by nitrate in the laboratory was by Lehmann in 1909 who observed a response to KNO_3 in the dark. A similar replacement of a light requirement by nitrate was observed by Gassner in 1915(a,b). Subsequent work showed a wide range of types of response including inhibition of germination by nitrate (Lugo 1955, on Vanilla planifolia). Examples of these responses include the replacement of requirements for light or glume removal, by KNO_3, in the stimulation of germination of Poa compressa (Andersen 1932) and stimulation of 2 out of 5 Sporobolus species but

only in particular temperature regimes (Toole 1941). This work made it clear that a response to nitrate was dependent on other environmental factors and that it was not a characteristic of all species.

Steinbauer and Grigsby (1957) tested 85 weed species and found that over 50% had higher percentage germination when wetted with nitrate solutions than with water. They also demonstrated varying degrees of interaction between nitrate and other dormancy breaking factors such as light and diurnally fluctuating temperatures. A similar interaction was observed by Englehardt *et al.* (1962) who showed that addition of KNO_3 to Amaranthus hybridus seeds enhanced their sensitivity to light stimulation.

Various compounds and concentrations of nitrate were used in these experiments (Table 1.1) and this may have contributed to some extent to the variation in the type and magnitude of response being observed. Walter (1963) used a range of nitrate concentrations in his experiments as well as testing species of both a nitrophilic and non-nitrophilic nature. He also reported the work of Mayser (1954, see Schimpf 1977) with seed buried in the soil, who concluded that nitrophilic species have higher nitrate optima and maxima for germination than do non-nitrophilic species.

Many more workers have continued to experiment with nitrates as dormancy breaking factors (Williams and Harper 1965, Biswas *et al.* 1972, Hendricks and Taylorson 1972, 1974, Evans and Young 1975, Moursi *et al.* 1977, Petersen and Bazzaz 1978, Schonbeck and Egley 1980, Dey and Choudhuri 1982) with varying results which often defy direct comparison. The interactions of nitrate with other dormancy breaking factors exacerbate the problem of comparing results from different experiments due to the variation in light and temperature regimes used. One feature common to the majority of these

Table 1.1 A summary of the plant species, salts and concentrations used in a cross section of previous laboratory germination experiments employing nitrates.

Author(s)	Date	Species	Nitrate salt(s)	Concentration(s)
Andersen	1932	*Poa compressa*	KNO_3	N/50
Benjamin	1974	*Capsella bursa-pastoris* *Chenopodium album* *Poa annua*	KNO_3 $NaNO_3$ NH_4NO_3	10^{-1}, 10^{-2}, 10^{-3} M
Bostock	1978	*Achillea millefolium* *Artemisia vulgaris* *Cirsium arvense* *Taraxacum officinale* *Tussilago farfara*	$NaNO_3$	10^{-3} M
Chavagnat and Jeudy	1981	*Primula obconica*	KNO_3	0.2%
Dey and Choudhuri	1982	*Ocimum sanctum*	KNO_3	10, 25, 50 ppm
Evans and Young	1975	*Bromus tectorum*	KNO_3	0.01, 0.1, 1, 10 mM
Frankland	1961	*Corylus spp.* *Fagus spp.* *Sorbus spp.*	KNO_3	2 g l^{-1}
Hay and Cumming	1959	*Avena fatua*	$NaNO_3$ KNO_3	0.2%
Henson	1970	*Chenopodium album*	KNO_3	10^{-1} M
Hilton	1983	*Senecio vulgaris*	KNO_3	$5x10^{-4}$, 1 x 10^{-3}, $2x10^{-3}$, $5x10^{-3}$, $1x10^{-2}$, $2x10^{-2}$ M
Lugo	1955	*Vanilla planifolia*	$Ca(NO_3)_2$	1, .1, .25 g l^{-1}
Nelson	1927	*Poa spp.*	KNO_3 $NaNO_3$ $Ca(NO_3)_2$ $Pb(NO_3)_2$	.01, .1, 1 %
Ogawara and Ono	1955	Tobacco	KNO_3, $NaNO_3$, $LiNO_3$, NH_4NO_3, $Ca(NO_3)_2$, $Mg(NO_3)_2$ $Ba(NO_3)_2$, $Bi(NO_3)_3$	0.001, 0.005, 0.01 N
Petersen and Bazzaz	1978	*Aster pilosus*	KNO_3 $NaNO_3$	.001, .005, .01, .05, 0.1, 0.5 M
Popay and Roberts	1970a	*Capsella bursa-pastoris* *Senecio vulgaris*	KNO_3 $NaNO_3$ NH_4NO_3	10^{-1}, 10^{-2}, 10^{-3} M
Roberts	1963	*Oryza sativa*	KNO_3	10^{-1}, 10^{-2}, 10^{-3} M
Roberts and Lockett	1975 1978b	*Stellaria media* *Solanum nigrum*	KNO_3	0.2%
Schonbeck and Egley	1980	*Amaranthus retroflexus*	KNO_3	0.02, 0.2%
Schwendiman and Shands	1943	*Avena spp.*	KNO_3 $Ca(NO_3)_2$ NH_4NO_3	0.2, 2 %
Srivastava, Oaks and Bakyta	1976	*Zea mays*	KNO_3 $NaNO_3$	5-50 mM
Toole	1941	*Sporobolus spp.*	KNO_3	0.2%
Walter	1963	*Urtica dioica* *Sisymbrium officinale* *Solanum nigrum* *Erigeron canadensis* *Chenopodium album* *Daucus carota*	KNO_3	0.1, 0.5, 1, 5, 50, 100 mM

experiments is the predominant use of either weed or crop species (Cook 1980). This is due to their economic importance and also to the great importance of dormancy in their life cycles. Consequently, comparisons with the germination responses of species from undisturbed habitats are difficult since experiments to date have largely neglected them.

E.H. Roberts reviewed the literature (1973) and has concluded that the physiological mechanism by which nitrate stimulates germination is as an oxidising substrate in a metabolic regulatory process involving the pentose phosphate pathway of glucose metabolism. This is supported by observations that other oxidising agents such as nitrite and hydroxylamine stimulate germination in the same way (Ogowara and Ono 1955, Hendricks and Taylorson 1974), although some more recent work has not supported the theory (Fuerst 1983). Murdoch (1982) considered the possible importance of nitrite in soil but concluded that its levels were too low and that it was too short lived to be of any significance in stimulating germination in the field.

The importance of the replacement of some dormancy breaking factors by nitrate and its interaction with others has been stressed and a number of workers have concentrated on the elucidation of these complex relationships. Henson (1970) illustrated this complexity in his experiments on the effects of light, alternating temperatures and nitrate on Chenopodium album. To successfully investigate these types of interaction a factorial experimental design is required and this was adopted in a number of studies (Vincent and Roberts 1977, 1979, Bostock 1978, Roberts and Benjamin 1979, Williams 1983a). In these factorial experiments the need for a variety of temperature regimes with different diurnal ranges created the practical difficulty of providing large numbers of controlled environments.

The use of thermogradient bars has provided a large number of temperature regimes in some germination experiments (e.g. Mason 1976, Thompson 1977). Larsen *et al.* (1973) used a thermogradient plate to demonstrate a small response to nitrate by seeds of dormant rescue grass and in experiments on a thermogradient bar by Williams (1983b) a nitrate stimulation was also observed for four other grass species; Holcus lanatus, Agrostis capillaris, Poa trivialis and Festuca rubra.

1.3 Nitrate literature review : Field experimentation and seed burial.

The stimulation of germination by nitrates in the laboratory and their widespread occurrence in many soils provides the opportunity for their involvement in the recognition of safe sites for seed germination. Bormann *et al.* (1968) reported that a flush of nutrients accompanied opening of the woodland canopy and Petersen and Bazzaz (1978) proposed that this might enhance seed germination and act as a gap detecting mechanism. Friejsen *et al.* (1980) also pointed out that a loose and disturbed surface soil layer favoured mineralization and nitrification and could have the same effect.

The seasonal variation in nitrate concentrations which has been observed by a number of workers (e.g. Davy and Taylor 1974, Taylor *et al.* 1982) may play a role in the identification of seasonal safe sites. Peaks in soil nitrate levels have generally been recorded in spring and autumn coinciding with flushes of seedling emergence at those times. In addition, the application of nitrate fertilisers may have the same effect. However, despite the implication that nitrate could be responsible for stimulating some seed germination in the field few experiments have been performed to assess its actual involvement.

One step removed from the completely artificial conditions of the laboratory experiments on collected seeds described above are those which involved burial in soil of collected seed, either fresh or after a period of laboratory storage. Sinyagin and Teper (1967, cited by Schimpf 1977) observed an increase in germination of Thlaspi arvense when KNO_3 was added to the soil in which it was buried although this was less marked than on filter paper in the laboratory. However, in the same experiment the germination of wild oat was depressed by addition of nitrate to the soil. Schimpf (1977) collected the seeds of six ruderal species and applied nitrate to them following burial. Half were laboratory stored prior to testing and the other half were subjected to simulated overwintering which included a period of chilling. Only one of the species, Setaria lutescens, responded to nitrate and then only in the laboratory stored state and not after overwintering.

Experiments by Friejsen *et al.* (1980) used seeds of Cynoglossum officinale and observed higher germination levels in calcareous dune soils. However, once again collected seeds were buried and retrieved to be tested under laboratory conditions and no changes in response to nitrate during periods of natural burial could be assessed. Hence the only work in which conditions began to approach those of the field situation failed to demonstrate any response to nitrate (Schimpf 1977).

Changes in the responses of seeds to nitrate have been studied in a number of experiments involving the burial of seeds in soil for varying periods of time and their subsequent retrieval for testing in the laboratory. Bostock (1978) showed that 6 months burial caused a loss of response to nitrate in Achillea millefolium, Artemisia vulgaris and Cirsium arvense. The responses of Stellaria media and Solanum nigrum to nitrate were shown by Roberts and Lockett (1975,

1978b) to change during burial although a nitrate response was still evident in some incubation environments after 9 months burial. Karssen (1980/81b), in a similar burial experiment, showed that a variation in soil nitrate levels during burial altered the response to temperature of seeds of Sisymbrium officinale. An annual cycle of response to dormancy breaking factors for Polygonum persicaria and Senecio vulgaris was also apparent in these experiments. It is clear from the experiments mentioned above that a response to nitrate is not a static property of seeds but may change during burial. Experiments in conditions as close as possible to natural ones are clearly necessary if changes in nitrate response due to burial in the field are to be explained. The experiments described above involved either the use of freshly collected seeds buried experimentally or the use of controlled environmental conditions during incubation.

Evidence of a response to nitrate in field conditions after soil burial is sparse. Popay and Roberts (1970b) presented indirect evidence for a stimulation of the germination of Capsella bursa-pastoris by nitrate in field conditions. They observed a correlation between elevated soil nitrate levels and a flush of germination in late July and early August. However, large diurnal temperature ranges occurred at the same time and they suggested that the nitrate was acting in synergism with them. A similar correlation between nitrate and germination flushes of Capsella bursa-pastoris in May and August-September was reported by Benjamin (1974). Nevertheless, due to the complexity of field conditions and the action of factors other than nitrate it was impossible to accurately assess its importance.

Murdoch (1982) made an extensive study of the factors controlling the depletion of Avena fatua and Chenopodium album seeds

in the soil. His experiments involved the burial of seeds at several depths and the observation of field emergence as well as laboratory tests on the remaining ungerminated seeds. He carefully monitored soil nitrate levels but did not attempt to control them in the field. He suggested that lower Avena fatua emergence during periods of low nitrate levels in the soil and laboratory nitrate stimulation of the remaining ungerminated seeds was good evidence that nitrate was a major factor in controlling its emergence in the field. The temperature requirement of three year old seeds was not as strict and the importance of nitrate appeared to decline with age, despite a rise in overall germination. Chenopodium album did, however, respond to alternating temperatures and light and consequently its germination varied with depth of burial. Murdoch (1982) observed that this species had a potential to respond to nitrate in May but that this remained unrealised in the field due to the low soil nitrate levels at that time. For a field response to nitrate to occur, soil nitrate levels must be elevated at the time of seed susceptibility to its stimulation.

Attempts to manipulate soil nitrate levels in order to stimulate germination have used various nitrate fertilisers, with the species observed once again being Avena fatua and Chenopodium album. In their experiments, Sexsmith and Pittman (1963) observed a fivefold increase in Avena fatua emergence following a 41 kg ha^{-1} application of NH_4NO_3. A fourfold increase in Avena ludoviciana germination was observed by Watkins (1966) when he applied 103 kg N ha^{-1} although differences were not significant and varied with the time of year. In 1971 however, he provided supporting evidence for a stimulation by showing greater germination with $CaNH_4(NO_3)_3$ than with NH_4SO_4 or urea. (Watkins 1971). Fay (1975), using Avena fatua, showed a twofold increase in germination with a 24 kg N ha^{-1} application of nitrate

fertiliser when this was applied following the "normal period of wild oat emergence".

The work of Fawcett and Slife (1978) in which they applied levels of NH_4NO_3 from 112-336 kg N ha^{-1} and observed the emergence of six species including Chenopodium album, failed to show any increases in germination. They did, however, record that Chenopodium album seeds collected from plants grown on the fertilised plots were less dormant than those from the untreated plots.

In summary, few experiments have been performed in field conditions and only with a restricted number of species. There is, however, indirect correlative evidence for a response to nitrate in some species and changes in the response to nitrate as a consequence of burial have been observed. Of the two species principally observed in the field Avena fatua clearly responds to nitrate in the field under some conditions whereas the importance of any response by Chenopodium album is still unclear.

1.4 The experiments conducted.

The organisation of experiments is shown in Table 1.2. They range from those performed in the most controlled conditions to those in the most natural ones. There were three experimental aims: to compare the laboratory responses to nitrate of a large number of ruderal species; to observe any field responses to nitrate and to assess their importance in timing field emergence; and to compare the responses, in the field and laboratory, of species from normally undisturbed environments with low nitrate concentrations (calcareous grassland and acid heathland) with those of ruderal species, including arable weeds, likely to experience higher concentrations.

Chapters 2 and 3 describe experiments designed to observe the

Table 1.2 A plan of experimental organisation indicating the source of soil and seeds used for each experiment.

Experimental Conditions	Type of Experiment	Chapter	Source of Seeds and Soil		
			Regularly disturbed land	Calcareous Grassland	Acid Heathland
Artificial: Collected seeds tested in the laboratory.	Experiments in petri dishes with: a) a range of nitrate concentrations	2	✓	✓	✓
	b) a nitrate or water pre-treatment		✓		
	Thermobar	3	✓	✓	✓
Intermediate: a) collected seeds tested in the laboratory after burial in the field.	Burial of sachets containing seeds in the field followed by germination in controlled environments.	4	✓		
b) Naturally buried seeds tested in the laboratory	Incubation of a natural seedbank in the laboratory	5	✓	✓	✓
Natural: Naturally buried seeds tested in field conditions	Field experiment	6	✓	✓	

laboratory responses of ruderal species. Collected seed was tested either in petri dishes in controlled environment cabinets or on a thermogradient bar. This enabled an optimum nitrate concentration to be found as well as permitting the testing of twenty-two ruderal species in eleven different temperature regimes in both the light and dark. An additional experiment to observe the effects of different types of storage on dormancy was performed using a single species, Cardamine hirsuta.

An experiment involving the observation of emergence from a natural seedbank, in field conditions, was performed to observe any field responses to soil nitrate. To provide a continuity between the laboratory and field experiments and to aid in the interpretation of field results, two types of intermediate experiment were performed. In the first type, collected seed was buried for varying periods in the soil before retrieval and incubation in controlled laboratory conditions. This allowed any changes in the dormancy and behaviour of seeds during burial to be observed. The second intermediate experiment, one step nearer to field conditions, was the incubation in controlled laboratory conditions of collected soil containing a naturally buried seedbank. A supplementary experiment was performed under laboratory conditions, with collected seed, to see if nitrate could have a residual effect (Chapter 2). This involved the application of nitrate in pre-incubation conditions unsuitable for germination, and subsequent removal to incubation conditions where any responses to residual nitrate could be observed.

To compare the responses of ruderal species from disturbed habitats with species from undisturbed habitats, some of the experiments listed above were performed on calcicole and calcifuge species from calcareous grassland and acid heathland respectively. The experiments which were performed using these are indicated in Table 1.2.

CHAPTER TWO

The Effects of Nitrate in Controlled Environments.

2. The Effects of Nitrate in Controlled Environments.

2.1 Introduction.

The effects of inorganic nitrate on seed germination have been investigated in the laboratory by a large number of workers (Table 1.1). A wide variety of compounds and concentrations of nitrate have been used in these investigations making the comparison of results difficult. Although even lead nitrate has been used (Nelson 1927) the most common compounds have been NH_4NO_3, $NaNO_3$ and KNO_3. Benjamin's work with a number of compounds showed that the nature of the associated cation had no effect on the response to nitrate of Chenopodium album, Capsella bursa-pastoris and Poa annua (Benjamin 1974).

Variation in the concentration of nitrate has proved to be more important, with only slight differences in concentration being responsible for large differences in germination of some species (Walter 1963). Solutions of 10^{-2} and 10^{-3}M nitrate have been common although more concentrated ones have also been employed.

The aim of these initial petri dish experiments was to observe the effects of a range of nitrate concentrations on germination in a number of species to ascertain an optimum concentration for later experiments. This optimum value could also be compared with field levels of nitrate to assess the similarity of field and laboratory conditions.

It is evident (Table 1.1) that in most previous experiments the species used have been either crops or weeds. Their disturbed arable habitats experience relatively high nitrate levels under normal conditions. The seeds of species from more natural, and less disturbed habitats with lower nitrate levels have been neglected.

Seeds from species in these habitats as well as ruderal species were tested to compare their responses to a range of nitrate concentrations. In particular the question arises as to whether species of high nitrate environments have higher nitrate optima, and whether species from low nitrate environments fail to respond to nitrate at all. That is, whether a response to nitrate is an adaption to a high nitrate environment or not.

Three groups of 4 species were selected;

a) "Ruderal" species from disturbed habitats. Nitrate concentrations in these conditions can regularly be above 10^{-3}M and may occasionally be as high as 10^{-2}M (e.g. Popay and Roberts 1970b, Benjamin 1974).

b) "Calcicole" species collected from calcareous grassland. The nitrate levels in such environments are considered to be higher than in most undisturbed soils but lower than those experienced by the ruderal species. For instance, Havill *et al.* (1977) measured nitrate levels of 20 mg NO_3-N l^{-1} of dry soil in a Festuca ovina dominated grassland. Assuming a 20% moisture content such levels would result in a concentration of 1.6 x 10^{-3}M in the soil solution. Taylor *et al.* in 1982 reported maximum nitrate concentrations of about 1 x 10^{-3}M in a Zerna erecta dominated grassland. Nitrate levels in calcareous soils are discussed in more detail in Chapter 6.

c) "Calcifuge" species from an acidic heathland. Their key feature being that they have an ammonium based nutrition (e.g. Bogner 1968, Gigon and Rorison 1972). This apparent adaptation, and nitrate measurments in a number of soils (e.g. Scurfield and Boswell 1953, Ellenberg 1964, Harmsen and Van Schreven, review, 1955) led to the belief that acid environments are characterised by low nitrate levels, the predominant form of nitrogen being

ammonium. More recently, however, it has been suggested that low nitrate availability is associated with poor aeration and low temperatures rather than acidity *per se* (Taylor *et al.* 1982). Indeed, there have been recent investigations in which substantial rates of nitrate formation have been demonstrated in some acidic soils (e.g. Davy and Taylor 1974, Runge 1974, Ellenberg 1977). The concentrations of nitrate in acidic soils are, however, much lower than those experienced by the ruderal species, for example almost zero in a podsolised brown earth dominated by Luzula spp. (Ellenberg 1964) and as high as 7 x 10^{-4}M in a soil supporting Deschampsia flexuosa (Taylor *et al.* 1982).

We have seen in Chapter 1 that the ability of a seed to germinate in the soil depends on a number of factors including the temperature regime, lighting, oxygen availability and moisture availability. Unsuitability of one or more of these factors may prevent immediate germination of the seed, depending upon its state of dormancy. The variability of nitrate levels in the soil (e.g. Popay and Roberts 1970b, Davy and Taylor 1974, Taylor *et al.* 1982) may mean that its optimum does not coincide with other conditions suitable for germination. The second experiment is designed to assess any residual effect of nitrate. That is, to determine whether nitrate experienced in the pre-incubation environment, when conditions prevent germination, will still be stimulatory when conditions become more favourable to germination. Seeds were therefore pre-incubated (in water or nitrate) in anoxic conditions and later incubated in conditions in which any residual effect of nitrate on germination could be observed.

The existence of such a residual response to nitrate would have

important implications in the field. A flush of nitrate early in the year would be able to satisfy the nitrate requirement of all seeds buried up to that time even if the conditions were not favourable for immediate germination. The lack of a residual response would result in the retention by seeds of a response to nitrate until a nitrate flush coincided with other conditions suitable for germination.

2.2 Materials and Methods.

2.2.1 General.

Seeds of ruderal species were collected from plants in disturbed habitats, the majority coming from wild plants in the University of Keele Botanical Gardens or from nearby arable farmland. Species nomenclature follows Clapham, Tutin and Warburg (1981).

Collection of the seeds of "calcifuge" species was from acid heathlands in the Gritstone Region of the Peak District in North Staffordshire (Grid ref. SK 033,634) and on Cannock Chase in South Staffordshire (Grid ref. SK 00,15). The sample of Digitalis purpurea is an exception to this and was collected near Ayr in Scotland. Seeds of "calcicole" species were collected from sites in the calcareous White Peak near Bakewell, Derbyshire, predominantly from a South facing slope in Monsal Dale (Grid ref. SK 184,717). The origin and collection time of every seed lot is listed in Appendix A.

The method of seed collection was consistent for all species. They were collected from at least ten individual plants in one population and allowed to dry in laboratory conditions for between three and five days. Only seeds approaching their natural time of dispersal from the parent plant were collected, ensuring that only ripe seed was used for experimentation. Following drying, the seeds

were cleaned of any investing structures and separated from waste material by combinations of sieving, rubbing and blowing. The cleaned seeds were then stored at 2°C to 4°C in screw top glass jars, to prevent significant ageing of the seed over the normally short periods of storage. Popay (1968) showed this method of storage to be successful for seeds of Capsella bursa-pastoris and Senecio vulgaris which were stored for 2 years in airtight conditions without loss of viability and with little appreciable loss of dormancy. The effect of varying periods of dry-storage at 5°C was tested for a large number of species by Grime *et al.* (1981), who found that only a few species were seriously affected by storage of less than 6 months. Mention of specific effects on particular species will be left to Section 2.3.

Counting of seeds into samples of 50 or 100 for experimentation was only done following thorough mixing of each batch to incorporate any smaller seeds which may have settled out in storage. The samples of seeds were then placed into small aluminium foil sachets which were selected at random for particular treatments.

2.2.2 Nitrate concentration experiment: Laboratory methods.

Four species from each of the three habitat types were selected for testing with a range of nitrate concentrations in petri dishes (Table 2.1).

The ruderal species were the four most common dicotyledonous weeds in the University Botanical Gardens: the annual (Cardamine hirsuta), the overwintering annuals (Senecio vulgaris and Stellaria media)and the perennial (Artemisia vulgaris). These representative ruderal species are used in a number of other experiments described in this and other chapters.

Table 2.1 A list of the species tested in the nitrate concentration experiment. The light regime employed was dependent upon the results of thermogradient bar experiments on the same species.

	Light conditions	
	Dark	12 hr photoperiod
Ruderals:		
Artemisia vulgaris	*	
Cardamine hirsuta	*	
Senecio vulgaris		*
Stellaria media		*
Calcicoles:		
Hypericum hirsutum		*
Inula conyza	*	
Leontodon hispidus	*	*
Origanum vulgare		*
Calcifuges:		
Calluna vulgaris		*
Digitalis purpurea		*
Erica tetralix		*
Juncus conglomeratus		*

A range of KNO_3 solutions (double glass distilled water being the solvent) at each molarity from 10^{-1}M to 10^{-6}M and water alone were used as moistening agents.

Germination tests were done in 9cm plastic petri dishes. Seeds were placed on two layers of Whatman No. 1 filter paper supported by a layer of 0.6cm diameter borosilicate glass beads containing the 17ml solution reservoir. Three replicates of 50 seeds were used for each treatment. Preliminary experiments without the reservoir had produced unacceptably large variation between replicates due to drying and concentration of the solutions. Unlike ordinary glass, borosilicate beads do not affect the pH of the solution (N. Peters 1981, pers. comm.). To prevent moisture loss the petri dish lids were sealed with a smear of soft white paraffin.

Results obtained from the thermogradient bar experiments (Chapter 3) determined that some species were tested only in the dark, some with a 12 hour photoperiod, and one, Leontodon hispidus in both of these conditions (Table 2.1). Warm White fluorescent tubes provided illuminance varying from 20 to 36 Wm^{-2}. To remove any effects of this variation from treatment differences each species was tested in only one cabinet and individual petri dishes were regularly re-randomised within it. For treatments in continuous darkness a supplementary light treatment was necessary to indicate the rate of germination and a suitable time for final counting. Dark treatments were wrapped in two layers of aluminium foil.

Thermogradient bar results showed that apart from Senecio vulgaris all of the species exhibit maximum germination in a temperature regime with a large diurnal range. Controlled environment cabinets were therefore used with a temperature regime of 25°C in the "day" and 5°C at "night", each for a 12hr period. Senecio vulgaris was found to have an optimum temperature regime in

the dark of 8/18°C and consequently it was tested under these conditions.

Germination was considered to have occurred when at least 1mm of radicle was visible. In light treatments seeds which had germinated were counted and removed every one or two days. In general, dark treatments were opened for counting when germination in the light control on any two consecutive days was less than 3% of the total number which had already germinated. However, some species which showed consistently slow germination, with perhaps only one or two seeds a day throughout, were incubated for longer than this simple rule would have permitted.

2.2.3 Nitrate concentration experiment: Statistical methods.

In all analyses germination percentages have been transformed to ensure normality using the arcsine of the square root of the proportion.

Curvilinear regressions were performed on the percentage germination in a range of nitrate concentrations by stepwise polynomial analysis producing linear, quadratic, cubic and quartic fitted curves. Goodness of fit was indicated by the significance of variance ratios. Using the Standard Errors from these analyses 95% confidence intervals for the lines of best fit were calculated, and drawn only at specific points for clarity. In calculating confidence intervals for the means of the water treatments, or any other isolated treatments, the standard error from an analysis of variance for all treatments from that particular species was used as the best estimate of error variation.

In the case of some of the calcicole and calcifuge species tested in the light, where all treatments produced similar high

germination percentages, the mean number of days to germination was calculated. A curvilinear regression was then also applied to these values.

2.2.4 Pre-incubation experiment: Laboratory methods.

In this experiment seeds of the four ruderals were submerged in either a nitrate solution or water as a "pre-incubation" treatment before being incubated in nitrate or water in petri dishes as described above. This was to determine whether pre-incubation exposure to nitrate in conditions unfavourable to germination (lacking oxygen) could have a residual effect on seeds later experiencing conditions favourable to germination.

For each species ten replicates of 50 seeds each were submerged in 8cm depth of either 10^{-2}M KNO_3 or double glass distilled water in airtight glass jars. It was expected that these conditions would be sufficiently anoxic to prevent germination. Each of these jars was then wrapped in two layers of aluminium foil and placed in a sealed black plastic container in a dark room at room temperature, to further reduce the likelihood of germination. After four days of storage the solution in all jars was replaced by double glass distilled water and the jars were left for a further 24hrs. After this period the water was itself replaced by fresh water to remove as much external nitrate solution from the seeds as possible.

Following removal of any seeds which had germinated under these conditons five replicates of each treatment were then placed in petri dishes in double glass distilled water or 10^{-2}M KNO_3 solution, as described above. This resulted in five replicates in each of four treatments;

1. Water during pre-incubation and incubation. W/W
2. Water during pre-incubation and nitrate in incubation. W/N
3. Nitrate during pre-incubation and water in incubation. N/W
4. Nitrate during pre-incubation and incubation. N/N

For Artemisia vulgaris in the dark and Stellaria media in the light germination in petri dishes was tested in a 5/25°C temperature regime. Senecio vulgaris was initially tested in the dark at 8/18°C but was later transferred to the light. Cardamine hirsuta seed was tested in the dark at 5/25°C but was from a batch of seed collected a year earlier and had been stored in the laboratory for that length of time. This seed was selected on the basis of a preliminary experiment (Chapter 3) which showed that although fresh seed only had a low germination percentage in the dark on the thermogradient bar, laboratory stored seed had both an increased percentage germination and also showed a response to nitrate.

Both the changing of the pre-incubation solutions in the darkroom and the placement of seeds of Senecio vulgaris, Cardamine hirsuta and Artemisia vulgaris into petri dishes was performed under green "safe light". This consisted of 3 layers of Cinemoid No. 39 Filter (Rank Strand Electrics Ltd.) surrounding a Daylight fluorescent tube sealed with insulation tape, to keep red and far-red emission to a minimum. Although this construction, recommended by Smith, (1975) is not "safe" for extended periods of use the short period of exposure of less than two minutes was considered "safe" because a photostationary state is only achieved over a long period of time at low irradiances by such a light. Bostock (1976) used a dim green "safelight" to monitor dark treatments of Artemisia vulgaris and Achillea millefolium and concluded that short exposures had no effect on the germination percentage of the two species in

water at a constant 25°C. Green light was shown to stimulate germination of Stellaria media following its burial in the soil for 16 or 18 months (Baskin and Baskin 1979), although the period of exposure was 5-15 min, substantially longer than in these experiments. Thus, although evidence for the use of "safe green" light is certainly equivocal (Baskin 1983, pers. comm.) its effect was considered to be negligible and its use necessary for short periods of exposure when operations could not be reliably performed in the dark.

2.2.5 Pre-incubation experiment: Statistical methods.

For both Artemisia vulgaris and Senecio vulgaris a "T-test" was used to compare the percentage germination which had occurred in the glass jars prior to petri dish incubation. A two way analysis of variance (pre-incubation versus incubation condition) was used to analyse the total germination percentages for each species. Differences between particular treatments were tested using Duncan's "*a posteriori*" multiple range test (referred to below as Duncan's MRT). This procedure, of an analysis of variance followed by Duncan's MRT, was also used in analysing the percentage germination occurring in incubation alone and in the case of Senecio vulgaris in incubation in the dark or light separately.

2.3 Results.

2.3.1 Nitrate concentration experiment: Ruderals.

The curves of best fit for the four ruderal species are shown in Figure 2.1. Artemisia vulgaris, which was tested in the dark, and

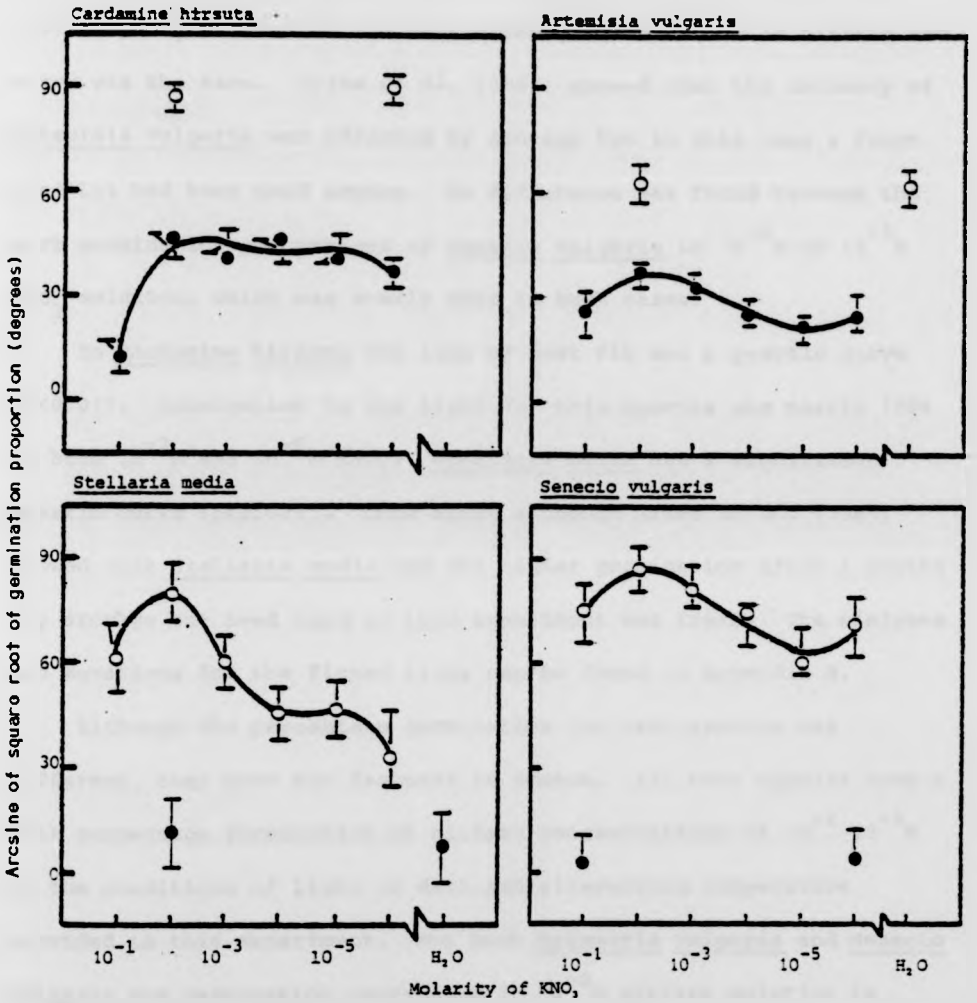


Figure 2.1 Nitrate concentration experiment : Ruderal species.
Fitted curves with $p \leq 0.05$ significance for the arcsine square root transformed proportion of germination over a range of KNO_3 concentrations. O, light; ●, dark. The temperature regime was 5/25°C except for Senecio vulgaris with 9/18°C.

Senecio vulgaris, which was tested in the light, produced cubic fitted curves ($p<0.01$). In the case of Artemisia vulgaris, germination percentages in additional treatments in the light were uniformly higher than in the dark although germination in nitrate and water was the same. Grime *et al.* (1981) showed that the dormancy of Artemisia vulgaris was affected by storage but in this case a fresh seed lot had been used anyway. No difference was found between the dark germination percentages of Senecio vulgaris in 10^{-6}M or 10^{-1}M KNO_3 solution, which was nearly zero in both cases.

In Cardamine hirsuta the line of best fit was a quartic curve ($p<0.01$). Germination in the light for this species was nearly 100% in both 10^{-2}M and 10^{-6}M KNO_3. Stellaria media has a significant quartic curve ($p<0.001$). Once again although Grime *et al.* (1981) showed that Stellaria media had 36% higher germination after 3 months dry storage the seed used in this experiment was fresh. The analyses and equations for the fitted lines can be found in Appendix B.

Although the percentage germination for each species was different, they have two features in common. All four species have a peak percentage germination at nitrate concentrations of 10^{-2}-10^{-3}M in the conditions of light or dark and alternating temperature provided in this experiment. For both Artemisia vulgaris and Senecio vulgaris the germination percentage in 10^{-2}M nitrate solution is different ($p<0.05$) from the germination in 10^{-4}, 10^{-5} or 10^{-6}M nitrate. In Stellaria media the response to higher nitrate concentrations is greater than for the other species and germination in 10^{-2}M solution is higher ($p<0.05$) than at any other concentration. The response of Cardamine hirsuta to nitrate is less marked but nevertheless the germination percentage in 10^{-3}M KNO_3 is greater than that in 10^{-6}M KNO_3 ($p < 0.05$).

The other common feature is a reduced level of germination in

10^{-1}M KNO_3. In all but Senecio vulgaris the mean percentage germination in 10^{-1}M nitrate is significantly lower ($p<0.05$) than that in 10^{-2}M nitrate, indicating that this concentration was supra-optimal. This was confirmed by the observation that, except in Senecio vulgaris, germination was abnormal in 10^{-1}M nitrate solution; the variability was greater and many of the seedlings were deformed and unhealthy.

2.3.2 Nitrate concentration experiment: Calcicoles.

The fitted curves for Hypericum hirsutum and Origanum vulgare in the light and for Inula conyza in the dark are shown in Figure 2.2. No significant curves could be fitted to the germination values for Leontodon hispidus in the light or dark and the mean values have been plotted, with 95% confidence intervals from an analysis of variance. Full analyses and equations are in Appendix B. Hypericum hirsutum had a straight line ($p<0.05$) dipping with increasing concentration, and Origanum vulgare a quadratic fitted curve ($p<0.01$). Inula conyza had a significant ($p<0.01$) quartic fitted curve but had extremely variable germination, possibly the result of a freak period of elevated temperature in the controlled environment cabinet when this species was being tested.

For both Origanum vulgare and Hypericum hirsutum when the values of germination in 10^{-1}M KNO_3 were omitted from the curvilinear regression, no significant fitted curves could be found. Since the significance of the fitted curves in these cases rely on the much lower germination percentages in 10^{-1}M nitrate, no evidence of stimulation or inhibition by the other nitrate concentrations can be extracted from this data.

Although significant graphs can be fitted to the mean days to

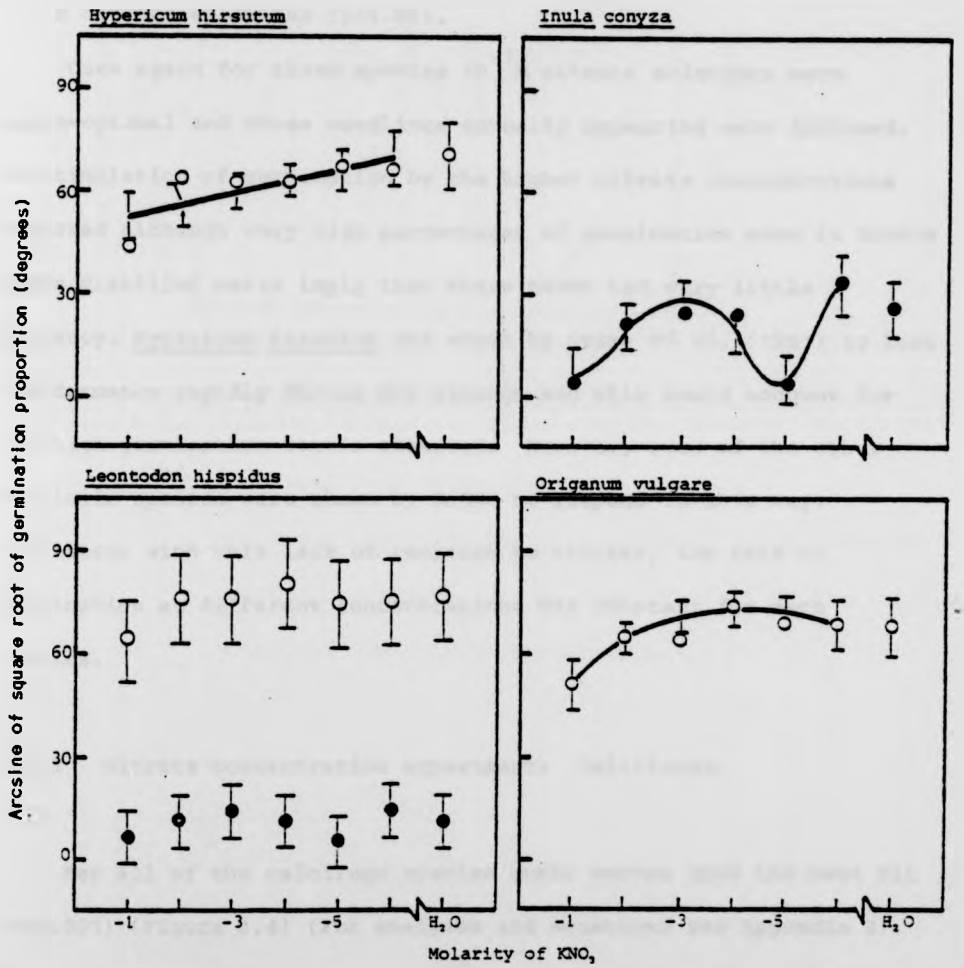


Figure 2.2 Nitrate concentration experiment : Calcicole species. Fitted curves with $p \leq 0.05$ significance for the arcsine square root proportion of germination over a range of KNO_3 concentrations, O, light; ●, dark. In the case of Leontodon hispidus a significant curve could not be fitted and confidence limits with a 0.05 significance were determined using an analysis of variance.

germination values for Origanum vulgare and Hypericum hirsutum (Appendix B), they too rely for their significance on the much slower germination rates in 10^{-1}M nitrate. Figure 2.3 shows the quadratic ($p<0.05$), mean day fitted curve for Hypericum hirsutum with a non-significant trend towards more rapid germination in 10^{-2}M to 10^{-4}M nitrate solutions ($p>0.05$).

Once again for these species 10^{-1}M nitrate solutions were supra-optimal and those seedlings actually appearing were deformed. No stimulation of germination by the higher nitrate concentrations occurred although very high percentages of germination even in double glass distilled water imply that these seeds had very little dormancy. Hypericum hirsutum was shown by Grime *et al.* (1981) to lose its dormancy rapidly during dry storage and this could account for the high germination levels observed. However, none of the other calcicole species were shown by Grime to respond in this way. Consistent with this lack of response to nitrate, the rate of germination at different concentrations was constant for each species.

2.3.3 Nitrate concentration experiment: Calcifuges.

For all of the calcifuge species cubic curves gave the best fit ($p<0.001$) (Figure 2.4) (For analyses and equations see Appendix B). However in every case only the 10^{-1}M nitrate treatment has 95% confidence intervals which do not overlap with the confidence interval for the other nitrate concentrations. Of these species Grime *et al.* (1981) showed that only Erica tetralix significantly responded to dry storage. This response was, however, only an 8% increase in germination over 3 months.

For Digitalis purpurea, Erica tetralix and Calluna vulgaris the

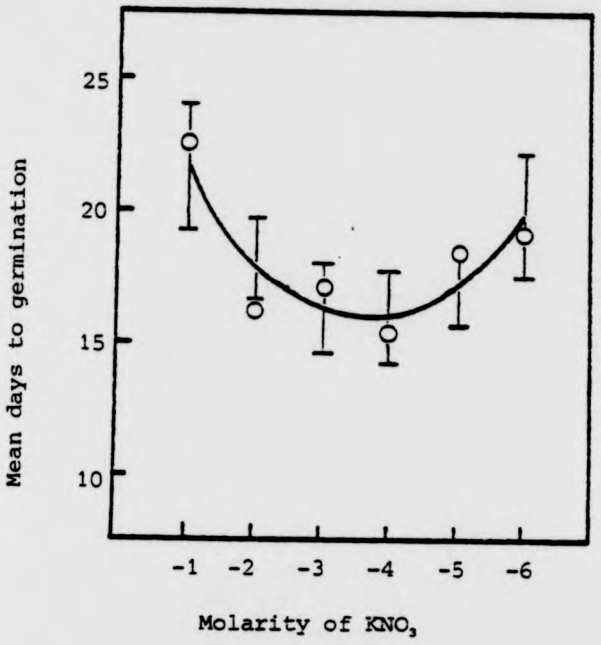


Figure 2.3 The mean days to germination of Hypericum hirsutum in a range of concentrations of KNO_3 solution, in the light at 5/25°C. The vertical bars are 95% confidence intervals.

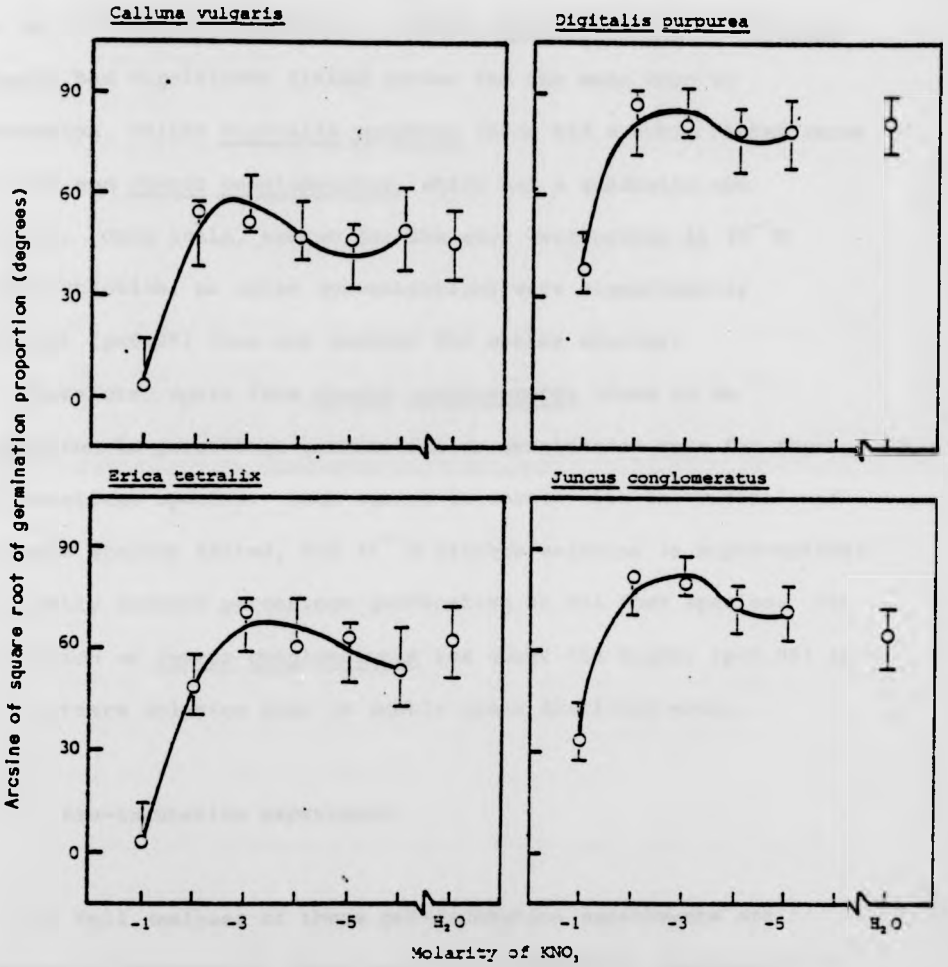


Figure 2.4 Nitrate concentration experiment : calcifuge species.
Fitted curves with $p \leq 0.05$ significance for the arcsine square root proportion of germination over a range of KNO_3 concentrations.
O, light; ●, dark.

mean percentage germination in double glass distilled water was not significantly different from that in the other nitrate concentrations, except in 10^{-1}M. The germination percentage in water for Juncus conglomeratus was, however, significantly different from that in 10^{-2}M nitrate solution. Neither Erica tetralix nor Calluna vulgaris had significant fitted curves for the mean days to germination, unlike Digitalis purpurea which had a cubic fitted curve ($p<0.05$) and Juncus conglomeratus, which had a quadratic one ($p<0.05$). Once again, except for the slow germination in 10^{-1}M nitrate solution, no other concentrations were significantly different ($p<0.05$) from one another for either species.

Therefore, apart from Juncus conglomeratus there is no stimulation in percentage germination or germination rate for these four calcifuge species. Once again, in common with the ruderal and calcicole species tested, the 10^{-1}M nitrate solution is supra-optimal and greatly reduced percentage germination in all four species. The germination of Juncus conglomeratus was about 15% higher ($p<0.05$) in 10^{-2}M nitrate solution than in double glass distilled water.

2.3.4 Pre-incubation experiment.

The full analyses of these pre-incubation experiments are presented in Appendix C. There was no pre-incubation germination of Cardamine hirsuta in either nitrate or water. Its germination in petri dish incubation (Figure 2.5) ranged from about 70 to 90 percent although no significant differences between the four treatments were observed. It is possible in this case that the seed had after-ripened in the laboratory storage and had entirely lost the requirement for light apparent in fresh seed. Further experiments on the effect of various types of storage on Cardamine hirsuta are

Figures 2.5-2.8

Percentage germination of

Cardamine hirsuta	Figure 2.5
Artemisia vulgaris	Figure 2.6
Stellaria media	Figure 2.7
and Senecio vulgaris	Figure 2.8

in four different combinations of pre-incubation and incubation solutions:

1. Water during pre-incubation and incubation W/W
2. Water during pre-incubation and 10^{-2}M KNO_3 solution during incubation W/N
3. 10^{-2} KNO_3 solution during pre-incubation and water during incubation N/W
4. 10^{-2} KNO_3 solution during both pre-incubation and incubation N/N


Germination in the pre-incubation environment


Germination during incubation in an environment with a 12 hour photoperiod.


Germination during incubation in the dark.

All incubation took place in a 5/25°C diurnal temperature alternation with a 12 hour thermoperiod except that of Senecio vulgaris which experienced an 8/18°C diurnal temperature alternation.

The significance of differences in germination between treatments is discussed in the text.

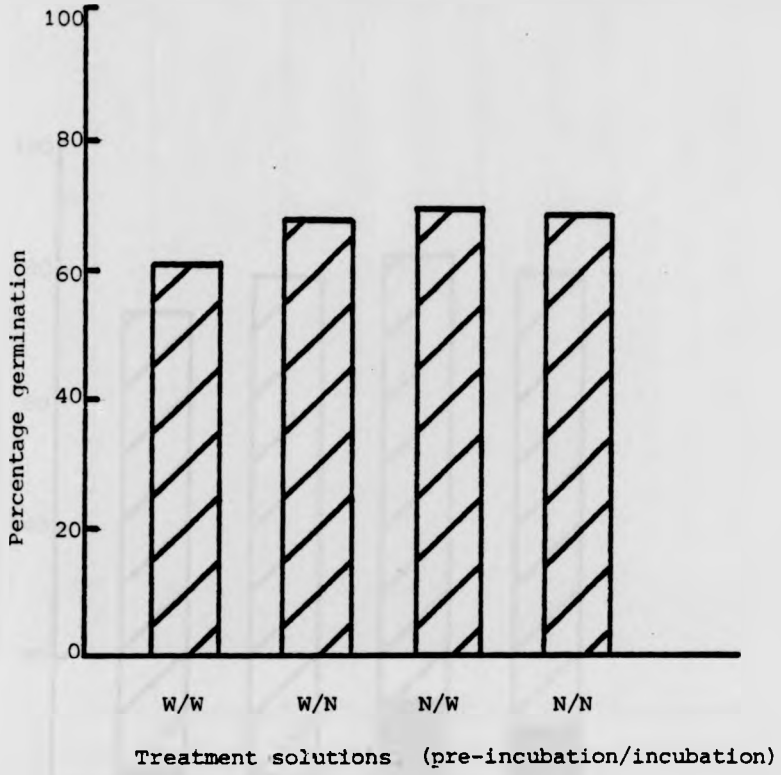


Figure 2.5 Cardamine hirsuta

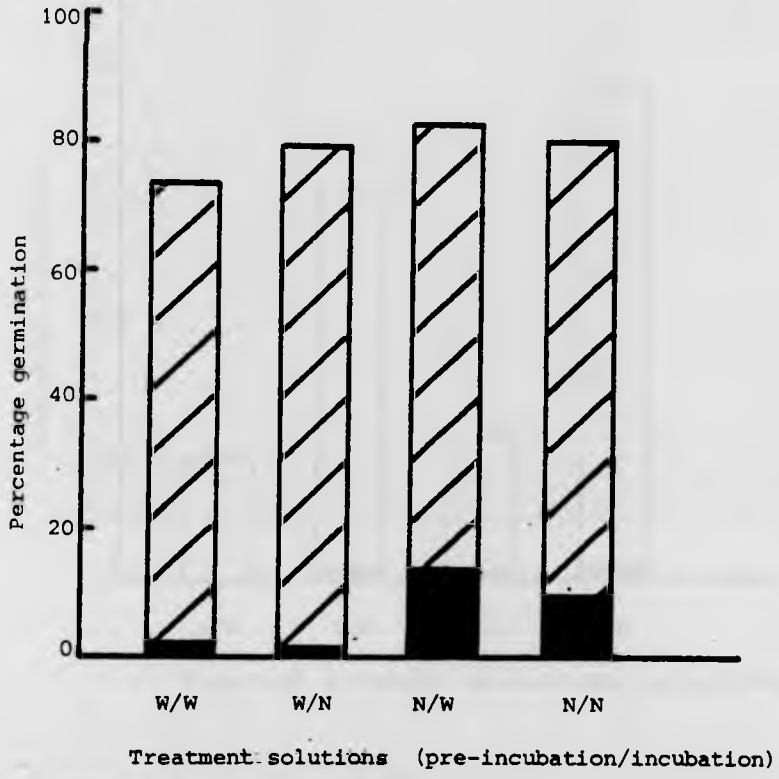


Figure 2.6 Artemisia vulgaris

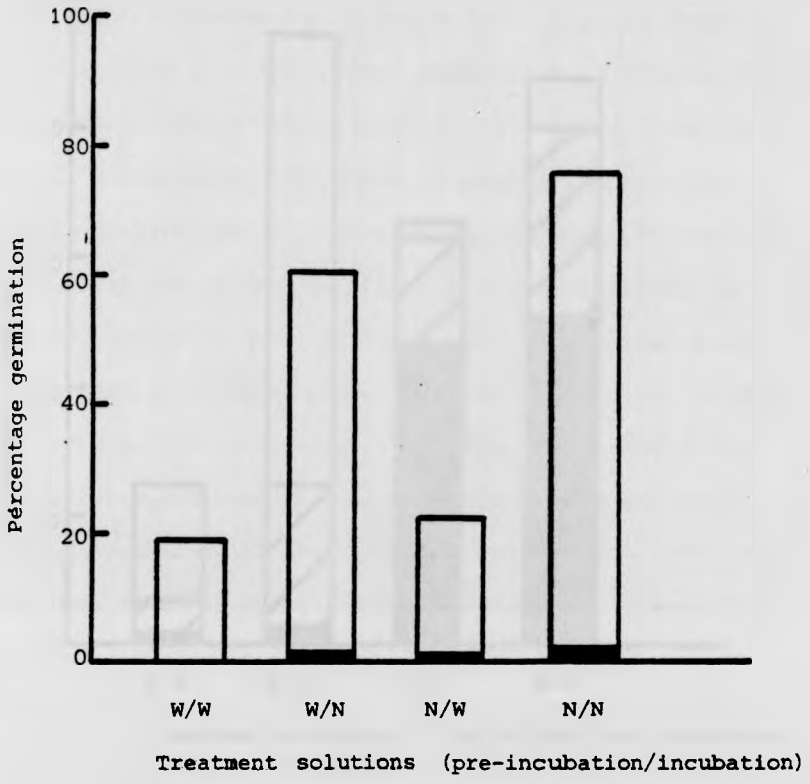


Figure 2.7 Stellaria media

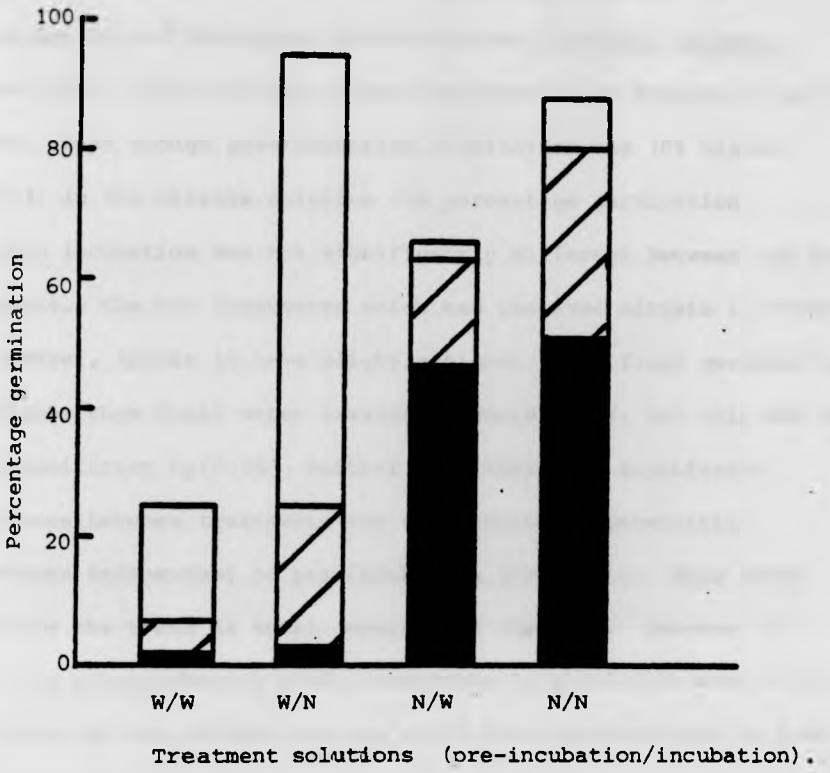


Figure 2.8 Senecio vulgaris

described in Chapter 3.

The other species tested entirely in the dark i.e. in pre-incubation and throughout incubation was Artemisia vulgaris (Figure 2.6), which also had a total germination of between 70 and 90 percent. Even though pre-incubation germination was 10% higher ($p<0.001$) in the nitrate solution the percentage germination following incubation was not significantly different between the four treatments. The two treatments which had received nitrate in storage did, however, appear to have slightly higher total final germination percentages than their water treatment counterparts, but this was not quite significant ($p<0.06$). Neither were there any significant differences between treatments for the incubation germination percentages independent of pre-incubation germination. This lends support to the trend in total germination observed. However, to enable the pre-incubation water treatments to attain the same total germination as the nitrate ones we would have expected them to have significantly greater germination during incubation. The only suggestive difference even approaching significance ($p<0.1$) between the individual totals using Duncan's MRT was between the W/W and N/W treatments. It seems that nitrate during incubation had therefore been sufficient to increase total germination in the W/N treatment above that of the W/W treatment, but not to bring it to the level of the treatments which had received pre-incubation nitrate; N/W and N/N. However, since no significant differences can be shown between the W/N and any other treatments this explanation cannot be statistically supported.

Pre-incubation germination of Stellaria media (Figure 2.7) was <2% and did not respond to nitrate. The analysis of variance for its incubation germination in the light, shows a higher mean germination ($p<0.001$) in those seeds incubated in nitrate solution than those in

water. The differences between those receiving nitrate and those receiving water pre-incubation was 4 and 16% which were non-significant differences ($p<0.1$). That is, if a pre-incubation effect occurred, it was small. No interaction was present between pre-incubation and incubation treatments.

The most complex responses to this range of treatments occurred with Senecio vulgaris (Figure 2.8), which was initially incubated in the dark and later moved into the light. Apart from the pre-incubation germination in the storage jars which was significantly ($p<0.001$) higher in nitrate than in water it is necessary to consider, firstly, the germination total following incubation in the dark and secondly the total germination following light incubation. We may also consider in a separate analysis the increments of increased germination due to both dark and light incubation individually.

The mean values for total germination following dark incubation are all significantly different from one another ($p<0.05$) although there is no interaction between pre-incubation and incubation nitrate treatments. The ANOVA for the numbers germinating during dark incubation, indicates that any of the treatments with nitrate, in either pre-incubation or incubation, gave significantly higher germination than the treatment that only received water ($p<0.05$). The magnitude of the carry-over effect of pre-incubation nitrate into incubation was determined by taking the germination during dark incubation as a percentage of the ungerminated seeds left after pre-incubation. In this way a significant carry-over of pre-incubation nitrate was demonstrated. Those treatments receiving it giving, on average, 33.9% greater germination of remaining seeds than those pre-incubated in water. That is to say, pre-incubation nitrate applied in conditions when 50% of the seeds were unable to

germinate had a stimulatory effect on the germination of a third of the remaining seeds when they were placed in more favourable conditions.

Following incubation in the light the importance of a residual response to nitrate was lost since the total germination of the W/N treatment exceeded that of the N/W treatment. Although the germination of seeds which did not receive nitrate at any time (i.e. the W/W treatment) was still substantially lower in the light than those which received nitrate during either pre-incubation or incubation, an additional 16% of germination occurred. There is also evidence of an interaction between nitrate and light, with the W/N treatment experiencing a 70% increase in germination following exposure to light. The N/N treatment did not show such an increase, but since 86% of these seeds had germinated it is probable that the maximum germination had been reached. In view of this strong response to nitrate in the presence of light it is suggested that the higher levels of germination with pre-incubation nitrate could have been caused by exposure to the green "safe light". This cannot be resolved from this experiment but does not alter the significance of the increased germination caused by a carry-over effect of nitrate (with or without light).

2.4 Discussion.

Past investigations into the effects of nitrate solutions on germination have concentrated on species such as arable weeds and crops from disturbed habitats with relatively high natural soil nitrate levels. Species of more stable habitats which have naturally lower nitrate concentrations have been neglected.

It is clear from the experiments using a range of nitrate

concentrations that the four ruderal species behaved in a very different way to the calcicoles and calcifuges. All four ruderal species showed a peak of germination at nitrate concentrations between 10^{-2} and 10^{-3}M. This optimum range of nitrate concentrations agrees with that found by Walter (1963) for a number of other ruderal species including Urtica dioica, Solanum nigrum and Chenopodium album. Hilton (1983) showed that at a constant 20°C with increasing nitrate concentration from 5×10^{-4}M up to 2×10^{-2}M, Senecio vulgaris gradually lost its light requirement. Other workers have also found 10^{-2}M and 10^{-3}M nitrate solutions to be optimal for the germination of ruderal species; e.g. Hesse 1924 reported in Toole *et al.* 1956, Williams and Harper 1965, Petersen and Bazzaz 1978. For these species, the nitrate optima for germination may correspond to the peak nitrate concentrations experienced in the soil (Popay and Roberts 1970b, Vincent 1974, Roberts and Benjamin 1979). Nitrate concentrations in calcareous and acidic soils are believed to be considerably lower, as discussed in Section 2.1.

For both the calcicole and calcifuge species there was no consistent response to high nitrate concentrations in a 5/25°C diurnal temperature range with the exception of Juncus conglomeratus. The rate of germination was also unaffected by the higher nitrate concentrations. It therefore appears that a response to high nitrate concentrations could be the result of the adaptation of species to environments experiencing intermittently high nitrate levels. The adaptation of ruderal species to such habitats could explain the difference in their response to the range of nitrate concentrations used, to that of the calcicole and calcifuge species. It is of course possible that these unresponsive species may react to high nitrate concentrations under different diurnal temperature conditions and these are therefore further investigated in Chapter 3.

One feature of germination common to ruderal, calcicole and calcifuge species was the detrimental effect of 10^{-1}M nitrate solution. In the case of the calcicoles and calcifuges it severely reduced germination percentages and was clearly toxic. Of the ruderal species only Cardamine hirsuta had reduced germination in 10^{-1}M nitrate solution but with the exception of Senecio vulgaris the seedlings of the other ruderals were unhealthy and deformed. Although supra-optimal for all twelve species, the ruderals thus appeared to be slightly more resistant to this concentration, another possible adaptive feature of species inhabiting potentially high nitrate environments. Work by both Henson (1970) and Williams and Harper (1965) on Chenopodium album successfully used 10^{-1}M KNO_3 although they eventually showed 10^{-3}M KNO_3 solution to be optimal.

The pre-incubation experiment was designed primarily to see if the nitrate responsible for an increase in germination has to be present in the medium during germination or whether it can fulfil its purpose prior to the onset of actual germination. If nitrate in a pre-germination environment was able to stimulate germination at a later time from that of its application, this would be an important consideration in any field studies (Chapter 6). The peaks of nitrate experienced in soil conditions may not immediately stimulate germination but could have a residual effect with a delayed increase in germination when other conditions become more favourable. In both Artemisia vulgaris and Cardamine hirsuta no such carryover of nitrate was evident although an unsuitable batch of aged seeds may account for this lack of response in Cardamine hirsuta. However, in Stellaria media the evidence for a carry-over of nitrate was on the borderline of statistical significance. Furthermore 33.9% more germination of Senecio vulgaris seeds which had received nitrate but remained ungerminated during pre-incubation, occurred during

incubation, than of seeds which had received water during the pre-incubation. This significant carry-over of nitrate occurred despite careful washing of the seeds after pre-incubation. Hence, this carryover which appears to exist in at least two out of the four ruderal species tested may affect our understanding of the field experiment results presented in Chapter 6.

It has been suggested that the mode of action of nitrate in stimulating germination may be in acting as an oxidising agent in the pentose phsophate pathway (Ogawara & Ono 1955, Roberts E.H. 1973). This role as an oxidising agent may explain the stimulation of germination of both Artemisia vulgaris and Senecio vulgaris under pre-incubation conditions. In a submerged, partially anaerobic, environment the nitrate may have been able to substitute for oxygen, accounting for the higher germination percentages in the nitrate treatments.

In the case of Senecio vulgaris there was an apparent interaction between light and nitrate in stimulating germination and for a small percentage of the seeds the light also appeared to be able to substitute for nitrate in its stimulation. The significance of these results may be clearer when thermogradient bar tests have been described. There have been reports of both light/nitrate interactions (e.g. Roberts, E.H. 1973, Vincent and Roberts 1977) and the substitution of nitrate for light but a more detailed discussion of these effects will follow in Chapter 3.

CHAPTER THREE

Thermogradient Bar Experiments.

3. Thermogradient Bar Experiments.

3.1 Introduction.

The importance of interactions between nitrate and other dormancy breaking factors has been stressed by a number of workers (Vincent and Roberts 1977, 1979, Roberts and Benjamin 1979, Roberts and Lockett 1975, 1978b and Bostock 1978). In many species, nitrate has been found to replace or interact synergistically with light and alternating temperatures in stimulating germination. These effects have been studied using a 2^N factorial experimental design. However, because temperature alternation is a continuous variable, there is a need for a variety of temperature regimes with different diurnal ranges, creating a practical difficulty in providing large numbers of controlled environments. Past experiments have consequently been restricted in the number of temperature treatments available.

In the experiments described below, a thermogradient bar (hereafter called a thermobar), of the type used by Thompson and Grime (1983), has been used to observe the responses to nitrate in a wide range of temperature regimes. Factorial experiments with eleven diurnal temperature ranges, with and without light and/or nitrate, were performed using this equipment. The interactions of these factors were observed and patterns of response in different species were categorised. Twenty-two common ruderal species were tested; the presence of a response to nitrate for some of these species having already been observed (Chapter 2). Representatives of the other two types of species used in Chapter 2, calcicoles and calcifuges, were also tested on the thermobar to investigate the possibility of a response to nitrate under a wider range of conditions.

During the course of these thermobar experiments a change in the response of Cardamine hirsuta to nitrate was observed following laboratory storage. Such changes in response have been observed in

different species by other workers (Bostock 1978, Roberts and Lockett 1975, 1978b) as a result of the widening of germination requirements in seeds after-ripening in storage (Vegis 1964). The nature of these changes with regard to nitrate was investigated in this species; dried seeds of Cardamine hirsuta were stored in a variety of conditions and later tested on the thermobar.

3.2 Materials and Methods.

3.2.1 Temperature control of the thermobar.

The thermobar used was constructed from two aluminium blocks through which was pumped glycol from three temperature controlled reservoirs. This temperature control was effected by an arrangement of aquarium heaters and cooling coils described in further detail in Appendix D. The larger of the two aluminium blocks (65 x 50 x 4 cm) had sixteen slots (56 x 2.3 x 2.5 cm) cut into its surface in which trays of seeds could be placed for incubation. Temperature control of this block provided a large diurnal temperature range of approximately 20 C° at one end and a relatively small alternating temperature range (of 3-5 C°) at the other. The large diurnal temperature range fluctuated between a "daytime" temperature of about 25°C and a "night-time" temperature of about 5°C. This regime was chosen as a compromise between the soil surface temperatures of open ground in spring (see Chapter 6) and the ability of the equipment to achieve low temperatures. The smaller temperature alternation was intended to correspond to the temperatures experienced at a depth of 4 cm in the same soil. Thus, intermediate points along the thermobar provided temperatures representing varying depths in the soil.

The smaller aluminium block (50 x 7 x 4 cm) with 16 similar

slots (5 x 2.3 x 2.5 cm) in it was maintained at as close to a constant 10°C as possible. Both blocks were surrounded by a layer of insulating polystyrene foam. The pipes connnecting them to the glycol reservoirs were also insulated.

During its three year period of operation the thermobar required three periods of maintenance which resulted in four distinct temperature regimes; A-D (Table 3.1). These were largely comparable and the small differences between them resulted from minor adjustments and the routine replacement of parts. Unfortunately, in regimes B and C it was not possible to attain a constant temperature and a slight alternation was recorded on the constant bar. The results for each species are expressed using the actual diurnal temperature ranges experienced by them, as monitored by thermistor beads embedded in the lower surface of the blocks and attached to a chart recorder. The accuracy of temperature control is also given in Table 3.1. By means of an electrically operated time clock the temperature was changed every twelve hours corresponding to the daylength in late March and September. This thermoperiod is that used by Williams (1983b) although various other workers have used a variety of other regimes (e.g. Thompson and Grime 1983). Following the changeover, the temperature took about ninety minutes to rise to its "daytime" level. It took a maximum of five hours to drop completely to its "night-time" level, but in some regimes this was reached much more rapidly.

Three sets of an additional petri dish treatment of the type described in Chapter 2 were placed in a constant environment cabinet in the dark with water at a constant 25°C. These behaved as a comparison with the 5/25°C end of the thermobar to check that the temperature alternation rather than just the elevation in temperature was responsible for any increases in germination.

Table 3.1 Thermobar temperature regimes. Values are the diurnal temperature ranges (C^{o}) at each station for the four temperature regimes. The accuracy of temperature control in each regime is also given.

	Regime A	Regime B	Regime C	Regime D
Constant bar	0	4.8	7.0	0
Station 1	4.2	8.6	5.0	3.0
2	5.9	9.8	7.1	5.0
3	7.6	11.1	9.2	7.0
4	9.2	12.3	11.3	9.0
5	10.9	13.6	13.4	11.0
6	12.6	14.8	15.6	13.0
7	14.3	16.0	17.7	15.0
8	15.9	17.3	19.8	17.0
9	17.6	18.5	21.9	19.0
10	19.3	19.8	24.0	21.0
Accuracy of control	$\pm 2^{o}C$	$\pm 1.5^{o}C$	$\pm 2^{o}C$	$\pm 1^{o}C$

3.2.2 Experimental design and techniques.

Groups of 50 seeds were placed at intervals along strips of Whatman 3MM chromatography paper which were supported by a layer of borosilicate beads within aluminium trays (55 x 2 x 2 cm). These trays were placed in the slots on the thermobar, and 25 ml of either KNO_3 or double glass distilled water was poured around the beads, where it acted as a reservoir of moistening agent for the seeds. Each tray accommodated 10 batches of seeds, each occupying 13 mm of the filter paper length and separated by 26 mm gaps. Similar smaller trays (4.5 x 2 x 2 cm) with one batch of 50 seeds were placed in the slots of the constant temperature bar. Eleven temperature treatments with a series of different diurnal ranges were thus provided.

Each slot in the bar was covered with either a transparent perspex lid or a tightly fitting aluminium lid, giving a light or dark treatment respectively. Four Warm White fluorescent tubes supported 30 cm above the bar by a frame, provided lighting of between 19.6 and 32.8 Wm^{-2} during the 12 hr "daytime" period of the cycle. A double glazed, transparent perspex lid was placed between the bar and the lighting. To check that no light was entering the dark treatments, three replicate petri dishes of seeds, each wrapped in 2 layers of aluminium foil, were placed in a controlled environment cabinet at 8/18°C for comparison with the same treatment on the thermobar; if the numbers germinating on the thermobar proved to be higher than in the cabinet a light leak would be suspected.

The air volume in the dark slots was flushed twice daily using small air pumps. This took place through 4 mm diameter piping and rubber tubing which was an integral part of the aluminium lids and connected the constant temperature bar slots with those on the main bar (Figure 3.1).

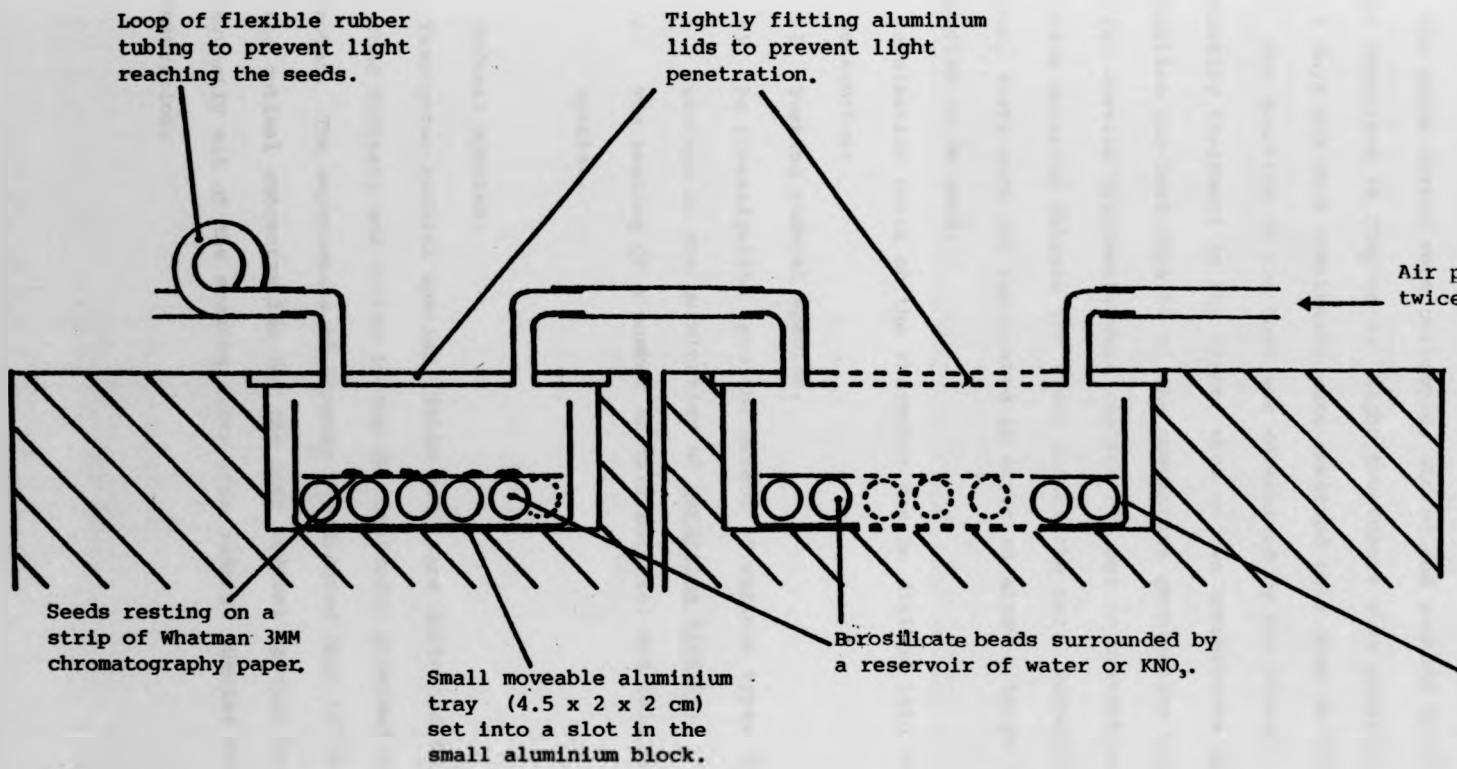


Figure 3.1 A longitudinal section of the thermobar illustrating the set up for germination testing of seeds in the dark. For germination in the light, the tightly fitting aluminium lids were replaced by clear perspex lids and there was no requirement for a loop of rubber tubing attached to the smaller lid. The diagram is life size but the lengths of the larger tray and block have been reduced for convenience.

Each species was thus tested under 11 temperature regimes, each in light and darkness and with water and KNO_3 solution (11 x 2 x 2 treatments).

The seeds tested were collected, stored and counted in the manner described in Chapter 2. Light treatments were observed every 2 or 3 days and dark treatments were observed only once at the end of test. The duration of the test was determined by the slowest germinating treatment in the light, when on two consecutive days its germination was less than 5% of the cumulative germination to date. In a few species treatments were replicated, but no systematic variation occurred between the slots across the bar. Normally, however, tests were not replicated in order to allow a larger number of species to be used.

Germination tests on the thermobar can be divided into three main categories:

a) Testing ruderal species.

b) An investigation into the effect of various types of storage on the germination of Cardamine hirsuta.

c) The testing of a number of calcicole (6) and calcifuge (4) species.

a) Ruderal species.

Twenty-two ruderal species (Table 3.2) were collected from disturbed habitats and tested in the 44 treatments provided by the thermobar. The experiments of Chapter 2 indicated that 10^{-2}M KNO_3 was the optimal concentration for the four ruderal species and consequently all of the ensuing tests with ruderal species used this concentration.

Table 3.2 A summary of the responses to nitrate, duration of tests and temperature regimes experienced on the thermobar by each of the 22 ruderal species.

Conditions in which a response to nitrate occurred.	Species	Temperature regime (see Table 3.1)	Duration of test (Days)
In the light and dark	*Artemisia vulgaris*	A	10
	Capsella bursa-pastoris	A	11
	Chenopodium album	B	11
	Cirsium arvense	A	10
	Papaver rhoeas	B	13
	Plantago major	B	12
	Polygonum convolvulus	C	18
	Silene alba	A	11
	Solanum nigrum	B	18
	Stellaria media	B	16
	Urtica dioica	B	12
In the light only	*Achillea millefolium*	A	10
	Cardamine hirsuta (fresh)	B	15
	Chamerion angustifolium	B	11
	Gnaphalium uliginosum	B	18
	Rumex obtusifolius (small response)	A	11
In the dark only	*Polygonum lapathifolium*	C	11
	Senecio vulgaris	B	6
	Silene nutans	B	13
No response	*Polygonum persicaria*	B	14
	Sagina procumbens	B	18
	Spergula arvensis	B	14

b) *Cardamine hirsuta* storage.

Preliminary experiments were performed on the thermobar, using just two layers of chromatography paper soaked in nitrate or water as a source of moisture, with no reservoir. This method produced unacceptably large error variation. The use of beads with a surrounding reservoir overcame the problem and all species were tested using this method. However, the early tests on *Cardamine hirsuta* revealed a change in its germination characteristics following storage. A batch of seeds stored for 4 months in airtight glass jars at room temperatures was retested after an additional 5 months storage and was found to have a higher germination percentage in darkness and a greater response to nitrate.

To investigate this after-ripening and the associated dormancy changes, a fresh collection of *Cardamine hirsuta* seed was made and tested immediately on the thermobar with a liquid reservoir. Seeds were then stored for 21 months in three environments before being retested on the thermobar. The environments were:

1. In a refrigerator at 2-4°C in a screwtop glass jar.
2. On the laboratory bench in an open glass jar.
3. In paperbags in a Stevenson screen at the University Botanical Gardens.

The first of these corresponds to, the conditions in which all other seeds were stored, although only for periods of up to 5 months. Storage in the soil was not examined in this series of experiments since its effects were investigated independently (Chapter 4).

c) Calcicole and calcifuge species.

Table 3.3 lists the six calcicole and four calcifuge species which were tested on the thermobar with and without 10^{-3}M KNO_3.

The only non-ruderal species to respond to nitrate in petri dish

Table 3.3 The calcicole and calcifuge species tested on the thermobar, with the duration of each test and the temperature regime used.

	Temperature regime used (see Table 3.1)	Duration of test (Days)
Calcicoles:		
Carex flacca	C	28
Hypericum hirsutum	C	28
Inula conyza	C	12
Leontodon hispidus	C	20
Linum catharticum	D	26 days after chilling
Origanum vulgare	D	14
Calcifuges:		
Calluna vulgaris	C	35
Digitalis purpurea	C	21
Erica tetralix	D	47
Vaccinium myrtillus	D	33

tests was Juncus conglomeratus which showed greatest germination with 10^{-3}M KNO_3 nitrate (Chapter 2). Unfortunately, adequate supplies of Juncus conglomeratus seeds were not available for this thermobar experiment. Species which did not respond to nitrate in petri dishes at 5/25°C, might, however, show a response in the other alternating temperature regimes provided by the thermobar. Digitalis purpurea seed was only tested in the light because no germination had occurred in darkness in petri dish tests.

Initially Linum catharticum failed to germinate in any of the treatments and was placed in a refrigerator at 2-4°C for a 41 day period of chilling, during which the dark treatments were wrapped in 2 layers of aluminium foil. When the trays were returned to the thermobar some germination occurred.

3.2.3 Analysis.

Transformed values (arcsine square root) of percentage germination for water treatments were subtracted from those for nitrate treatments for each regime. In most cases this produced a nitrate minus water (N-W) graph with eleven values to which a curvilinear analysis of the type described in Chapter 2 could be applied, although in some cases fewer values were available. These N-W graphs enabled direct comparison between species, of the types of nitrate response experienced.

The mean number of days to germination (see Chapter 2) were calculated for the light treatments of species with similar high germination values in both nitrate and water. This was also done for species with variable germination and a slight nitrate effect. These mean day values could also be used to produce N-W graphs, but few significant curves could be fitted.

3.3 Results

The good agreement between the replicates of Linum catharticum following chilling (Figure 3.2) and between those of Achillea millefolium and Capsella bursa-pastoris in supplementary tests (Figure 3.3) illustrate the small variation between slots across the bar.

Of the species not already discussed in Chapter 2 only five were shown by Grime *et al.* (1981) to have significantly greater germination after 3 months dry storage (Achillea millefolium, Polygonum lapathifolium, Polygonum persicaria, Solanum nigrum and Vaccinium myrtillus). A further two, Plantago major and Urtica dioica, were shown to have reduced germination levels after the same period. In these experiments the time of storage was, however, relatively short and any changes would be slight.

3.3.1 Ruderal species.

In Table 3.2 the ruderal species tested are divided into groups according to whether they responded to nitrate in the light only, in the dark only, in both or in neither. The duration of each particular test is also given. Graphs of percentage germination for each species are contained in Appendix E with the mean percentage germination in the supplementary petri dishes at constant 25°C and 8/18°C in the dark. In all cases there was a good agreement betwen the 8/18°C, dark petri dish treatment and the germination percentage under the same conditions on the thermobar, which indicates that the dark treatments on the thermobar were sufficiently "light tight". The germination at 25°C in the dark was only slightly greater than at a constant 10°C, and was lower than on the thermobar at 5/25°C, which

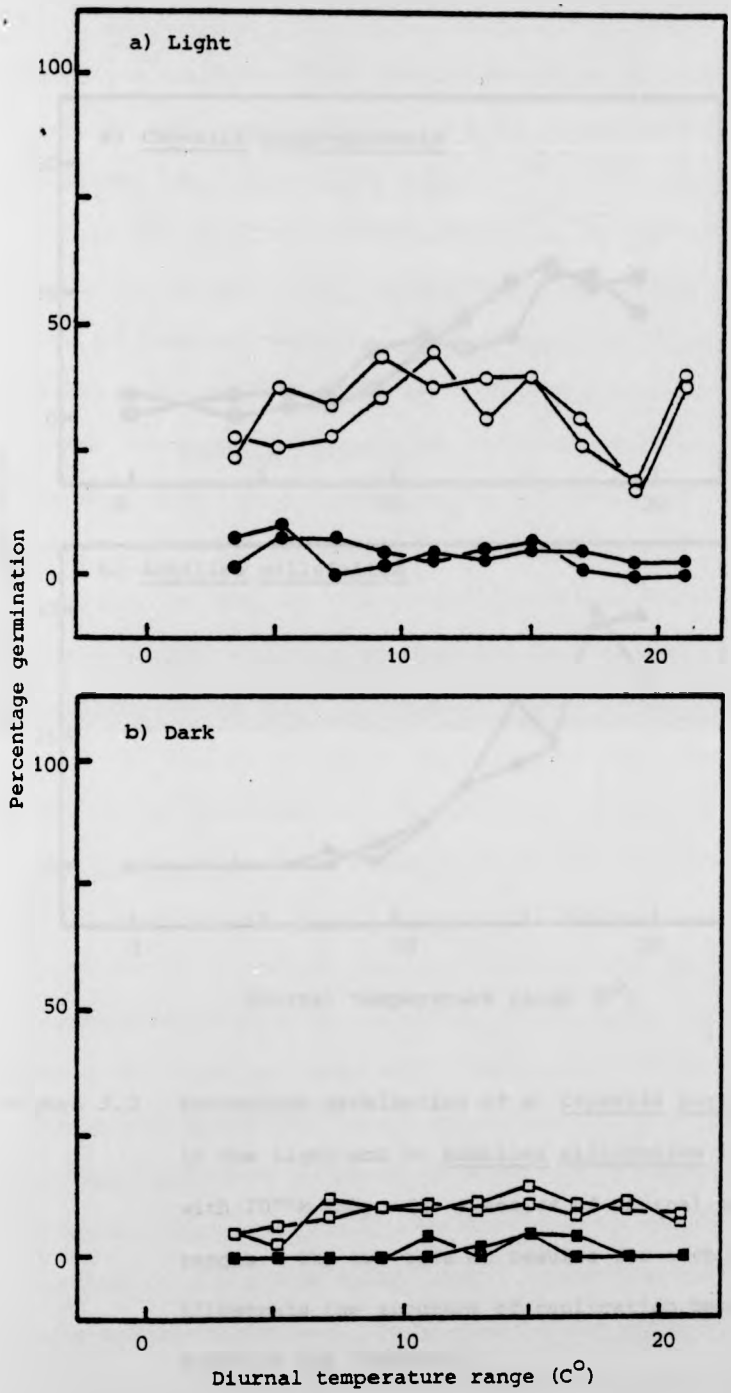


Figure 3.2 Percentage germination of Linum catharticum at a series of diurnal temperature ranges, following a 41 day period of chilling. The two sets of results in each environment, illustrate the accuracy of replication on the thermobar.

● 10^{-3} M KNO_3, light; ○ water, light;
■ 10^{-3} M KNO_3, dark; □ water, dark;

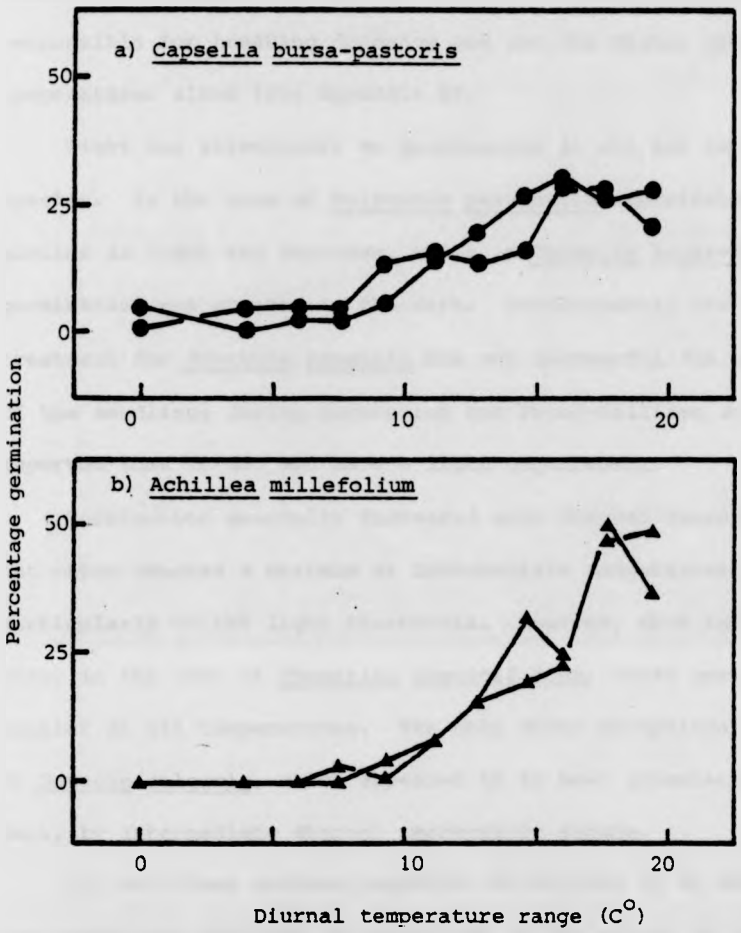


Figure 3.3 Percentage germination of a) Capsella bursa-pastoris in the light and b) Achillea millefolium in the dark, with 10^{-3} M KNO_3, for a series of diurnal temperature ranges. The two sets of results for each species illustrate the accuracy of replication between different slots on the thermobar.

supports the assumption that it was temperature alternation that was responsible for breaking dormancy and not the higher mean or absolute temperatures alone (see Appendix E).

Light was stimulatory to germination in all but two of the species. In the case of Polygonum persicaria, germination was similar in light and darkness, while in Capsella bursa-pastoris germination was greater in the dark. Unfortunately the dark treatment for Spergula arvensis was not successful due to mortality of the seedlings during incubation but Froud-Williams *et al.* (1984) reported that it did not have a light requirement.

Germination generally increased with diurnal temperature range, but often reached a maximum at intermediate temperatures, particularly in the light treatments. However, this feature is not clear in the case of Chamerion angustifolium, where germination was similar at all temperatures. The only other exceptional case is that of Senecio vulgaris, which appeared to be most stimulated in the dark, by intermediate diurnal temperature ranges.

All but three species responded to nitrate in at least some of the conditions although the magnitude of the effect of nitrate varied in different light and temperature treatments. Eleven of the N-W curves showed significant linear regressions (Figure 3.4 and Table 3.4), indicating that the response to nitrate increased linearly with the diurnal temperature range. In three species the N-W values gave statistically significant fitted curves rather than straight lines (Figure 3.5 and Table 3.4). In the case of Urtica dioica, for example, this was because both nitrate and water germination reached maximum values (corresponding to 100% possible germination) in the treatments with large diurnal ranges. Similar effects could be observed in the N-W graphs of a number of other species (Figure 3.6), but the curves fitted did not achieve statistical significance.

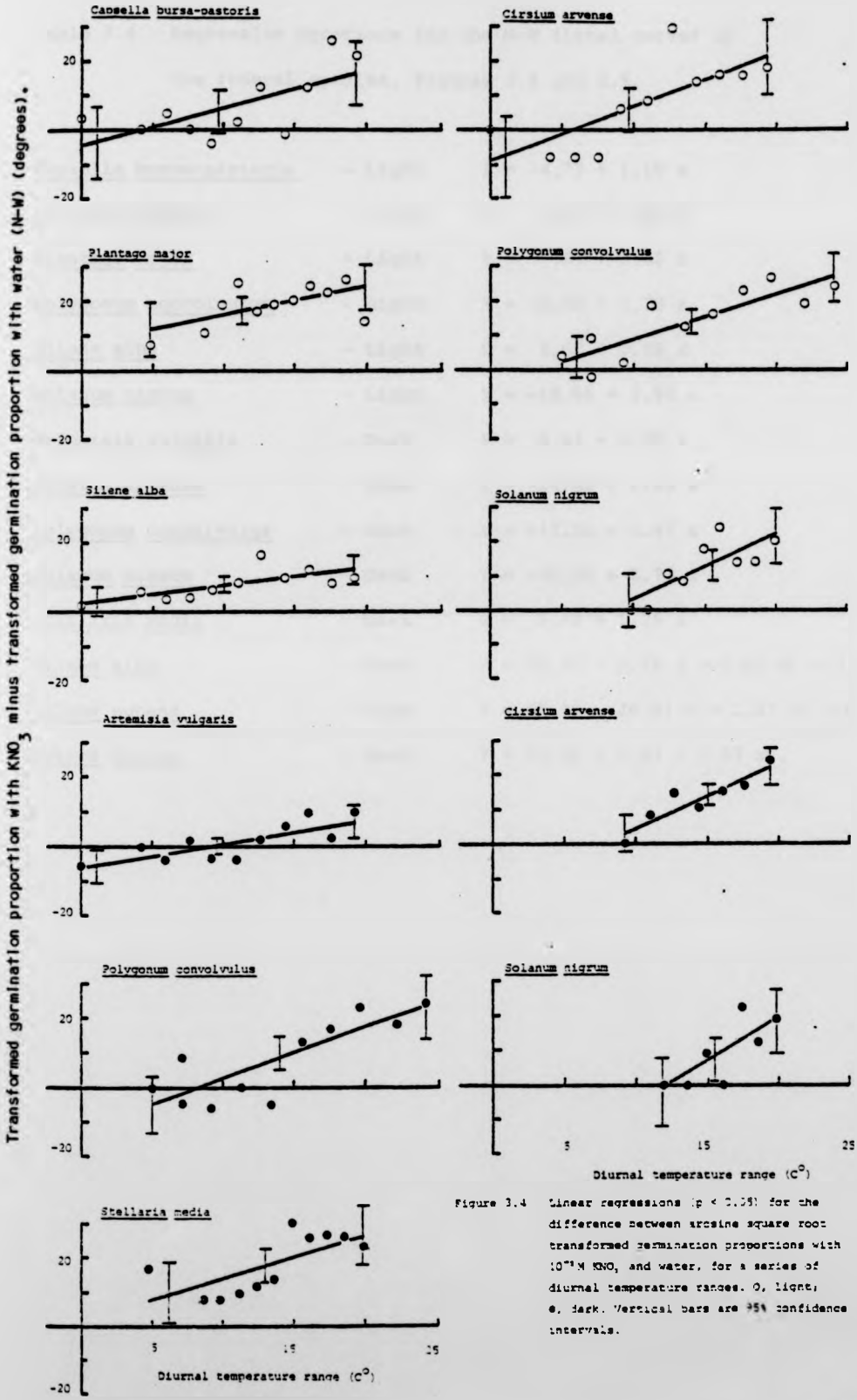


Figure 3.4 Linear regressions ($p < 0.05$) for the difference between arcsine square root transformed germination proportions with 10^{-1}M KNO_3 and water, for a series of diurnal temperature ranges. ○, light; ●, dark. Vertical bars are 95% confidence intervals.

Table 3.4 Regression equations for the N-W fitted curves of the ruderal species, Figures 3.4 and 3.5.

Capsella bursa-pastoris	- Light	$Y = -4.73 + 1.10\ x$
Cirsium arvense	- Light	$Y = -9.24 + 1.58\ x$
Plantago major	- Light	$Y = 7.64 + 0.82\ x$
Polygonum convolvulus	- Light	$Y = -4.02 + 1.28\ x$
Silene alba	- Light	$Y = 2.66 + 0.49\ x$
Solanum nigrum	- Light	$Y = -15.95 + 1.90\ x$
Artemisia vulgaris	- Dark	$Y = 6.43 + 0.70\ x$
Cirsium arvense	- Dark	$Y = -14.48 + 1.90\ x$
Polygonum convolvulus	- Dark	$Y = -12.52 + 1.47\ x$
Solanum nigrum	- Dark	$Y = -35.05 + 2.73\ x$
Stellaria media	- Dark	$Y = 1.45 + 1.26\ x$
Silene alba	- Dark	$Y = 10.57 - 5.16\ x + 0.61\ x^2 - 0.02\ x^3$
Silene nutans	- Dark	$Y = 82.46 - 26.01\ x + 2.37\ x^2 - 0.06\ x^3$
Urtica dioica	- Dark	$Y = 25.36 + 5.82 - 0.23\ x^2$.

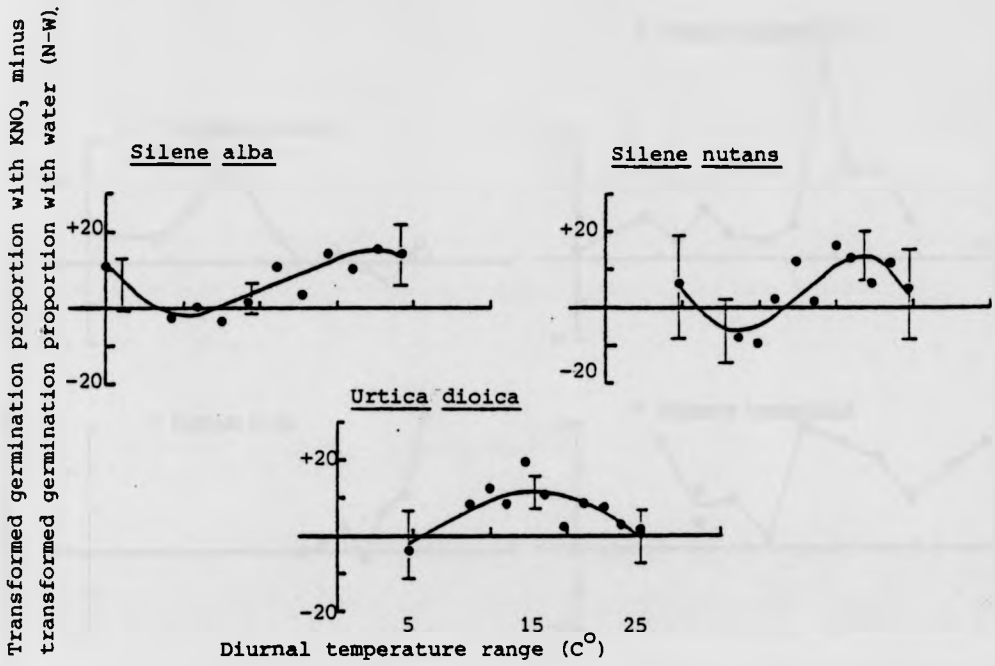


Figure 3.5 Non-linear regressions ($p \leq 0.05$) for the difference between arcsine square root transformed germination proportions with 10^{-2} M KNO_3 and water, in the dark, for a series of diurnal temperature ranges. Vertical bars are 95% confidence intervals.

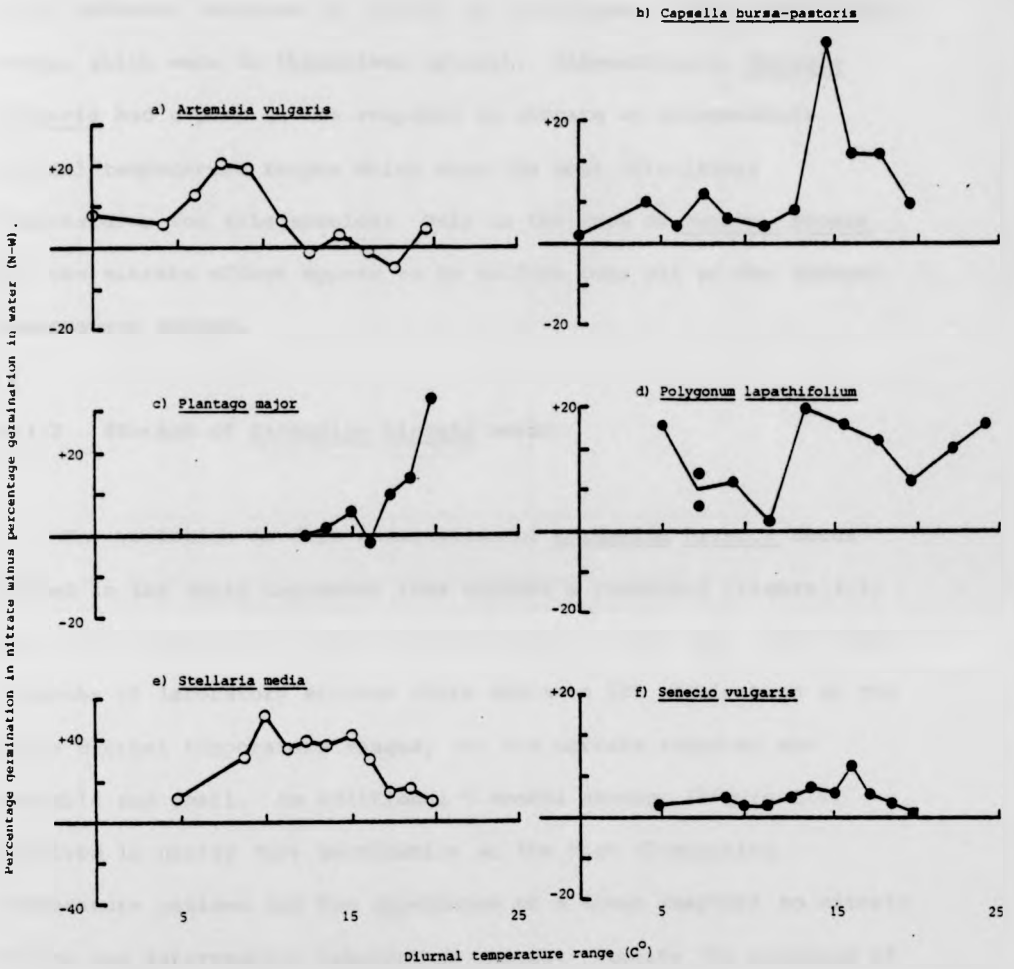


Figure 3.6 The difference between percentage germination of six ruderal species in 10^{-2}M KNO_3 and water, for a series of diurnal temperature ranges. Although statistically significant fitted curves were not available for these results, the graphs of raw data provide additional examples of the types of nitrate response that may occur.

Thus, most species which did not achieve 100% germination showed their greatest response to nitrate at the largest diurnal temperature ranges which were in themselves optimal. Interestingly, Senecio vulgaris had a peak in its response to nitrate at intermediate diurnal temperature ranges which were the most stimulatory temperatures for this species. Only in the case of Papaver rhoeas did the nitrate effect appear to be uniform over all of the diurnal temperature ranges.

3.3.2 Storage of Cardamine hirsuta seeds.

The variation in dark germination of Cardamine hirsuta seeds tested in the early thermobar runs without a reservoir (Figure 3.7) was lower than for the other species tested in that way. After just 4 months of laboratory storage there was some 60% germination at the large diurnal temperature ranges, but the nitrate response was variable and small. An additional 5 months storage (Figure 3.7) resulted in nearly 100% germination at the high alternating temperature regimes and the appearance of a clear response to nitrate at low and intermediate temperature ranges. Despite the presence of this nitrate response over most of the temperature regimes, a statistically significant N-W curve could not be fitted. However, after-ripening clearly reduced its dormancy and made a nitrate response evident.

The germination of fresh seeds of Cardamine hirsuta (Figure 3.8) was 100% in the light with large temperature ranges so that a nitrate effect was evident only with smaller ranges. Thus, the response to nitrate was restricted to a narrow band of temperature conditions which failed to give complete germination. In the dark, however, germination was very low (up to 15%) and no nitrate response could be

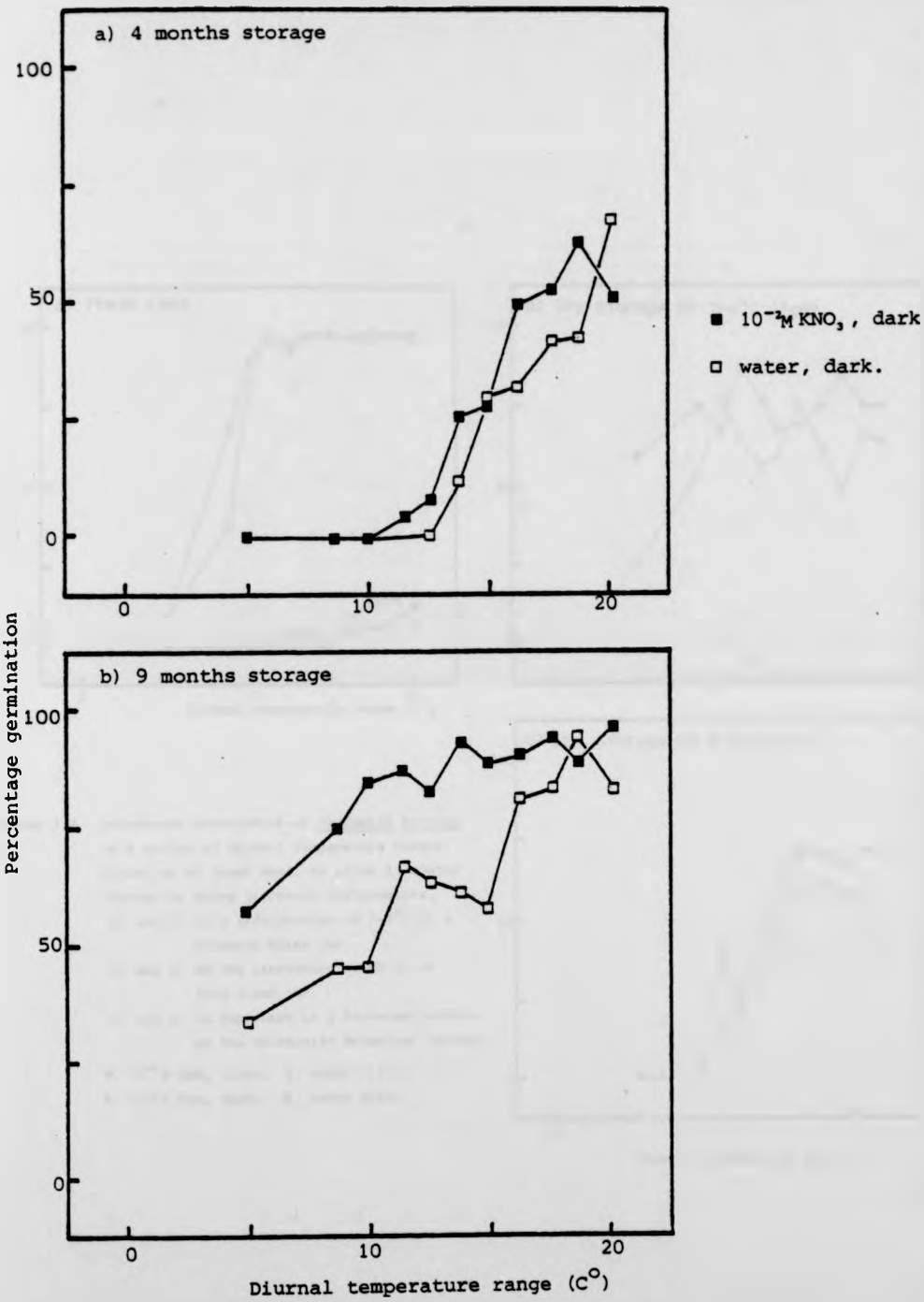


Figure 3.7 Percentage germination of Cardamine hirsuta, after a) 4 months storage and b) 9 months storage, at a series of diurnal temperature ranges. These germination tests were performed on the thermobar using two layers of chromatography paper, soaked in nitrate or water as a substrate.

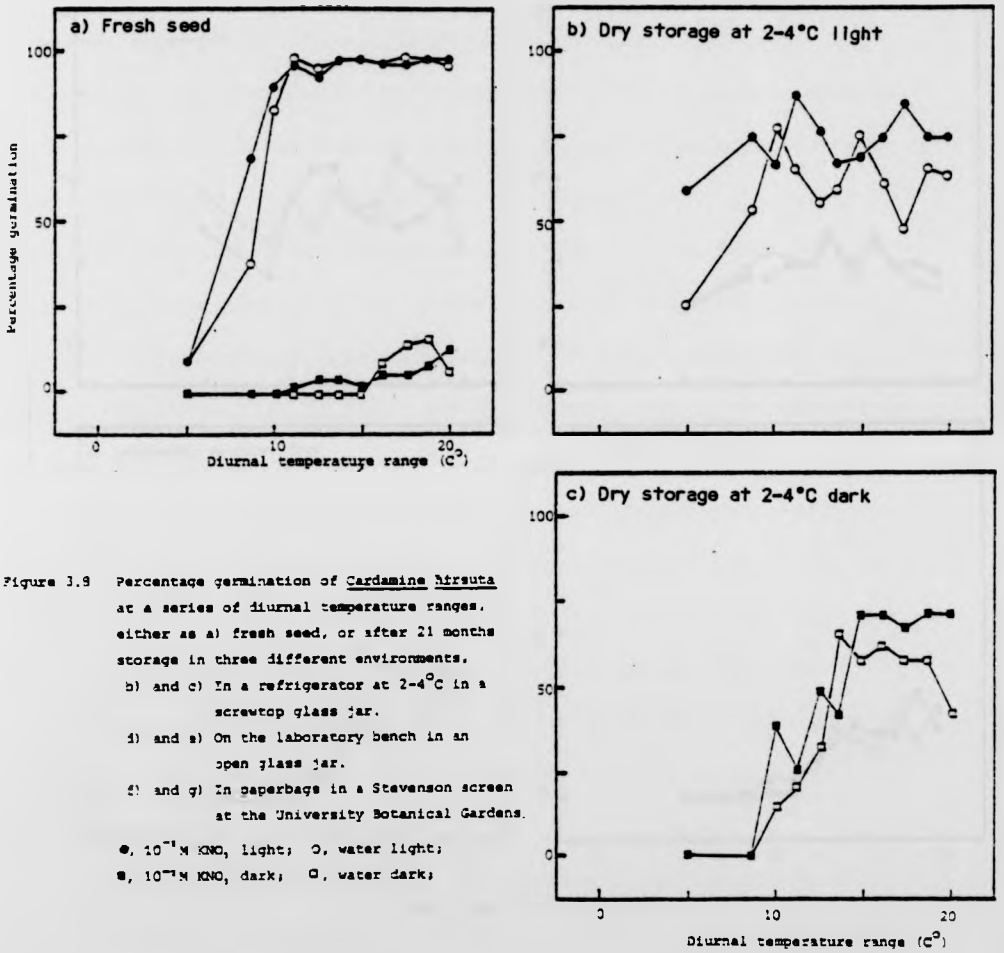
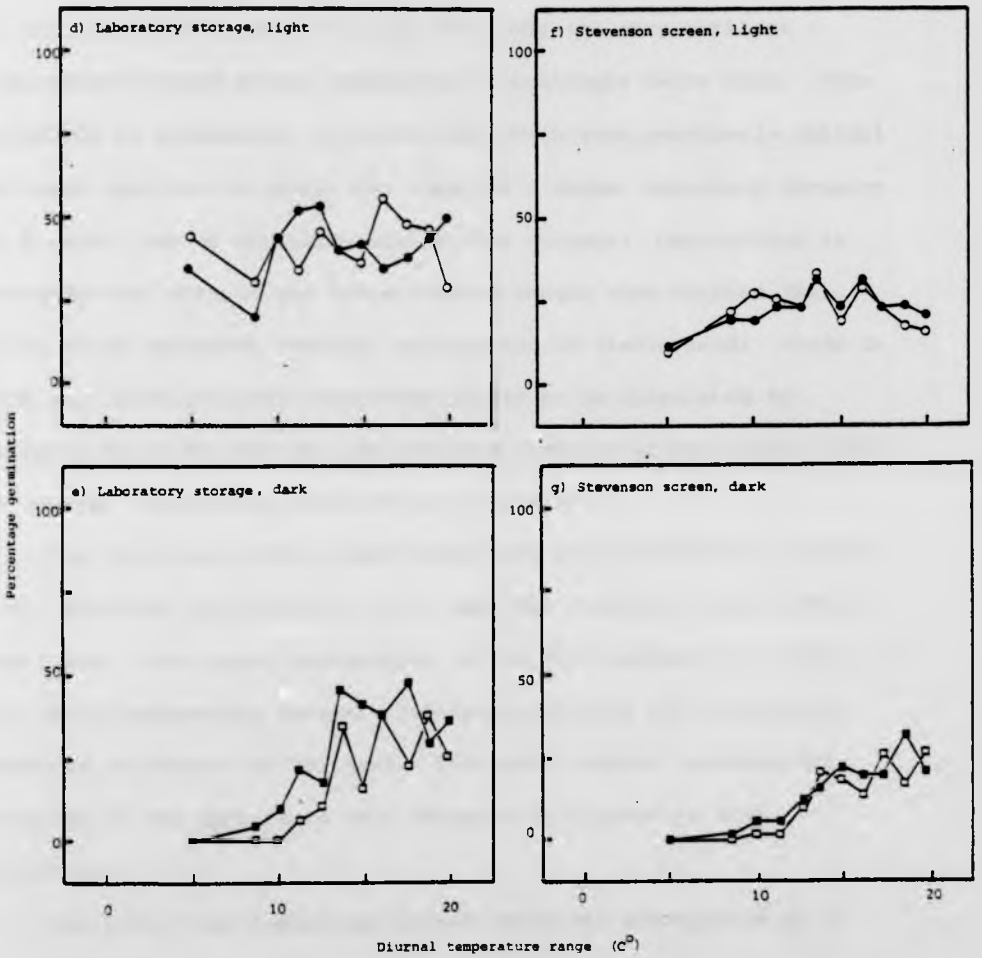


Figure 3.9 Percentage germination of Cardamine hirsuta at a series of diurnal temperature ranges, either as a) fresh seed, or after 21 months storage in three different environments.
b) and c) In a refrigerator at 2-4°C in a screwtop glass jar.
d) and e) On the laboratory bench in an open glass jar.
f) and g) In paperbags in a Stevenson screen at the University Botanical Gardens.
●, 10^{-1}M KNO, light; ○, water light;
■, 10^{-1}M KNO, dark; □, water dark;

continued


d) Laboratory storage, light
f) Stevenson screen, light
e) Laboratory storage, dark
g) Stevenson screen, dark
Percentage germination
Diurnal temperature range (C°)
100
50
0
0
10
20

observed.

After 21 months of storage in an airtight jar in the refrigerator, germination at the larger diurnal temperature ranges in the dark had risen by about 55% (Figure 3.8). However, germination in the light had dropped to about 70%, only the very smallest temperature ranges giving germination percentages below that. This reduction in germination in conditions which were previously optimal may have been due to either the onset of a deeper secondary dormancy or a c.20% loss of viability during this storage. Germination in darkness with nitrate and large diurnal ranges also reached 70%, which might represent complete germination of viable seed. Seeds in both the light and dark treatments appear to be stimulated by nitrate, by an average of 7.4%, but the response is especially clear at greater alternating temperatures in the dark.

The laboratory stored seed shows much the same pattern (Figure 3.8), although germination in this case was reduced to about 40% in the light. Once again germination in the dark reached this level in the large temperature ranges. Laboratory storage also produced a response to nitrate in the dark. Thus both types of storage had resulted in the appearance of a response to nitrate in some treatments.

Testing of the laboratory stored seeds was accompanied by a fungal infection around them which had not previously been observed. A similar fungal growth occurred to a greater extent on the seeds stored in the Stevenson screen, consistent with a greater loss of viability. These had a maximum germination of 30% in the light which was also reached at high alternating temperatures in the dark (Figure 3.8). In this case, however, there was no response to nitrate. The overall effect of all of these storage treatments was to reduce the maximum numbers germinating in the light and, in all but the

Stevenson screen storage, to produce a nitrate response in at least some treatments.

3.3.3 Calcicole and Calcifuge species.

Light was stimulatory to the germination of all of the calcicoles (Figure 3.9). All but the chilled Linum catharticum, which is dealt with separately, responded to increased diurnal temperature ranges. A small response to nitrate in the light at the higher diurnal temperature ranges was evident for Leontodon hispidus, although a curve could not be fitted to the N-W graph (Figure 3.10). Germination, however, occurred between 2 and 3 days earlier in nitrate than water at these temperatures, and a significant quartic curve was fitted to the N-W meanday figures (Figure 3.10). Inula conyza did not respond to nitrate in either its germination rate or its total germination percentage in either light or dark (Figure 3.9). An inhibition by nitrate was experienced in the dark for Carex flacca, Origanum vulgare and Hypericum hirsitum. The only one of these to produce a significant fitted N-W curve ($p < 0.01$) was Hypericum hirsutum (Figure 3.11) which had a maximum reduction in germination of about 48%.

The seeds of Linum catharticum, which failed to germinate before being chilled, had greater germination in the light at moderate rather than large diurnal temperature ranges, following chilling (Figure 3.2). A marked inhibition by nitrate was observed in both light and dark (Figure 3.2), the former producing a significant ($p<0.01$) quartic N-W fitted curve (Figure 3.12). A puzzling depression in germination with light and water occurred in the penultimate temperature treatment, and was observed in both replicates which were closely similar throughout (Figure 3.2).

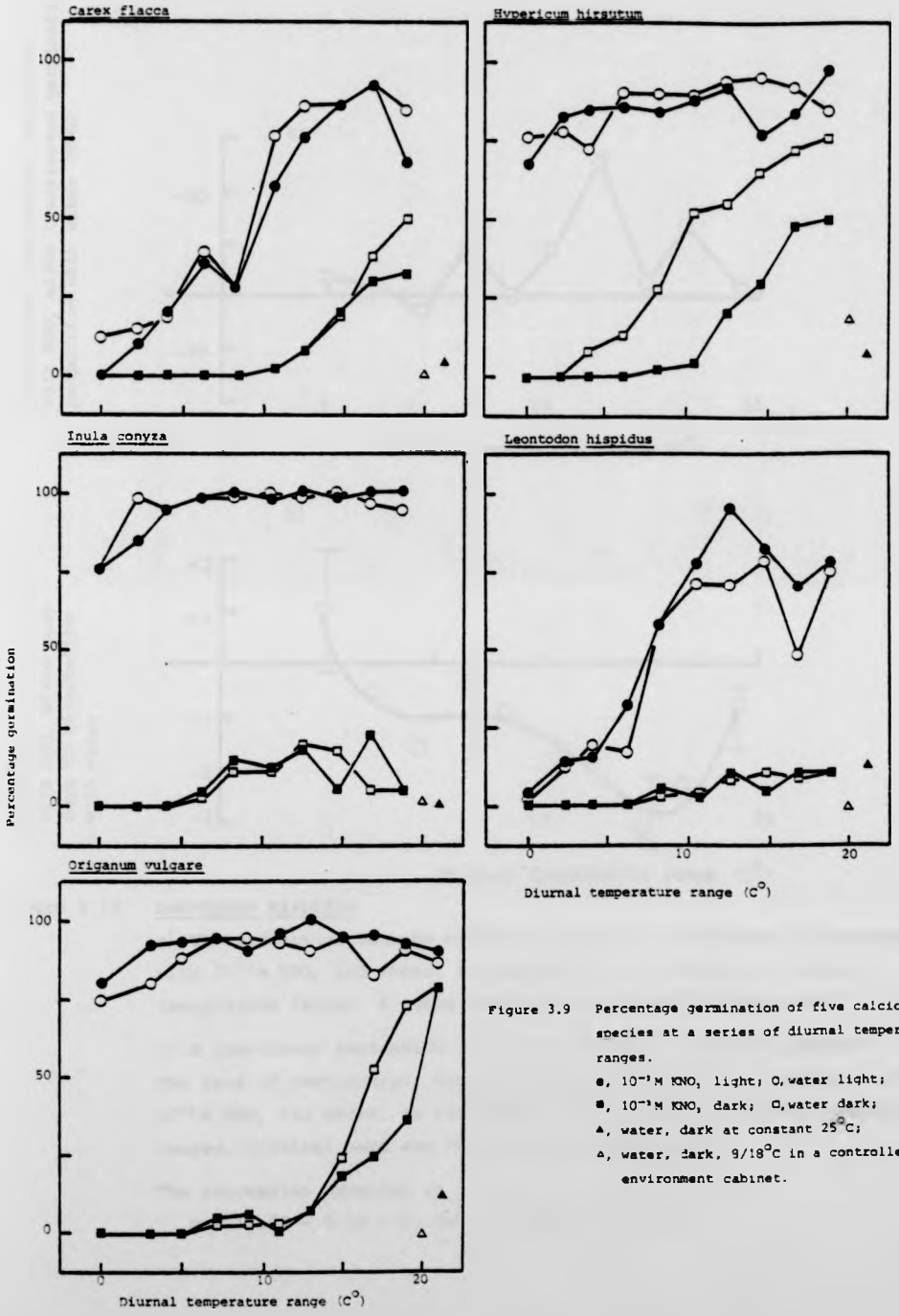


Figure 3.9 Percentage germination of five calcicole species at a series of diurnal temperature ranges.
●, 10^{-3}M KNO_3, light; ○, water light;
■, 10^{-3}M KNO_3, dark; □, water dark;
▲, water, dark at constant 25°C;
△, water, dark, 9/18°C in a controlled environment cabinet.

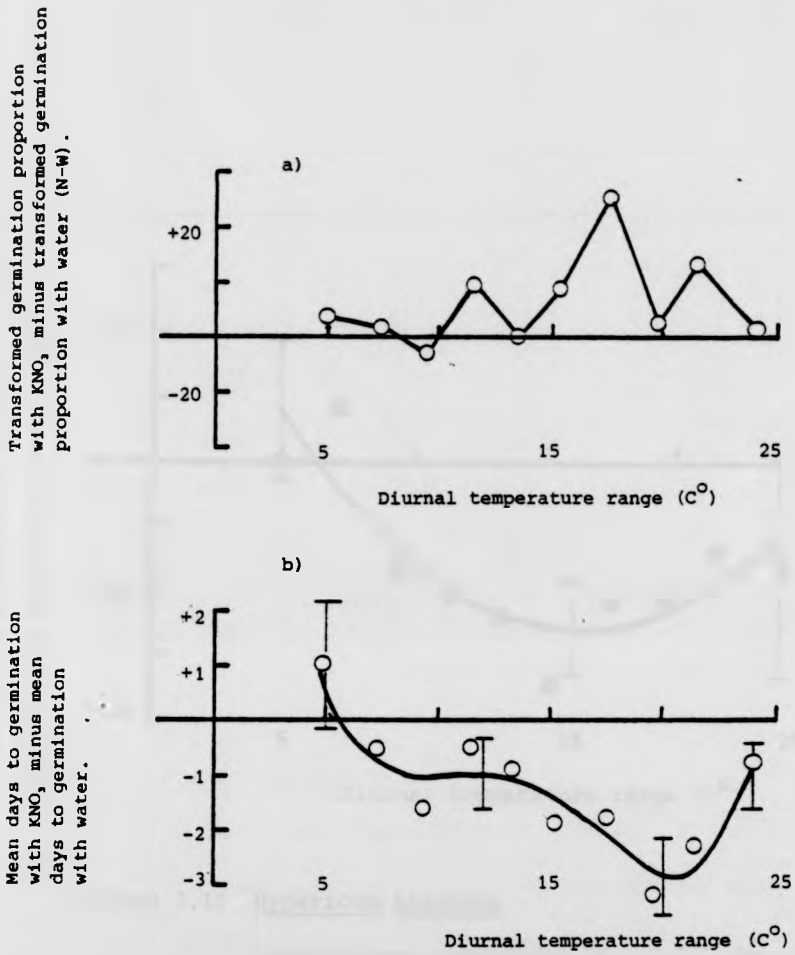


Figure 3.10 Leontodon hispidus

a) The difference between arcsine square root transformed germination with 10^{-3}M KNO_3 and water, in the light, for a series of diurnal temperature ranges. A curve could not be fitted to this data.

b) A non-linear regression ($p < 0.01$) for the difference between the rate of germination, determined as mean days to germination, with 10^{-3}M KNO_3 and water, in the light, for a series of diurnal temperature ranges. Vertical bars are 95% confidence intervals.

The regression equation is

$$y = 17.72 - 6.16 + 0.73x^2 - 0.04x^3 + 0.0007x^4$$

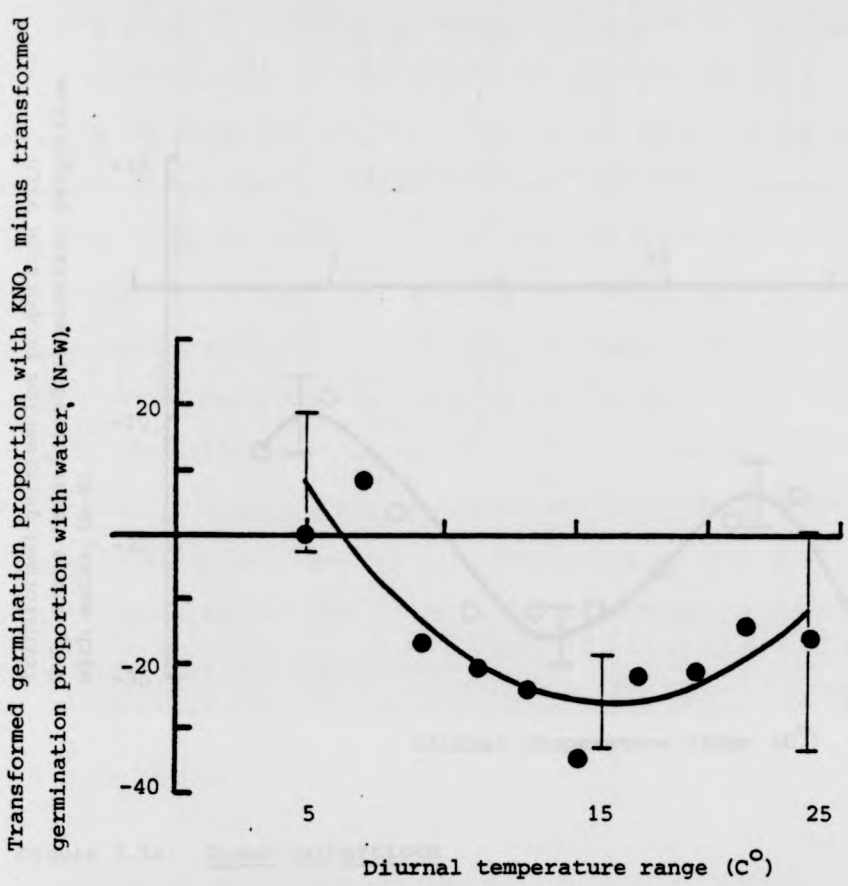


Figure 3.11 Hypericum hirsutum

A non-linear regression ($p < 0.01$) for the difference between arcsine square root transformed germination proportions with 10^{-3} M KNO_3 and water, in the dark, for a series of diurnal temperature ranges. Vertical bars are 95% confidence intervals.

The regression equation is

$$y = 43.42 - 8.42x + 0.26x^2$$

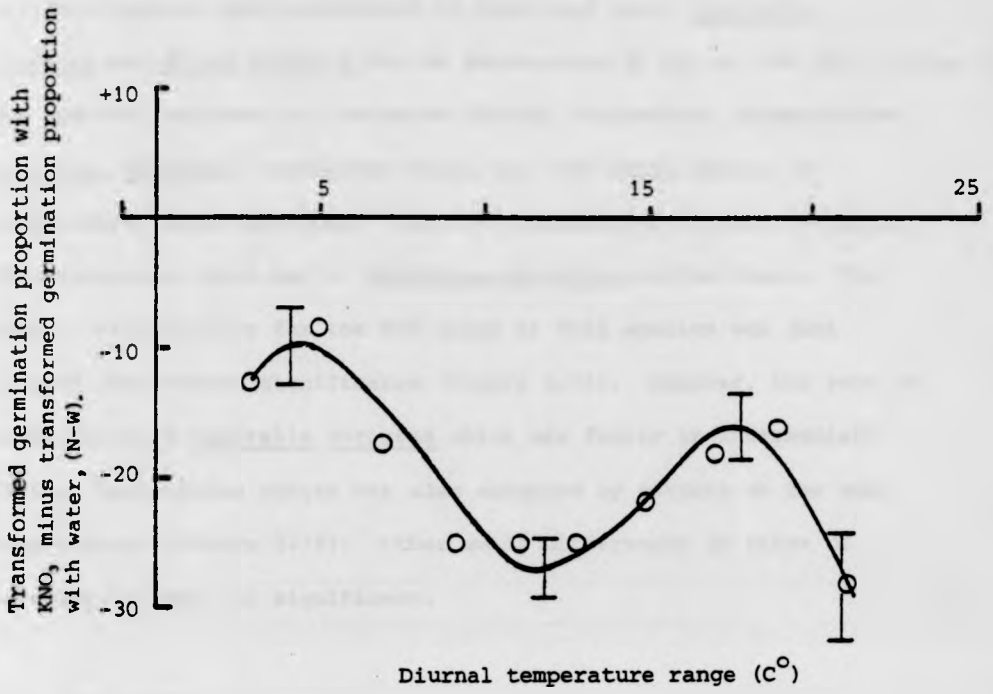


Figure 3.12 Linum catharticum

A non-linear regression ($p < 0.01$) for the difference between arcsine square root transformed germination proportions with 10^{-3} M KNO_3 and water, in the light, for a series of diurnal temperature ranges.
Vertical bars are 95% confidence intervals.
The regression equation is

$$y = -43.21 + 19.78x - 3.80x^2 + 0.26x^3 - 0.006x^4$$

The graphs of calcifuge germination (Figure 3.13) indicate that all four species were stimulated by light and both Digitalis purpurea and Erica tetralix had no germination at all in the dark. Three of the species responded to increased diurnal temperature ranges whilst Digitalis purpurea, germinated fully over the whole series of temperature ranges provided. The only response to nitrate in number of germinating seeds was in Vaccinium myrtillus in the light. The quartic fitted curve for the N-W graph of this species was just outside statistical significance (Figure 3.14). However, the rate of germination of Digitalis purpurea which was faster at intermediate diurnal temperature ranges was also enhanced by nitrate at the same temperatures (Figure 3.15). Other small differences in rates of germination were not significant.

3.4 Discussion.

Treatment replication was not used in these experiments so as to gain the alternative advantage of wider comparisons with more species. Previous thermobar screening programmes have successfully adopted the same policy (Mason 1976, Thompson 1977). In the few cases where replicates were performed there was close agreement, with an average standard error for transformed data of only ±2.9 degrees around the means. The use of curvilinear regression has overcome some of the problems of significance testing caused by this policy.

The thermobar results for the ruderal species were similar to those observed in the petri dish tests of Chapter 2. In Artemisia vulgaris nitrate produced approximately 15% higher germination in darkness in both experiments, while on the thermobar germination at the same temperature was some 25% higher. This was probably the

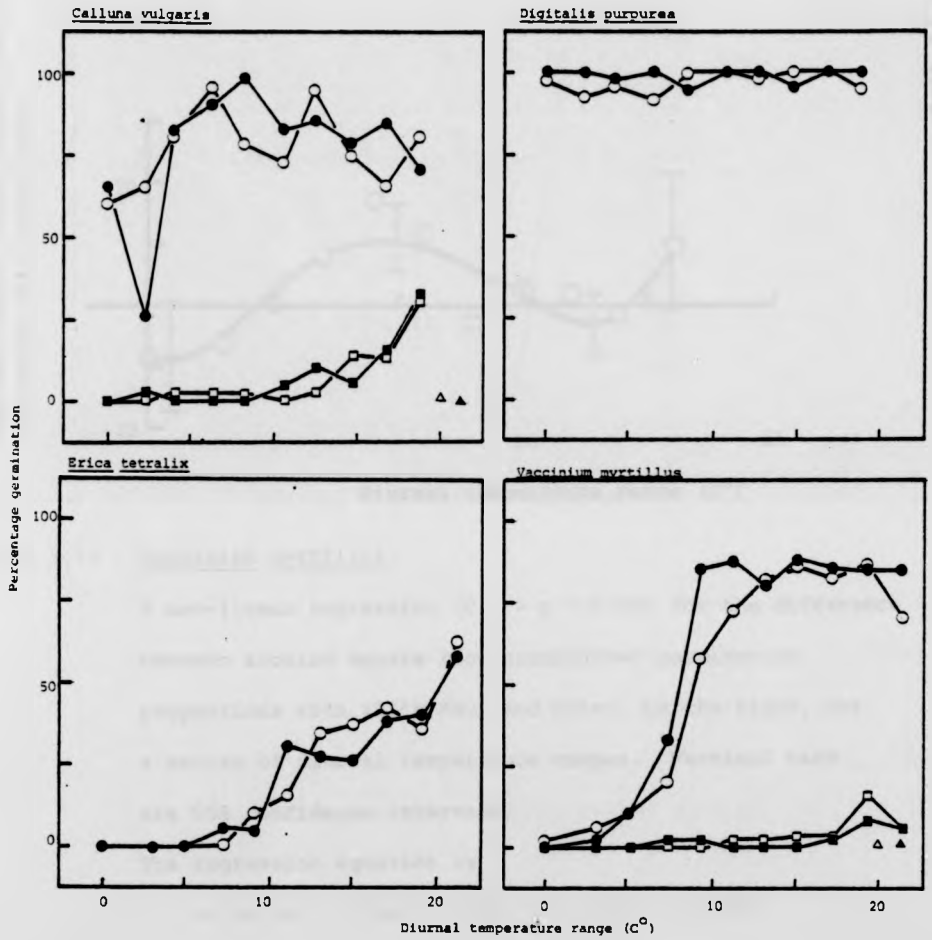


Figure 3.13 Percentage germination of four calcifuge species at a series of diurnal temperature ranges.

●, 10^{-3}M KNO, light; ○, water light;
■, 10^{-3}M KNO, dark; □, water dark;
▲, water, dark at constant 25°C;
△, water, dark, 18°C in a controlled environment cabinet.

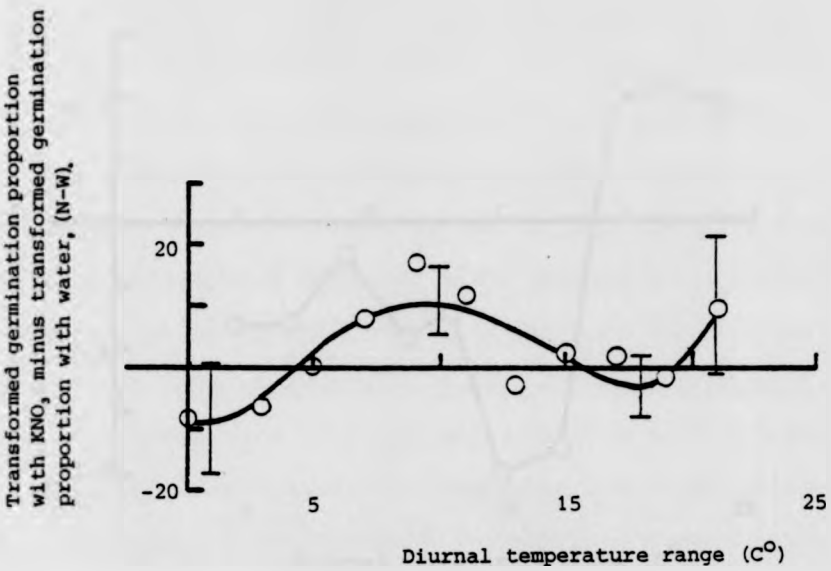


Figure 3.14 Vaccinium myrtillus

A non-linear regression ($0.1 > p > 0.05$) for the difference between arcsine square root transformed germination proportions with 10^{-3}M KNO_3 and water, in the light, for a series of diurnal temperature ranges. Vertical bars are 95% confidence intervals.

The regression equation is

$$y = -8.86 - 1.15x + 1.16x^2 - 0.12x^3 + 0.003x^4$$

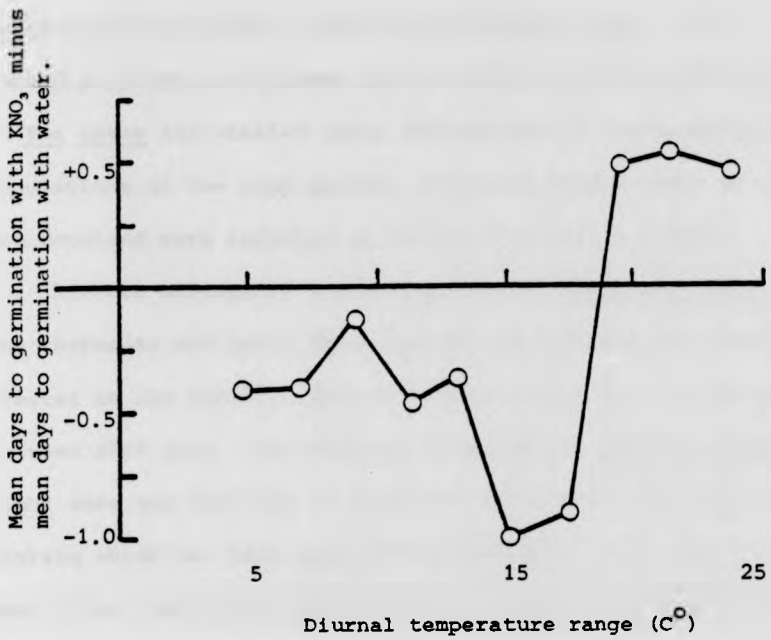


Figure 3.15 Digitalis purpurea

The difference between the rate of germination, determined as mean days to germination, with 10^{-3} M KNO_3 and water, in the light, for a series of diurnal temperature ranges. This indicates that germination was marginally faster in KNO_3 than water at intermediate temperature ranges around (15 C°).

result of differences between the two seed lots which had been collected at different times from different sites. Such interpopulation differences were observed by Wells (1974) in his work on Poa annua and similar large differences in the germination of populations of the same species collected from a range of sites in N-E Scotland were reported by Naylor and Abdalla (1982).

Nitrate stimulated the germination of Stellaria media in both the thermobar and petri dish experiments although the response was greater in the latter. This too was probably due to the differences between seed lots. The thermobar results for Senecio vulgaris in the light were too variable to allow the detection of a clear response to nitrate which had been apparent in the petri dish tests. However, apart from these small differences the responses observed on the thermobar were of the same type as those described in Chapter 2.

The thermobar experiments of Thompson and Grime (1983) investigated the effects of alternating temperatures on the germination of many of the species used in these experiments, but did not include nitrate as an experimental factor. They observed responses to larger diurnal temperature ranges in the light for Gnaphalium uliginosum, Polygonum lapathifolium and Polygonum persicaria, in the dark for Rumex obtusifolius and Stellaria media and in both light and dark for Urtica dioica. Similar responses were observed in these experiments although in the case of Urtica dioica stimulation by alternating temperatures was more apparent in the dark.

The experiments of Thompson and Grime (1983) did not, however, show any response of Chenopodium album to alternating temperatures in the dark, of the type observed in these experiments and confirmed by more recent work (Bostock pers. comm.), although this may have been due to the storage of their seed at 20°C. The temperature regimes

they used were not directly comparable to those used in these experiments which explains their failure to observe the response of Senecio vulgaris to intermediate diurnal temperature ranges in the dark. Murdoch (1982) did, however, observe such a response.

For some of the species, comparisons can be made with previous experiments which have actually used nitrate and in general the results are similar. The thermobar results for Capsella bursa-pastoris showed a synergism between nitrate and alternating temperatures in both light and dark which was also found by Popay and Roberts (1970a). The work of Benjamin (1974), however, showed the greatest germination to be produced by the combination of light and nitrate. This does not agree with the rather unexpected reduction of light germination in these tests. A useful review of previous germination work on both Capsella bursa-pastoris and Chenopodium album was prepared by Murdoch (1982) who concluded that alternating temperature was the principal governing factor upon which the response to light and nitrate depended.

In Chenopodium album, Henson (1970) found the response to 10^{-1}M nitrate to be greater than that to 10^{-2}M. However, Walter (1963) found that germination in 0.5×10^{-1}M KNO_3 exceeded that in either 10^{-3}M or 10^{-2}M KNO_3 which had equivalent effects. Therefore, 10^{-2}M may be sub-optimal and this may explain the only slight response to nitrate on the thermobar. Experiments by Williams and Harper (1965) investigated the polymorphic characteristics of Chenopodium album and showed that its brown or black and reticulated or smooth seed behaved differently. The seed used on the thermobar was a randomly selected population unlike that used by Benjamin (1974) or Henson (1970) who selected only black seed. However the responses to nitrate observed did agree with their earlier work. An interaction between nitrate and alternating temperatures of the type found by

Vincent and Roberts (1977) occurred, although the thermobar did not produce an interaction between light and alternating temperature or between all three factors. These were probably masked by the high levels of germination in the light at the larger alternating temperatures.

The thermobar results for Papaver rhoeas agreed with those of Vincent and Roberts (1977) in which positive light x nitrate interactions were found. The slight interaction between light and alternating temperatures which they observed was not, however, apparent. In Solanum nigrum, Roberts and Lockett (1978b) found a nitrate, alternating temperature syngergism and the thermobar test shows this occurring in both light and darkness, germination in the light being higher. Roberts and Lockett (1975) found that light stimulated germination of Stellaria media and in the thermobar test a light-nitrate synergism was observed in the intermediate temperature regimes.

Thus, the thermobar results have confirmed some of the more complex interactions between nitrate, light and alternating temperatures which were observed by other workers.

Light sensitivity of the type exhibited by all but two of the species tested has been investigated by many past workers (e.g. Toole 1973, Grime *et al.* 1981) and its ecological importance has been widely discussed. Thompson and Grime (1983) illustrate that seeds exhibiting light sensitivity are those which are normally encountered in the buried seed bank, particularly in arable soils. It was suggested by Wooley and Stoller (1978) that cultivation could expose such weed seeds to light, facilitating their germination, and other work (e.g. Roberts and Dawkins 1967, Wesson and Wareing 1969) supports this hypothesis. Wooley and Stoller (1978) conclude that light is unlikely to induce germination of light-sensitive seeds

buried at greater than 2mm depth in the soil. These thermobar results for the twenty-two ruderal species, which are all normally incorporated to varying extents in buried seed banks (Thompson 1977, Grime *et al.* 1981, Thompson and Grime 1983) confirm such a light requirement.

Thompson and Grime (1983) stress that the importance of alternating temperatures in breaking dormancy must also be considered. They discuss in detail the importance of larger fluctuating temperatures as both depth-sensing and gap-detecting mechanisms enabling seeds to germinate into safe sites. Clearly the species in these thermobar experiments respond to alternating temperatures in much the same way as those of Thompson and Grime.

The work of Murdoch (1982) showed that the diurnal temperature range also changes seasonally and in the year of his study increased from relatively small fluctuations in late winter and early spring to greater ones in late spring and early summer. A response to alternating temperatures could, therefore, also allow seed recognition of the most suitable times of year for germination. This could partially explain the flushes in germination of some species (Roberts and Feast 1970, Lawson *et al.* 1974, Froud-Williams *et al.* 1984) during these periods of optimal temperature range.

Nitrate solutions stimulated the germination of 19 of the 22 species tested. The magnitude of the effect and the conditions in which the effect was greatest varied in different species, often increasing with increased diurnal temperature range. More generally, it increased as the temperature conditions became more favourable to germination in the absence of nitrate. This became particularly clear in the case of Senecio vulgaris with its optimum response to nitrate appearing in the dark at the optimal intermediate temperature regimes. In 5 species in the dark and in 6 species in the light

there was a markedly linear synergism between the effects of nitrate and alternating temperatures. When maximum germination was reached in greater temperature ranges the response to nitrate appeared to be maximal at the intermediate ranges below the point at which germination in water approached 100% of the viable seeds present (e.g. Artemisia vulgaris and Plantago major).

Nitrate levels in soils have been shown to fluctuate naturally (e.g. Williams 1968, 1969, Popay and Roberts 1970b) and in arable situations can reach 10^{-2}M. Eagle (1961) determined that the microbial release of nitrogen in the soil was greatest in spring and late autumn, and a spring increase of nitrogen release was also observed by Seifert (1962) in the bare soil of a vegetable bed. Taylor *et al.* (1982) observed such nitrate flushes in acidic and calcareous soil although the actual nitrate levels were not as high. The addition of fertiliser to arable land is also a common cause of elevated nitrate levels in the spring. Increased spring nitrate levels may be successful in the recognition of safe sites in the same way that alternating temperatures are thought to indicate both the temporal and spatial suitability of sites for germination. These seedlings, which could be killed by cold conditions following premature spring germination, would benefit from a response to nitrate in late spring and early summer.

In the experiments of Murdoch (1982) the soil nitrate levels in early June and thereafter were substantially higher than those in mid-April. In early June he recorded higher nitrate concentrations at 2.5cm and 7.5cm depth which were approximately double those at the surface or at 23cm. Similarly in July the nitrate concentrations at the surface were almost double those at 2.5cm and five times greater than those at 23cm. Ellenberg (1964) showed a gradual decline in nitrate concentrations with depth, the most marked example being in a

Brown earth soil in which nitrate levels at 10cm depth were half of those at the surface.

Burns (1977) showed that a soil profile following fertiliser addition, had a distinct concentration of nitrate at the surface with a large drop in levels down to 15cm depth. Leaching gradually moved this nitrate down the profile with a resultant reduction in the surface nitrate concentrations. Ammonium fertiliser which became nitrified also produced a concentration of nitrate in the surface layers which was eventually leached, although in this case the actual nitrate values were lower. It is clear from these reports that changes in nitrate concentrations with depth vary considerably and are strongly influenced by the soil water relations. Indeed the high surface nitrate concentrations observed by Murdoch (1982) in July could have been caused by evaporation during a drier period. Thus, although there are distinct variations of nitrate concentration with soil depth, their dependence on the soil moisture content makes them unpredictable and their involvement in any seed "depth-sensing" mechanism unlikely.

Petersen and Bazzaz (1978) suggested that nitrate may play a role in gap detection (Thompson and Grime 1983). Bormann *et al.* (1968) showed that disturbance resulting from canopy removal was responsible for nitrate release which could stimulate the germination of early successional species responsive to it. Gap formation is normally likely to be associated with disturbance and Friejsen *et al.* (1980) suggested that loose and disturbed conditions, "May be regarded as favouring mineralisation and nitrification." Nitrification appears to be inhibited by climax ecosystems (Rice and Pancholy 1972, Kurkin 1977) with the highest nitrate levels being present in the early successional stages associated with disturbance and gap formation. The uptake of nitrate by established vegetation also seems to keep

nitrate levels low in soils with plant cover.

Thus, it seems that in the natural situation the temporal variations in nitrate could be important in the recognition of temporal safe sites. Similarly nitrate may be involved in the recognition of gaps in vegetation and areas of disturbance, suitable for colonization. However, its involvement in "depth-sensing" seems unlikely due to the apparent unpredictability of the vertical distribution of nitrates in the soil.

It might be predicted from the above discussion that the increased nitrate level in spring and early summer could be partly responsible for causing flushes of germination. The increase in diurnal temperature range which occurs at the same time of year and itself stimulates germination, has been shown by these thermobar tests to interact with nitrate responses. The synergistic effect of nitrate and alternating temperatures may, therefore, have a very pronounced effect on the distribution of field germination. Because of their interaction the response to nitrate may be difficult to isolate from that of the alternating temperatures in its effect on seedling emergence.

Stored seeds of Cardamine hirsuta showed a general reduction in dormancy, which conforms to the view of Vegis (1964) that, as seeds after-ripen in storage, their requirements for germination generally become less strict. Changes in response to nitrate have been observed for some species (e.g. Roberts and Lockett 1975, 1978b, Bostock 1978). Such changes occurred in Cardamine hirsuta with the appearance of a response to nitrate after 9 months and 21 months laboratory storage. Popay and Roberts (1970a) reported that over a 2 year period of dry storage at 4-2°C in Kilner jars there was no loss of viability or dormancy of Capsella bursa-pastoris and only a small loss of dormancy in Senecio vulgaris. 21 months of similar storage

of Cardamine hirsuta in these tests caused a reduction in its viability and a loss of dormancy with the appearance of a response to nitrate in the dark. The other species in these tests which had been stored in the same way were all used within 5 months of collection and losses in dormancy or viability were probably small. It is clear that a response to nitrate, like other dormancy-breaking factors, is not an unchanging characteristic of the species. Storage in a Stevenson screen for 21 months which more closely approached the conditions of natural storage caused a 70% loss of viability although a nitrate response did not develop. Burial of seeds in the soil may also cause similar changes in seed dormancy and response to nitrate. These are discussed in more detail in Chapter 4, with specific reference to the burial of Cardamine hirsuta seeds.

Figure 3.16 shows an idealised series of curves which represent the types of response to nitrate and alternating temperatures observed for most of the ruderal species tested on the thermobar. The position on this family of curves of the actual response curve for each species will depend on its absolute dormancy and the presence or absence of light. The response of nearly all of the species tested could fit into this diagram, although the actual magnitude of the nitrate response cannot be predicted. From the evidence of Cardamine hirsuta, after-ripening of seeds during storage would cause their response curve to move across the range of curves towards the left.

Some general relationships between seed size and germination behaviour in response to light and temperature have been described (Grime *et al.* 1982, Thompson and Grime 1983). A possible relationship between seed size and nitrate response was sought but not found.

The germination of calcicole and calcifuge species on the thermobar compared closely with that observed in the petri dish tests

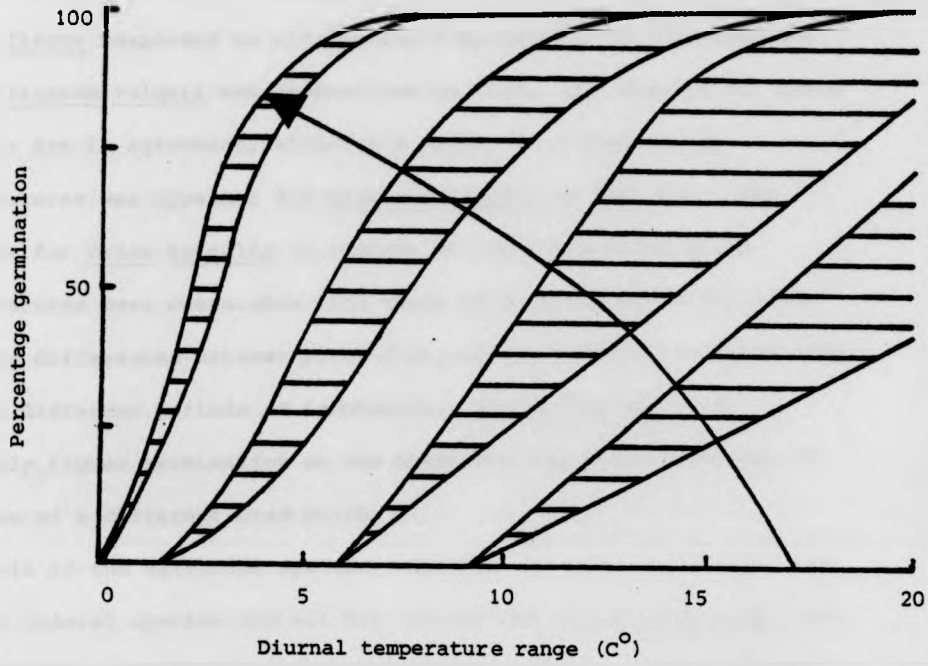


Figure 3.16 An idealised series of curves representing the types of response to nitrate and alternating temperatures observed for most of the ruderal species tested on the thermobar. The shaded zones indicate stimulation of germination by nitrate. The arrow indicates the direction in which the response of seeds would move following either after-ripening or under the influence of light.

of Chapter 2. Apart from the germination of Inula conyza which was once again variable, the calcicole germination percentages were the same in both experiments. Thompson and Grime (1983) observed that Carex flacca responded to alternating temperatures in the light and that Origanum vulgare was insensitive to them. The results for these species are in agreement, although a response to alternating temperatures was apparent for Origanum vulgare in the dark. The results for Erica tetralix in respect of light and alternating temperatures were comparable with those of Thompson and Grime (1983) and any differences between petri dish and thermobar percentages were due to different periods of incubation. Calluna vulgaris had slightly higher germination on the thermobar but this may be due to the use of a different seed batch.

All of the calcicole species responded to light in the same way as the ruderal species and all but the chilled Linum catharticum were stimulated by the higher diurnal temperature ranges. After 41 days chilling Linum catharticum had greater germination in intermediate temperature ranges (10C° range) in the same way as Senecio vulgaris.

Only Leontodon hispidus appeared to respond to nitrate, in the light, and in the darkness four of the calcicole species were actually inhibited by nitrate: a maximum inhibition of 48% for Hypericum hirsutum, 36% for Origanum vulgare, 26% for Linum catharticum and 18% for Carex flacca. In Chapter 2, 10^{-3}M KNO_3 had not been supra-optimal for Hypericum hirsutum or Origanum vulgare at 5/25°C. There was also no inhibition of Origanum vulgare germination in nitrate on the thermobar at 5/25°C and it only became evident in some of the other treatments. An explanation could not be found for the reduction in germination of 26% for Hypericum hirsutum in nitrate at 5/25°C on the thermobar.

The calcifuges all showed a strong light requirement which was

obligate in the case of Digitalis purpurea and Erica tetralix. Such a requirement is a characteristic of small seeded species (Thompson and Grime 1983) and was also observed for Juncus conglomeratus in Chapter 2. All of the calcifuges except Digitalis purpurea responded to alternating temperatures but once again only one species; Vaccinium myrtillus, responded to nitrate.

Thus, although light and alternating temperatures appear to be fulfilling the same purpose of safe site recognition for these calcicole and calcifuge species as they are thought to do for most other species, nitrate is not clearly involved. It would seem that unlike the ruderals there would be no advantage for these species in having a response to high nitrate concentrations in their native environments with lower nitrate levels. In species possessing it, the germination response to nitrate therefore appears to be an adaptation to their particular soil environments rather than an arbitrary by-product of seed metabolism. Such species are for the most part ruderals or crops. This is in agreement with the work of Mayser (1954, reported in Schimpf and Palmblad 1980) who concluded that the seeds of nitrophilic species have higher nitrate optima and maxima for germination in the soil than do non-nitrophilic species.

CHAPTER FOUR

Nitrate Responses of Four Ruderal Species Following Burial.

4. Nitrate Responses of Four Ruderal Species Following Burial.

4.1 Introduction.

The dry storage of Cardamine hirsuta seeds under a variety of conditions (Chapter 3) showed that the response of seeds to nitrate could vary over time in both form and magnitude. Responses to other dormancy breaking factors including alternating temperatures and light have been shown to change during dry storage (e.g. Roberts and Lockett 1975, 1978b), normally resulting in a gradual loss of dormancy.

Burial of seeds in the soil also produces changes in their response to dormancy breaking factors (Karssen 1980/81a,b, Baskin and Baskin 1981, Roberts and Lockett 1975, 1978b, Bostock 1978) although this is not usually in the form of a straightforward loss of dormancy. In some species cyclic seasonal changes in dormancy have been shown to occur (Courtney 1968, Taylorson 1970, Stoller & Wax 1974, Karssen 1980/81a,b, Roberts & Neilson 1982, Froud-Williams *et al.* 1984) and Baskin and Baskin (1981) suggested that "these are the rule rather than the exception". These usually take the form of an annual rhythm in the depth of dormancy with induction of dormancy leading up to periods in the year with little or no germination followed by a loss of dormancy prior to flushes of germination. Karssen (1980/81b) demonstrated this behaviour in Polygonum persicaria and Senecio vulgaris which both had flushes of germination in spring and a reduction of germination between July and January over the two year period of his experiment. In the same experiment Chenopodium album showed a high level of germination in late spring and early summer in both light and dark but was capable of responding to a light stimulus earlier on in spring. Murdoch's (1982) work revealed a similar cycle of dormancy induction and loss in Avena

fatua and Chenopodium album but these responses were overshadowed by an overall long-term loss of dormancy. Clearly the changes in response to dormancy breaking factors, brought about by seed burial, are complex and have a dominant influence on field emergence patterns.

The aim of the experiments described here was to observe the changes in response to nitrate of four ruderal species following varying periods of burial in the soil. This information should contribute to an understanding of any nitrate responses observable in the field. If a cyclical change in nitrate response is observed this may produce a similar annual cycle of response in the field. Alternatively if a gradual loss in dormancy of the type observed by Murdoch (1982), overrides any other changes in the nitrate response, the presence of seed buried in previous years may partially or completely mask the responses to nitrate of seed more recently incorporated into the seedbank. Although nitrate itself could be involved in the actual process of modifying changes in dormancy (Karssen 1980/81b) these experiments are not intended to specifically investigate such effects.

The experiments of Murdoch (1982) and Karssen (1980/81b) involved the burial of seeds in nylon sachets but in both cases this has meant that the seeds were not in direct contact with the soil. Murdoch (1982) has since stressed the requirement for this direct contact and advocated inclusion of soil in the sachets to develop a continuity with that in the surroundings. The seeds in this experiment were buried in sachets following mixing with a small amount of soil. The sachets were removed on three occasions over 15 months to monitor the changes in response to dormancy breaking factors brought about by burial. The influence of such changes on the effect of nitrate flushes and applications in the field experiment (Chapter 6) can then be considered.

4.2 Materials and Methods.

4.2.1 Experimental methods.

Seed lots of the four ruderal species were divided into groups of 100 seeds. Each group was then mixed into 7g of dry soil which had been collected from the University Botanical Gardens and sorted to remove stones and plant roots. The soil had been previously steam-sterilised to kill any seeds from its natural seedbank. The mixture of soil and seed was then heat sealed into a 5 x 5 cm nylon micromesh sachet.

The 53 μm mesh of the sachet was chosen as a compromise between protection from all but the very smallest soil organisms and the creation of a good contact between the sachet soil and external soil following burial. Whilst fungal and bacterial influx from the outside soil could not be avoided it was hoped that predation of the seeds by soil invertebrates would be prevented. The mesh was, however, large enough to allow the moisture conditions of the sachet to equilibrate with that of the surrounding soil.

Once sealed, each sachet was attached by a length of nylon fishing twine to a label which remained at the surface. Small coloured wires were then attached to the labels to identify the species in each particular sachet.

Fifty four sachets of each species were buried at 15 cm depth in a bed at the University Botanical Gardens in December 1981. It was expected that burial at this depth would prevent any *in situ* germination. Murdoch's (1982) results showed little difference between the effects of burial at 7.5 or 23 cm although in Chenopodium album *in situ* germination was lower with increased burial depth. Groups of four sachets with a representative of each of the species

were randomised within a flat area of $6.5m^2$ with a 30 cm margin around its edge.

Eighteen sachets of seeds which were not buried were placed in a sieve and leached with tap water in darkness for 12 hours to remove as much nitrate as possible. Half which were to become nitrate treatments were then washed through with 250 ml of 10^{-2}M KNO_3 whilst the water treatments received the same volume of water. They were cut open and laid onto a single layer of Whatman No. 1 filter paper and the soil was spread over its area. This paper was supported by two plastic petri dish lids within a 13.5 x 7.5 x 5 cm clear perspex box. The lids were surrounded by 80 ml of either 10^{-2}M KNO_3 solution or double glass distilled water into which the paper dipped at both ends. This provided moisture for the seeds without waterlogging the soil.

In preliminary experiments gravel was used to support the seed sachets and they were not leached following removal from the field. This resulted in higher measured nitrate levels in the water treatments (Appendix F), and consequently the method described above was adopted.

Boxes of water and nitrate treatments were placed in controlled environment cabinets (see Chapter 2) in one of three regimes:

a) In a 5/25°C alternating diurnal temperature range and 12 hour photoperiod and thermoperiod. This represents soil surface conditions in spring.

b) In darkness with a 5/25°C diurnal temperature regime and a 12 hour thermoperiod. This represents the conditions experienced by seed buried just below the soil surface in spring.

c) Dark with an 8/18°C diurnal temperature range and 12hr thermoperiod. This represented the conditions experienced by seed at approximately 4 cm below the soil surface in spring.

The six treatments were replicated three times.

Dark treatments were created by double wrapping the perspex boxes in aluminium foil. The procedures between leaching of the sachets and wrapping of the boxes normally took less than 3 minutes and were performed under the green "safe light" described in Chapter 2. Although Baskin and Baskin (1979) showed that buried Stellaria media seed responded to 5 minutes or more of exposure to green light its use could not be avoided but was kept to a minimum.

Seedling emergence was counted every one or two days in the light treatments and the criterion used in Chapter 2 for removal of dark treatments was employed. These sachets which had not been buried acted as a control treatment in which the laboratory germination of fresh seed in soil rather than on filter paper could be observed.

The buried sachets were removed in

i) April 1982 after 3 months burial.

ii) September 1982 after 9 months burial.

iii) April 1983 after 15 months burial.

These removal times were chosen to fall just before the occurence of natural flushes of germination in the field. The sachets were selected randomly and placed directly into a black plastic bag following exhumation. They were then treated in exactly

the same way as the control sachets.

Variation between replicates was high in both September 1982 and April 1983 removals. Consequently, following counting of the dark treatments all dishes were moved into a light 5/25°C environment and water was replaced by nitrate. This stimulated the germination of any seeds which had remained dormant in either of the two dark treatments as well as allowing extra germination of slower seeds in the light. Total germination following this procedure was used as an estimate of the number of germinable seeds present.

Results for Senecio vulgaris germination after 3 months of burial (April 1982 exhumation) were unavailable due to damping off of seedlings in the dark treatments before light germination had finished. Replacement sachets of Senecio vulgaris were therefore buried in December 1982 to provide two additional times of removal. The first batch was exhumed in early May 1983 and the second in September 1983, after 3½ and 9 months burial respectively. Each of these removals had four replicates per treatment but procedures were otherwise identical with those of the previous experiment.

4.2.2 Statistical methods.

The transformed data (arcsine square root) for each species at each removal were analysed separately using a factorial analysis of variance. This directly showed the significance of any overall difference between the nitrate and water treatments. If an overall nitrate versus water comparison was significant, tests between nitrate effects in different incubation environments were made using a test of least significant difference. If differences between incubation conditions were significant in the ANOVA they were compared using Duncan's MRT. Values of germination, as percentages

of germinable seed, for the September 1982 and April 1983 removal were also calculated and analysed in the same way.

Comparisons between different removal times were only analysed if they appeared to be of particular importance, using a t-test for the comparison of two means with different variances (Sokal and Rohlf 1969).

4.3 Results.

Analyses of these results appear in full in Appendix G.

4.3.1 Artemisia vulgaris.

The germination in soil of fresh Artemisia vulgaris seed was not affected by nitrate. Germination was lowest ($p<0.01$) at 8/18°C in the dark and germination in the light at 5/25°C was greater ($p<0.01$) than dark germination in the same temperature regime (Figure 4.1a).

Following 3 months burial, a significant ($p<0.001$) overall response to nitrate was found. It was, however, only individually significant ($p<0.05$) for the two dark treatments. A difference in germination between the two temperature regimes in the dark no longer existed although they still produced significantly ($p<0.01$) lower levels of germination than the light treatment (Figure 4.1b). It appears that these Artemisia vulgaris seeds developed a response to nitrate during burial which has replaced the requirement for a larger diurnal temperature range in the dark and also partially substituted for the presence of light. Germination in water in the dark treatments was also somewhat greater ($p<0.001$) after 3 months burial than for fresh seeds. By September 1982, after 9 months burial, germination did not respond to nitrate (Figure 4.1c), the only treatment difference being a higher percentage germination of total

Figures 4.1, 4.2 and 4.3

Germination of Artemisia vulgaris (4.1), Cardamine hirsuta (4.2), Stellaria media (4.3), in three environments.

Light with a 5/25°C diurnal temperature alternation

Dark with a 5/25°C diurnal temperature alternation

and Dark with an 8/18°C diurnal temperature alternation

with 10^{-2} M KNO_3 

or distilled water 

after varying periods of burial in nylon sachets in the soil,

a) Fresh seeds.

b) 3 months burial.

c) 9 months burial.

d) 9 months burial - percentage of germinable seeds remaining.

e) 15 months burial.

f) 15 months burial - percentage of germinable seeds remaining.

All germination percentages have been transformed using the arcsine square root transformation.

The error bars represent the Least Significant Differences ($p < 0.05$) for comparison of nitrate and water treatments within each environment.

Comparison between environments was made using Duncan's Multiple Range test and within each burial period those environments carrying different letters were significantly different ($p < 0.05$).

Figure 4.4

Germination of Senecio vulgaris following burial in the soil. The information presented is the same as for Artemisia vulgaris, Cardamine hirsuta and Stellaria media except that graph d) represents a second experiment involving burial for 9 months rather than the percentage of germinable seeds which emerged after that time.

Figure 4.1 Artemisia vulgaris

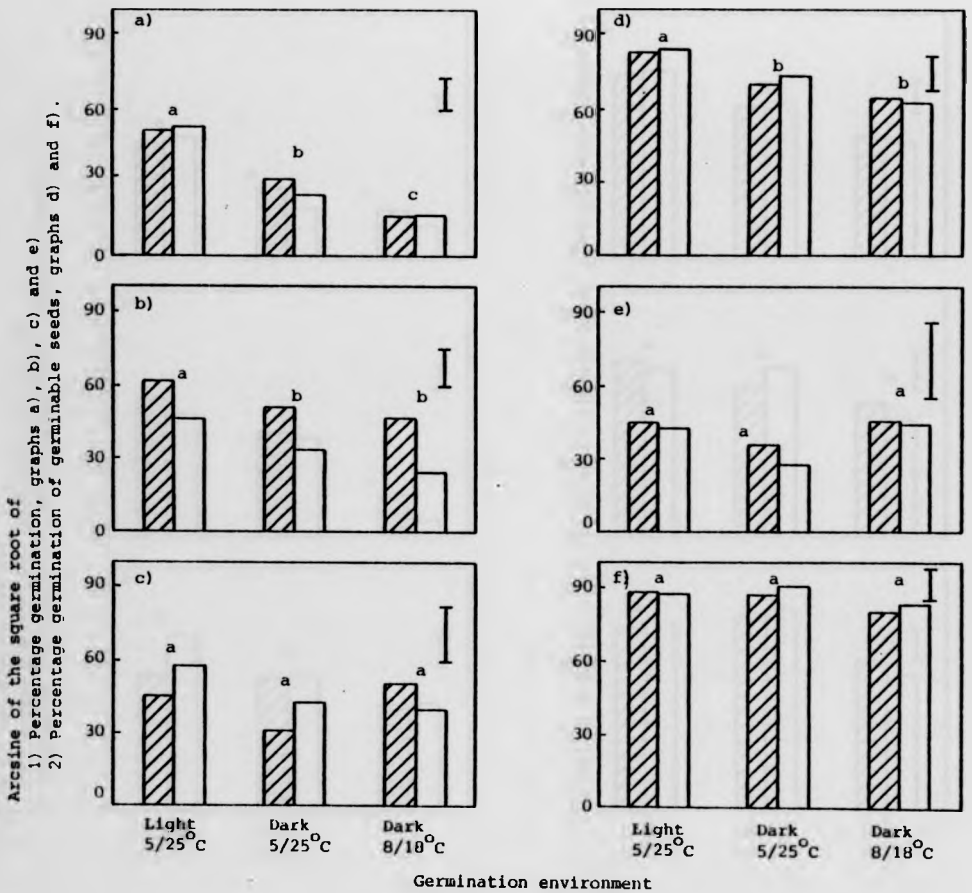


Figure 4.2 Cardamine hirsuta

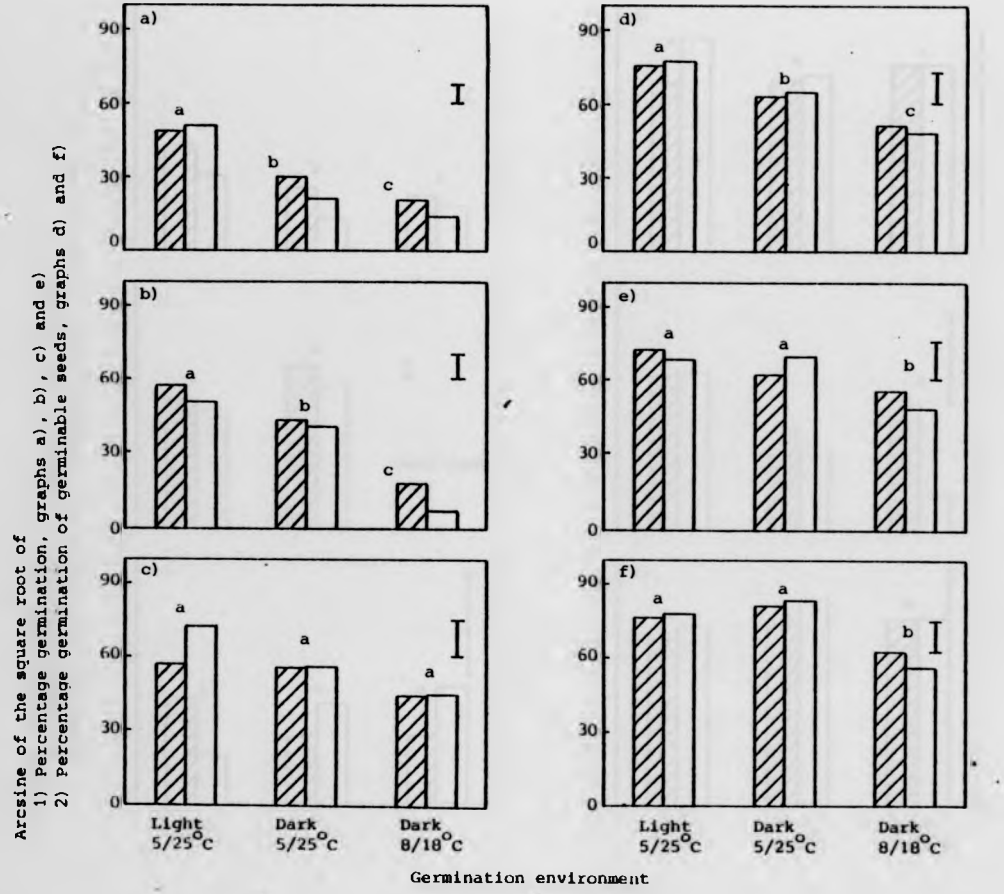


Figure 4.3 Stellaria media

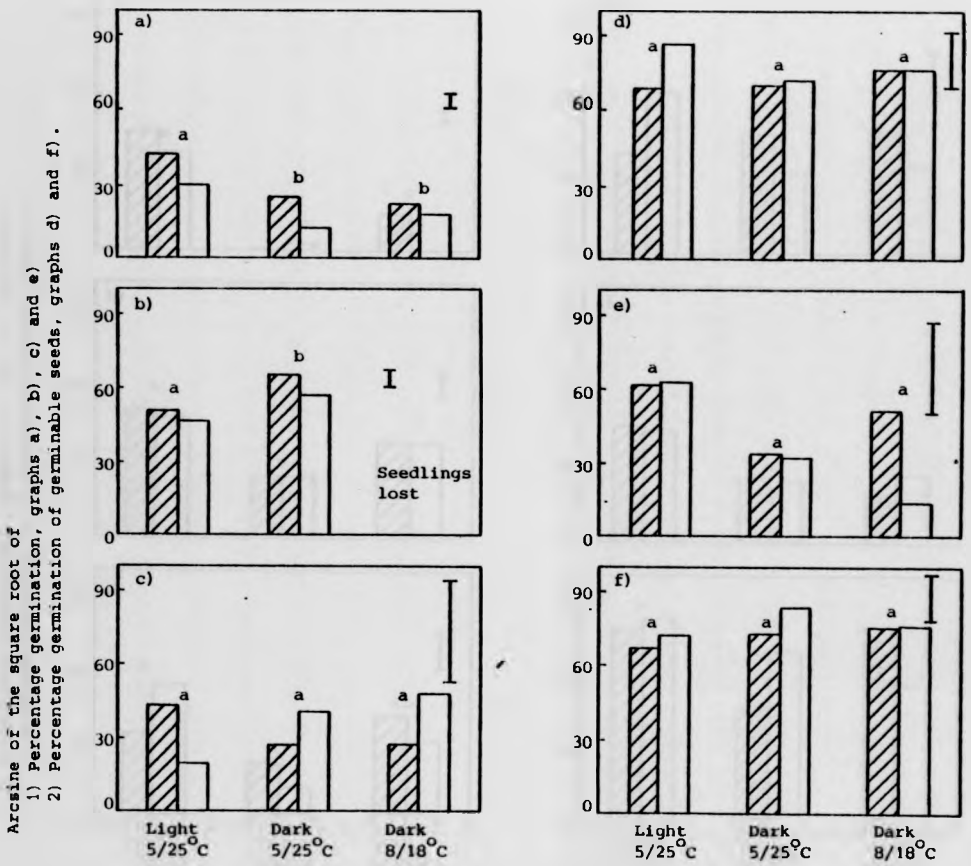
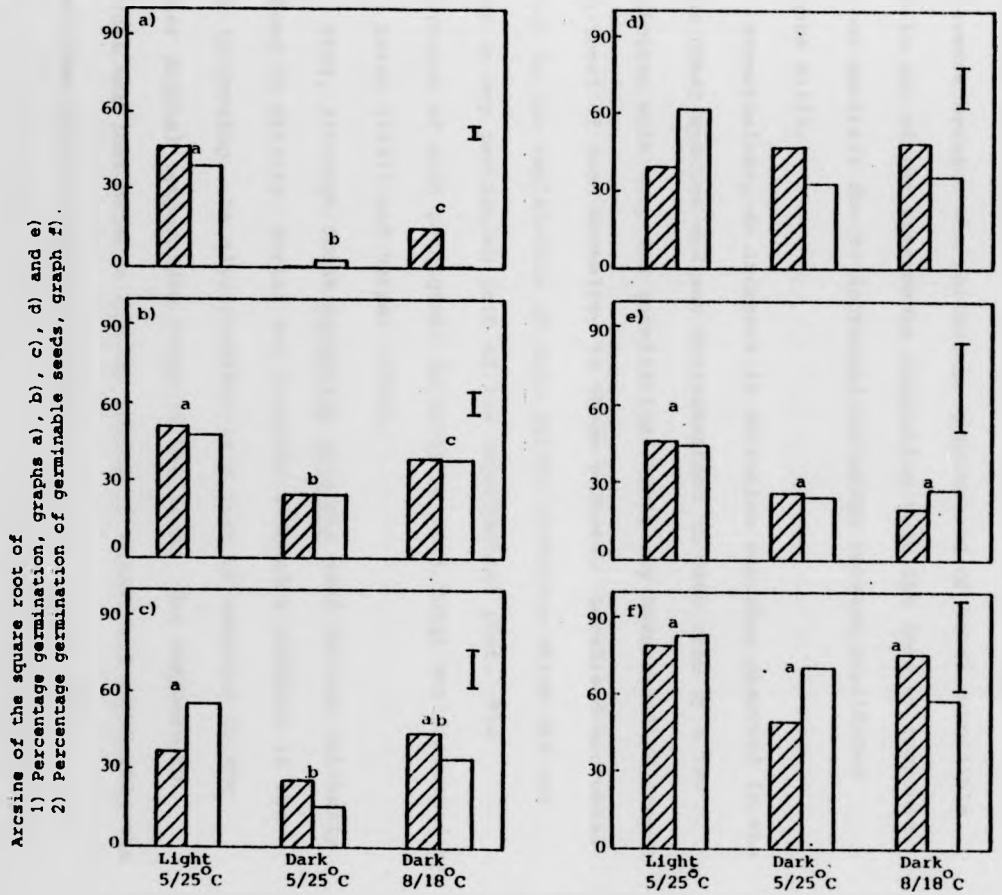


Figure 4.4 Senecio vulgaris



germinable seed ($p<0.05$) in the light than in the two dark environments (Figure 4.1d).

The variability between replicates gradually increased with the burial period. In April 1983, when the last removal took place, the variation was particularly large and no differences between treatments were significant (Figure 4.1e). There was also no difference between the percentage germination of total germinable seed in any of the treatments suggesting that the loss of response was not entirely due to increased variation between replicates (Figure 4.1f).

Nevertheless, an increase in variation was also observed in the three other species and was characterised in each case by a few replicates with very low germination. This may have resulted from a high level of seed mortality in those sachets. Localised microbial attack is one explanation of this patchy mortality which did not occur in any particular part of the experimental plot. The importance of such pathogenic activity of soil fungi was discussed by both Leach (1947) and Harper (1955).

Thus, although fresh Artemisia vulgaris seed did not initially respond to nitrate, burial for 3 months allowed a response in the dark to develop. It also resulted in a loss of response to the larger diurnal temperature range in the dark. The response to nitrate was lost after a further period of burial and eventually the seeds lost their response to light.

4.3.2 Cardamine hirsuta.

The germination of fresh Cardamine hirsuta before burial was stimulated by nitrate ($p<0.05$) in both dark treatments (Figure 4.2a). Although there was no response to nitrate in the light, germination

was still significantly ($p<0.01$) greater than in the dark. For this reason the interaction between nitrate and incubation environment was significant ($p<0.05$). Germination in the dark at 5/25°C was also significantly higher ($p<0.01$) than that at 8/18°C.

After 3 months burial the overall response to nitrate was still significant ($p<0.05$) but within individual treatments this response was only significant at 8/18°C in the dark ($p<0.05$) (Figure 4.2b). Once again average germination percentages in the incubation treatments were all different ($p<0.05$) from one another.

Cardamine hirsuta did not respond to nitrate in any of the environments following 9 months of burial in the soil (Figure 4.2c). The dark 8/18°C treatment produced lower germination than both of the 5/25°C treatments ($p<0.05$), with the increase in between replicate variation being less evident in this species. The percentage germination of germinable seed was sufficiently less variable than the raw data for the mean values for all three incubation environments to be significantly different ($p<0.05$) (Figure 4.2d).

After 15 months burial germination percentages in the 8/18°C environment were still lower ($p<0.05$) than in 5/25°C and the seeds did not respond to nitrate (Figure 4.2e). Percentage germination of germinable seeds was significantly lower ($p<0.05$) in the dark at 8/18°C than in either of the other two incubation environments (Figure 4.2f). This is a strong indication that a larger diurnal temperature range was still stimulatory after this period of burial. There was no apparent difference between the light 5/25°C and dark 5/25°C treatments. Germination after 15 months burial was significantly ($p<0.01$) higher than in fresh seed in all three environments.

Thus, burial caused a gradual loss of response to nitrate which was accompanied by a loss of response to light at the higher

alternating temperatures. Even after 15 months burial a response to the larger diurnal temperature range was retained although it was reduced in magnitude.

4.3.3 *Stellaria media*.

The fresh *Stellaria media* seed responded ($p<0.05$) to nitrate at 5/25°C in both the light and the dark (Figure 4.3a). There was also significantly ($p<0.05$) higher germination in the light than in the dark.

After 3 months burial the 8/18°C dark treatment was unfortunately lost due to damping off of the seedlings but nitrate stimulation was still significant in the dark 5/25°C treatment ($p<0.05$) (Figure 4.3b). The germination under those conditions was also significantly ($p<0.05$) higher than in the light. After both 9 and 15 months burial the germination of *Stellaria media* had become extremely variable (Figures 4.3c and d) and there were no significant differences between any of the treatments. This apparent loss of response was also confirmed by the values of percentage germination of germinable seed which also showed no significant differences (Figures 4.3e and f).

The germination of *Stellaria media* in the dark at 5/25°C appeared to reach a peak value after 3 months burial and decline to its original level after further periods in the soil.

Stellaria media, therefore, responded to nitrate when fresh but had completely lost this response after 9 months burial. After this time it had also lost its response to light and wider temperature ranges.

4.3.4 Senecio vulgaris.

The germination of fresh Senecio vulgaris seed showed similar responses to that germinated in petri dishes and on the thermobar (Figure 4.4a). It had significantly higher germination in the light than in the dark ($p<0.01$) and germination at 8/18°C was greater ($p<0.01$) than that at 5/25°C in the dark. An overall response to nitrate was significant ($p<0.001$) as was an interaction ($p<0.001$) between nitrate and the light/temperature treatments. The response to nitrate was individually significant in the light at 5/25°C and in the dark at 8/18°C.

Damping off of seedlings resulted in the loss of all of the results for the April 1982 removal. The repeat experiment in May 1983 following 3½ months burial showed a similar series of responses to the light/temperature treatments although any response to nitrate had been lost (Figure 4.4b).

The results of the original September 1982 removal and those of the repeat experiment for the same burial time (September 1983) were similar (Figures 4.4c and d) and were analysed together in a three-way analysis of variance (Appendix G). An overall response to nitrate was not found but the interaction between nitrate and the light/temperature treatments was significant ($p<0.01$). Nitrate appeared to be inhibitory in the light but to promote germination in both temperature regimes in the dark. The nitrate inhibition in the light was significant ($p<0.05$) when both sets of results were combined as was the stimulation in the dark ($p<0.05$) when the results from both temperature regimes and both experiments were analysed together.

The analysis of variance showed that the light/temperature treatments were significantly different ($p<0.01$). However, when

Duncan's MRT was applied the only observable difference ($p<0.05$) was between the light 5/25°C and dark 5/25°C treatments in the original experiment. The low germination in the dark 5/25°C treatment was in agreement with that observed in fresh seed although in this case despite its appearance it was not significantly lower than in the dark 8/18°C treatments. There were no significant differences between these treatments in the repeat experiment.

The final April 1983 removal of the original experiment produced very variable results and there were no observable differences between any of the treatments (Figure 4.4e). Analysis of the percentage germination of germinable seed did not provide any additional information (Figure 4.4f).

Germination was greater in the dark after 9 and 15 months burial than in fresh seed ($p<0.05$) although no significant differences in dark germination were present between 9 and 15 months burial themselves. There was no significant change in the percentage of germination in the light between fresh seed and that buried for 9 months.

4.4 Discussion.

Germination of the four species in soil without burial was similar to that observed in the thermobar experiments. The responses of Artemisia vulgaris to the nitrate and light/temperature treatments were comparable to those on the thermobar although the germination percentages were lower. In that respect they were comparable to the levels of germination observed in the petri dish experiments. In Stellaria media the overall germination and magnitude of its responses were lower than on the thermobar but the types of responses were the same. The responses of Senecio vulgaris were very similar

to those on the thermobar and in the petri dish tests, with germination in the dark at 8/18°C being greater than that in the dark at 5/25°C. The germination percentage in soil in the light was, however, about 16% lower than in the other experiments. This could be due to the burial of a small proportion of the seeds beneath the thin layer of soil in the plastic boxes preventing their exposure to light. The germination of Cardamine hirsuta in the soil was higher in the dark than on the thermobar, although its germination in the light was lower than in the other experiments. The response to nitrate of fresh Cardamine hirsuta seed in the soil in darkness appeared to be of the type developed by seed stored in the laboratory. Any response to nitrate was also lost after 15 months burial in the same way that 21 months storage in the Stevenson screen resulted in a loss of response. The percentage germination did not, however, decline as much after 15 months in the soil as it did in the laboratory or Stevenson screen after 21 months storage. The responses observed for each burial time are summarised in Table 4.1.

There were no striking cycles of response to the dormancy breaking factors although the increased variability of germination after 9 and 15 months burial could be responsible for masking them. If, however, such variation in germination was also a feature of groups of seeds in the field it would clearly reduce the apparent importance of individual dormancy breaking factors such as nitrate. Failure to observe a cyclic germination pattern may have been due to the infrequency of removal times and the experiment would perhaps have benefitted from later removals, although once again the variability of germination would have been an overriding factor. Murdoch (1982) observed that the dormancy of Chenopodium album was greatest in November and December. It is possible that the September removal in these experiments was prior to the induction of dormancy

Table 4.1 Summary of the response to dormancy breaking factors of four ruderal species following different lengths of burial in the soil.

D = Dark
L = Light
AT = A large diurnal temperature range (20 C^{o} rather than 10 C^{o}) in the dark.
N = Nitrate (10^{-2} M KNO_3)
+ve = Stimulation
-ve = Inhibition
= = No response

Species	Burial period (months) 0	3	9	15
Artemisia vulgaris	L +ve AT +ve N =	L +ve AT = N +ve (partly replacing L)	L +ve AT = N =	L = AT = N =
Cardamine hirsuta	L +ve AT = N +ve (only in D)	L +ve AT +ve N +ve (only in 8/18°C,D)	L +ve (%values) AT +ve N = N x L -ve	L = AT +ve N =
Senecio vulgaris	L +ve AT -ve N +ve	L +ve AT -ve N =	L +ve AT = L x N -ve D x N +ve	L = AT = N =
Stellaria media	L +ve AT = N +ve AT x N +ve	L -ve N +ve (only in 5/25°C,D)	L = AT = N =	L = AT = N =

and consequently too early to observe any period of deeper dormancy of that type. A similar increase in the dormancy of Senecio vulgaris during October and November was recorded by Karssen (1980/81b) but was not apparent in these experiments.

The only slight evidence for a period of induction of dormancy was with Stellaria media in which the germination after 9 months burial was significantly lower than after 3 months. This is in agreement with the results of Roberts and Lockett (1975) who observed maximum germination after 3½ months burial and a partial return of dormancy after 9 months. The germination of fresh seed in this experiment was, however, substantially higher than they had observed under a similar temperature regime in the light. Germination of Stellaria media after 15 months was not significantly higher than in the previous removal and does not consequently support the evidence for an annual rhythm of dormancy change. Froud-Williams *et al.* (1984) observed a peak of Stellaria media germination in the light in autumn, after 3 months burial, followed by a reduction in germination in spring after 9 months burial. A second smaller increase in germination occurred in summer after 12 months burial, followed by lower germination in autumn, after 15 months burial. The increased germination after 3 months, and decreases after 9 and 15 months burial, are similar to the results described here for the same periods of burial despite the fact that the seasons of burial and recovery were different. This is contrary to the view that the degree of dormancy is controlled by the prevailing soil conditions (Froud-Williams 1984, Roberts & Lockett 1978a,b) to produce annual cyclical changes in dormancy.

Although there was no clear cycle of dormancy it was obvious in all four species that the length of burial altered their response to dormancy breaking factors. Artemisia vulgaris did not respond to

nitrate as fresh seed but by April a nitrate response was evident which replaced the requirement for alternating temperatures in the dark. This response to nitrate also partially substitutes for the presence of light in some of the seeds. The effect of this in the field would be to encourage germination of buried seed in the spring when nitrate levels are high. By September, of the dormancy breaking factors only light and alternating temperature together produced higher germination. The following spring did not see a return of the response to nitrate and seeds in all three light/temperature environments showed similar germination. Thus during the course of burial the Artemisia vulgaris seeds initially developed a response to nitrate but finally lost their response to any of the dormancy breaking factors.

Germination after 15 months burial was lower than after 3 months which could be explained either by seed mortality (including loss of dormancy and *in situ* germination) or by the onset of a deeper dormancy which could not be broken by any of the dormancy breaking factors being used. Although no *in situ* germination was observed when sachets were removed from the gardens, in view of the high variability of the results the former explanation seems more likely. In some of the replicates more than 80% germination was recorded whilst we would expect that the onset of deeper dormancy would have reduced their germination levels as well. Murdoch (1982) reported that in Chenopodium album there was a gradual loss of dormancy which would correspond in this experiment to the reduction in response to dormancy breaking factors rather than an absolute increase in the germination percentage after 15 months burial.

In Cardamine hirsuta the response to nitrate which was evident in fresh seed in the dark had become restricted to just the dark 8/18°C treatment after 3 months burial and was entirely lost after 9

months. Once again the response to other dormancy breaking factors was lost during burial and after 15 months only the larger alternating temperature in the dark produced a clear stimulation, the light only appearing stimulatory when the values of percentage of germinable seed were used. Cardamine hirsuta showed a gradual increase in germination with higher levels after 15 months burial which represent a loss of dormancy of the type observed by Murdoch (1982).

Stellaria media responded to nitrate and light when fresh but after 9 months burial there were no responses to either. After 3 months of burial, germination in the dark at 5/25°C was greater than in the light at the same temperatures although this cannot be related to any field effects. Again there was a reduction in the response to dormancy breaking factors, although the high variability after 9 and 15 months burial prevents conclusions being drawn from them.

The fresh seed of Senecio vulgaris responded to light and nitrate. There was also an interaction between nitrate and diurnal temperature range in the dark, the stimulation being greatest in the 8/18°C treatment. After 3 months the response to nitrate had been lost whilst light and 8/18°C temperatures were still stimulatory. After 9 months, however, the nitrate was inhibitory in the light and stimulatory in the dark at 5/25°C. These inhibitory and stimulatory effects would evidently counteract one another in the field and no overall response to nitrate would be evident. The light itself was still stimulatory after this period of burial. After 15 months the germination percentages were lower, but once again the responses to the dormancy breaking factors being tested had been lost.

Despite lower germination in all but Cardamine hirsuta after 15 months, without evidence for a cyclical response, the observed reduction in the response of all four species to the three dormancy

breaking factors suggests an overall reduction in dormancy of the type observed by Murdoch (1982). The lower levels of germination could themselves be due to loss of viability which was not measured directly. The alternative explanation of the lower germination could be the onset of deeper dormancy which could not be broken by light, temperature alterations or nitrate. If such a dormancy had developed it would have perhaps been broken by variation in the oxygen concentration or the use of growth hormones. This explanation is less likely considering the occurrence of individually high (80%+) germination percentages in the otherwise variable results for all four species in their final removal.

It is thus clear from these results that periods of burial of greater than 3 months duration have reduced and eventually completely removed any response to nitrate. Such a loss of response to nitrate was observed by Bostock (1978) for the seeds of Achillea millefolium, Artemisia vulgaris and Cirsium arvense following their burial for 6 months. Thus the presence of a seedbank consisting of seed from different seed falls, being buried for different periods of time may not show a response to nitrate despite the ability of its younger seed component to respond to it. Hence seed mortality in the field and the gradual loss of dormancy would reduce the ecological and agricultural importance of a nitrate response and make field observations of it difficult. However, such field responses may be discernible in conditions when and where the germination of freshly fallen seed is dominant.

CHAPTER FIVE

Germination of a Natural Seedbank in Controlled Conditions.

5. Germination of a Natural Seedbank in Controlled Conditions.

5.1 Introduction.

Experiments were designed to observe the response to nitrate of a natural seedbank in controlled conditions. These experiments provide a link between those described on collected seed and those in the field situation (Chapter 6). A sample of garden soil with a seedbank dominated by the ruderal species studied in Chapter 4, was collected and incubated with and without nitrate, in the laboratory. Calcareous soil from a permanent grassland and an acid soil from a heathland were also tested in the same way. These seedbanks included seeds of some of the calcicole and calcifuge species tested in Chapters 2 and 3.

5.2 Materials and Methods.

5.2.1 Garden soil.

A large volume of soil containing a seedbank of mainly arable weed species was removed from a garden bed in the University Botanical Gardens in 1981. After thorough mixing and sieving to remove large stones and roots, 750 ml of soil was placed directly into each of thirty two 26 x 14 x 9 cm perspex boxes. The soil in half of the boxes was then moistened with 10^{-2}M KNO_3 solution and the other half with double glass distilled water. Moistening agent was added until the soil appeared completely wet without causing waterlogging and was consequently not the same volume in each box. Lids were placed on the boxes to minimise evaporation.

The boxes were randomly divided into groups of four nitrate and four water replicates which were placed in different environments in

growth cabinets (Table 5.1). Dark treatments were wrapped in two layers of aluminium foil. Germination in the light was recorded every one or two days and the dark treatments were removed and counted when no further germination occurred in the light. The species of seedlings in the light were identified using books by Chancellor (1978) and Hanf (1971). Such identification was not possible for the etiolated seedlings of the dark treatments and they were divided into just two groups; monocotyledons and dicotyledons (hereafter referred to as monocots and dicots).

Following counting, a subsample of soil from each box was weighed and then reweighed after oven drying at 100°C so that their percentage moisture contents could be calculated. The nitrate content of a 50 g subsample of soil from two replicates of each treatment were determined using the colorimetric, phenoldisulphonic acid method used by Popay (1968) (after Metson, 1956). The concentration of nitrate in the soil water was then calculated from the above values.

The results were analysed using two-way analyses of variance and Duncan's multiple range tests on the total figures and the figures for the monocots and dicots individually. An analysis of covariance was also performed using the percentage moisture contents, variation in which appeared to influence the amount of germination.

5.2.2 Calcareous soil.

Calcareous soil was collected from a South facing slope in Monsal Dale (Grid ref. SK 184, 717) firstly in March and then in July 1983. A refinement of the techniques used to test the garden soil was used in this experiment. Subsamples of 250 ml of the thoroughly mixed and coarsely sieved soil were placed on a single layer of

Table 5.1 The laboratory environments, degree of replication and duration of tests used to investigate germination from the naturally buried seedbanks of three soil types, with and without the application of nitrate.

Treatment	Light/Dark	Temperature (°C) (12 hr. thermoperiod)	Garden	Calcareous	Acid
A	L	5/25	✓	✓	✓
B	D	5/25	✓	✓	
C	D	8/18	✓	✓	
D	D	18 const.	✓		
Replication			4 replicates per treatment	4 blocks per treatment 2 replicates per block	4 blocks per treatment 2 replicates per block
Total number of boxes			32	48	16
Duration of tests (days)			25	22	28

Whatman No. 1 filter paper supported by three upturned 13.5 x 7.5 x 5 cm perspex boxes within a large 26 x 14 x 9 cm perspex box. The filter paper was then able to dip into a 750 ml reservoir of either 10^{-3}M KNO_3 or double glass distilled water surrounding the upturned boxes (Figure 5.1). A small amount of moistening agent was also poured onto the surface of the soil to ensure its initial wetting but was able to drain freely into the reservoir. Two replicates of the nitrate and water treatments were placed in three different environments (see Table 5.1) directly after each of the two soil collections. A second pair of replicates were placed in each of the six treatments directly after germination of the first two was complete. The soil was stored in black plastic bags at room temperature for the short period of the initial run. For the purpose of analysis the four runs (2 for each collection) were considered to be experimental blocks each containing two replicates and a two-way analysis of variance with blocks was used. The procedure for counting the seedlings was the same as that used for the garden soil. In this case due to the presence of a reservoir of solution the individual soil moisture contents were not calculated. The nitrate concentrations in the reservoirs of three randomly selected nitrate and three randomly selected water treatments were determined using the same method employed for the garden soil.

5.2.3 Acid heathland seedbank.

The surface litter and a small amount of soil was collected in March and July 1983 from a Calluna vulgaris dominated hillside in North Staffordshire (Grid ref. SK 033, 634). A preliminary experiment showed that the majority of seed in such an organic soil is in the litter. Any large Calluna vulgaris stems were removed from

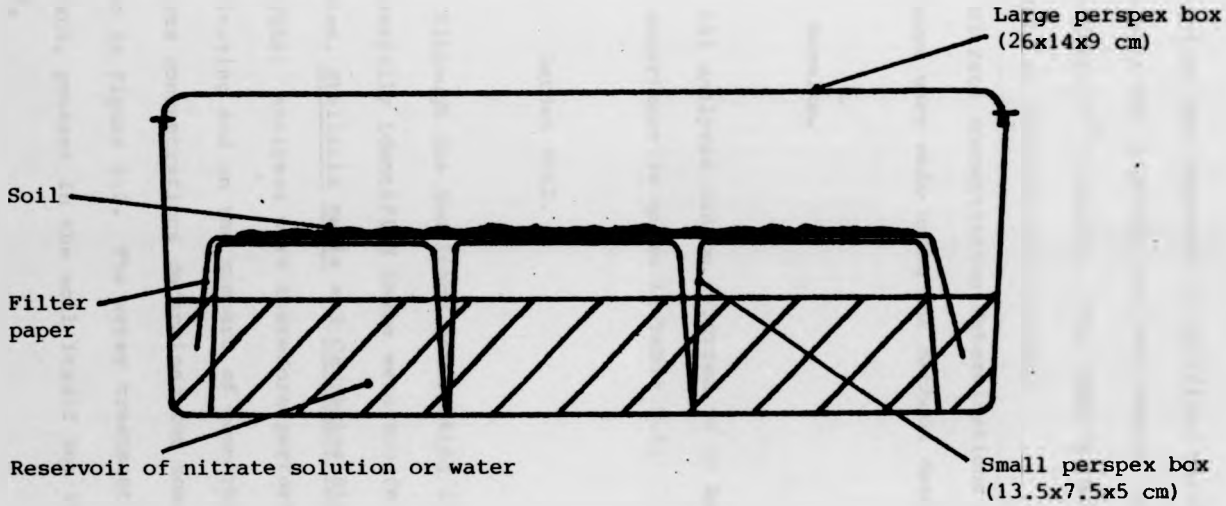


Figure 5.1 The arrangement used to provide a reservoir of nitrate or water allowing germination from a soil seedbank in laboratory conditions.

the litter which was thoroughly mixed and dealt with in the same way as the calcareous soil. Since germination of the calcifuge species had been low in the dark on the thermobar and since little germination was observed in the first dark treatments of this experiment the seedbank was only tested in the light at 5/25°C with or without 10^{-3}M nitrate. The results were analysed using a one-way analysis of variance with blocks.

Nitrate concentration determinations of the reservoirs of all of the boxes were made using the methods described above.

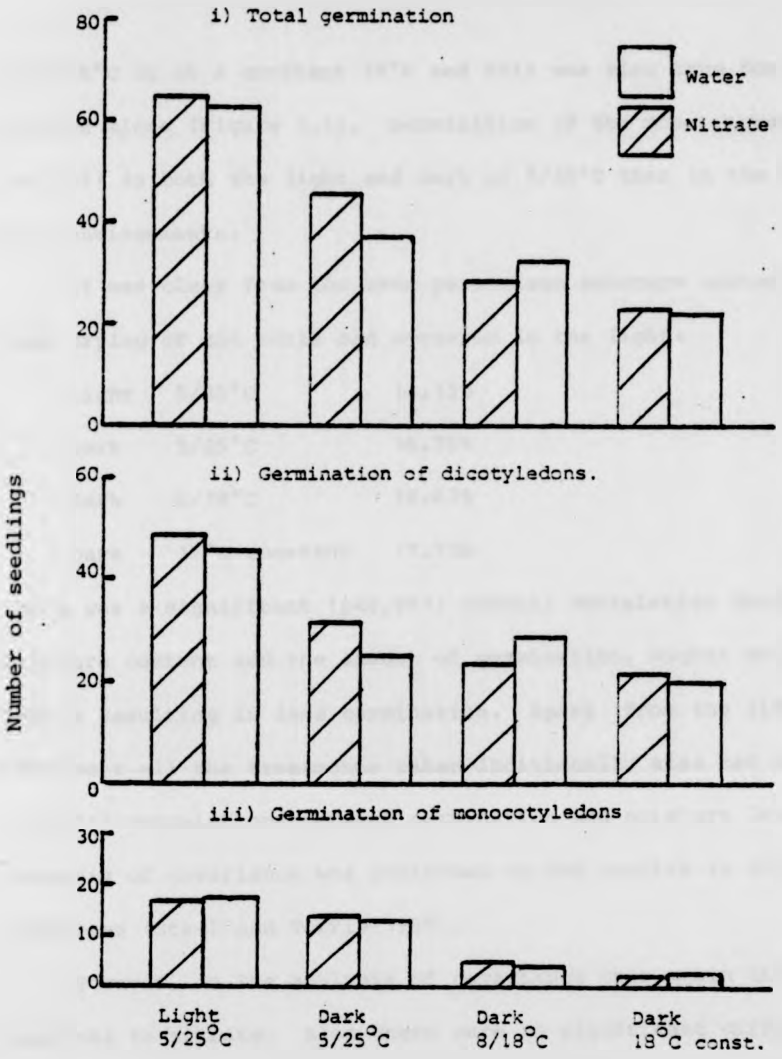
5.3 Results.

All analyses can be referred to in Appendix H. The duration of each experiment is given in Table 5.1.

5.3.1 Garden soil.

Although the seedlings germinating in the light were successfully identified there were too few of even the most frequent species, Stellaria media and Cardamine hirsuta for individual analysis. Analyses were therefore performed on the total numbers germinating and on the numbers of monocots and dicots separately. The nitrate concentrations determined for some of the treatments are given in Figure 5.2. The water treatments evidently contained some nitrate, present in the soil itself but this did not exceed 9 x 10^{-4}M.

Nitrate did not significantly increase germination of either the monocots or dicots although the light/temperature environments did produce different levels of germination. Total germination in the light at 5/25°C was significantly greater ($p<0.05$) than in the dark



Treatment	Water	Nitrate
Light 5/25°C	7.4×10^{-4}	1.3×10^{-2}
	8.9×10^{-4}	1.5×10^{-2}
Dark 5/25°C	6.8×10^{-4}	1.1×10^{-2}
	8.3×10^{-4}	1.4×10^{-2}
Dark 8/18°C	5.6×10^{-4}	1.2×10^{-2}
	7.3×10^{-4}	1.4×10^{-2}
Dark 18°C Const.	5.2×10^{-4}	1.1×10^{-2}
	6.5×10^{-4}	1.2×10^{-2}

Figure 5.2 The numbers of seedlings emergent from the garden soil when placed in four different laboratory environments, with and without nitrate. Each column represents the mean value of four replicates. The significance of any differences between treatments is discussed in the text. The nitrate concentrations recorded in the reservoirs of two randomly selected boxes from each treatment are also given.

at 8/18°C or at a constant 18°C and this was also true for the dicot species alone (Figure 5.2). Germination of the monocots was greater ($p<0.05$) in both the light and dark at 5/25°C than in the other two dark environments.

It was clear from the mean percentage moisture contents that some drying of the soils had occurred in the light:

Light	5/25°C	14.13%
Dark	5/25°C	16.75%
Dark	8/18°C	18.63%
Dark	18°C constant	17.73%

There was a significant ($p<0.001$) overall correlation between the moisture content and the amount of germination, higher moisture levels resulting in less germination. Apart from the light 5/25°C treatment all the treatments taken individually also had significant ($p<0.01$) correlations between germination and moisture levels and an analysis of covariance was performed on the results to allow for such responses (Steel and Torrie 1980).

However, in the analysis of covariance once again there was no response to nitrate. Also there were no significant differences between germination in the light/temperature treatments using this form of analysis (Table 5.2).

5.3.2 Calcareous soil.

The number of seedlings which emerged from the calcareous soil was much lower than that observed in the garden soil. The total numbers of seedlings observed are represented in Figure 5.3 but in this case they were not grouped into monocots and dicots. The measured nitrate concentrations are also given in Figure 5.3.

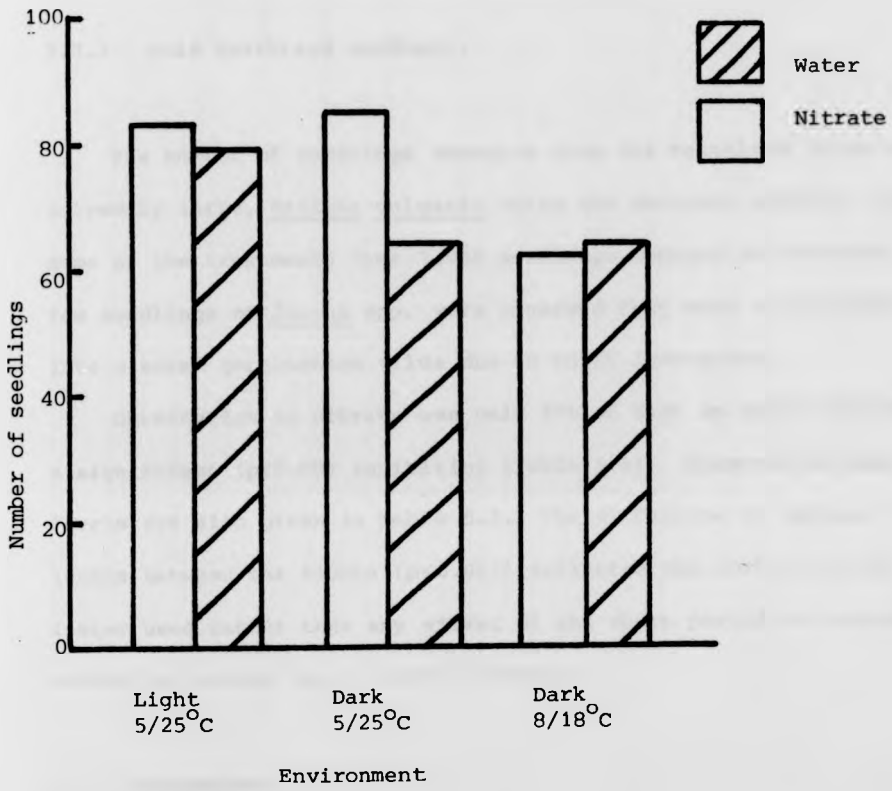
Table 5.2 Analysis of covariance for the seedling emergence and soil moisture content of the garden soil containing a naturally buried seedbank.

		ENVIRONMENT									
		L 5/25°C		D 5/25°C		D 8/18°C		D 18°C const.		Totals	
Replicate	Nitrate/ Water	X	Y	X	Y	X	Y	X	Y	X	Y
1	N	12.3	93	16.4	44	19.9	14	18.3	19	136.2	271
	W	12.9	46	19.1	19	19.7	18	17.6	18		
2	N	14.3	78	16.4	39	18.2	30	17.2	23	133.1	329
	W	13.7	63	16.3	43	19.6	18	17.4	35		
3	N	15.4	39	16.8	42	18.5	28	18.9	8	137.6	288
	W	14.5	81	16.1	48	18.3	32	19.1	10		
4	N	13.8	48	15.6	58	18.3	42	16.1	42	131.0	377
	W	16.1	61	17.3	39	16.5	61	17.3	26		
Subtotals	N	55.8	258	65.2	183	74.9	114	70.5	92	266.4	647
	W	57.2	251	68.8	149	74.1	129	71.4	89	271.5	618
Totals		113.0	509	134.0	332	149.0	243	141.9	181	537.9	1265

Y = Emergence numbers.

X = Moisture content (%).

	Sum of products				Y adjusted for X			
Source of variation	df	X,X	X,Y	Y,Y	df	SS	MS	F-ratio
Total	31	125.250	-1097.560	13618.000				
Environments	3	90.953	- 776.872	7632.344				
Nitrate/Water	1	0.816	- 4.622	26.281				
Env. x Nit./Water	3	1.230	- 13.740	153.594				
Error	24	32.250	- 302.326	5805.751	20	2971.612	148.581	
Environment + error.	27	123.203	-1079.198	13438.095	23	2984.849		
Difference for testing adjusted environment means.					3	1013.237	337.745	2.273 Non-significant at $p < 0.05$.
Nitrate/Water + error.	25	33.066	306.948	5932.032	21	2982.668		
Difference for testing adjusted nitrate/water means.					1	11.056	11.056	0.074 Non-significant
Env. x Nitrate/Water + error.	27	33.480	316.066	5959.345	23	2975.542		
Difference for testing adjusted Env. x N/W means.					3	3.930	1.310	0.009 Non-significant.



Nitrate concentrations in the treatment reservoirs

Treatment	Water	Nitrate
Nitrate Conc. (M)	3.0×10^{-6}	1.5×10^{-3}
	5.0×10^{-6}	1.6×10^{-3}
	9.0×10^{-6}	2.0×10^{-3}
Mean	5.7×10^{-6}	1.7×10^{-3}

Figure 5.3 The total numbers of seedlings emergent from the calcareous soil when placed in three different laboratory environments, with and without nitrate. The nitrate concentrations recorded in the reservoirs of three randomly selected water and three randomly selected nitrate treatments are also given.

There was no significant response to nitrate and only a non-significant trend ($p<0.1$) for differences between the light/temperature treatments.

5.3.3 Acid heathland seedbank.

The number of seedlings emerging from the heathland litter was extremely large, Calluna vulgaris being the dominant species. In some of the treatments over 1,000 seedlings emerged and although a few seedlings of Juncus spp. were observed they were incorporated into a total germination value due to their infrequency.

Germination in nitrate was only 87% of that in water indicating a significant ($p<0.05$) inhibition (Table 5.3). Measured nitrate levels are also given in Table 5.3. The difference in germination levels between the blocks ($p<0.001$) reflected the portion of the litter used rather than any effect of the short period of storage before the second run of each collection.

5.4 Discussion.

Nitrate concentrations of 5-9 x 10^{-4}M in the water treatments of the garden soil experiment may have been partially responsible for the failure to observe any response to added nitrate. However, judging by the nitrate optima for the four ruderal species in Chapter 2 such nitrate levels would not have been capable of satisfying the entire nitrate requirements of at least fresh seeds of the species present in the seedbank tested. No such problem was encountered in either the experiments with calcareous or acid soil since nitrate levels in the water treatments were substantially lower due to the large reservoirs of solution present.

Table 5.3 Germination figures and nitrate concentrations for the acid heathland seedbank. In each case there were two replicates per block contributing to the mean value for each treatment.

		Block number				
	Treatment	1	2	3	4	Nitrate/Water Means
Mean emergence figures	Nitrate	1060.0	311.5	946.5	306.5	656.1
	Water	1108.0	359.5	1111.0	430.0	752.1
	Block mean	1084	335,5	1028.8	368.3	
Mean Nitrate concentration (M)	Nitrate	1.5×10^{-3}	1.2×10^{-3}	1.1×10^{-3}	1.2×10^{-3}	
	Water	$<1 \times 10^{-6}$	3×10^{-6}	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	

The failure of nitrate to stimulate germination from the garden soil seedbank provides supporting evidence for the loss of response to dormancy breaking factors observed in Chapter 4. The naturally buried seeds present in the garden soil had probably been buried for varying periods of time and the overall effect of this range of ageing periods was to mask any possible response to nitrate of the more recently incorporated seeds. This would clearly reduce the possibility of observing a response to fluctuating nitrate levels in the field. However, it may be that a response could become evident directly after periods of heavy seed fall when fresh seeds are predominant. A discussion of this possibility is left for Chapter 7 following consideration of the field experiment results.

The apparent reduction of germination by higher percentage moisture contents emphasised the importance of careful control over factors other than those being directly tested in these experiments. All other experiments were therefore carried out using standard volumes of moistening agent and evaporation losses were kept to a minimum.

It was difficult to assess the importance of the lack of significant stimulation by light or alternating temperatures, following the analysis of covariance. The reduction in moisture levels in the light treatments in particular, meant that the higher germination in them could be due to either the moisture levels, a slight concentration of nitrate due to drying or the light itself. Similarly, the slightly higher germination in the dark at 5/25°C rather than at 8/18°C could have been due to the temperature regime or variations in moisture level, which appeared to be related. It is, however, evident that these naturally buried seeds have to some extent lost their ability to respond to the light/temperature treatments in the same way that their response to nitrate was lost.

Seed buried in the calcareous soil behaved in the same way as fresh seed in petri dishes, the responses to light/temperature treatments being less evident than those observed on the thermobar. It is not surprising that there was no response to nitrate in buried seed when freshly collected seed did not respond in the thermobar tests.

The large numbers of seedlings emerging from the acid heathland litter were sufficient to provide evidence for an actual inhibition by nitrate. The same tendency was observed for smaller sample sizes of the calcifuge species on the thermobar. With the exception of Juncus conglomeratus and Vaccinium myrtillus, all failed to respond to nitrate in previous experiments. This may be related to the restricted ability of certain calcifuge species to utilize nitrate (Havill *et al.* 1977). The most extreme examples of this are those in which nitrate actually retarded the growth of some cultivated species of the Ericacae (Cain 1952, Townsend 1966, Griedanus *et al.* 1972).

CHAPTER SIX

Field Experiments.

6. Field Experiments.

6.1 Introduction.

None of the experiments described so far have demonstrated responses to nitrate in field conditions; they have all used collected seeds, the incubation of seeds in controlled conditions, or both. The natural burial and ageing of seeds in the soil and the complexity of the environment in the field, is likely to influence the magnitude and form of any responses to nitrate. The experiments described in this chapter were attempts to observe any responses to nitrate in conditions as near as possible to those of the natural environment of garden weeds.

In order to attribute germination responses in the field to the direct influences of inorganic nitrates it is, however, necessary to exert some control over soil conditions. This becomes evident from the work of Popay and Roberts (1970b), who recorded seedling emergence and environmental variables at two sites, over a period of a year, without attempting to control soil conditions. They observed flushes of germination of Senecio vulgaris and Capsella bursa-pastoris corresponding to increased levels of soil nitrate in July. However, this could only be used as circumstantial evidence for a field response to nitrate, since these germination flushes also coincided with a period of relatively large diurnal temperature ranges, themselves known to stimulate germination. Thus, although any form of environmental control may influence the responses observed, a degree of control is necessary to isolate those responses attributable to nitrate. Consequently, these experiments involve attempts at controlling nitrate levels in the soil, under otherwise natural environmental conditions.

Although mention has been made in Section 3.4 of the possible role of nitrate as a gap indicator, these experiments concern

seasonally occurring safe sites. The few previous germination experiments which have been performed in the field using added nitrates were reviewed in Section 1.3. The natural fluctuations of soil nitrate concentration throughout the year make its control under experimental conditions difficult. Peaks of nitrate availability associated with increased nitrification rates have been observed by many workers (Ellenberg 1964, Williams 1968, 1969, Davy and Taylor 1974, Taylor *et al.* 1982), and it is the reduction of these to a background level that presents a major problem. Schimpf and Palmblad (1980) used a nitrification inhibitor to prevent the production of nitrate from ammonium, having already depleted the soil of nitrate by planting a nitrogen demanding crop of maize. The same nitrification inhibitor was used in the 1981 field experiment to control the production of soil nitrates in some treatments. Leaching of the soil with water was also used to deplete background nitrate levels in some treatments.

Apart from reducing nitrate concentrations to a background level in some treatments, it was necessary to elevate nitrate levels in others to simulate flushes of nitrification in the soil. This was achieved by the addition of nitrate to the soil in a series of monthly treatments. It was then hoped that any flushes of germination could be related to the changing nitrate levels in the soil. No attempts were made to control any other environmental variables such as temperature or rainfall. Thus, the aim of this field experiment was to control soil nitrate levels in otherwise natural conditions and observe the resultant seedling emergence from a naturally formed seedbank.

A second experiment was performed in 1982 with the same aims, but with some differences in methods, following consideration of the 1981 results. In 1982 parallel experiments were performed using soil

from a calcareous grassland and soil from an acid heathland. This was intended to compare the response to nitrate of species from a soil which was expected to have naturally lower nitrate levels with that of the relatively fertile garden soil (Section 2.4). Unfortunately, the heathland experiment was unsuccessful due to drying out of the litter and low numbers of emergent seedlings. The calcareous soil did produce sufficient seedlings, of different species, for useful comparison.

6.2 **Materials and Methods.**

6.2.1 1981 Experiment : Methods.

In the last week of March 1981, a 3 cm thick layer of topsoil, containing a buried seedbank of mainly arable weeds, was removed from a 6.6 x 2.2 m plot at the University Botanical Gardens. It was spread out on a polythene sheet, thoroughly mixed and any large stones were removed. Two hundred and eighty, 10 cm long plastic pipes of 15 cm diameter were sunk into the plot to a depth of 7 cm, leaving a 3 cm rim protruding above the surface. Each pipe was separated by 5 cm from its nearest neighbour and 33 cm wide walkways were left at six intervals down the plot to allow access. Into each of the pipes was then placed 300 ml of the mixed soil, giving a soil depth of about 2 cm. The whole plot was then surrounded by rabbit netting to prevent disturbance and slug pellets were periodically sprinkled between the pipes. Five treatments were applied to the soil in the pipes at each of seven monthly intervals between April and October (Table 6.1).

In some of the treatments a nitrification inhibitor, 2-chloro-6-(trichloromethyl)pyridine, was used to prevent the production of nitrate by soil bacteria, by specifically inhibiting

Table 6.1 Timetable of treatments and monitorings for the 1981 field experiment.

Treated	Volume of nitrate or water applied (ml)	Soil mixed before treatment	Germination monitored
March 30th	250	x	April 14th
			April 28th
May 1st	250	x	May 13th
			May 22nd
June 4th	500	✓	June 12th
			June 29th
July 2nd	500	✓	July 15th
			July 31st
August 4th	500	✓	August 18th
			August 30th
August 31st	500	✓	September 22nd
			October 2nd
October 9th	500	✓	October 22nd
			November 5th

the oxidation of ammonium by Nitrosomonas spp. (Gasser 1970). The same chemical, marketed by Dow Chemical Co. as "N-serve", had previously been used in association with ammonium sulphate in the experiments of Schimpf and Palmblad (1980). In all cases the N-serve was applied to the pipes at a rate equivalent to 2 l ha^{-1}; the commercially recommended dose rate. Fifteen replicates of the five treatments were assigned randomly to pipes within the plot.

The five treatments applied each month were:

i) A nitrate "flush" treatment, (henceforth referred to as NO_3- flush), intended to provide a single, short lived, rise in soil nitrate concentrations. This involved the application of 10^{-2}M potassium nitrate solution and N-serve to a different series of pipes each month. Those pipes awaiting this treatment in later months received an equivalent volume of N-serve without nitrate.

ii) A series of pipes which received 10^{-2}M potassium nitrate and N-serve every month (NO_3- monthly). This was intended to provide elevated nitrate concentrations throughout the year.

iii) An application of N-serve every month to the same pipes, to deplete the natural soil nitrate levels through leaching. This is identical to the treatment of NO_3-flush pipes in months other than that of their flush.

iv) Application of water without N-serve, to act as a control for the N-serve treatment.

v) A "no treatment" control.

Two hundred and fifty millilitres of solution was applied for each treatment in each of the first two months, but this was doubled for subsequent applications (Table 6.1). This was poured slowly over the soil to prevent waterlogging. A comparison between these application rates and the volume of rainfall experienced during each

month is left for the results, Section 6.3.

Seedlings were counted, identified using books by Chancellor (1978) and Hanf (1971), and removed from the treatment pipes twice each month (Monitoring dates. Table 6.1). Some seedlings required transplanting before identification became possible. All other pipes and the gaps between them were also kept clear of emergent seedlings.

For the first two applications the soil remained undisturbed, but due to an apparent decline in seedling numbers, the soil was removed from each pot and individually mixed before subsequent treatments. This redistributed the remaining seeds, bringing more into the otherwise seed depleted surface layer of the soil.

Additional pipes were given the same treatments and used for monitoring of the soil moisture and nitrate levels. Percentage moisture content was calculated from the weights of freshly collected soil samples and their dry weights following an 18 hour period in an oven at 105°C. Nitrate was extracted and measured using the phenol-disulphonic acid, colorimetric method described in detail by Popay (1968, after Metson 1956). Based upon the assumption that all nitrate in soil is dissolved in the soil water (Russell 1973, p. 335), its concentration in the soil was calculated from the moisture content and nitrate extracted using these methods. Some monitorings of nitrate concentration in the NO_3-flush treatments were replicated, but due to the consistency of results, later replication was omitted.

The soil temperature on the surface and at a depth of 2.5 cm in a pipe, was recorded hourly, by a Grant chart recorder. Additional monitorings at depths of 0.5, 1.5 and 5 cm were made throughout April, to illustrate the change in diurnal temperature range with depth. The University of Keele Meteorological Station provided daily records of other climatic variables: rainfall, maximum and minimum air temperatures and maximum and minimum soil temperatures at 10 cm

and 20 cm depth.

6.2.2 1981 Experiment : Analysis.

The experiment was designed to be analysed using a two-way analysis of variance of the five treatments and seven months. For this purpose the numbers of seedlings from each of the two monitorings per month were combined. However, due to the low numbers of emergent seedlings in October, the final analysis was only performed for the first six months. Treatment variances of raw data were heterogeneous between months. This was overcome by transformation. An "Fmax" test (Sokal and Rohlf 1969), and in borderline cases the more stringent Bartlett's test, were applied, to test homogeneity after data transformation. Consequently the total numbers of emergent seedlings were analysed following a $\sqrt{x + 0.5}$ transform.

Only the individual numbers of Poa spp. and Stellaria media seedlings were sufficiently high to warrant independent analysis. The former were analysed following a $\sqrt{x + 0.5}$ transform. However, neither a logarithmic or square root transform was capable of making the Stellaria media variances homogeneous. In this case, therefore, each month's data was analysed separately, using one-way analyses of variance. The data for May, July and September all still required a log (x + 1) transform, and insufficient seedlings germinated in August to justify analysis.

Duncan's multiple range test was used in conjunction with these ANOVAs, to make individual comparisons between treatments. For such comparisons involving the April treatments, it was possible to lump the NO_3-flush and NO_3-monthly values, since at that stage there were no differences between the two treatments. Further analysis,

however, then involved the use of a Student-Newman-Keuls test for multiple comparisons based on unequal sample size.

6.2.3 1982 Experiment : Methods.

This experiment differed in two major respects from that of 1981. Firstly, due to the apparently undesirable affect of N-serve in the 1981 experiment, no attempt was made to artifically reduce nitrification in the soil. Secondly the total treatment area was increased so that larger numbers of seedlings could be recorded.

An additional aspect of the experiment was the use of calcareous grassland soil, collected in the last week of April 1982, from Monsal Dale (Grid ref. SK 184, 717). This was treated in the same way, described below, as the garden soil. An attempt was also made to use soil from an acid heathland but this was unsuccessful due to drying out and will not be described in detail.

At the end of April 1982 two adjacent plots (17.6 x 2.1 m and 7 x 2.5 m) in the University Botanical Gardens were divided up into one hundred and seventeen, 0.5 x 0.5 m quadrats. These were 10 cm apart and were separated from the surrounding grass sward by a 20 cm gap. Each quadrat was delimited by string attached to four seed tags, one at each of its corners. The soil from each quadrat was dug out to a depth of 10 cm and the base of the resulting hole was spiked using a garden fork, to facilitate drainage. An 80 x 80 cm square of nylon mesh was then placed into each of the holes. One cubic metre of thoroughly mixed soil of each type; garden soil, calcareous grassland soil and acid heathland soil was then divided into 2.5 litre portions. These were then randomly distributed amongst the quadrats, placed on the mesh and carefully levelled off.

Once again slug pellets were sprinkled between the quadrats at

suitable intervals. Rabbit fencing was not erected due to the adequate repair of such fencing surrounding the whole of the Botanical Gardens.

In this experiment, treatments were performed at three intervals during the year; 7th May, 14th July and 28th August. The treatments were:

i) A nitrate flush treatment on different quadrats at each treatment time. This was applied as 10^{-2} M potassium nitrate in two doses; a 4 litre application followed by a further 2 litres after two days. This was intended to extend the duration of the nitrate flush, the total volume applied being equivalent to that of the 1981 experiment.

ii) A NO_3-every treatment, receiving 10^{-2}M nitrate at the same rate, but at all of the three treatment times.

iii) A water treatment receiving the same volume of water each time.

iv) An untreated control.

Four replicates of each treatment were used, providing almost four times the area per treatment compared to the 1981 experiment (i.e. 1 m^2 compared to 0.265 m^2 in 1981). With the aid of the nylon mesh, the soil from each quadrat was removed and mixed in a bucket before the second and third applications. Monitoring once again took place twice for each application, but the numbers were combined in the analysis of results. Seedlings were counted, identified and removed, many of those from the calcareous soil requiring transplanting before identification. Soil in additional quadrats was provided with the same treatments so that nitrate levels could be monitored using the methods described for the 1981 experiment. Meteorological reports were used to provide additional environmental data.

6.2.4 1982 Experiment : Analysis.

Results were analysed using two-way analyses of variance (treatment x month) and Duncan's multiple range tests. It was necessary to transform some of the data to overcome the problem of heterogeneity of treatment variances. The figures for total germination in the garden soil were analysed following a logarithmic transform. In this case a Student-Newman-Keuls test was used to compare treatments in the first application, after combination of the NO_3-flush and NO_3-monthly figures.

Adequate numbers of Senecio vulgaris, Stellaria media, Poa spp. and Cardamine hirsuta emerged to justify individual species analyses. Analysis of the raw data was possible for Senecio vulgaris and the Poa spp., but Cardamine hirsuta figures required a logarithmic transformation. The data for Stellaria media could not be analysed either in a two-way ANOVA or as separate ANOVAs for each application, even after logarithmic or square root transformation.

In the case of the calcareous soil the occasional emergence of Stellaria media, Senecio vulgaris or Cardamine hirsuta seedlings was assumed to be due to contamination by seed fall in the gardens. The total numbers of seedlings, excluding those of the above species, were therefore analysed separately and no transform was necessary. The only species which emerged from the calcareous soil to be analysed separately was Holcus lanatus. This was only done for the results from the first application and no transformation was necessary.

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6.3 Results.

6.3.1 1981 Experiment : Environmental factors.

In the first two months, April and May, the amount of solution applied to the pipes was equivalent to 1.4 cm of rainfall. This was only about one quarter of the volume that actually fell as rainfall in those months. Between July and October the solution applied was equivalent to 2.8 cm of rain. This was a substantially greater volume compared to the rainfall values for June, July and August, but for September and October was again only about a quarter (Table 6.2).

After the first few days following each application, the moisture content of the treated soils did not differ greatly from that of the untreated soils (Figure 6.1). Indeed, the natural rainfall distribution (Figure 6.2) appeared to be the overriding factor governing soil moisture content in the treated soil, even in the driest months; June, July and August.

The effect of rainfall on the nitrate concentration of the soil at different times throughout the year was also evident. Concentrations of nitrate in the monitored pipes are shown in Figure 6.3. Nitrate applications during June, July, August and September produced soil nitrate concentrations of between 9×10^{-3}M and 2×10^{-2}M. The October flush of nitrate was not as effective, probably due to higher rainfall at its time of application. Unfortunately, the concentration was not monitored after the April and May flushes but it is assumed that levels of nitrate were similar to those produced in the following four months. The levels of nitrate in the untreated pipes were relatively low during May and early June (between 3×10^{-3}M and 4.5×10^{-3}M), but reached the levels of the nitrate treated pipes between late June and early September. This corresponds to the driest part of the experimental period and was probably caused by concentration of the soil solution due to

Table 6.2 Comparison between actual rainfall and the volume of nitrate applied in the 1981 field experiment.

Month	Rainfall equivalent of volume applied in each month (cm).	Actual monthly rainfall (cm).
April	1.4	4.90
May		6.93
June	2.8	4.17
July		4.12
August		4.18
September		11.33
October		9.77

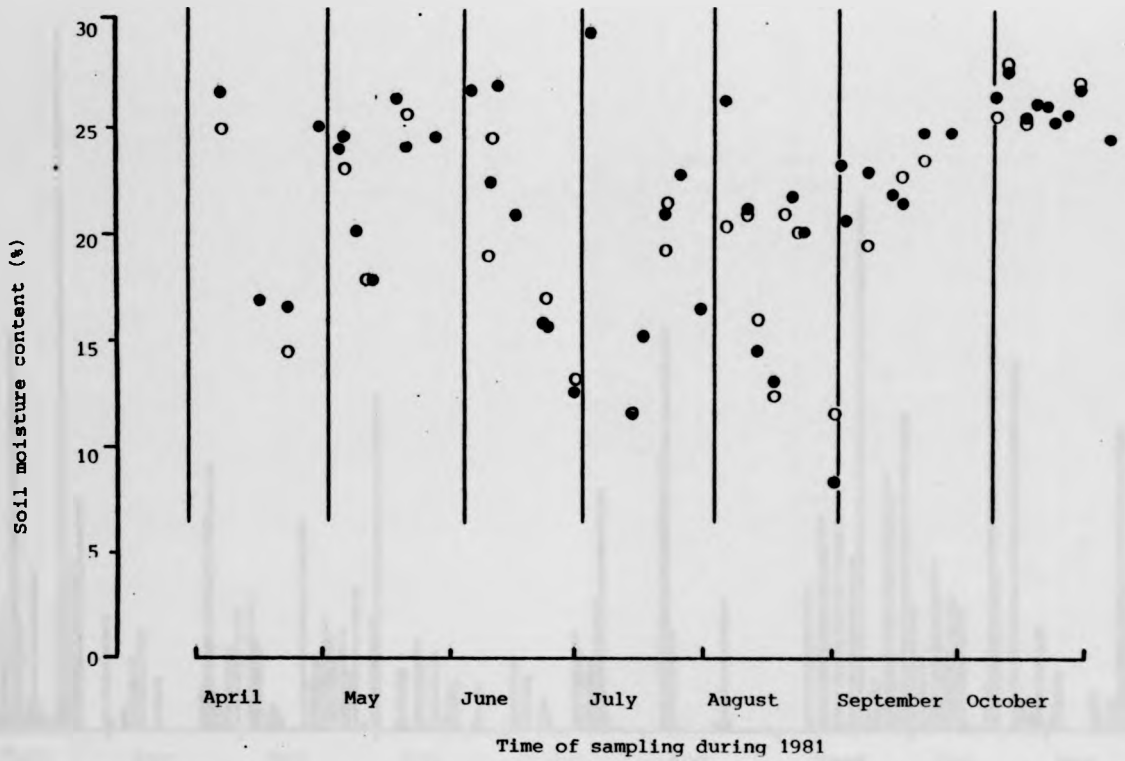


Figure 6.1 Percentage moisture content of soil samples taken at intervals throughout the 1981 field experiment.

● moisture content of thesoil in pipes which received a nitrate or water application.

O moisture content of the soil in pipes which did not receive any treatment.

Treatment application times are marked by the vertical dividing lines.

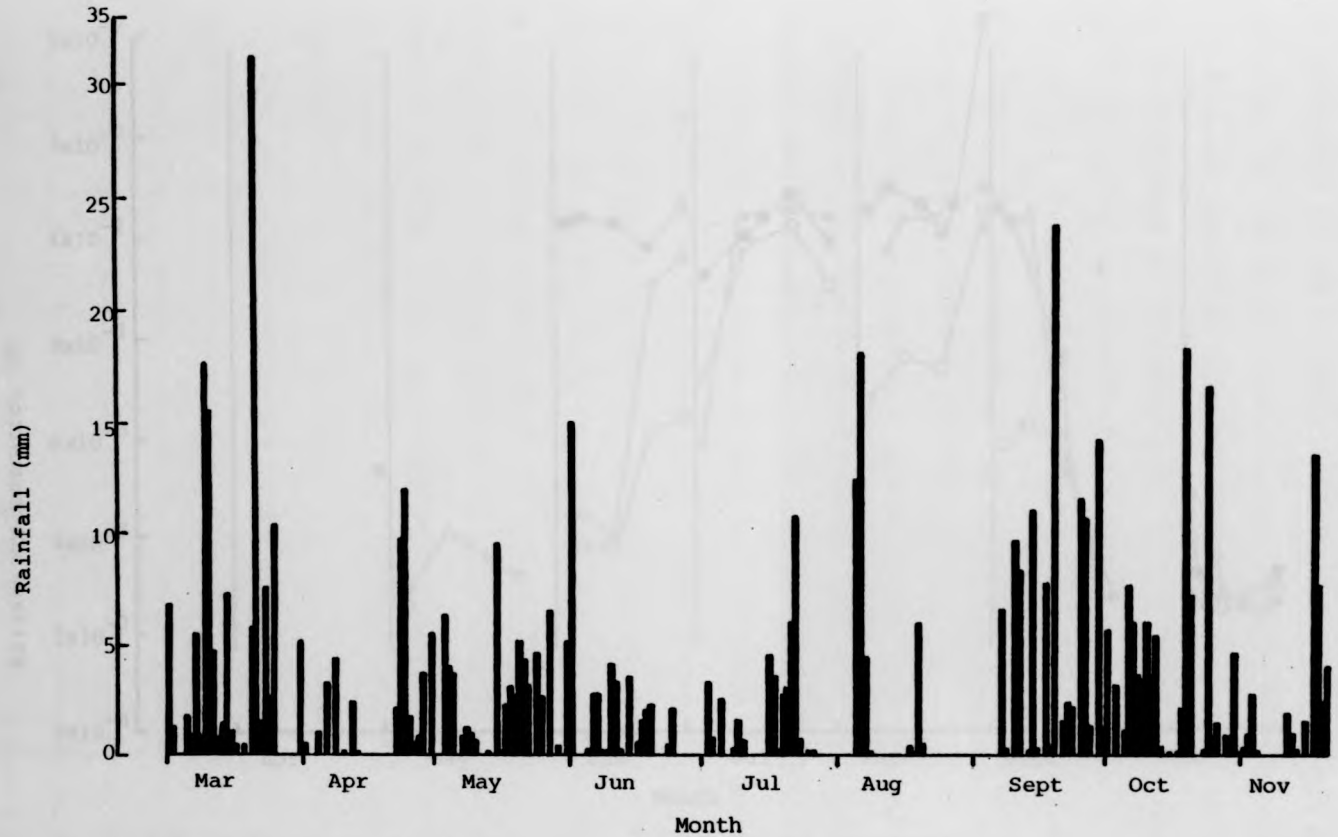


Figure 6.2 Natural rainfall distribution between March and November 1981 from records provided by the University of Keele Meteorological Station.

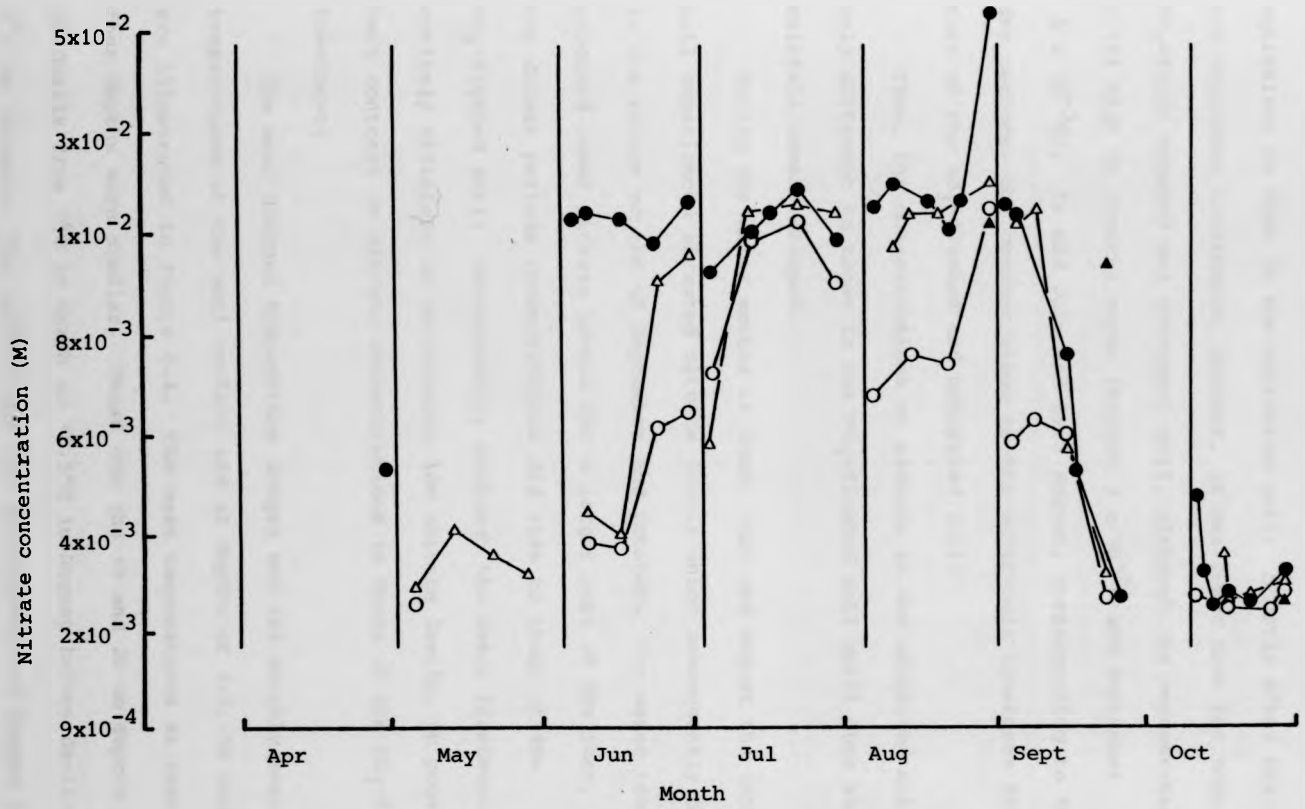


Figure 6.3 Soil nitrate levels throughout the 1981 Field experiment.

● Nitrate application ○ Water application

△ No treatment ▲ N-serve

The vertical lines indicate the times of treatment application.

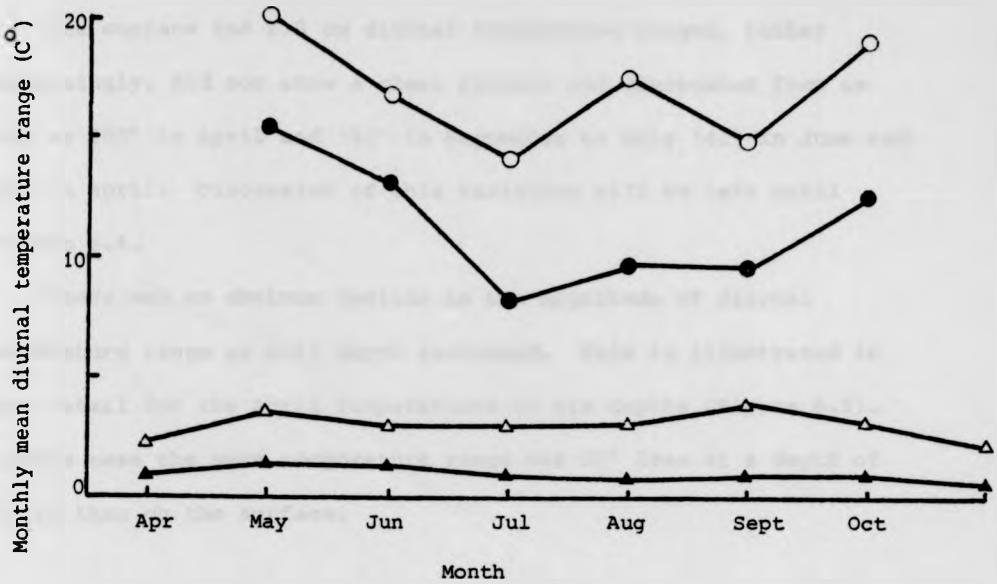
evaporation.

The concentration of nitrate in the water treatment (4×10^{-3}M) was lower than for the NO_3-flush treatment in early June, but was equivalent to that in the untreated soil. Shortly after the August and September treatments, however, it was lower than for both the NO_3-flush treated and untreated soil, although its concentration was still high in general terms (August 7×10^{-3}M and September 6×10^{-3}M). In mid July and late August, corresponding to two very dry periods, the concentration in the water-only treatment did reach that of the NO_3-flushed and untreated soil.

Thus, the concentrations of nitrate in the untreated soil were only different to those in the NO_3-flushed soil until June when the rainfall levels dropped.

During the drier months of June, July and August the untreated soil experienced elevated nitrate levels which subsequently declined in the wetter months of September and October. The water treatment produced lower nitrate levels for a larger part of the year, but in the driest periods concentrations did rise to those of the NO_3-flushed soil. Consequently, although the water treatment was not entirely efficient in maintaining low nitrate levels, it provides the best contrast in nitrate concentrations to those of the NO_3-flush treatment.

The mean diurnal temperature ranges and the monthly mean temperatures at the soil surface and at depths of 2.5, 10 and 20 cm are illustrated in Figure 6.4. The mean temperatures at each of the four depths were similar. Those for the 10 and 20 cm depths rose gradually from 7°C in March to 17.5°C in August before declining to 8°C in October. The surface and 2.5 cm temperatures showed a similar pattern between May and August, but temperatures were higher on the surface in April (14°C) and did not drop as low in September.



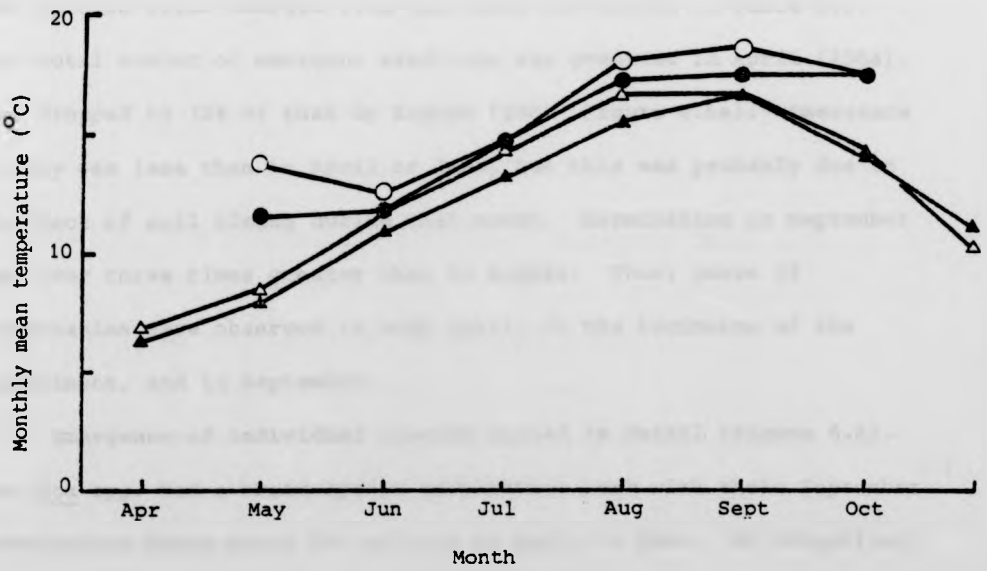


Figure 6.4 The monthly mean diurnal temperature ranges and monthly mean temperatures at the soil surface (O) and at depths of 2.5 (●), 10(Δ) and 20(▲) cm.

The surface and 2.5 cm diurnal temperature ranges, rather surprisingly, did not show a clear pattern and fluctuated from as wide as 20C° in April and 19C° in September to only 14C° in June and 15C° in April. Discussion of this variation will be left until Section 6.4.

There was an obvious decline in the magnitude of diurnal temperature range as soil depth increased. This is illustrated in more detail for the April temperatures at six depths (Figure 6.5). In this case the mean temperature range was 5C° less at a depth of 2.5 cm than on the surface.

6.3.2 1981 Experiment : Seedling emergence.

Analyses of the seedling emergence data are shown in Appendix I. The species which emerged from this soil are listed in Table 6.3. The total number of emergent seedlings was greatest in April (2584), but dropped to 10% of that by August (258) (Figure 6.6a). Emergence in May was less than in April or June, but this was probably due to the lack of soil mixing during that month. Germination in September was over three times greater than in August. Thus, peaks of germination were observed in both April, at the beginning of the experiment, and in September.

Emergence of individual species varied in detail (Figure 6.6). The *Poa* spp. had a broad spring germination peak with their September germination being about 75% of that in April to June. In comparison *Stellaria media* had little September emergence.

In *Senecio vulgaris* and *Sagina procumbens* the September peak of germination was greater than the spring one and emergence was lowest in July. *Cardamine hirsuta* showed germination peaks of equal magnitude in June and September, with a low in August. Germination

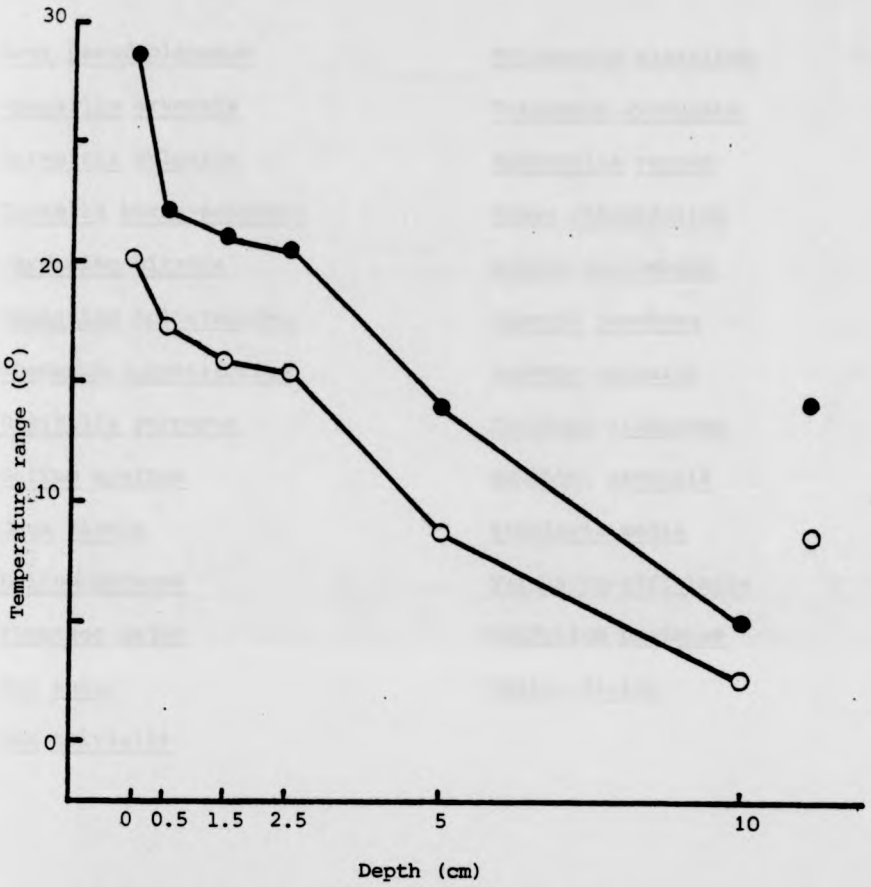


Figure 6.5 The mean and maximum diurnal temperature ranges at five depths in the soil and on the soil surface during April 1981.

● maximum diurnal temperature range

○ mean diurnal temperature range

The two additional points give comparable values for air temperatures in the shade.

Table 6.3 Species list of emergence from the garden soil.

Acer pseudoplatanus

Anagallis arvensis

Artemisia vulgaris

Capsella bursa-pastoris

Cardamine hirsuta

Cerastium holosteoides

Chamerion angustifolium

Digitalis purpurea

Galium aparine

Geum rivale

Lolium perenne

Plantago major

Poa annua

Poa trivialis

Polemonium caerulium

Polygonum aviculare

Ranunculus repens

Rumex obtusifolius

Sagina procumbens

Senecio jacobaea

Senecio vulgaris

Solidago virgaurea

Sonchus arvensis

Stellaria media

Taraxacum officinale

Trifolium pratense

Urtica dioica

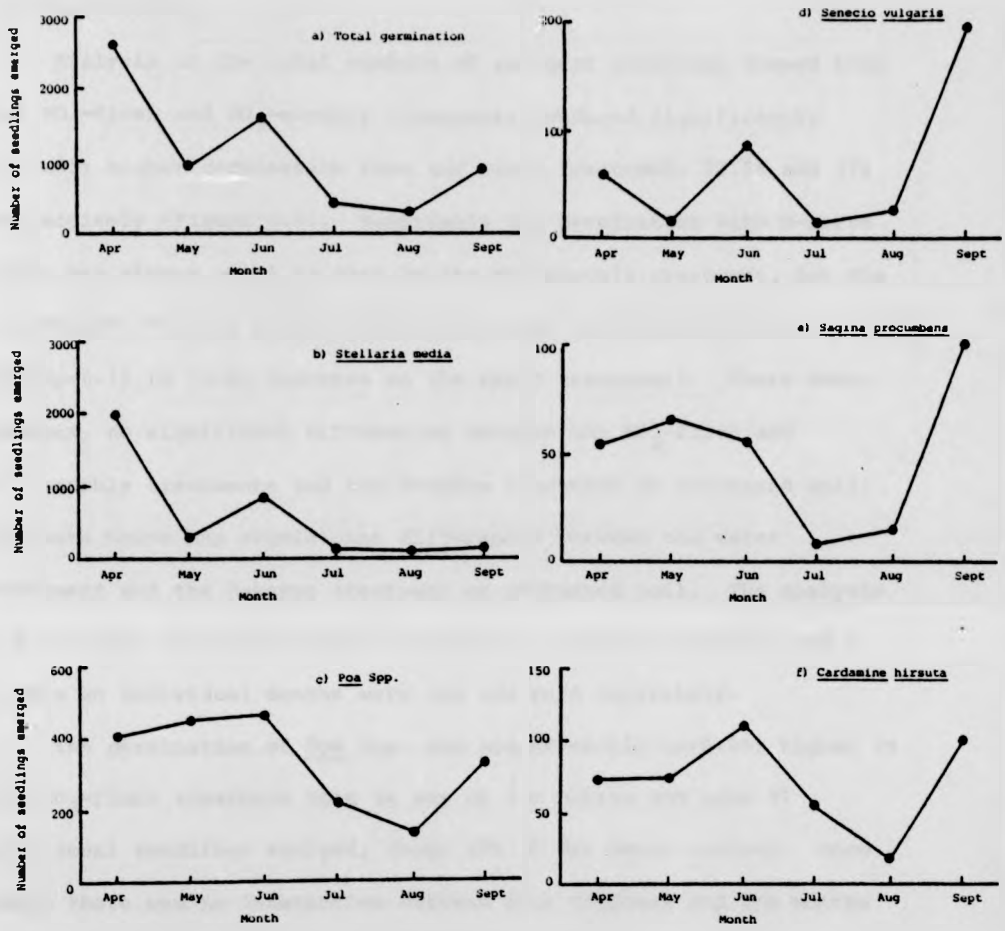


Figure 6.6 The total monthly emergence figures for the 1981 Field experiment, incorporating the germination from all treatments. This illustrates the overall emergence pattern for the year and the individual emergence patterns of the five most abundant species.

in the other months including October only dropped to about 75% of the peak values.

Analysis of the total numbers of emergent seedlings showed that the NO_3-flush and NO_3-monthly treatments produced significantly ($p<0.05$) higher germination than the water treatment; 20.5% and 17% respectively (Figure 6.7). Remarkably the germination with N-serve alone was almost equal to that in the NO_3-monthly treatment, but the difference from the water treatment was not quite significant ($0.5<p<0.1$),(a 14.6% increase on the water treatment). There were, however, no significant differences between the NO_3-flush and NO_3-monthly treatments and the N-serve treatment or untreated soil. Nor were there any significant differences between the water treatment and the N-serve treatment or untreated soil. The analysis did not show any significant interactions between treatments and months so individual months were not analysed separately.

The germination of Poa spp. was significantly ($p<0.05$) higher in the NO_3-flush treatment than in any of the others and some 97 additional seedlings emerged, about 25% of the water control. Once again there was no interaction between this response and the months over which the pipes were treated (Figure 6.8).

Analysis of the Stellaria media results was performed separately for each month and only April showed any significant differences between treatments; the lumped NO_3-flush and NO_3-monthly germination was greater than germination in the water treatment but not in the N-serve treatment (Figure 6.9).

Thus, the total germination was greater in the NO_3-flush and NO_3-monthly treatment than in the water treatment for the year as a whole, and in the case of Stellaria media was greater in just April for those treatments. It is possible that such a stimulation was due to the joint action of nitrate and N-serve, a combination peculiar to

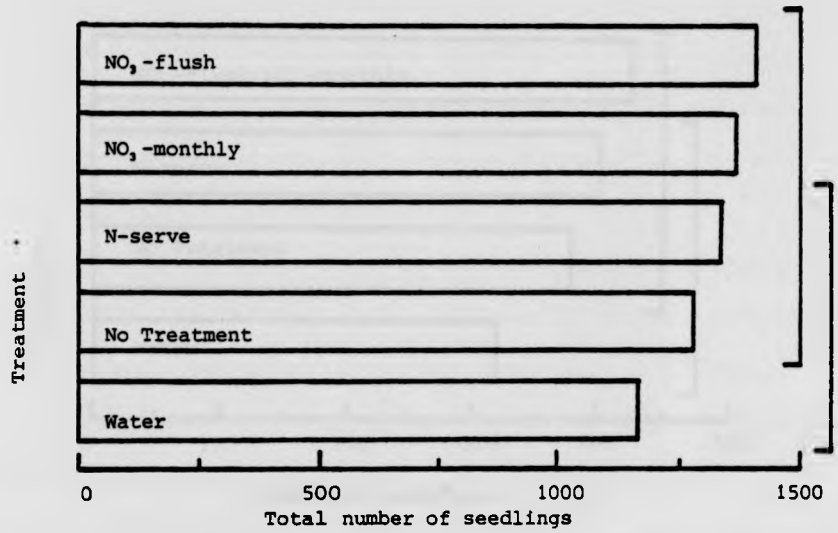


Figure 6.7 The total number of seedlings emergent from each of the five treatments during the 1981 Field experiment. Treatments which are not within the same bracket are significantly different at the p = 0.05 level.

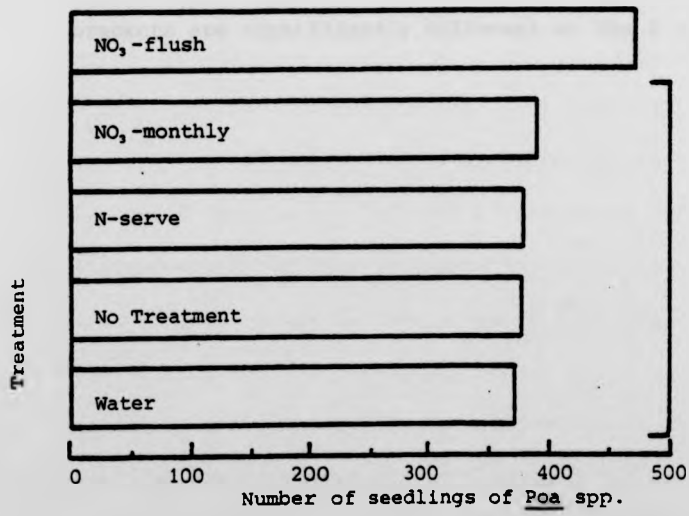


Figure 6.8 The total number of seedlings of Poa spp. emergent from each of the five treatments during the 1981 Field experiment. Treatments which are not within the same bracket are significantly different at the p = 0.05 level.

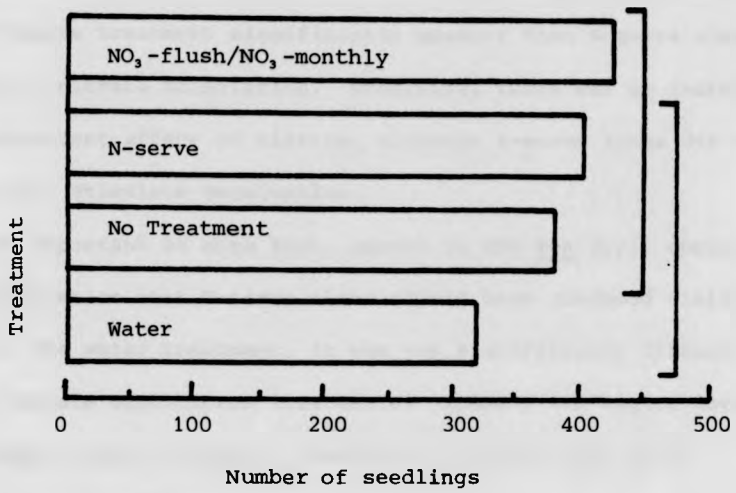


Figure 6.9 Number of seedlings of Stellaria media emergent during April of the 1981 Field experiment. A mean was taken of the NO_3-flush and NO_3-monthly figures since during the first month of the experiment their treatment was the same. Treatments which are not within the same brackets are significantly different at the P = 0.05 level.

the NO_3-flush and NO_3-monthly treatments. Only in Poa spp. was a nitrate/N-serve treatment significantly greater than N-serve alone, indicating a nitrate stimulation. Otherwise, there was no indication of an independent effect of nitrate, although N-serve alone did not significantly stimulate germination.

It is important to note that, except in the Poa spp., contrary to the expectation that N-serve alone should have produced similar results to the water treatment, it was not significantly different from the nitrate application treatments. Indeed, the higher levels of germination with N-serve in comparison to water were only marginally non-significant.

6.3.3 1982 Experiment : Garden soil, environmental factors.

The rainfall figures for 1982 are illustrated in Figure 6.10, showing that May was drier than in 1981 and August substantially wetter. The May application of nitrate was followed by rainfall but nitrate levels in the treated plots were still successfully elevated above levels in both the water treated and untreated plots (Figure 6.11). Nitrate levels immediately following application rose to 1×10^{-2}M in the treated plots, whereas levels in the water treatment only reached 4×10^{-3}M and those in the untreated soil had a maximum recorded level of 7×10^{-3}M.

Due to the drier conditions following application in mid-July, the nitrate levels in the untreated quadrats reached the same levels as those treated with nitrate (2×10^{-2}M). Although lower than for the nitrate treated quadrats, nitrate levels in the untreated quadrats rose to 9×10^{-3}M within two days of treatment and were identical to those in the untreated quadrats after only five days. Unfortunately in the water treated quadrats it appears that any

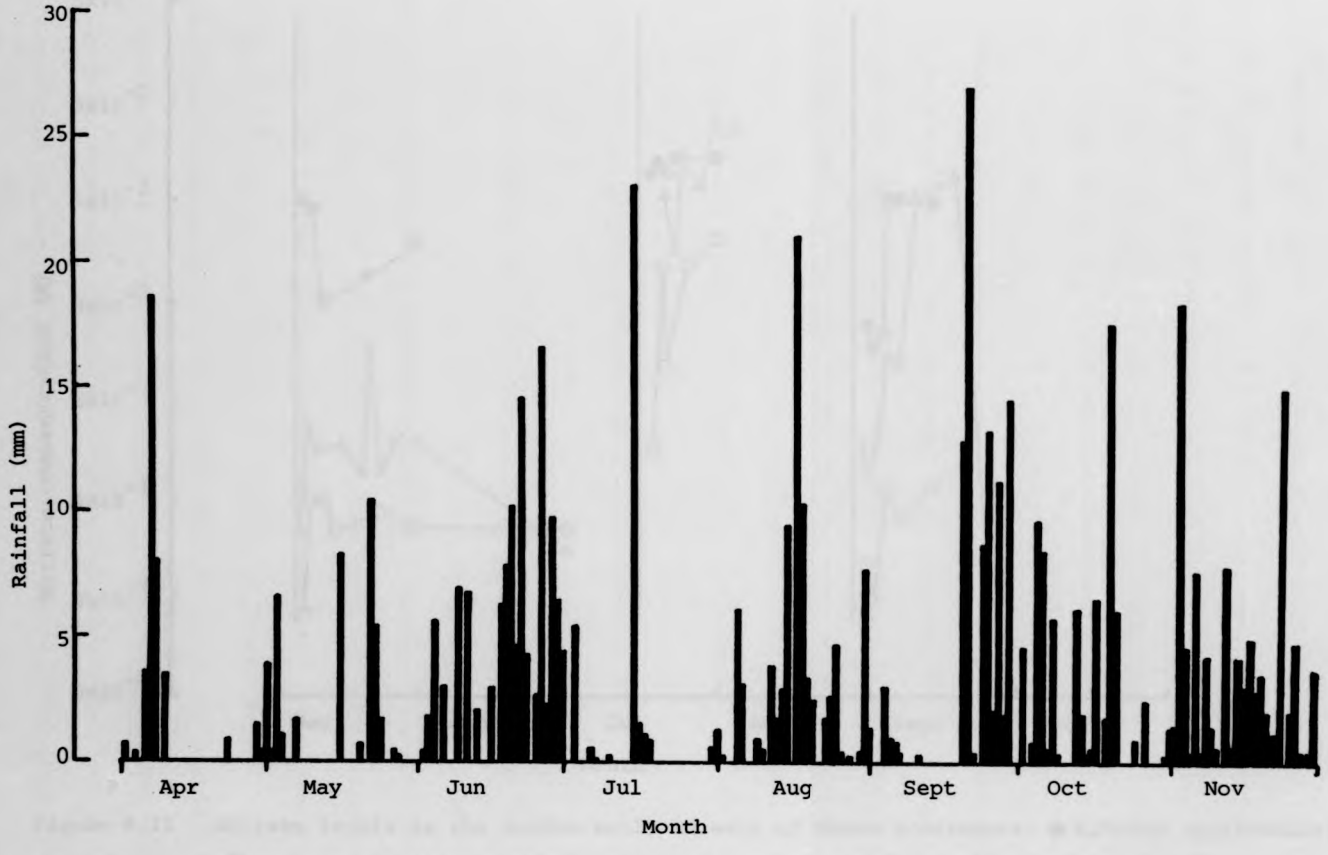


Figure 6.10 Natural rainfall distribution between April and November 1982 from records provided by the University of Keele Meteorological Station.

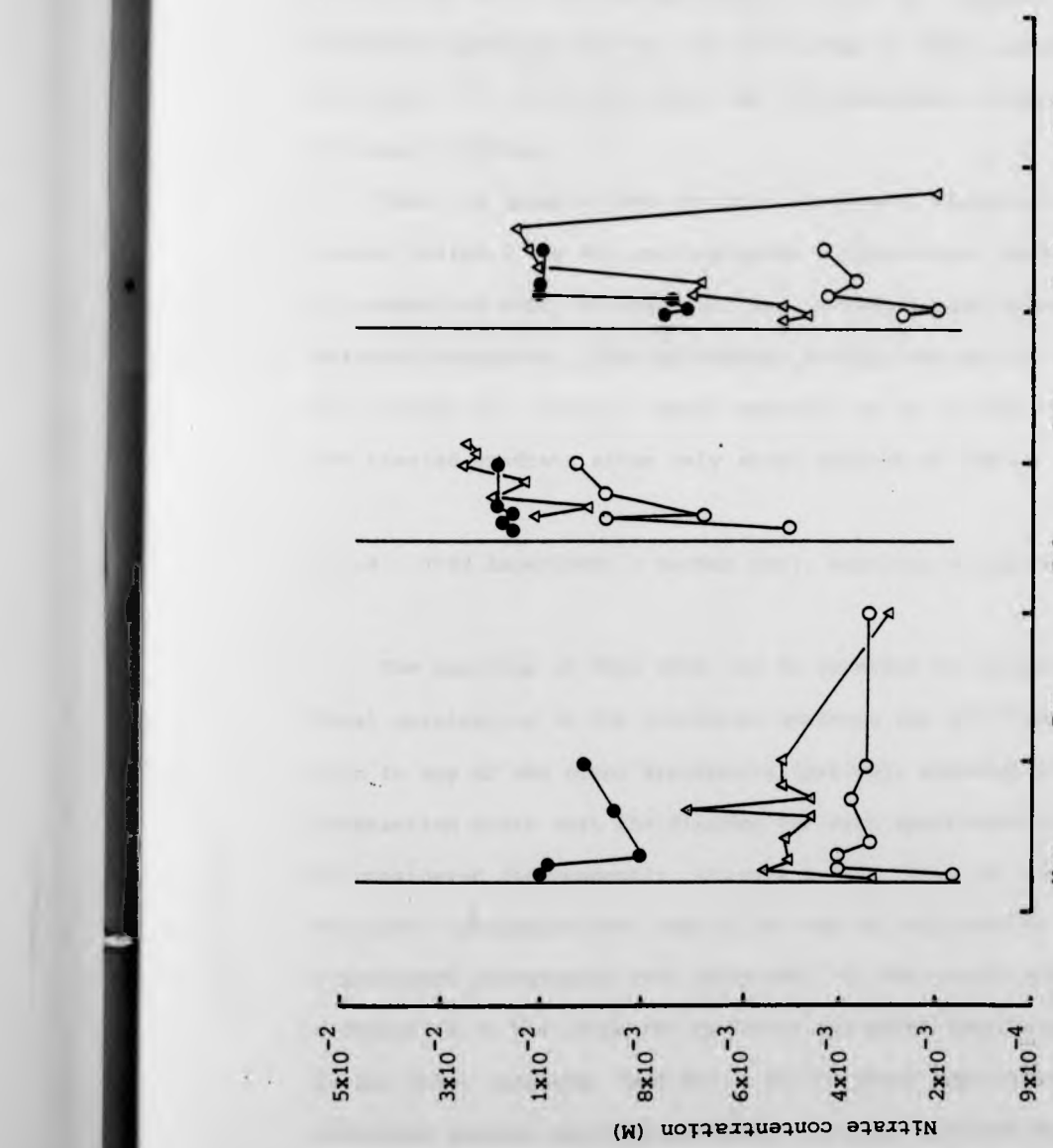


Figure 6.11 Nitrate levels in the garden soil, in each of three treatments; ● nitrate application; O water application and Δ untreated control, during the 1982 Field experiment. The three vertical lines indicate the times of treatment application.

differences in nitrate levels from those of the other treatments were probably short lived.

The nitrate levels following the application of nitrate in late August were higher in the treated quadrats (1×10^{-2}M) (Figure 6.11), than in the untreated plots (7×10^{-3}M), which were in turn greater than in the water treated quadrats (4×10^{-3}M). Levels in the untreated quadrats rose to 1×10^{-2}M after 12 days, probably due to the onset of a short dry spell and the subsequent concentration of the soil solution.

Thus, it appears that the most successful elevation in nitrate levels followed the May and September applications, when both the untreated and water treated soil had nitrate levels below that of the nitrate treatments. The application in July was not as successful and natural soil nitrate levels appeared to be as high as those of the treated quadrats after only short periods of time.

6.3.4 1982 Experiment : Garden soil, seedling emergence.

The analyses of this data can be referred to in Appendix J. Total germination in the untreated quadrats was significantly greater than in any of the other treatments ($p<0.05$), although a significant interaction meant that the figures for each application time had to be considered independently (Figure 6.12). When the NO_3-flush and NO_3-every treatments were lumped for the May application no significant differences were observed. In the second application germination in the untreated quadrats was significantly greater than in any other treatment ($p<0.05$). In the final application the untreated control was significantly different ($p<0.05$) from the water and NO_3-every treatment but the same as the NO_3-flush treatment. The NO_3-flush treatment was not itself significantly different from

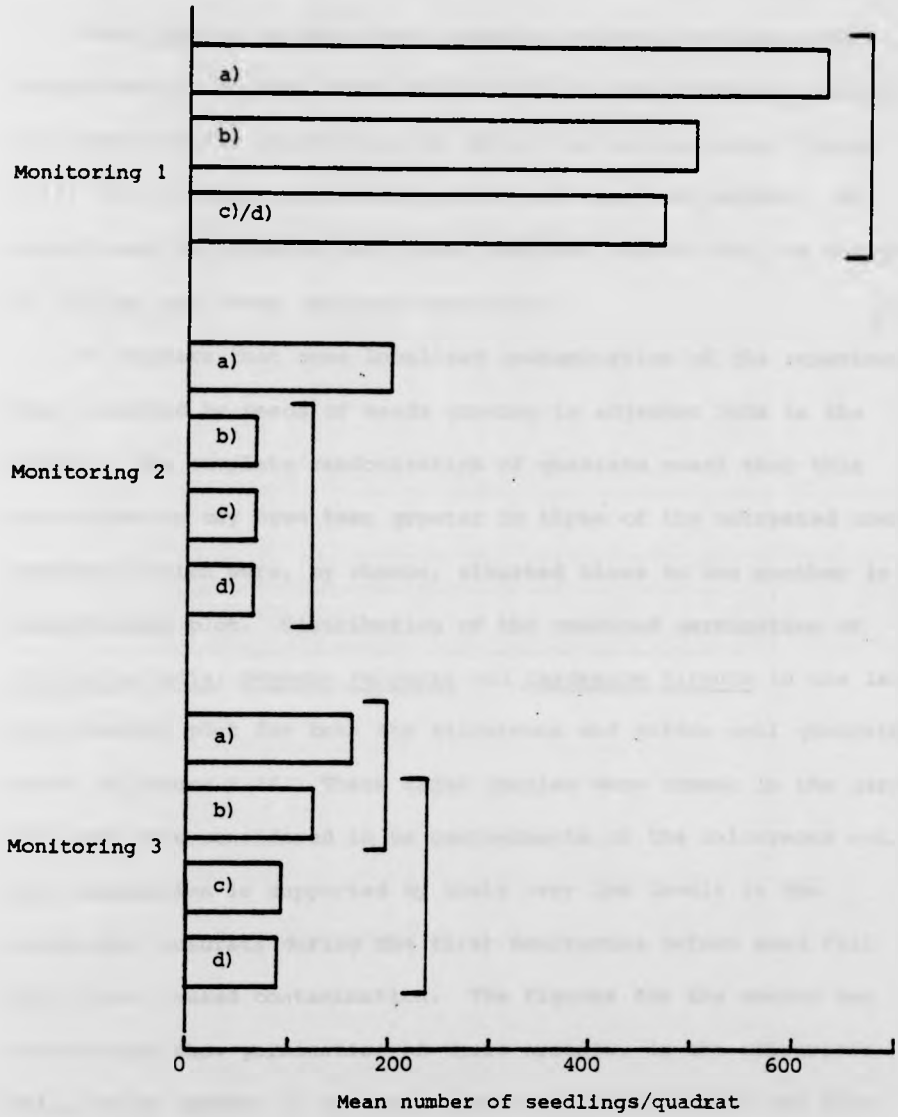


Figure 6.12 The mean number of seedlings per quadrat at each of three monitoring times during the 1982 Field experiment.

Treatments:
- a) Untreated control,
- b) Water application,
- c) Nitrate application at that treatment time alone,
- d) Nitrate application at all three times,
- c)/d) The combined figures for c) and d) after the first monitoring.

Treatments not within the same bracket are significantly different at the p = 0.05 level.

either the water or NO_3-every treatments.

When *Senecio vulgaris* and *Cardamine hirsuta* were analysed independently, in both cases germination in the untreated quadrats was significantly higher than in any of the treated ones (Figure 6.13) and no interaction with application time was evident. No significant differences were found when the figures for the emergence of the *Poa* spp. were analysed separately.

It appears that some localised contamination of the experimental plot occurred by seeds of weeds growing in adjacent beds in the garden. The complete randomisation of quadrats meant that this contamination may have been greater in three of the untreated control quadrats, which were, by chance, situated close to one another in the experimental plot. Distribution of the combined germination of *Stellaria media*, *Senecio vulgaris* and *Cardamine hirsuta* in the larger experimental plot for both the calcareous and garden soil quadrats is shown in Figure 6.14. These three species were common in the garden soil and were considered to be contaminants of the calcareous soil. This assumption is supported by their very low levels in the calcareous quadrats during the first monitoring before seed fall could have caused contamination. The figures for the second two monitorings show germination of those species, in the calcareous soil, to be greater in quadrats nearer to the middle of the plot. Similarly, numbers were higher in garden soil quadrats in the same parts of the plot. Unfortunately those quadrats correspond to three of the no treatment controls in the second monitoring and to three of the no treatment controls and to two of the NO_3-flush treatments in the third monitoring.

Hence, it is possible that contamination of the quadrats by seeds from an external source could be responsible for the significantly higher levels of germination in the untreated controls.

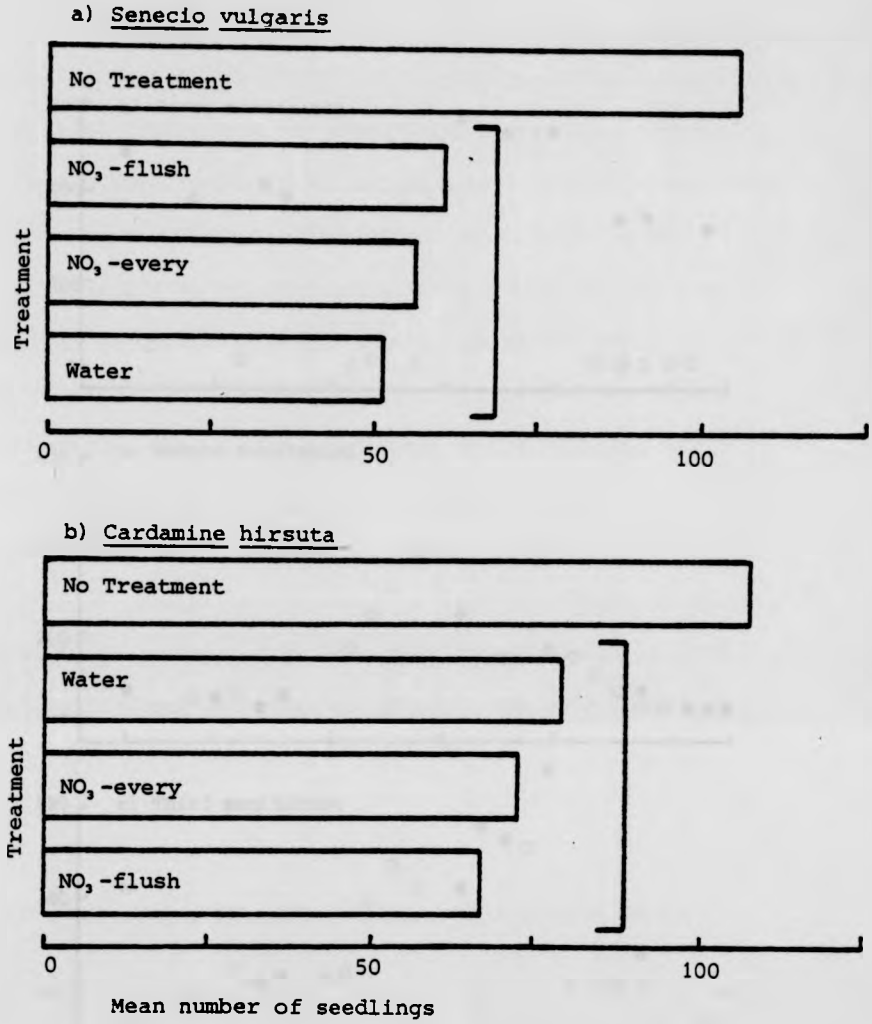


Figure 6.13 The mean number of seedlings per treatment for a) Senecio vulgaris and b) Cardamine hirsuta emergence from the garden soil during the 1982 Field experiment. Treatments not within the same bracket are significantly different at the p = 0.05 level.

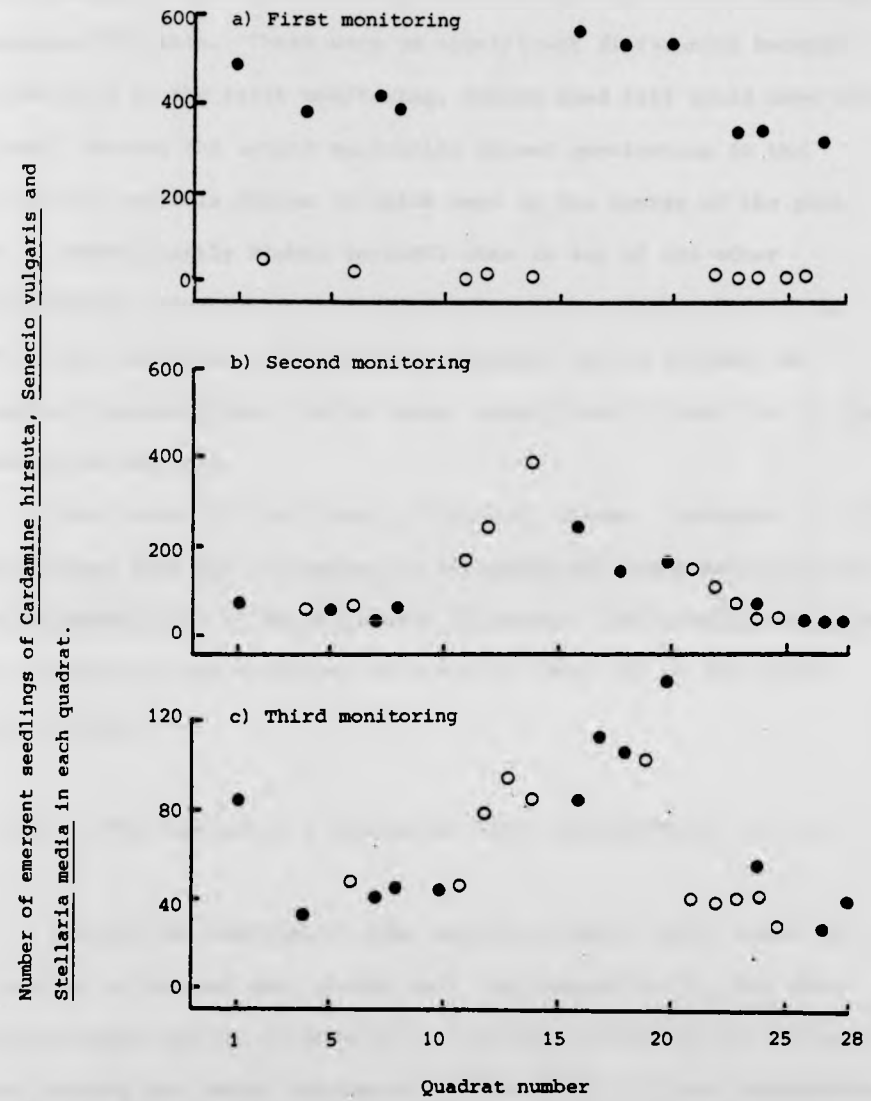


Figure 6.14 The combined numbers of Cardamine hirsuta, Senecio vulgaris and Stellaria media seedlings emergent from recorded quadrats in the large experimental plot during the 1982 Field experiment. Emergence figures for both garden soil ● and calcareous soil ○ are given for each of the three monitorings. The quadrat number indicates its position along the plot and thus illustrates the distribution of germination within the plot.

The analysis for the individual monitorings also provides supporting evidence for this. There were no significant differences between treatments in the first monitoring, before seed fall could have taken place, whereas the second monitoring showed germination in the untreated controls (three of which were in the centre of the plot), to be significantly higher ($p<0.05$) than in any of the other treatments. Finally, in the third monitoring, germination in the NO_3-flush treatment, which had two quadrats in the vicinity of maximum contamination, was no longer significantly lower than in the untreated controls.

There were no significant differences between treatments in this experiment that did not appear to be caused by contamination of the experimental plot by seedfall from elsewhere. The nitrate treatments themselves did not stimulate germination after any of the three applications.

6.3.5 1982 Experiment : Calcareous Soil, environmental factors.

Despite the expectation that natural nitrate levels would be lower in calcareous than garden soil (see Section 6.1), they were surprisingly similar (Figure 6.15). Nitrate levels in the untreated soil during May ranged between 4 and 8 x 10^{-3}M, the same magnitude as those observed in the garden soil over that period. The NO_3-flush treatment increased the nitrate concentration to 1 x 10^{-2}M and the water treatment reduced levels to 2 x 10^{-3}M, immediately following treatment, these levels being the same as those observed in the garden soil.

Following the July application, nitrate levels in the untreated soil and the NO_3-flushed soil were about the same as those in the garden soil with the same treatments. However, the water treatment

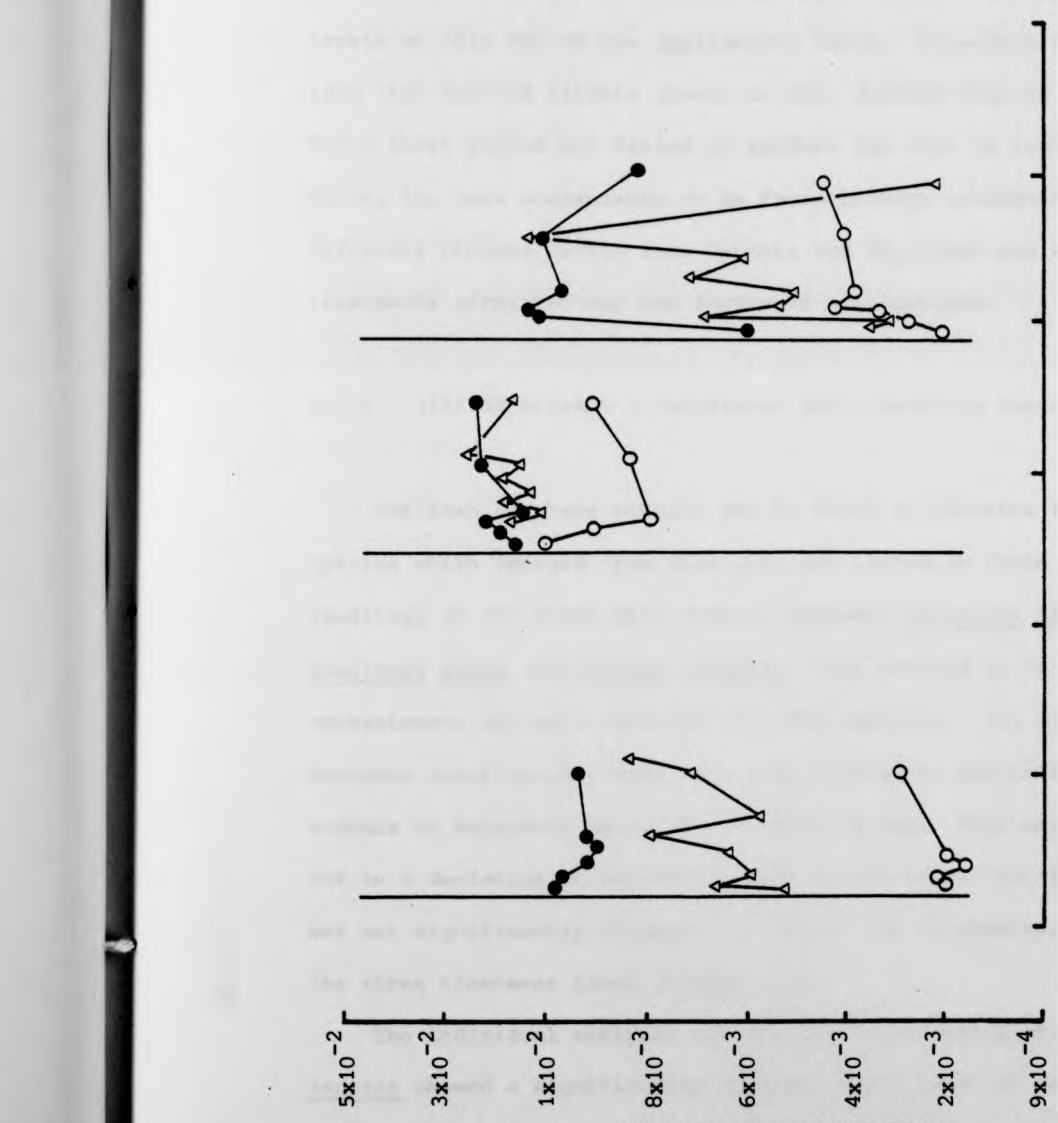


Figure 6.15 Nitrate levels in calcareous soil, in each of three treatments; ● nitrate application; ○ water application and △ untreated controls, during the 1982 Field experiment. The three vertical lines indicate the times of treatment application.

was less effective and nitrate levels remained just above 10^{-2}M for several days. Concentrations after the last application were similar to those in the garden soil, except that levels in the untreated soil took slightly longer to rise to above 10^{-2}M once the dry spell had begun.

Thus, the water treatments were successful in reducing nitrate levels at only two of the application times. NO_3-flush treatments less than doubled nitrate levels in May, doubled them in September for a short period and failed to produce any rise in levels in July. Hence, the best comparisons to be found between treatments with different nitrate levels were between the NO_3-flush and water treatments after the May and September applications.

6.3.6 1982 Experiment : Calcareous soil, seedling emergence.

Analyses of these results can be found in Appendix K. The species which emerged from this soil are listed in Table 6.4. Any seedlings of the three main ruderal species, Cardamine hirsuta, Stellaria media and Senecio vulgaris, were assumed to be external contaminants and were excluded from the analysis. The number of emergent seedlings declined with each successive monitoring, the numbers in September being 20% of those in May. This may have been due to a depletion of available seeds in the soil. Total emergence was not significantly different in any of the treatments, at any of the three treatment times (Figure 6.16).

The individual analysis for the first monitoring of Holcus lanatus showed a significantly ($p<0.05$) lower level of germination in the untreated quadrats than with any of the other treatments. This could be attributed to the lower moisture levels in the untreated quadrats during the relatively dry May of 1982.

Table 6.4 Species list of emergence from the calcareous grassland soil.

Artemisia vulgaris	*	*Plantago lanceolata*	
Atriplex hastata		*Pimpinella saxifraga*	
Cardamine hirsuta	*	*Poa spp.*	
Carduus nutans		*Polygonum aviculare*	
Centaurea nigra		*Ranunculus repens*	
Chenopodium album	*	*Rumex obtusifolius*	
Cirsium vulgare		*Sagina procumbens*	*
Dactylis glomerata		*Sanguisorba minor*	
Festuca ovina		*Senecio jacobaea*	
Galium verum		*Senecio vulgaris*	*
Geranium dissectum		*Sonchus oleraceus*	
Geranium robertianum		*Stellaria media*	*
Holcus lanatus		*Taraxacum officinale*	
Lolium perenne		*Trifolium repens*	
Medicago lupulina		*Urtica dioica*	*
Lotus corniculatus			

*Species marked with an asterisk may have been contaminants from the surrounding garden.

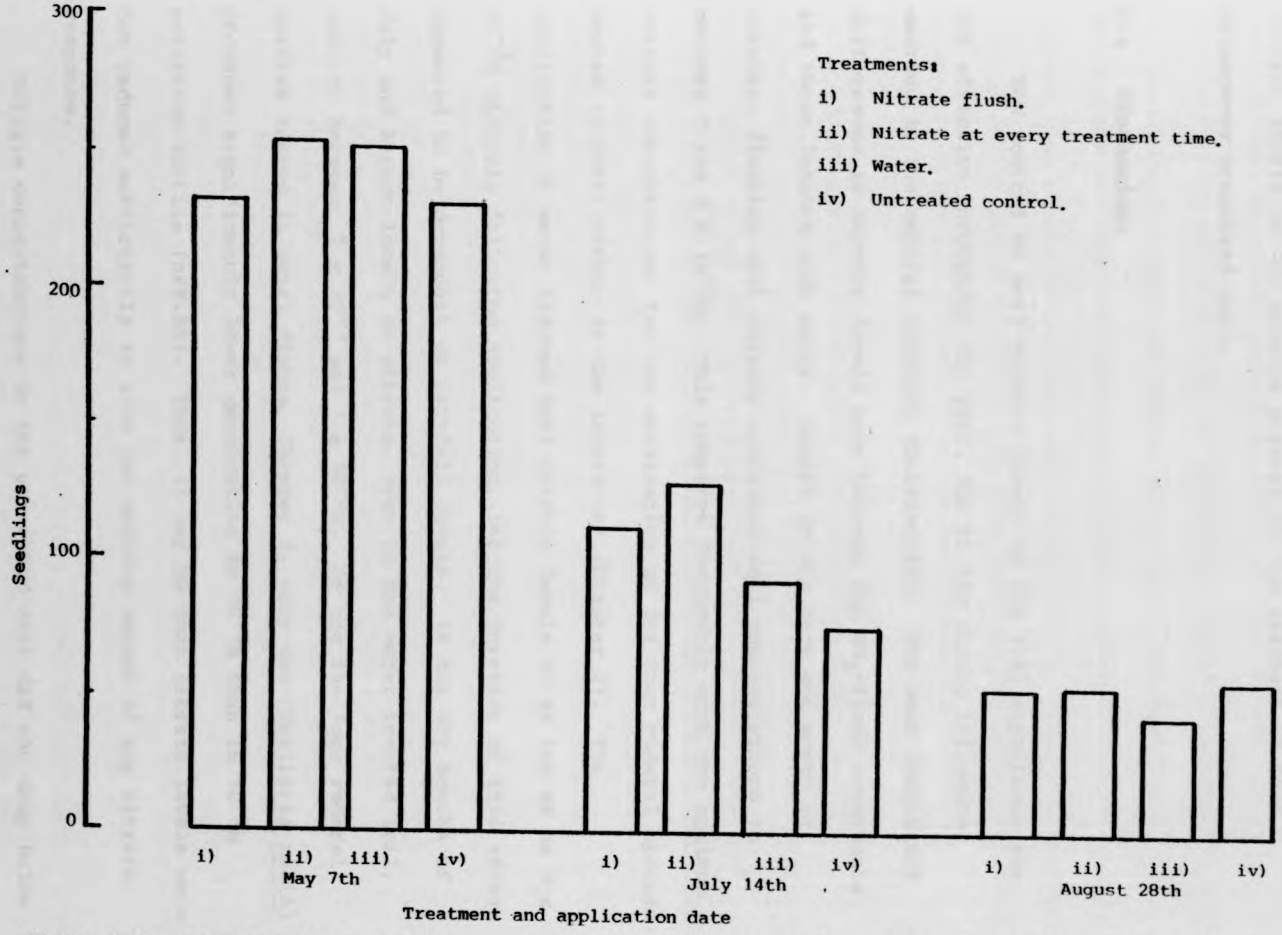


Figure 6.16 Total numbers of seedlings per treatment emerging from the calcareous soil after each of the three applications during 1982.

Thus, there did not appear to be any response to elevated nitrate levels by the species present in the seedbank of this calcareous grassland soil.

6.4 Discussion.

The control of soil nitrate levels in the 1981 experiment was not effective throughout the year, due to the strong influence exerted by the natural rainfall distribution. The most consistent differences in nitrate levels were between the NO_3-flush treatments and those leached with water. Except in the very wet month of October, flushing with nitrate increased soil concentrations to between 1 and 2 x 10^{-2}M. This compares favourably with the optimum nitrate concentration for the germination of the four ruderal species tested in petri dishes in the laboratory (Chapter 2). The application of water reduced soil nitrate levels to as low as as 3 x 10^{-3}M directly following application, but the duration of this effect appeared to be dependent on rainfall levels. In the dry months of July and August levels of nitrate, even in the water treated soil, rose to between 7 x 10^{-3} and 1 x 10^{-2}M. Of the the four ruderal species tested in petri dishes, Chapter 2, only one (Stellaria media) produced significantly lower germination in 10^{-3}M than in 10^{-2}M potassium nitrate ($p<0.05$). Thus, it may be that nitrate levels were not reduced sufficiently to show the maximum extent of any nitrate response.

Nitrate concentrations in the untreated soil did not drop below those of the water treatment, the lowest recorded levels being 3 x 10^{-3}M in May and late-September onwards. From late June through to the beginning of September nitrate concentrations in the untreated soil were as high as those in the nitrate treated soil. Such high

nitrate concentrations in the untreated soil may have a number of possible explanations. Friejsen *et al.* (1980) and Russell (1973) mention that loose and disturbed soil conditions favour mineralization and nitrification which could assist in elevating soil nitrate levels. Thus, the mixing of the soil, necessary to produce sufficient emergent seedlings and replenish those in the surface layers, may itself have been partly responsible for the high nitrate levels. This mixing could also be considered to be a characteristic of arable situations and the types of environment inhabited by ruderal species.

Kurkin (1977) has reported that vegetation cover is capable of impeding nitrification and keeping nitrate levels low. The lack of such vegetation and the open nature of the experimental site may therefore also have contributed to the high nitrate levels observed. Once again, open spaces and gaps are characteristic of arable and ruderal environments and are consequently an integral part of the experimental design.

The other major influence on nitrate concentrations in the untreated soil was rainfall. There were a number of dry spells during July, August and early September and these resulted in drying of the soil, and concentrations of the soil nitrate solution. In view of the relatively low numbers of seedlings emergent during July and August, however, although nitrate concentrations were high the low soil moisture level may have limited germination. The importance of this period of imperfect control over nitrate conditions is therefore reduced. A response to nitrate in the earlier months, when control was better, might still be expected providing the high nitrate levels (1×10^{-2}M) were sufficiently different from those of the water treatment (3×10^{-3}M).

Total germination over the whole experimental period was

significantly greater in the NO_3-flush and NO_3-monthly treatments than in the water treatment. However, the ANOVA did not show any interaction between the treatments and the times of application, as we might have expected from the above discussion on nitrate levels. Both nitrate treatments also received N-serve and the lack of a nitrate-only treatment makes it impossible to unequivocally attribute any responses to the nitrate alone. Overall, the N-serve treatment only just failed to be significantly different from the water treatment. Indeed it produced similar levels of germination to the NO_3-monthly treatment (NO_3-monthly 20.8% of total, N-serve 20.4% of total). This treatment provides the best comparison since the NO_3-flushes were applied to new plots each month, making fresh susceptible seeds available. Remarkably, therefore, it appears that any significant responses may be due to the N-serve applied, or an interaction between the N-serve and nitrate, rather than to the nitrate alone.

Although Schimpf and Palmblad (1980) used N-serve in association with ammonium in their experiments, they did not perform an N-serve control treatment. Consequently, their failure to observe any differences between a nitrate application and one of ammonium and N-serve may have been due to a similar response to both N-serve and nitrate. Unfortunately, in his review on nitrification inhibitors, Gasser (1970) does not mention any effects of N-serve on germination. In consequence, although the 1981 experiment produced treatment responses, the influence of nitrate could not be isolated, except in the case of the Poa spp. where germination was significantly greater ($p<0.05$) in the NO_3-flush treatment than in any of the others.

It became clear in Chapter 3, that responses to nitrate were dependent on both the diurnal temperature range and the presence or absence of light. It has been shown (Popay and Roberts 1970b,

Benjamin 1974, Murdoch 1982) that the average diurnal temperature range increases in the summer and declines in the winter. The ranges experienced on the surface in this experiment were greatest (20°C) in April and September and there was no evidence of a peak diurnal temperature range in midsummer. Murdoch (1982) also recorded a number of large diurnal temperature ranges in April and it was only prior to these that he observed smaller ranges on the surface. Thus, during our experimental period, April to September, only the monthly average temperatures, rather than the diurnal temperature ranges, produced a clear pattern, with a peak in August. A detailed comparison of the temperature regimes provided by the thermobar (Chapter 3), with those experienced in the field, is made in Chapter 7.

It was not possible to know whether emergent seedlings had germinated in response to light or not, but the process of mixing obviously increased their chances of exposure. This is clear from the immediate decline in numbers of emergent seedlings during May when the soil was not mixed. Subsequent mixing in June then produced an increase in germination. The process of mixing appeared to have its most serious effect on the numbers of Stellaria media seedlings. Initial mixing produced a massive flush of what were probably light sensitive seeds. A much smaller secondary peak of germination was observed in September, which was in much better agreement with the emergence pattern described by Roberts H.A. (1982). The germination of Senecio vulgaris was also in agreement with that described by Roberts H.A. (1982), showing two peaks of emergence; one in April-June and the other in September. The decline in germination of Poa spp. during July and August may be attributed to the dry conditions prevalent in that period. In general, the process of soil mixing did not appear to have seriously altered the expected seasonal

patterns of emergence, although initial mixing and placement of the soil stimulated a large amount of germination of mainly Stellaria media seeds.

Fawcett and Slife (1978) suggested that the natural fertility of the soil in their experiment may have provided enough nitrate to saturate any mechanism affecting seed germination. This could also be the case for the soil used in these experiments, since depletion of soil nitrate levels rather than addition of exogenous nitrate produced the most consistent differences in nitrate concentrations between treatments. Even when endogenous nitrate levels were below those of the treatments with added nitrate, it was not possible to isolate a response. Those responses which were observed between the NO_3 treatments and the water treatment could have been the result of the N-serve used and consequently this experiment failed to provide any clear cut evidence for a nitrate response in the field.

The 1982 experiment had a reduced number of replicates, but an increase in plot size meant that the total area occupied by each treatment was increased fourfold. This had the added advantage of reducing the importance of any possible edge effects. N-serve was not used, so that any resultant responses could be entirely attributed to the nitrate treatments. Due to practical constraints, treatments were only applied three times, which proved to be disadvantageous in comparison to the monthly 1981 intervals. Furthermore, contamination by weed seeds from an adjacent garden bed probably occurred, which made interpretation of the results more difficult. It transpired that the only significant differences between treatments, (untreated quadrats producing higher germination that treated ones), were probably due to this contamination.

The control of nitrate levels in the soil was similar to that in 1981. Once again the background concentrations of nitrate in the

untreated soil appeared to be sufficient to satisfy any nitrate requirements of the seeds and were actually comparable to the levels in the NO_3-treated soil. The water treatment was efficient in reducing nitrate concentrations in May and September, but in the dry period of July concentrations were almost as high as in the other treatments (9×10^{-3}M).

Although the numbers of Senecio vulgaris seedlings in this experiment were greater than in 1981, contrary to the correlative evidence for a field response provided by Popay and Roberts (1970b) they did not show any significant response to nitrate.

Thus, in 1982 a response to nitrate was not observed, although again this may be due to the high endogenous nitrate concentrations in the soil and the difficulties encountered in reducing them. A second factor which may have resulted in the failure to detect a nitrate response was the infrequency of treatment applications. Any short lived responses to nitrate could have been missed, although their effects, if any, would probably be overshadowed by that of the naturally high nitrate concentrations in the soil.

A calcareous soil was also used in the 1982 experiment to test the seedbank of a normally undisturbed environment. The nitrate concentrations measured in calcareous soils by previous workers have usually been lower than in agricultural soils and those experiencing regular disturbance. Davy and Taylor (1974) reported maximum levels in a Chiltern chalk soil, of 6 mg l^{-1} of NO_3-N, which at a moisture content of 20% would create a soil solution of about 5×10^{-4}M. In a Festuca ovina dominated grassland Havill *et al.* (1977) recorded levels of 20 mg NO_3-N l^{-1} of dry soil; about 1.6×10^{-3}M concentration, at 20% moisture levels. The work of Taylor *et al.* (1982) also showed concentrations of about 10^{-3}M in a Zerna erecta dominated, calcareous soil. However, in more loose and disturbed

calcareous dune soils, Friejsen *et al.* (1980) reported nitrate concentrations rising to about 10^{-2}M. Thus, although nitrate levels in undisturbed calcareous grasslands appear to be low in comparison to those in disturbed ruderal dominated sites, disturbance may make them comparable. This disturbance inevitably occurred in these experiments and the nitrate concentrations recorded in the calcareous soil were similar to those in the garden soil.

Once again the problem of reducing nitrate levels for experimental comparisons was encountered. The untreated quadrats had nitrate levels comparable to those in the NO_3-flush treated quadrats. This again was probably due to the regular disturbance, and removal of vegetation which would normally utilise the nitrate, and consequently reduce its concentration. The water treatment provided the best contrast in nitrate concentrations, but reduction in levels was only successful in two out of the three applications. Even after the other two applications, nitrate concentrations were still above 10^{-3}M.

There were no significant differences in emergence between treatments, which is in agreement with the lack of a response to nitrate by the calcicole species tested in Chapters 2 and 3. The importance of these results is somewhat reduced, due to the only small reductions in nitrate levels achieved. The reduction of endogenous nitrate concentrations in experimentally disturbed conditions was a major practical difficulty. Experimentation of this type inevitably changes the situation being observed, yet it was a requirement that nitrate levels should be manipulated, whilst altering other environmental variables as little as possible. Consequently, large scale interference with moisture levels and regular leaching of the soil would not have been suitable. Similarly, it was a requirement of the experiment that the soil was

disturbed by mixing and kept free of vegetation, to encourage observable levels of emergence.

Any future experiments may have to rely on the use of an artificial soil substitute, for instance sand, unless more efficient methods of control over nitrate concentrations in natural soil can be devised. It would, however, be necessary to balance this step away from natural conditions by ensuring that the seeds used were buried directly after seedfall and allowed to age naturally in the soil. This could be done with the use of sachets of seeds which would have the added advantage of reducing variation by providing more consistent numbers of seeds within each volume of soil/sand. It would also be important to remember that a natural seedbank consists of seeds of a variety of ages from different seedfalls and account would have to be made of this, either in the experiment, or in interpretation of results.

CHAPTER SEVEN

Discussion.

7. Discussion.

The experiments described in this thesis have been of three types: those performed in the laboratory, those performed in close to natural conditions in the field and a series of experiments in intermediate conditions which were designed to help with interpretation of the field experiment results. The aim of these experiments was to observe the effects of inorganic nitrates on seed germination and assess their importance in identifying spatial and temporal safe sites for germination and survival.

The laboratory experiments served three purposes: to find an optimal concentration of nitrate solution for germination, to compare the responses to nitrate of a number of ruderal species in a range of incubation environments and to compare the responses to nitrate of species from relatively undisturbed environments (calcareous grassland and acid heathland) with those of ruderal species (sensu Grime 1979).

The optimum concentration of potassium nitrate solution for the germination of four ruderal species was found to be between 10^{-2}M and 10^{-3}M, corresponding to the peak nitrate concentrations observed in some soils (Popay and Roberts 1970b, Vincent 1974, Roberts and Benjamin 1979). This concentration was therefore used on the thermogradient bar to compare the germination of responses of twenty-two ruderal species. The thermogradient bar provided eleven temperature regimes and allowed seeds to be tested with or without nitrate, in the presence or absence of light. Previous work has shown that these factors commonly influence germination and often interact with one another (Roberts E.H. 1973, Vincent and Roberts 1977, 1979, Bostock 1978, Roberts and Benjamin 1979).

Of the twenty-two ruderal species used all but two responded to

light and only three did not respond to nitrate in any of the environments. A number of interactions between factors were observed and there was a significant linear synergism between nitrate and increasing diurnal temperature range for five species in the light and six species in the dark. Other species also appeared to have a greater response to nitrate in the larger diurnal temperature ranges, but their regressions were not statistically significant. In some species, where germination reached 100% of viable seeds in the widest diurnal temperature ranges, a response to nitrate became apparent in intermediate ranges where water rather than nitrate solution produced sub-maximal germination. Although Senecio vulgaris appeared to have an optimal temperature range of 8/18°C rather than 5/25°C, its greatest response to nitrate still coincided with the most stimulatory temperatures. Hence, the comparison of a large number of ruderal species has shown that a response to nitrate is widespread amongst them and that it commonly acts synergistically with stimulatory alternating temperatures.

In view of these results, the complexity of changes in temperature and nitrate levels in the field could make the interpretation of field experiments difficult and complicate the evaluation of a possible role of nitrate in identifying temporal safe sites.

In addition to the ruderal species normally inhabiting disturbed sites with relatively high nitrate levels, the seeds of a number of calcicole and calcifuge species from less disturbed habitats were also tested in the laboratory. Measurements of nitrate levels in these other environments (Chapter 2) have shown them to be generally lower than in areas of large scale disturbance such as those of arable conditions. The majority of calcicole and calcifuge species tested did not respond to nitrate either in petri dish tests or on

the thermogradient bar. All the calcicole species responded to light and all, except chilled seeds of Linum catharticum, responded to larger diurnal temperature ranges. However, only Leontodon hispidus responded to nitrate in the light and indeed four of the species were actually inhibited by a 10^{-3}M nitrate solution in the dark.

The smaller seeded calcifuge species from an acid heathland environment showed a strong light requirement which was obligate for both Digitalis purpurea and Erica tetralix. All the calcifuges except for Digitalis purpurea responded to larger diurnal temperature ranges, whereas only Juncus conglomeratus and Vaccinium myrtillus showed any response to nitrate. This lack of response by the calcicole and calcifuge species, compared to the large number of ruderals which were stimulated by nitrate, suggests that such responses were the result of adaptation to environments with naturally high nitrate levels. Seeds of species of undisturbed environments would be less likely to encounter elevated nitrate levels and would therefore gain no advantage from the ability to respond to it.

The large scale disturbance in the field experiment produced nitrate levels in the calcareous soil as high as those observed in the garden soil. This is in agreement with the results of Friejsen *et al.* (1980) who observed nitrate levels of about 1×10^{-2}M in a calcareous dune soil which was also considerably disturbed. Disturbances in a calcareous grassland are much smaller than in those sites colonised by ruderal species and therefore nitrate levels would probably be lower due to the proximity of the vegetation. It might also be suggested that the smaller scale of disturbance could itself result in reduced rates of mineralization. Future experiments to measure the nitrate levels in gaps of different sizes within established vegetation would be particularly useful for comparison

with those in areas of large scale disturbance.

The buried seedbank in the calcareous soil taken into the laboratory failed to respond to nitrate and the seeds in an acid heathland seedbank were actually inhibited by it. This is consistent with the tests on collected seeds discussed above. There was not, however, any response to nitrate by the seeds from the garden soil seedbank used in these field experiments, a feature which will be discussed later.

The seeds of Cardamine hirsuta, which were stored for varying periods of time in a number of artificial environments, showed changing responses to nitrate in the laboratory. Other workers have also shown that as seeds age their responses to dormancy breaking factors change (Karssen 1980/81 a,b, Baskin and Baskin 1981, Roberts and Lockett 1975, 1978b, Bostock 1978) and this will further complicate their behaviour in the field. Laboratory storage of Cardamine hirsuta resulted in the development of a response to nitrate in the dark whereas, after storage in a Stevenson screen, the seeds were unresponsive to nitrate. Thus, changes in dormancy appear to differ according to the storage conditions and the results of laboratory storage experiments can only be used with caution in the detailed interpretation of field experiments.

In the experiments involving burial of seeds in the soil and subsequent germination testing in the laboratory (Chapter 4), three of the four ruderal species used, Cardamine hirsuta, Senecio vulgaris and Stellaria media, gradually lost their responses to nitrate following burial. In the fourth, Artemisia vulgaris, some seeds actually developed a nitrate response after 3 months burial, but this was lost during the next 6 months. If seeds of these species were buried in autumn, by April all but the Artemisia vulgaris seeds would already have a smaller response to nitrate than when they were fresh.

This would itself reduce the likelihood of being able to observe a response to nitrate in the field.

Both Vincent and Roberts (1977) and Roberts and Benjamin (1979) have shown that a period of chilling may alter the response of seeds to dormancy breaking factors. They suggest that in some cases this chilling can result in increased responses to alternating temperatures and interactions between light and nitrate. In these experiments the first three months of burial, over the winter, will have included a period of chilling, which would itself affect the seeds dormancy. Only in Artemisia vulgaris and Cardamine hirsuta, however, were there changes of the types observed by the above authors; Artemisia vulgaris developed a response to nitrate and Cardamine hirsuta a response to alternating temperatures after 3 months burial in the soil. In the other species it would still be expected that the cold winter conditions played some part in changing the dormancy characteristics of the seeds. As well as the changes in dormancy due to stratification over the winter, it is probable that burial during the summer will have other influences on seed dormancy.

One of the reasons why the response of some seeds to nitrate is lost during burial might be the satisfaction of a nitrate requirement whilst other environmental factors are unfavourable for germination. If the nitrate were to have a residual effect, then later, when environmental conditions such as temperature became suitable, the seeds would be able to germinate irrespective of the current nitrate levels. In experiments in the laboratory (Chapter 2) 33.9% of the seeds of Senecio vulgaris showed such a carry-over response to nitrate and a similar non-significant response was observed for Stellaria media. This could mean that a nitrate flush during periods of small diurnal temperature ranges could result in increased germination when the temperature range increased later and it may

partially explain the loss of dormancy of these two species during burial. This will make it difficult to identify nitrate response merely by correlating emergence with natural variations in environmental conditions. Experiments manipulating nitrate levels in the soil are therefore clearly necessary.

To further complicate the situation some species have been shown to have a cyclical response to dormancy breaking factors (Courtney 1968, Taylorson 1970, Stoller and Wax 1974, Roberts and Neilson 1982, Froud-Williams *et al.* 1984) and Murdoch (1982) has shown that even when nitrate is present the seeds may have to be in a susceptible state to respond. No assessment of the importance of these cyclical changes in dormancy on the manifestation of a response to nitrate in the field can be made from these experiments.

With the progressive death, continued dormancy or emergence of seeds, a species' seedbank will consist of a population of seeds of mixed age, many having been buried for 3 months or more (Barton 1962, Chancellor 1981). The results above show that, due to the variety of changes in dormancy during burial, it is likely that only a small proportion of the seedbank, normally the most recently buried seeds, will be responsive to nitrate at any one time. This will in turn reduce the likelihood of observing a nitrate response in the field. In the cases of Cardamine hirsuta, Senecio vulgaris and Stellaria media this would mean that periods when fresh seed burial was coincident with high nitrate levels and suitable temperatures would provide the greatest opportunities for observing any responses to nitrate in the field. For Artemisia vulgaris any response would probably be most apparent after a few months burial, which would be over the winter. The field experiments described in Chapter 6 did not include fresh seed falls during the year and it was in April that seeds had least soil storage but had experienced overwintering. If,

as was the case in 1981, stimulating nitrate levels and temperature ranges occurred in spring then it is at this time that any emergence response to nitrate is most likely to be observed.

The garden soil containing a natural seedbank, which was tested in the laboratory, (Chapter 5), would be expected to consist of a population of mixed age seeds. It could be for this reason that no responses to nitrate were observed. There was a reduction of germination in wetter conditions which indicates another factor, soil water levels, which affect germination in the field. Failure to show a response to nitrate in this experiment made it unlikely that a field response could be observed.

In two years, 1981 and 1982, field experiments were performed in an attempt to observe the effects of nitrate in the field. These involved the monitoring of environmental variables and the manipulation of soil nitrate levels whilst emergence was being recorded.

Figure 7.1 shows the relationship between diurnal temperature range, monthly mean temperature and depth in the soil for the six months of the 1981 experiment. The thermogradient bar temperature regime is also plotted for comparison. Temperatures in the winter months were not recorded, but average Keele data and similar recordings by Popay and Roberts (1970b), Benjamin (1974) and Murdoch (1982) all illustrate that means and ranges are considerably smaller than in the spring and summer. The results of the above workers show that there is a large amount of variation between years, as to the month in which diurnal temperature ranges at the soil surface are greatest. In some years this is as early as April and in others it is July or August. Examples of the former include measurements from Fallowfield and Jodrell in 1966 (Popay and Roberts 1970b) and Shinfield in 1971 (Benjamin 1974). In four other recordings the

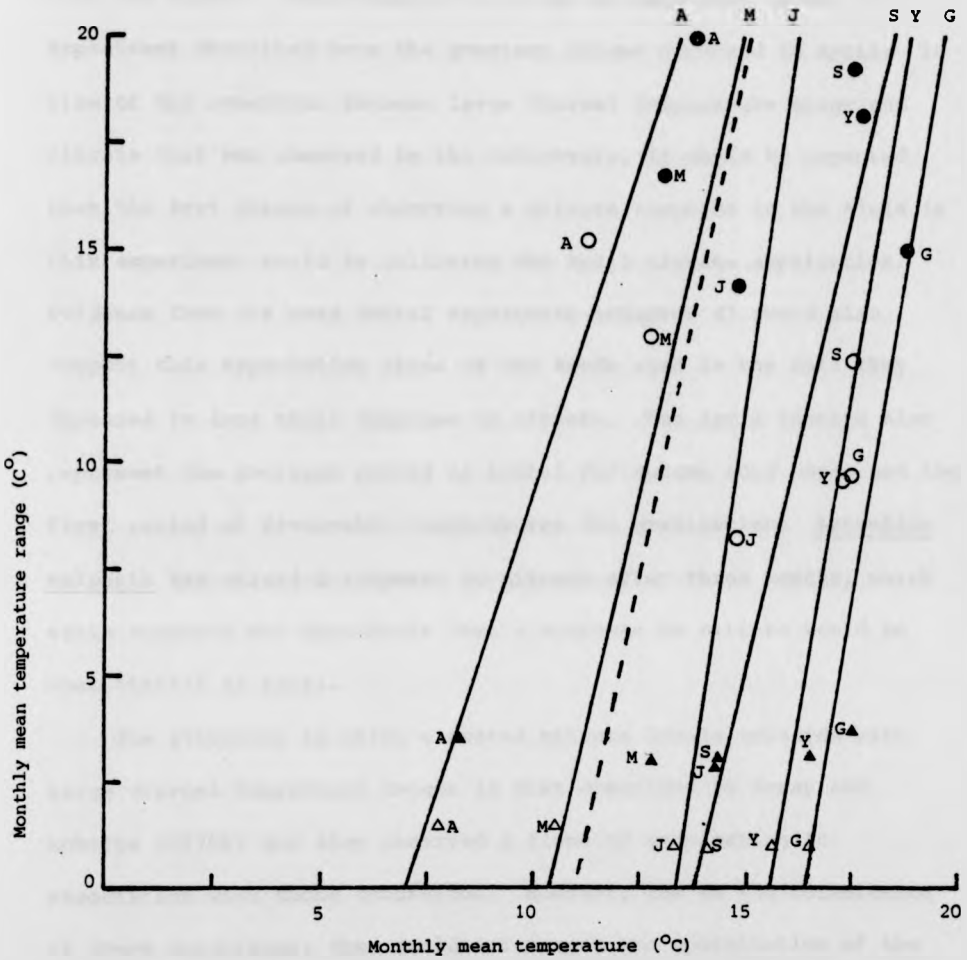


Figure 7.1 Monthly mean diurnal temperature range plotted against monthly mean temperature from readings taken at four depths in the soil each month between April and September 1981. Individual points represent the readings at each of the four depths: ● surface; O, 2.5 cm; ▲, 10 cm; △, 20 cm; the month being identified by a letter; April, A; May, M; June, J; July, Y; August, G and September, S. The dashed line represents the temperature regime created by the thermobar and the solid lines are linear regressions for each month based upon the individual points.

diurnal temperature ranges were greatest in later months; between July and August. From Figure 7.1 it can be seen that in the 1981 experiment described here the greatest ranges occurred in April. In view of the synergism between large diurnal temperature range and nitrate that was observed in the laboratory, it would be expected that the best chance of observing a nitrate response in the field in this experiment would be following the April nitrate application. Evidence from the seed burial experiment (Chapter 4) would also support this expectation since as the seeds aged in the soil they appeared to lose their response to nitrate. The April results also represent the shortest period of burial for autumn shed seeds and the first period of favourable temperatures for germination. Artemisia vulgaris has gained a response to nitrate after three months, which again supports the hypothesis that a response to nitrate would be most visible in April.

The situation in which elevated nitrate levels coincide with large diurnal temprature ranges is that described by Popay and Roberts (1970b) and they observed a flush of germination in association with those conditions. However, due to the coincidence of these conditions, they could not assess the contribution of the nitrate. A situation in which a nitrate flush occurs without wider diurnal temperature ranges, from the evidence of the thermogradient bar, would seem much less likely to result in increased levels of germination. Such conditions may not exist naturally at any particular time in the year, but levels of nitrate may be as high as 2×10^{-3}M and diurnal temperature ranges small as far down as 230 mm in the soil (Murdoch 1982).

Seedling emergence in 1981 was significantly greater in the treatments receiving nitrate every month and those receiving nitrate flushes in individual months, compared to the treatments receiving

only water. The interpretation of these results was, however, complicated by the use of a nitrification inhibitor, N-serve, which was applied in addition to the nitrate in both nitrate treatments to prevent natural nitrate production complicating experimental conditions. There was no treatment of nitrate without N-serve and consequently any responses could be attributed equally well to the nitrate, N-serve, or an interaction of the two. An N-serve only treatment only just failed to be significantly different from the water treatment and this suggests that N-serve was involved in some way in the increase of germination. Thus, germination in the nitrate/N-serve treatment was greater than in N-serve alone, which in turn exceeded that in water. Although this might be the expected order of response, the N-serve was not significantly different from the nitrate/N-serve and its difference to the water was only just outside significance. The involvement of nitrate cannot be entirely discounted, however, since it may equally well have been able to stimulate germination in the absence of N-serve. It is fortunate that an N-serve control treatment was performed, because the results would otherwise have been construed as evidence for a large nitrate response. Except in the case of the Poa species, where a nitrate response was demonstrated, much of the apparent nitrate treatment effect must be attributed to the effects of N-serve. For this reason, N-serve was not used in the 1982 experiment.

Although a response to nitrate was expected to be most likely in April, in 1981 the analysis of the total germination figures did not show an interaction between the treatments and the months and consequently the figures for each month were not analysed separately.

Both the water treatment and the untreated plots were intended to give a comparison of germination levels in low nitrate conditions with those in the nitrate treated plots. The reduction in nitrate

levels was most successful in the water flushed treatment and levels were as low as 3 x 10^{-3}M in May, compared to levels of over 1 x 10^{-2}M in the nitrate treatments. However, later in the year drier conditions led to levels of up to 1 x 10^{-2}M (July) in the water treated soil. The overall reductions in nitrate levels were not as great as was intended and consequently none of the treatments exhibited very low nitrate concentrations. This could be a result of the regular disturbance of the plots and will be discussed later.

In the laboratory experiments of Chapter 2 where the germination of four ruderal species was tested with a range of nitrate concentrations, only the Stellaria media had significantly greater germination in 10^{-2}M as opposed to the 10^{-3}M potassium nitrate solution. This means that the background nitrate levels in the field experiment were sufficiently great to satisfy the nitrate requirement of most of the seeds, although it is possible that they were actually lower in the wetter month of April, before monitoring had begun.

The numbers of Stellaria media seedlings were sufficiently great to allow an individual analysis of them, for the month of April 1981 alone. Stellaria media, which even with the high background nitrate levels might be expected to respond to the nitrate application, did show significantly greater germination in the nitrate treatments than in the water treatment. However, once again interpretation of the results is complicated by the use of N-serve.

In the 1982 experiment N-serve was not used, which simplifies the interpretation of results. In May the water treated plots had a concentration of 4 x 10^{-3}M nitrate which is comparable to the 1981 experiment although once again the concentration rose due to drying out of the soil later in the year. There were no significant differences in germination between the treatments which could not be attributed to seed contamination from outside the plots. Although

the figures for Stellaria media in May could not be analysed separately it was clear from the raw data that even this species did not show any response to the nitrate treatments. This lack of response in May 1982 with nitrate alone suggests that, in the 1981, experiment the N-serve, or an N-serve x nitrate interaction, had indeed been responsible for the stimulation of germination, rather than the nitrate alone. A second complicating factor which might also contribute to the lack of response in 1982 in comparison to 1981 might have been the prevailing weather conditions. However, it is difficult to assess their relative importance.

In both 1981 and 1982 the background nitrate levels consistently exceeded 1×10^{-3}M, which is in agreement with the work of Benjamin (1974) who rarely observed nitrate levels below 1×10^{-3}M over the two year period of his experiments. In contrast, in the experiment of Popay and Roberts (1970b) background nitrate levels in the soil were much lower and only exceeded 1×10^{-3}M at a few specific peaks during the year. Nitrate levels vary considerably between soils and sites and it has also been shown that they are dependent on the degree of disturbance and the amount of vegetation cover present (Russell 1973, Kurkin 1977, Friejsen *et al.* 1980). The relatively high background levels of nitrate in these experiments were probably due to the regular disturbance and the lack of vegetation cover which would also occur in the natural situation and be characteristic of some of the environments colonised by ruderals. These background nitrate levels would have been sufficient to satisfy any requirements, thus removing treatment differences. This possibility was also suggested by Fawcett and Slife (1978) in their experiments on weed seed germination in the field.

In Chapter 1, three types of safe site for germination were identified; temporal safe sites, spatial safe sites in relation to

depth of burial and those spatial safe sites associated with open conditions and gaps in vegetation. The importance of nitrate in helping to identify each of these types of safe site can now be considered in the light of the experimental results.

The experiments on the thermobar showed an interaction between nitrate and alternating temperatures which would clearly complicate the observation of nitrate involvement in identifying safe sites in the field. The month in which diurnal temperature ranges begin to increase may vary from year to year, although it has been shown that they are normally much smaller in the winter months. This would suggest that germination would be stimulated in spring as they begin to increase, although the widest diurnal temperature ranges may actually be experienced later in the year. This stimulation would of course be much greater if the increase in diurnal temperature ranges was accompanied by higher nitrate levels.

A spring peak in nitrogen mineralization has been reported by many workers, (e.g. Eagle 1961, Seifert 1962, Taylor *et al.* 1982), although much of this work was performed on natural rather than agricultural soils. Transiently high nitrate levels of about 5×10^{-3}M were also recorded in June and July by Popay and Roberts (1970b) and levels of up to 1×10^{-2}M at intervals throughout the year by Benjamin (1974) in agricultural soils. Although the presence of stimulating alternating temperatures and nitrate levels may occur at many times in the year, low moisture contents in the soil may at some times restrict the actual levels of germination (Roberts H.A. 1982).

The results of Chapter 4 showed that older seeds began to lose their response to nitrate and in some cases alternating temperatures. This makes it probable that freshly fallen seeds of some species could respond to the levels of nitrate and wide alternating

temperature ranges, which might follow the shedding of these seeds later in the year. Other seeds, which were buried over the winter, would be expected to respond to elevated nitrate levels and wider diurnal temperature ranges in spring in the years in which they coincide. These field experiments did not show any such flushes in germination which could be attributed to the added nitrate, but this is partly due to the complication of the N-serve nitrification inhibitor. A response may also have been observed if the experiments had been started slightly earlier in the year when alternating temperature ranges were still small. The high background nitrate levels throughout the year and the lack of fresh seed also reduced the chance of observing any germination flushes during the rest of the experimental period. Future experiments could indeed benefit from the incorporation of fresh seed in additional treatments which would more closely parallel the natural situation.

Thus, due to the variation in seasonal patterns of temperature and nitrate in the field, it is difficult to predict the presence of a temporal response to nitrate. In sites with high natural nitrate levels of above 1×10^{-3}M, such as those used in these experiments and also those monitored by Benjamin (1974), such responses would be restricted to the early spring. These high nitrate levels do, however, appear to be characteristic of disturbed conditions and are thus an integral part of many ruderal environments. In environments with lower background nitrate levels a response to elevated nitrate levels at particular times of the year is a possibility. The contribution of the nitrate to a flush of germination is often difficult to assess since the nitrate and alternating temperatures have been shown to interact. This interaction may cause flushes of germination when high nitrate levels coincide with large diurnal temperature ranges but it will always make it difficult to evaluate

the individual contribution of the nitrate.

The involvement of nitrate in helping to indicate the depth of burial of seeds was discussed in Chapter 3. It appeared to be unlikely that nitrate would have an influence since its vertical distribution in the soil profile appeared to be unpredictable and was affected by the drying out of the surface layers. The pattern of greater nitrate response at higher diurnal temperature ranges would, however, reduce the risks to seeds of stimulation to germination by nitrate at depths unfavourable to emergence. The remaining type of safe site in which nitrate may be involved is provided by open areas and gaps in vegetation. It has been suggested that disturbed conditions will increase the rate of mineralization and nitrification (Russell 1973, Friejsen *et al.* 1980) and that conversely established vegetation cover will result in lower nitrate levels (Rice and Pancholy 1972, Kurkin 1977). The nitrate levels in the disturbed conditions of the field experiments were indeed relatively high and often exceeded 1×10^{-3}M, even in the untreated plots. Another factor which contributed to these high nitrate concentrations was drying of the soil surface, but this was itself a result of the disturbance and lack of vegetation cover. Thus, high nitrate levels appear to be characteristic of many of the disturbed sites colonised by ruderals and could be a key factor in encouraging their germination. This is supported by the laboratory experiments which suggested that a response to nitrate was an adaptive feature of ruderal species, since most of the calcicole and calcifuge species that were also tested did not respond to nitrate. Hence, nitrate seems to be involved in indicating gaps in vegetation and areas of disturbance suitable for the germination of ruderal species and a response to it would clearly be an advantage to species with potential emergence throughout the year. Such a response was

demonstrated in a small proportion of seeds of Poa species, but because of the complexity of the factors influencing germination in the field it has proved difficult to demonstrate a clear response to nitrate by natural seedbanks of other ruderal species *in situ*.

APPENDICES

Appendix A: Date and site of collection of seeds for each experiment.

Experiments: C = Concentration (Chapter 2).

P = Pre-treatment (Chapter 2).

T = Thermobar (Chapter 3).

S = Sachet (Chapter 4).

Sites: Gardens - University of Keele Botanical Gardens.

Cannock Chase, Ayr and Goyts moss - Heathland sites.

Monsal Dale - Calcareous grassland site.

Species	Source	Experiment	Collection time
Achillea millefolium	Roadside verge	T	Sept. 1980
Artemisia vulgaris	Gardens	C	Sept. 1981
		P	Aug. 1982
		T	Oct. 1980
		S	Sept. 1981
Calluna vulgaris	Cannock Chase	C	Aug. 1981
		T	Aug. 1981
Capsella bursa-pastoris	Arable fields	T	Sept. 1980
Cardamine hirsuta	Gardens	C	Nov. 1982
		P	Nov. 1982
		T	Sept. 1981
		S	Sept. 1981
Carex flacca	Monsal dale	T	Sept. 1982
Chamerion angustifolium	Edge of woodland	T	Oct. 1980
Chenopodium album	Arable fields	T	Sept. 1980
Cirsium arvense	Roadside verge	T	Sept. 1980
Digitalis purpurea	Ayr	C	Sept. 1982
		T	Sept. 1982
Erica tetralix	Cannock Chase	C	Sept. 1983
		T	Sept. 1983
Gnaphalium uliginosum	Arable fields	T	Sept. 1980
Hypericum hirsutum	Monsal dale	C	Sept. 1982
		T	Sept. 1982
Inula conyza	Monsal dale	C	Sept. 1981
		T	Sept. 1981
Juncus conglomeratus	Goyts moss	C	July 1981

Species	Source	Experiment	Collection time
Leontodon hispidus	Monsal dale	C	Sept. 1981
		T	Sept. 1981
Linum catharticum	Monsal dale	T	July 1981
Origanum vulgare	Monsal dale	C	Sept. 1981
		T	Sept. 1981
Papaver rhoeas	Arable fields	T	Sept. 1980
Plantago major	Edge of woodland	T	Oct. 1981
Polygonum convolvulus	Arable fields	T	Sept. 1981
Polygonum lapathifolium	Arable fields	T	Sept. 1981
Polygonum persicaria	Arable fields	T	Sept. 1980
Rumex obtusifolius	Roadside verge	T	Sept. 1980
Sagina procumbens	Gardens	T	Sept. 1981
Senecio vulgaris	Gardens	C	Sept. 1981
		P	Oct. 1982
		T	July 1981
		S	Sept. 1981
		S(repeat)	Oct. 1982
Silene alba	Roadside verge	T	Sept. 1980
Silene nutans	Roadside verge	T	Sept. 1980
Solanum nigrum	Arable fields	T	Sept. 1980
Spergula arvensis	Arable fields	T	Sept. 1980
Stellaria media	Gardens	C	Oct. 1981
		P	Nov. 1982
		T	Aug. 1981
		S	Oct. 1981
Urtica dioica	Edge of woodland	T	Oct. 1981
Vaccinium myrtillus	Cannock Chase	T	Sept. 1983

Appendix B: Analyses for concentration experiments Chapter 2.

1. Ruderal species - Curvilinear analysis and ANOVA.

   a) *Artemisia vulgaris*

   b) *Cardamine hirsuta*

   c) *Senecio vulgaris*

   d) *Stellaria media*

2. Calcicole species.

   a) Curvilinear analyses and ANOVA.

      i) *Hypericum hirsutum*

      ii) *Inula conyza*

      iii) *Origanum vulgare*

   b) ANOVA

      i) *Leontodon hispidus* - Light

      ii) *Leontodon hispidus* - Dark

   c) Curvilinear analysis for mean days to germination.

      i) *Hypericum hirsutum*

      ii) *Origanum vulgare*

3. Calcifuge species - Curvilinear analysis and ANOVA.

   a) *Calluna vulgaris*

   b) *Digitalis purpurea*

   c) *Erica tetralix*

   d) *Juncus conglomeratus*

In all curvilinear regressions x(M) = nitrate concentration (molarity)

y(Germ.) = arcsine of the square root of percentage germination.

1a) *Artemisia vulgaris*

	X(M)	Y(Germ.)		X(M)	Y(Germ.)
	1.000	26.560			
	1.000	21.970			
	1.000	27.970			
	2.000	39.230		2.000	78.460
	2.000	33.210		2.000	75.820
	2.000	42.700		2.000	78.460
Dark	3.000	27.970	Light		
	3.000	33.210		H_2O	71.560
	3.000	35.670		H_2O	75.820
	4.000	20.270		H_2O	81.870
	4.000	23.580			
	4.000	29.330			
	5.000	21.970			
	5.000	18.430			
	5.000	27.970			
	6.000	25.100			
	6.000	20.270			
	6.000	27.970			

Y= -3.99778 + 42.80498 X -13.84729 XX + 1.253920 XXX

LIN EFFECT X	1	153.9088019	153.9088019	
QUAD EFF X	1	51.9612099	51.9612099	
CUBIC EFF X	1	305.6579869	305.6579869	16.4981250
OVERALL EFF X	3	511.5279987		
UNACCOUNTABLE	14	259.3756458	18.5268318	
TOTAL	17	770.9036444		
CORRELATION COEFFICIENT =		0.814582		p < 0.01

ANOVA for all treatments

Error mean square 17.1179 Error D.F. 16

Probability of F = 94.941 with 7 and 16 D.F. = p < 0.001

95% Confidence intervals = ±5.07.

1b) *Cardamine hirsuta*

	X(M)	Y(Germ.)		X(M)	Y(Germ.)
	1.000	14.180			
	1.000	11.540			
	1.000	11.540			
	2.000	42.710		2.000	78.460
	2.000	50.770		2.000	90.000
	2.000	45.000		2.000	90.000
Dark	3.000	42.710	Light		
	3.000	40.400		6.000	90.000
	3.000	42.710		6.000	90.000
	4.000	41.550		6.000	90.000
	4.000	48.450			
	4.000	48.450			
	5.000	42.710			
	5.000	41.550			
	5.000	41.550			
	6.000	35.670			
	6.000	36.870			
	6.000	39.230			

Y= -95.53556 + 166.78630 X -70.15801 XX + 12.47738 XXX -0.80069444 XXXX

LIN EFFECT X	1	573.9381376	573.9381376	
QUAD EFF X	1	1317.5773349	1317.5773349	
CUBIC EFF X	1	312.3905896	312.3905896	
QUART EFF X	1	158.2629762	158.2629762	11.8103723
OVERALL EFF X	4	2362.1690384		
UNACCOUNTABLE	13	174.2043894	13.4003376	
TOTAL	17	2536.3734278		p < 0.01
CORRELATION COEFFICIENT =		0.965048		

ANOVA for all treatments

Error mean square 10.6719 Error D.F. 16

Probability of F = 186.368 with 7 and 16 D.F. = p < 0.001

95% Confidence intervals = ±4.00.

1c) Senecio vulgaris

	X(M)	Y(Germ.)		X(M)	Y(Germ.)
	1.000	71.570			
	1.000	78.460			
	1.000	73.570			
	2.000	90.000		1.000	8.100
	2.000	90.000		1.000	0.000
	2.000	78.460		1.000	0.000
Light	3.000	90.000	Dark		
	3.000	78.460		6.000	0.000
	3.000	71.570		6.000	0.000
	4.000	81.870		6.000	11.500
	4.000	81.870			
	4.000	60.670			
	5.000	56.790			
	5.000	58.050			
	5.000	64.900			
	6.000	71.570			
	6.000	71.570			
	6.000	69.730			

Y= 34.71889 + 56.60230 X - 18.82019 XX + 1.727994 XXX

LIN EFFECT X	1	444.2261186	444.2261186	
QUAD EFF X	1	51.2191750	51.2191750	
CUBIC EFF X	1	580.4711424	580.4711424	11.1944816
OVERALL EFF X	3	1075.9164360		
UNACCOUNTABLE	14	725.9466140	51.8533296	
TOTAL	17	1801.8630500		
CORRELATION COEFFICIENT =		0.772731		p < 0.05

ANOVA for all treatments

Error mean square 47.4531 Error D.F. 16

Probability of F = 72.080 with 7 and 16 D.F. = p < 0.001

95% Confidence intervals = ±8.44.

1d) Stellaria media

	X(M)	Y(Germ.)		X(M)	Y(Germ.)
	1.000	62.030			
	1.000	68.030			
	1.000	53.130			
	2.000	75.820		2.000	8.130
	2.000	90.000		2.000	8.130
	2.000	73.570		2.000	14.180
Light	3.000	63.440	Dark		
	3.000	53.130		H_2O	8.130
	3.000	63.440		H_2O	14.180
	4.000	31.950		H_2O	0.000
	4.000	47.290			
	4.000	58.050			
	5.000	55.550			
	5.000	39.230			
	5.000	45.000			
	6.000	46.150			
	6.000	27.970			
	6.000	31.950			

Y= -76.58167 + 232.68319 X -115.82368 XX + 22.26088 XXX -1.48368056 XXXX

LIN EFFECT X	1	2517.8434405	2517.8434405	
QUAD EFF X	1	162.0328766	162.0328766	
CUBIC EFF X	1	431.2120417	431.2120417	
QUART EFF X	1	543.4086013	543.4086013	7.0238502
OVERALL EFF X	4	3654.4969600		
UNACCOUNTABLE	13	1005.7606011	77.3662001	
TOTAL	17	4660.2575611		
CORRELATION COEFFICIENT =		0.885541		p < 0.001

ANOVA for all treatments.

Error mean square 70.6299 Error D.F. 16

Probability of F = 26.633 with 7 and 16 D.F. = p < 0.001

95% Confidence intervals = ±10.28.

2a)i) Hypericum hirsutum

	X(M)	Y(Germ.)		
	1.000	34.400		
	1.000	53.100		
	1.000	42.700		
	2.000	62.000		
	2.000	69.700	H_2O	68.000
	2.000	59.300	H_2O	68.000
Light	3.000	59.300	H_2O	73.600
	3.000	58.000		
	3.000	68.000		
	4.000	68.000		
	4.000	45.000		
	4.000	73.600		
	5.000	68.000		
	5.000	68.000		
	5.000	63.400		
	6.000	66.400		
	6.000	64.900		
	6.000	64.900		

Y= 48.60000 + 3.39523810 X

LIN EFFECT X	1	605.2011905	605.2011905	7.6703394
OVERALL EFF X	1	605.2011905		
UNACCOUNTABLE	16	1262.4238095	78.9014881	
TOTAL	17	1867.6250000		
CORRELATION COEFFICIENT =		0.569253		$p < 0.05$

ANOVA for all treatments.

Error mean square 64.0 Error D.F. 14

Probability of F = 3.44 with 6 and 4 D.F. = $p < 0.05$

95% Confidence intervals = ±9.84.

2a)ii) Inula conyza

	X(M)	Y(Germ.)		
	1.000	0.000		
	1.000	11.500		
	1.000	0.000		
	2.000	28.000		
	2.000	18.400	H_2O	26.6
	2.000	16.400	H_2O	25.1
Dark	3.000	29.300	H_2O	25.1
	3.000	23.600		
	3.000	18.400		
	4.000	33.200		
	4.000	14.200		
	4.000	23.600		
	5.000	8.100		
	5.000	0.000		
	5.000	0.000		
	6.000	33.200		
	6.000	29.300		
	6.000	35.700		

LIN EFFECT X	1	344.8324286	344.8324286	
QUAD EFF X	1	33.2958730	33.2958730	
CUBIC EFF X	1	1237.9069630	1237.9069630	
QUART EFF X	1	392.6019048	392.6019048	7.9953942
OVERALL EFF X	4	2008.6371694		
UNACCOUNTABLE	13	638.3456084	49.1035083	
TOTAL	17	2646.9827778		
CORRELATION COEFFICIENT =		0.871114		$p < 0.01$

ANOVA for all treatments.

Error mean square 39.13 Error D.F. 14

Probability of F = 11.13 with 6 and 14 D.F. = $p < 0.001$

Confidence intervals = ±7.70.

2a)iii) Origanum vulgare

	X(M)	Y(Germ.)		
	1.000	42.700		
	1.000	48.500		
	1.000	59.300		
	2.000	73.600	H_2O	81.900
	2.000	63.400	H_2O	68.000
	2.000	64.900	H_2O	68.000
Light	3.000	64.900		
	3.000	78.500		
	3.000	69.700		
	4.000	66.400		
	4.000	71.600		
	4.000	81.900		
	5.000	66.400		
	5.000	63.400		
	5.000	75.800		
	6.000	71.600		
	6.000	66.400		
	6.000	64.900		

Y= 36.30333 + 18.18655 X -2.21726 XX

LIN EFFECT X	1	373.0667143	373.0667143	
QUAD EFF X	1	550.6200397	550.6200397	12.2083432
OVERALL EFF X	2	923.6867540		
UNACCOUNTABLE	15	625.3093571	41.6872905	
TOTAL	17	1548.9961111		
CORRELATION COEFFICIENT =		0.772213		p < 0.01

ANOVA for all treatments.

Error mean square 44.22 Error D.F. 14

Probability of F= 4.61 with 6 and 14 D.F. = p < 0.01

95% Confidence intervals = ±8.82.

2b)i) Leontodon hispidus - Dark

Analysis of Variance Table

Source	SS	DF	MS
Grand Total	235.9999	21	
Grand Mean	11.2381	1	
Treatments	111.2424	6	18.5404
Error	518.8462	14	37.0604
Total	630.0886	20	

F = 0.500 on [6, 14] degrees of freedom.

Probability of F greater than or equal to 0.500
with [6, 14] degrees of freedom is 0.798

2b)ii) Leontodon hispidus - Light

Analysis of Variance Table

Source	SS	DF	MS
Grand Total	1555.3992	21	
Grand Mean	74.0666	1	
Treatments	413.4375	6	68.9063
Error	1472.3750	14	105.1696
Total	1885.8125	20	

F = 0.655 on [6, 14] degrees of freedom.

Probability of F greater than or equal to 0.6555
with [6, 14] degrees of freedom is 0.686.

2c)i) Hypericum hirsutum - Mean days to germination

	X(M)	Y(Germ.)
	1.000	25.310
	1.000	23.300
	1.000	18.830
	2.000	17.690
	2.000	17.070
Light	2.000	13.590
	3.000	18.140
	3.000	17.870
	3.000	15.330
	4.000	14.840
	4.000	17.020
	4.000	14.280
	5.000	18.700
	5.000	18.270
	5.000	18.150
	6.000	19.480
	6.000	20.100
	6.000	17.630

Y= 26.50467 =5.75407 X 0.77298 XX

LIN EFFECT X	1	6.1851505	6.1851505	
QUAD EFF X	1	66.9191254	66.9191254	14.6217828
OVERALL EFF X	2	73.1042759		
UNACCOUNTABLE	15	68.6501019	4.5766735	
TOTAL	17	141.7543778		
CORRELATION COEFFICIENT = 0.718130				p < 0.01

2c)ii) Origanum vulgare - Mean days to germination.

	X(M)	Y(Germ.)
	1.000	6.040
	1.000	5.930
	1.000	6.000
	2.000	4.520
	2.000	4.450
	2.000	4.830
Light	3.000	4.590
	3.000	4.830
	3.000	4.520
	4.000	4.690
	4.000	4.640
	4.000	4.730
	5.000	4.710
	5.000	4.700
	5.000	4.600
	6.000	4.490
	6.000	4.500
	6.000	4.290

Y= 10.39222 =6.67576 X + 2.69079 XX -0.44978 XXX 0.0265278 XXXX

LIN EFFECT X	1	2.4537619	2.4537619	
QUAD EFF X	1	1.0725143	1.0725143	
CUBIC EFF X	1	1.1947407	1.1947407	
QUART EFF X	1	0.1737190	0.1737190	10.8750067
OVERALL EFF X	4	4.8947360		
UNACCOUNTABLE	13	0.2076640	0.0159742	
TOTAL	17	5.1024000		
CORRELATION COEFFICIENT = 0.979439				p < 0.001

3a) Calluna vulgaris

	X(M)	Y(Germ.)		
	1.000	8.100		
	1.000	0.000		
	1.000	0.000		
	2.000	59.300		
	2.000	56.800		
	2.000	46.100		
	3.000	39.200	H_2O	51.900
Light	3.000	62.000	H_2O	41.500
	3.000	51.900	H_2O	41.500
	4.000	50.800		
	4.000	59.300		
	4.000	29.300		
	5.000	49.600		
	5.000	49.600		
	5.000	39.200		
	6.000	40.400		
	6.000	50.800		
	6.000	50.800		

Y = -87.48889 + 122.73510 X -33.15807 XX + 2.749691 XXX

LIN EFFECT X	1	1626.3017143	1626.3017143	
QUAD EFF X	1	2057.7143254	2057.7143254	
CUBIC EFF X	1	1469.8200185	1469.8200185	14.9754414
OVERALL EFF X	3	5153.8360582		
UNACCOUNTABLE	14	1374.0817196	98.1486943	
TOTAL	17	6527.9177778		
CORRELATION COEFFICIENT =		0.888542		p < 0.001

ANOVA for all treatments.

Error mean square 78.3993 Error D.F. 14

Probability of F = 11.771 with 6 and 14 D.F. = p < 0.001

95% Confidence intervals = ±10.45.

3b) Digitalis purpurea

	X(M)	Y(Germ.)		
	1.000	47.300		
	1.000	29.300		
	1.000	36.900		
	2.000	90.000		
	2.000	78.500		
	2.000	90.000		
	3.000	90.000	H_2O	81.9
Light	3.000	75.800	H_2O	81.9
	3.000	75.800	H_2O	78.5
	4.000	75.800		
	4.000	81.900		
	4.000	78.500		
	5.000	73.600		
	5.000	90.000		
	5.000	73.600		

Y= -74.66667 + 157.33333 X -48.65833 XX + 4.675000 XXX

LIN EFFECT X	1	1689.0003333	1689.0003333	
QUAD EFF X	1	1820.2916667	1820.2916667	
CUBIC EFF X	1	944.1630000	944.1630000	14.1184532
OVERALL EFF X	3	4453.4550000		
UNACCOUNTABLE	11	735.6183333	66.8743939	
TOTAL	14	5189.0733333		
CORRELATION COEFFICIENT =		0.926411		p < 0.001

ANOVA for all treatments.

Error mean square 49.3021 Error D.F. 12

Probability of F = 19.381 with 5 and 12 D.F. = p < 0.001

95% Confidence intervals = ±8.83.

3c) Erica tetralix

	X(M)	Y(Germ.)		
	1.000	0.000		
	1.000	0.000		
	1.000	8.100		
	2.000	59.300		
	2.000	51.900		
	2.000	34.400		
	3.000	62.000	H_2O	59.3
Light	3.000	71.600	H_2O	59.3
	3.000	78.500	H_2O	68.0
	4.000	50.800		
	4.000	62.000		
	4.000	68.000		
	5.000	50.800		
	5.000	68.000		
	5.000	71.600		
	6.000	60.700		
	6.000	47.300		
	6.000	53.100		

Y= -82.86667 + 108.91521 X -25.00516 XX + 1.779630 XXX

LIN EFFECT X	1	3588.5600476	3588.5600476	
QUAD EFF X	1	4472.2006349	4472.2006349	
CUBIC EFF X	1	615.6806667	615.6806667	7.0279279
OVERALL EFF X	3	8676.4413492		
UNACCOUNTABLE	14	1226.4680952	87.6048639	
TOTAL	17	9902.9094444		
CORRELATION COEFFICIENT = 0.936029				p < 0.001

ANOVA for all treatments.

Error mean square 74.8887 Error D.F. 14

Probability of F = 20.685 with 6 and 14 D.F. = p < 0.001

95% Confidence intervals = ±10.72.

3d) Juncus conglomeratus

	X(M)	Y(Germ.)		
	1.000	34.400		
	1.000	32.000		
	1.000	33.200		
	2.000	90.000	H_2O	71.6
Light	2.000	71.600	H_2O	60.7
	2.000	81.900	H_2O	60.7
	3.000	81.900		
	3.000	81.900		
	3.000	73.600		
	4.000	67.200		
	4.000	75.800		
	4.000	78.500		
	5.000	73.600		
	5.000	75.800		
	5.000	65.700		

Y= -79.26000 + 155.87063 X -47.26548 XX + 4.430556 XXX

LIN EFFECT X	1	1456.0333333	1456.0333333	
QUAD EFF X	1	2294.0038095	2294.0038095	
CUBIC EFF X	1	848.0083333	848.0083333	22.5009766
OVERALL EFF X	3	4598.045762		
UNACCOUNTABLE	11	414.5638571	37.6876234	
TOTAL	14	5012.6093333		
CORRELATION COEFFICIENT = 0.957756				p < 0.001

ANOVA for all treatments.

Error mean square 35.3438 Error D.F. 12

Probability of F = 26.584 with 5 and 12 D.F. = p < 0.001

95% Confidence intervals = ±7.48.

Appendix C: Analysis for the pre-incubation experiments Chapter 2.

1. *Cardamine hirsuta* ANOVA for germination during dark incubation.

2. *Artemisia vulgaris*

   a) T-test for germination during pre-incubation

   b) ANOVA and Duncan's MRT for total germination following pre-incubation and incubation in the dark.

   c) ANOVA for germination during dark incubation.

3. *Stellaria media* ANOVA and Duncan's MRT for total germination in the light.

4. *Senecio vulgaris*

   a) T-test for germination during pre-incubation

   b) ANOVA and Duncan's MRT for combined pre-incubation germination and incubation germination in the dark.

   c) ANOVA and Duncan's MRT for germination during dark incubation alone.

   d) ANOVA and Duncan's MRT for total germination; pre-incubation, dark incubation and light incubation combined.

   e) ANOVA and Duncan's MRT for germination during light incubation alone.

W = Water N = 10^{-2}M KNO_3 solution

Pre-inc = the solution in which seeds were placed prior to incubation

Inc = the solution in which seeds were incubated.

1. Cardamine hirsuta - incubation in the dark.

Table of Mean Values

		Pre-inc. W	Pre-inc. N	
Inc.	W	60.8600	67.7999	64.3299
	N	69.0799	68.6800	68.8799
		64.9699	68.2400	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	103.5000	1	103.5000	2.7832
Columns	53.4375	1	53.4375	1.4370
Interaction	67.3750	1	67.3750	1.8118
Subtotal	224.3125	3		
Within	595.0000	16	37.1875	
Total	819.3125	19		

Probability of F >= 2.7832 with 1 and 16 D.F. is 0.1147
Probability of F >= 1.4370 with 1 and 16 D.F. is 0.2481
Probability of F >= 1.8118 with 1 and 16 D.F. is 0.1971

2a. T-test Analysis for Artemisia vulgaris germination during pre-incubation.

F= 1.0724 D.F.(numerator) = 9
D.F.(denominator) = 9

Mean X = 4.7300 St. dev. X = 4.8744
Mean Y = 20.0800 St. dev. Y = 5.0479

T= 6.5624 D.F.= 18

P= 0.001

2b. Artemisia vulgaris Total germination.

Table of Mean Values

		Inc. W	Inc. N	
Pre-Inc.	W	59.1400	62.0999	60.6200
	N	65.6800	64.2599	64.9700
		62.4100	63.1799	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	94.6250	1	94.6250	4.2232
Columns	3.0000	1	3.0000	0.1339
Interaction	23.9375	1	23.9375	1.0783
Subtotal	121.5625	3		
Within	358.5000	16	22.4063	
Total	480.0625	19		

Probability of F >= 4.2232 with 1 and 16 D.F. is 0.0566
Probability of F >= 0.1339 with 1 and 16 D.F. is 0.7192
Probability of F >= 1.0683 with 1 and 16 D.F. is 0.3167

Duncan's MRT

p < 0.05

W/W	W/N	N/N	N/W

2c. *Artemisia vulgaris* germination during dark incubation.

Table of Mean Values

		Inc. W	Inc. N	
Pre-Inc.	W	58.1600	61.6200	59.8900
	N	56.6600	57.4200	57.0400
		57.4100	59.5200	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	40.6250	1	40.6250	2.2208
Columns	22.2500	1	22.2500	1.2163
Interaction	9.1250	1	9.1250	0.4988
Subtotal	72.0000	3		
Within	292.6875	16	18.2930	
Total	364.6875	19		

Probability of F >= 2.2208 with 1 and 16 D.F. is 0.1556
Probability of F >= 1.2163 with 1 and 16 D.F. is 0.2864
Probability of F >= 0.4988 with 1 and 16 D.F. is 0.4902

3. *Stellaria media* Total germination.

Table of Mean Values

		Inc. W	Inc. N	
Pre-Inc.	W	22.8200	51.0000	36.9100
	N	28.0600	60.7999	44.4300
		25.4400	55.9000	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	282.7344	1	282.7344	3.0547
Columns	4639.0469	1	4639.0469	50.1213
Interaction	26.0195	1	26.0195	0.2811
Subtotal	4947.8008	3		
Within	1480.9023	16	92.5564	
Total	6428.7031	19		

Probability of F >= 3.0547 with 1 and 16 D.F. is 0.0997
Probability of F >= 50.1213 with 1 and 16 D.F. is 0.0000
Probability of F >= 0.2811 with 1 and 16 D.F. is 0.6032

Duncan's MRT

P < 0.05

W/W
N/W

W/N
N/N

4a. Seneció vulgaris Pre-incubation germination.

F= 6.7788 D.F.(numerator) = 9
D.F.(denominator) = 9

Mean X = 8.3100 St. dev. X = 3.1599
Mean Y = 44.2399 St. dev. Y = 8.2272

T = 12.2306 D.F. = 18

P < 0.001

4b. Senecio vulgaris Pre-incubation and dark incubation germination.

Table of Mean Values

		Inc. W	Inc. N	
Pre-inc.	W	14.5600	29.5200	22.0400
	N	52.8400	63.9400	58.3900
		33.7000	46.7300	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	6606.6016	1	6606.6016	141.5217
Columns	848.8828	1	848.8828	18.1841
Interaction	18.6406	1	18.6406	0.3993
Subtotal	7474.1250	3		
Within	746.9219	16	46.6826	
Total	8221.0469	19		

Probability of F >= 141.5217 with 1 and 16 D.F. is 0.0000
Probability of F >= 18.1841 with 1 and 16 D.F. is 0.0000
Probability of F >= 0.3993 with 1 and 16 D.F. is 0.5364

Duncan's MRT

P < 0.05

All treatments significantly different.

4c. Senecio vulgaris germination during dark incubation alone.

Table of Mean Values

		Inc. W	Inc. N	
Pre-inc.	W	11.2600	27.4200	19.3400
	N	23.7400	32.2600	28.0000
		17.5000	29.8400	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	374.9727	1	374.9727	6.002
Columns	761.3867	1	761.3867	13.4017
Interaction	72.9727	1	72.9727	1.2844
Subtotal	1209.3320	3		
Within	909.0039	16	56.8127	
Total	2118.3359	19		

Probability of F >= 6.6002 with 1 and 16 D.F. is 0.0206
Probability of F >= 13.4017 with 1 and 16 D.F. is 0.0021
Probability of F >= 1.2844 with 1 and 16 D.F. is 0.2738

Duncan's MRT

P < 0.05

W/W
N/W
W/N
N/N

4d. *Senecio vulgaris* Total germination.

Table of Mean Values

		Inc. W	Inc. N	
Pre-inc.	W	29.0800	79.4400	54.2600
	N	54.8200	78.6400	66.7300
		41.9500	79.0400	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	777.5000	1	777.5000	10.8139
Columns	6878.3750	1	6878.3750	95.6679
Interaction	880.4375	1	880.4375	12.2456
Subtotal	8536.3125	3		
Within	1150.3750	16	71.8984	
Total	1150.3750	19		

Probability of F >= 10.8139 with 1 and 16 D.F. is 0.0046
Probability of F >= 95.6679 with 1 and 16 D.F. is 0.0000
Probability of F >= 12.2456 with 1 and 16 D.F. is 0.0030

Duncan's MRT

P < 0.05

W/W
N/W
N/N
W/N

4e. *Senecio vulgaris* germination during light incubation.

Table of Mean Values

		Inc. W	Inc. N	
Pre-inc.	W	23.1800	57.2599	40.2200
	N	8.8200	22.9000	15.8600
		16.0000	40.0800	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	2967.0625	1	2967.0625	32.1613
Columns	2899.2305	1	2899.2305	31.4261
Interaction	499.9844	1	499.9844	5.4196
Subtotal	6366.2773	3		
Within	1476.0898	16	92.2556	
Total	7842.3672	19		

Probability of F >= 32.1613 with 1 and 16 D.F. is 0.0000
Probability of F >= 31.4261 with 1 and 16 D.F. is 0.0000
Probability of F >= 5.4196 with 1 and 16 D.F. is 0.0334

Duncan's MRT

P < 0.05

N/W
N/N
W/W
W/N

Appendix D: Thermobar structure and control.

a) The arrangement of water reservoirs, coolers, pumps and heating coils used to effect temperature control on the thermobar.

b) The phases of operation of the thermobar;

A_1 Daytime - 25°C at the alternating end;

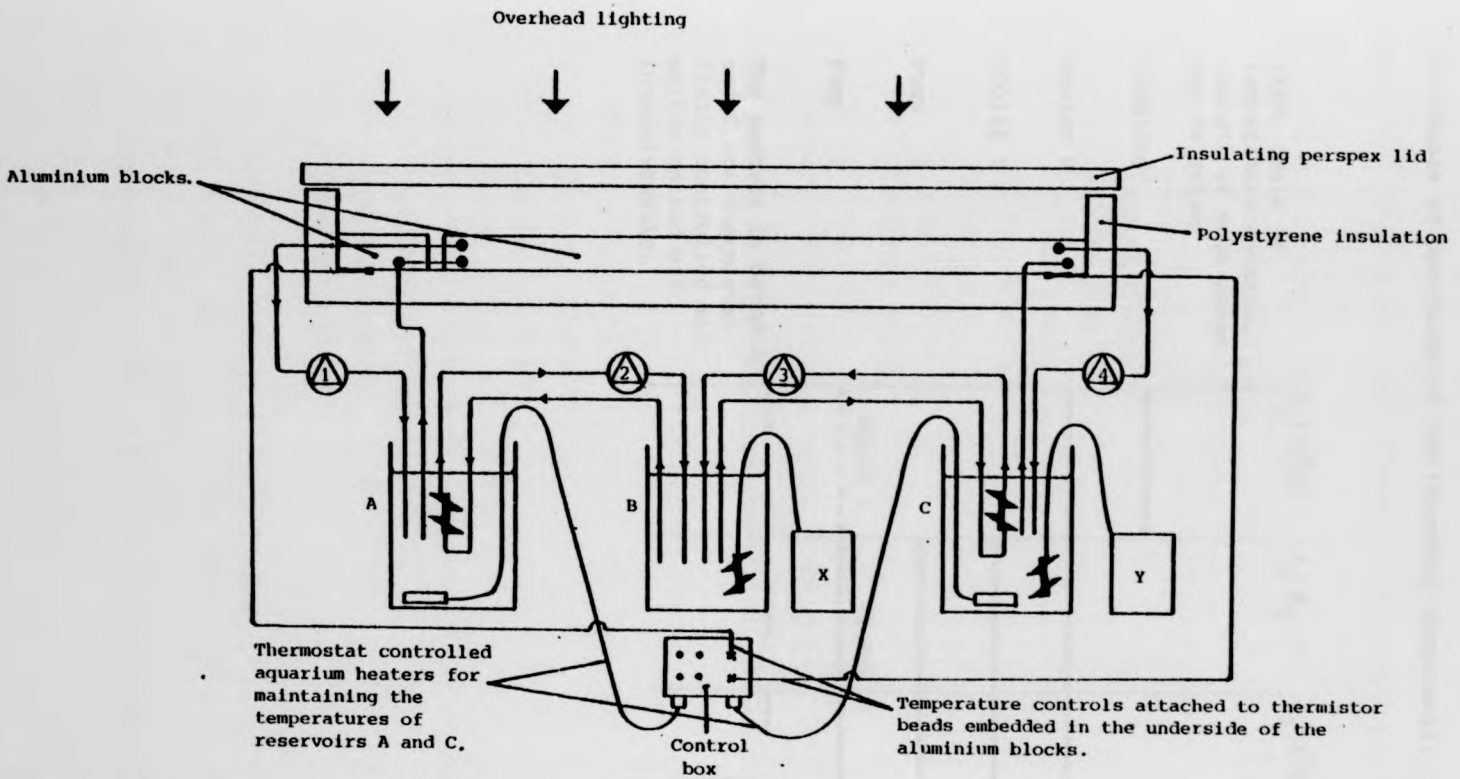
A_1-A_2 Changeover from day to night;

A_2 Nighttime - 5°C at the alternating end;

A_2-A_1 Changeover from night to day;

and the corresponding periods of operation of each respective pump and cooler.

Pumps 1 and 4 were in operation continuously, passing temperature controlled water from the reservoirs through the aluminium block, as a means of maintaining the temperature regimes. The temperatures of reservoirs A and C are about the same as the bar itself and B was maintained at just over 0°C.



a) Thermobar structure.

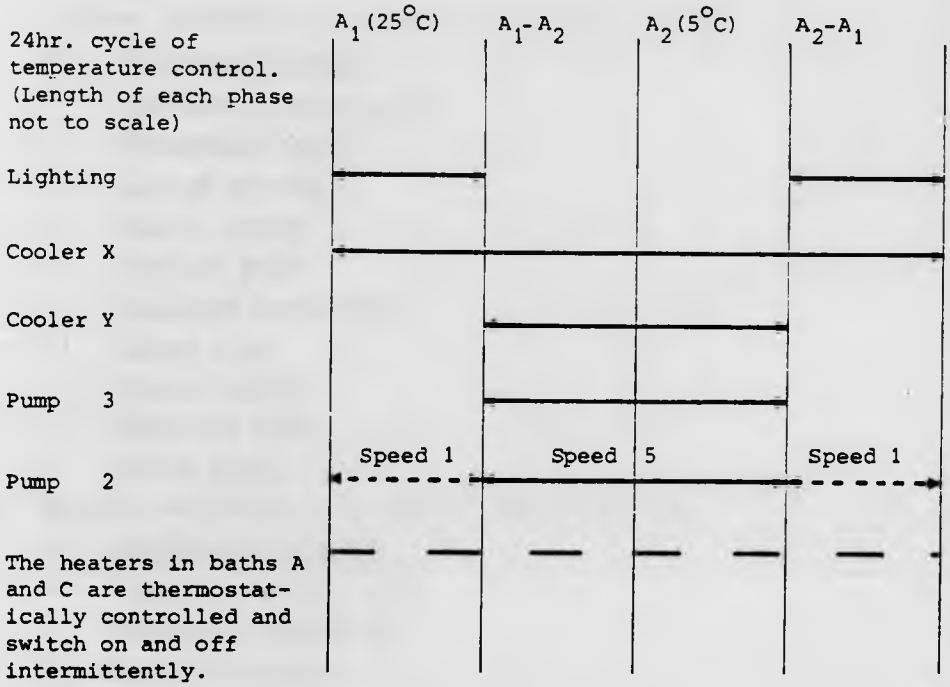
Pumps for passing glycol through the aluminium blocks and between the glycol reservoirs.

A,B and C reservoirs containing glycol to prevent freezing.

Cooling coils for assisting temperature control in the reservoirs

Direction of glycol flow.

b) Phases of operation of the thermobar components.

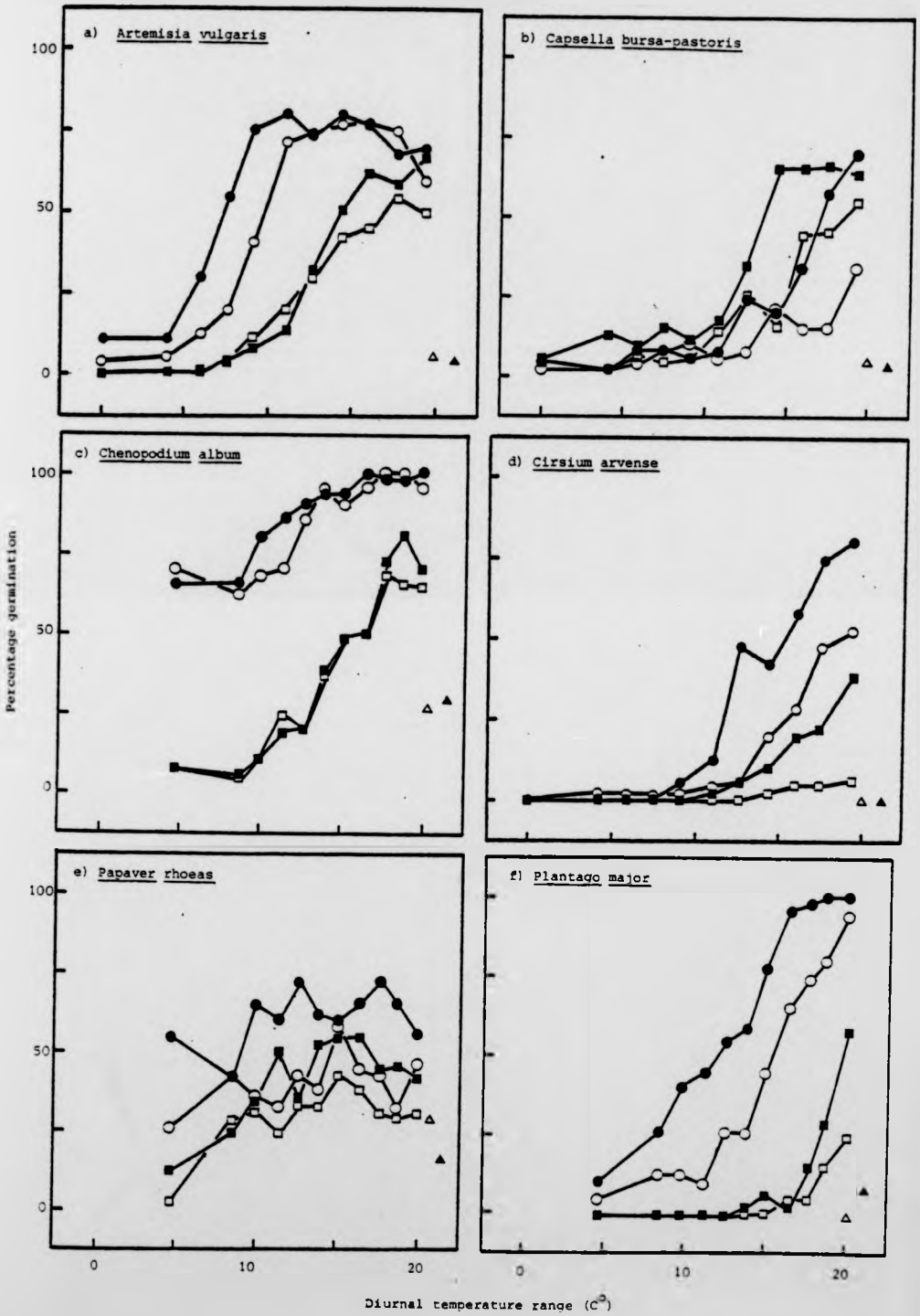


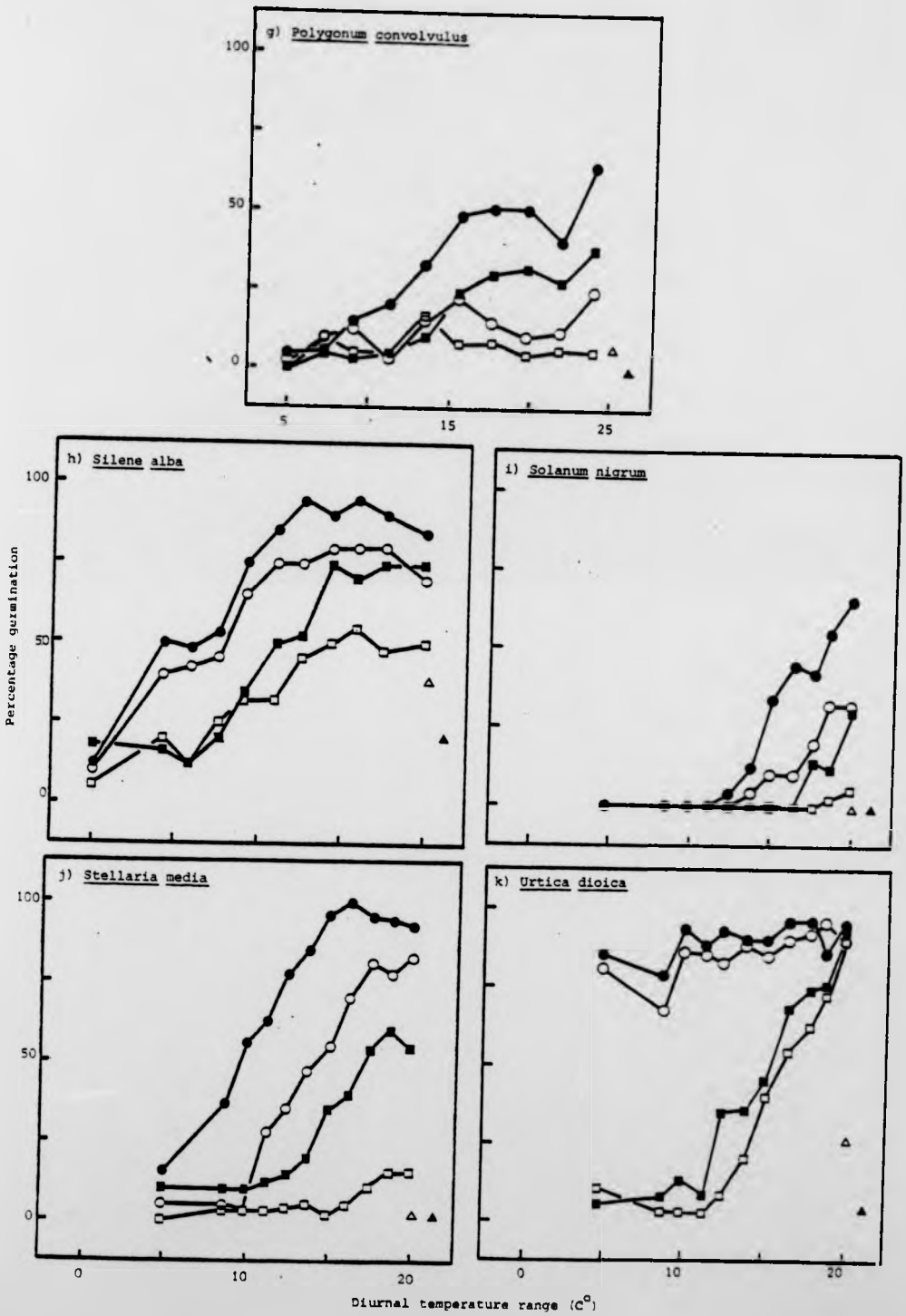
Appendix E: Percentage germination at a series of diurnal temperature ranges for the ruderal species tested on the thermobar (Chapter 3).

1. Species responding to nitrate in the light and dark.
   a) *Artemisia vulgaris*
   b) *Capsella bursa-pastoris*
   c) *Chenopodium album*
   d) *Cirsium arvense*
   e) *Papaver rhoeas*
   f) *Plantago major*
   g) *Polygonum convolvulus*
   h) *Silene alba*
   i) *Solanum nigrum*
   j) *Stellaria media*
   k) *Urtica dioica*
2. Species responding to nitrate in the light only.
   a) *Achillea millefolium*
   b) *Chamerion angustifolium*
   c) *Gnaphalium uliginosum*
   d) *Rumex obtusifolius*
3. Species responding to nitrate in the dark only.
   a) *Polygonum lapathifolium*
   b) *Senecio vulgaris*
   c) *Silene nutans*
4. Species with no response to nitrate in light or dark.
   a) *Polygonum persicaria*
   b) *Sagina procumbens*
   c) *Spergula arvensis*

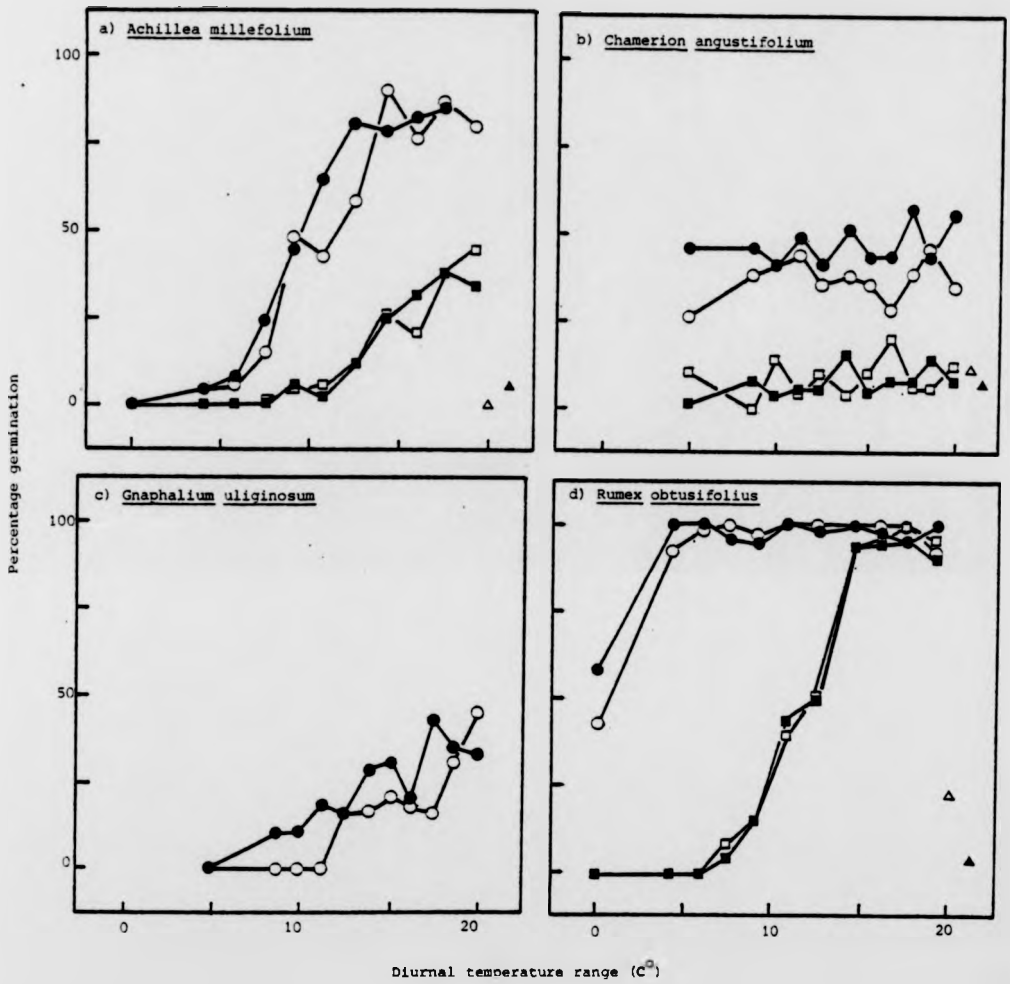
Key ●, 10^{-2} M KNO_3, light; ○, water, light;
■, 10^{-2} M KNO_3, dark; □, water, dark;
▲, water, dark, at constant 25°C;
△, water, dark, 8/18°C in a controlled environment cabinet.

1. Species responding to nitrate in the light and dark.

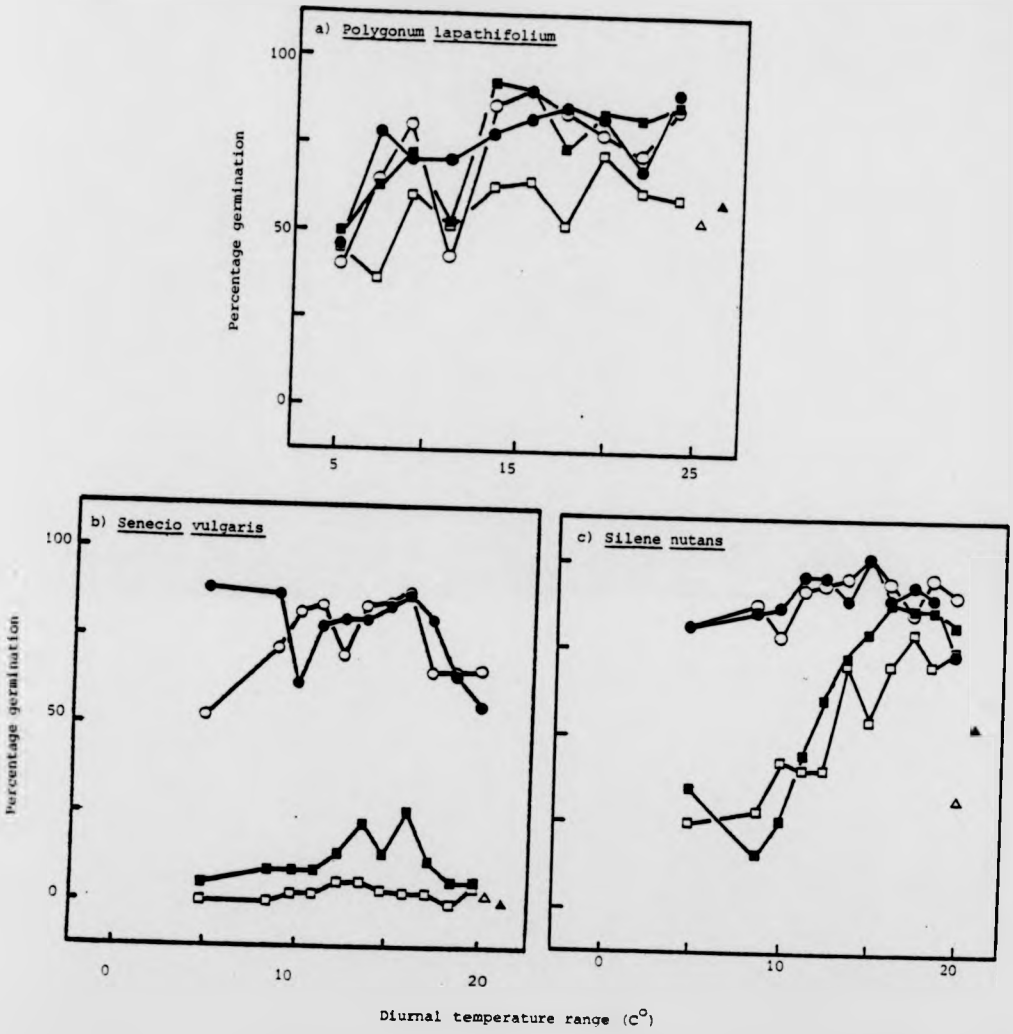



g) Polygonum convolvulus
h) Silene alba
i) Solanum nigrum
j) Stellaria media
k) Urtica dioica
Percentage germination
Diurnal temperature range (C°)
100
50
0
5
15
25
10
20

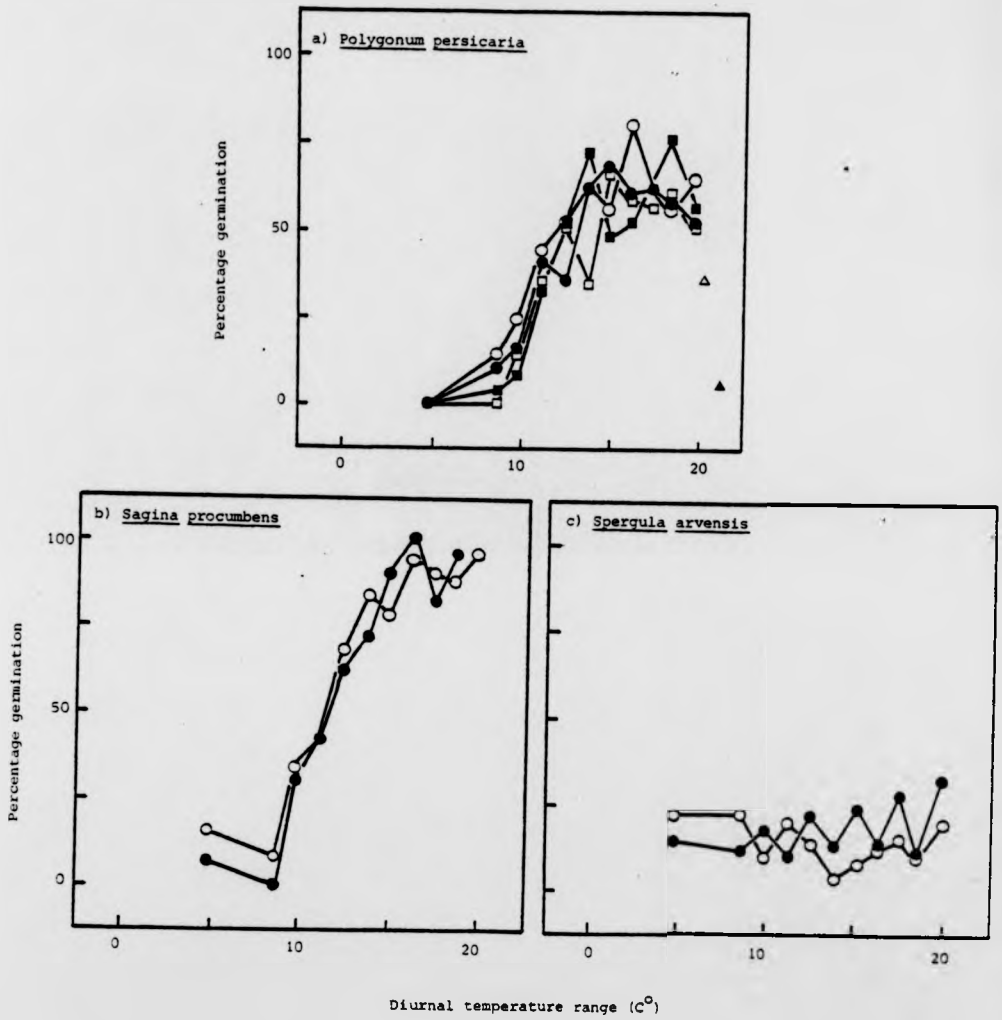
2. Species responding to nitrate in the light only.



3. Species responding to nitrate in the dark only.



4. Species with no response to nitrate in the light or dark.



Appendix F: A preliminary experiment to determine the effects upon nitrate levels during incubation of:

i) the nature of the incubation medium, gravel or petri dish lids.

ii) the leaching of sachets prior to incubation.

Nitrate levels were determined in six replicate boxes of each of four different treatments:

1. Unleached sachets placed on gravel.
2. Leached sachets placed on gravel.
3. Unleached sachets supported by petri dish lids.
4. Leached sachets supported by petri dish lids.

Treatment	Nitrate concentration (M)		
	Mean	Minimum	Maximum
1)	6.6×10^{-4}	5.7×10^{-4}	8.4×10^{-4}
2)	2.4×10^{-4}	1.9×10^{-4}	3.4×10^{-4}
3)	2.0×10^{-4}	0.5×10^{-4}	2.6×10^{-4}
4)	2.3×10^{-5}	1.2×10^{-5}	4.7×10^{-5}

Appendix G: Analyses for Chapter 4. Nitrate responses of four ruderal species following burial.

1. *Artemisia vulgaris*

   a) Fresh seeds.

   b) 3 months burial.

   c) 9 months burial.

   d) 9 months - Percentage of germinable seeds.

   e) 15 months burial.

   f) 15 months - Percentage of germinable seeds.

   g) t-test for comparison of means with different variances.

2. *Cardamine hirsuta*

   a) Fresh seeds.

   b) 3 months burial.

   c) 9 months burial.

   d) 9 months - Percentage of germinable seeds.

   e) 15 months burial.

   f) 15 months - Percentage of germinable seeds.

   g) t-tests for comparison of means with different variances.

3. *Stellaria media*

   a) Fresh seeds.

   b) 3 months burial.

   c) 9 months burial.

   d) 9 months - Percentage of germinable seeds.

   e) 15 months burial.

   f) 15 months - Percentage of germinable seeds.

4. Senecio vulgaris

a) Fresh seeds.

b) 3½ months burial.

c) 9 months burial (Three-way ANOVA).

d) 15 months burial.

e) 15 months - Percentage of germinable seeds.

f) t-tests for comparison of means with difference variables.

(All data is Arcsin $\sqrt{}$ transformed).

Abbreviations used in analyses.

Treatment A = Light 5/25°C

B = Dark 5/25°C

C = Dark 8/18°C

N = Nitrate

W = Water

1a) Artemisia vulgaris Fresh seeds.

Table of Mean Values

	A	B	C	
W	52.8867	23.9267	15.0133	30.6089
N	51.6835	30.8033	15.2400	32.5755
	52.2850	27.3650	15.1267	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	17.4180	1	17.4180	0.4429
Columns	4304.0664	2	2151.5332	54.7145
Interaction	55.7617	2	27.8809	0.7090
Subtotal	4376.2461	5		
Within	471.8750	12	39.3229	
Total	4848.1211	17		

Probability of F >= 0.4429 with 1 and 12 D.F. is 0.5183
Probability of F >= 54.7145 with 2 and 12 D.F. is 0.0000
Probability of F >= 0.7090 with 2 and 12 D.F. is 0.5116

Duncan's MRT.

P = 0.01

All three environments significantly different.

1b) Artemisia vulgaris 3 months burial.

Table of Mean Values

	A	B	C	
W	50.8133	26.3100	33.4900	36.8711
N	61.9467	51.0333	55.9566	56.3122
	56.3800	38.6716	44.7233	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	1700.8125	1	1700.8125	23.8260
Columns	972.1797	2	486.0898	6.8094
Interaction	159.1133	2	79.5566	1.1145
Subtotal	2832.1055	5		
Within	856.6172	12	71.3848	
Total	3688.7227	17		

Probability of F >= 23.8260 with 1 and 12 D.F. is 0.0000
Probability of F >= 6.8094 with 2 and 12 D.F. is 0.0106
Probability of F >= 1.1145 with 2 and 12 D.F. is 0.3598

Least significant difference between nitrate and water at p = 0.05 15.038

Duncan's MRT

p = 0.05 p = 0.01

A A
B B
C C

1c) Artemisia vulgaris 9 months burial.

Table of Mean Values

	N	W	
A	45.9333	57.5333	51.7333
B	31.1333	48.0333	39.5833
C	55.1667	44.6333	49.9000
	44.0778	50.0667	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	514.8350	2	257.4180	1.7021
Columns	161.4375	1	161.4375	1.0674
Interaction	635.2695	2	317.6348	2.1002
Subtotal	1311.5430	5		
Within	1814.8438	12	151.2370	
Total	3126.3867	17		

Probability of F >= 1.7021 with 2 and 12 D.F. is 0.2235
Probability of F >= 1.0674 with 1 and 12 D.F. is 0.3219
Probability of F >= 2.1002 with 2 and 12 D.F. is 0.1652

1d. Artemisia vulgaris 9 months burial - percentage of germinable seeds.

Table of Mean Values

	A	B	C	
N	81.9333	69.2767	64.0667	71.7589
W	83.0000	72.9333	62.4333	72.7889
	83.4666	71.1050	63.2500	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	4.8750	1	4.8750	0.0885
Columns	1120.2500	2	560.1250	10.1658
Interaction	20.8750	2	10.4375	0.1894
Subtotal	1146.0000	5		
Within	661.1875	12	55.0990	
Total	1807.1875	17		

Probability of F >= 0.0885 with 1 and 12 D.F. is 0.7712
Probability of F >= 10.1658 with 2 and 12 D.F. is 0.0026
Probability of F >= 0.1894 with 2 and 12 D.F. is 0.8299

Duncan's MRT.

p = 0.05

A
B
C

1e) Artemisia vulgaris 15 months burial.

Table of Mean Values

	A	B	C	
N	44.9333	35.6667	45.8000	42.1333
W	42.3333	27.4667	44.2333	38.0111
	43.6333	31.5667	45.0166	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	76.4805	1	76.4805	0.2621
Columns	656.8633	2	328.4316	1.1254
Interaction	38.2188	2	19.1094	0.0655
Subtotal	771.5625	5		
Within	3502.1367	12	291.8447	
Total	4273.6992	17		

Probability of F >= 0.2621 with 1 and 12 D.F. is 0.6180
Probability of F >= 1.1254 with 2 and 12 D.F. is 0.3565
Probability of F >= 0.0655 with 2 and 12 D.F. is 0.9370

1f) Artemisia vulgaris 15 months - percentage of germinable seeds.

Table of Mean Values

	A	B	C	
N	88.0999	86.6667	79.7333	84.8333
W	87.3000	90.0000	82.3333	86.5443
	87.6999	88.3333	81.0333	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	13.0625	1	13.0625	0.2767
Columns	196.3125	2	98.1563	2.0792
Interaction	14.6875	2	7.3438	0.1556
Subtotal	224.0625	5		
Within	566.5000	12	47.2083	
Total	790.5626	17		

Probability of F >= 0.2767 with 1 and 12 D.F. is 0.6085
Probability of F >= 2.0792 with 2 and 12 D.F. is 0.1678
Probability of F >= 0.1556 with 2 and 12 D.F. is 0.8576

1g) Artemisia vulgaris t-test comparing germination in water in the dark of fresh seeds and those buried for 3 months.

$$t = \frac{22.97 - 53.48}{\sqrt{\frac{39.32 + 71.38}{12}}} = 10.05 \qquad p < 0.001$$

2a) Cardamine hirsuta Fresh seeds.

Table of Mean Values

	A	B	C	
W	50.2300	20.7666	13.7267	28.2411
N	48.4566	29.7000	20.5233	32.8933
	49.3433	25.2333	17.1250	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	97.4023	1	97.4023	8.4652
Columns	3370.1172	2	1685.0586	146.4481
Interaction	96.3398	2	48.1699	4.1864
Subtotal	3563.8549	5		
Within	138.0742	12	11.5062	
Total	3701.9336			

Probability of F >= 8.4652 with 1 and 12 D.F. is 0.0131
Probability of F >= 146.4481 with 2 and 12 D.F. is 0.0000
Probability of F >= 4.1864 with 2 and 12 D.F. is 0.0418

Least Significant Difference between nitrate and water at p = 0.05 6.04

Duncan-s MRT.

P = 0.01

All three environments significantly different.

2b) Cardamine hirsuta 1 months burial.

Table of Mean Values

	A	C	B	
W	50.0667	6.5567	19.7966	12.1700
N	57.1100	17.3167	42.4833	38.9700
	53.5883	11.9367	41.1100	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	211.1445	1	211.1445	6.1602
Columns	5483.3125	2	2741.656.1	79.9881
Interactions	48.2500	2	24.1250	0.7039
Subtotal	5742.7070	5		
Within	411.3086	12	34.2757	
Total	6154.0156	17		

Probability of F >= 6.1601 with 1 and 12 D.F. is 0.0289
Probability of F >= 79.9881 with 2 and 12 D.F. is 0.0000
Probability of F >= 0.7039 with 2 and 12 D.F. is 0.5140

Least Significant Difference between nitrate and water at p = 0.05 10.42

Duncan's MRT,

P = 0.01

All three environments significantly different.

2c) Cardamine hirsuta 9 months burial.

Table of Mean Values

	N	W	
A	57.3000	72.1000	64.6000
B	55.3667	55.9667	55.6667
C	43.6000	44.2333	43.9167
	52.0889	57.4333	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	1303.1992	2	651.5996	9.4272
Columns	128.5430	1	128.5430	1.8597
Interaction	201.1953	2	100.5977	1.4554
Subtotal	1632.9375	5		
Within	829.4297	12	69.1191	
Total	2462.3672	17		

Probability of F >= 9.4272 with 2 and 12 D.F. is 0.0035
Probability of F >= 1.8597 with 1 and 12 D.F. is 0.1977
Probability of F >= 1.4554 with 2 and 12 D.F. is 0.2717

Duncan's MRT.

P = 0.05

A
B
C

2d) Cardamine hirsuta 9 months-percentage of germinable seeds.

Table of Mean Values

	A	B	C	
N	74.9333	62.8000	50.7333	62.8222
W	76.2333	64.4000	47.7000	62.778
	75.5833	63.6000	49.2166	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	0.0625	1	0.0625	0.0015
Columns	2091.3750	2	1045.6875	24.3213
Interaction	20.1250	2	10.0625	0.2340
Subtotal	2111.5625	2		
Within	515.9275	12	42.9948	
Total	2627.5000	17		

Probability of F >= 0.0015 with 1 and 12 D.F. is 0.9702
Probability of F >= 24.3213 with 2 and 12 D.F. is 0.0000
Probability of F >= 0.2340 with 2 and 12 D.F. is 0.7949

Duncan's MRT.

P = 0.05

All three environments significantly different.

2e) Cardamine hirsuta 15 months burial.

Table of Mean Values

	A	B	C	
N	71.9667	61.9333	54.9667	62.9555
W	67.8333	69.1667	47.2666	61.4222
	69.9000	65.5500	51.1167	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	10.6250	1	10.6250	0.1323
Columns	1160.1875	2	580.0938	7.2244
Interaction	182.4375	2	91.2188	1.1360
Subtotal	1353.2500	5		
Within	963.5625	12	80.2969	
Total	2316.8125	17		

Probability of F >= 0.1323 with 1 and 12 D.F. is 0.7224
Probability of F >= 7.2244 with 2 and 12 D.F. is 0.0087
Probability of F >= 1.1360 with 2 and 12 D.F. is 0.3533

Duncan's MRT.

P = 0.05

A
B
C

2f) Cardamine hirsuta 15 months - Percentage of germinable seeds.

Table of Mean Values

	A	B	C	
N	75.9667	80.8333	61.7333	72.8444
W	77.1667	82.8667	55.5667	71.8667
	76.5667	81.8500	58.6500	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	4.3125	1	4.3125	0.0937
Columns	1774.3750	2	887.1875	19.2845
Interaction	61.1250	2	30.5625	0.6643
Subtotal	1839.8125	5		
Within	552.0625	12	46.0052	
Total	2391.8750	17		

Probability of F >= 0.0937 with 1 and 12 D.F. is 0.7647
Probability of F >= 19.2845 with 2 and 12 D.F. is 0.0000
Probability of F >= 0.6643 with 2 and 12 D.F. is 0.5326

Duncna's MRT.

P = 0.05

A
B
C

2g) Cardamine hirsuta t-tests for comparison of germination of fresh seeds and those buried for 15 months.

Environment

Light 5/25°C $t = \frac{49.34 - 69.9}{\sqrt{\frac{11.50 + 80.3}{12}}} = 7.42$ $p < 0.01$

Dark 5/25°C $t = \frac{25.23 - 65.55}{2.77} = 14.60$ $p < 0.01$

Dark 8/18°C $t = \frac{17.12 - 51.11}{2.77} = 12.27$ $p < 0.01$

3a) Stellaria media Fresh seeds.

Table of Mean Values

	A	B	C	
W	29.4600	10.3400	17.3167	19.0389
N	42.1133	24.7800	22.1967	29.6967
	35.7867	17.5600	19.7567	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	511.1602	1	511.1602	42.6383
Columns	1188.0195	2	594.0098	49.5492
Interaction	77.4961	2	38.7480	3.2322
Subtotal	1776.6758	5		
Within	143.8594	12	11.9883	
Total	1920.5352	17		

Probability of F >= 42.6283 with 1 and 12 D.F. is 0.0000
Probability of F >= 49.5492 with 2 and 12 D.F. is 0.0000
Probability of F >= 3.2322 with 2 and 12 D.F. is 0.0754

Least Significant Difference between nitrate and water p = 0.05 6.16

Duncan's MRT.

P = 0.01

A
B]
C]

3b) Stellaria media 3 months.

Table of Mean Values

	A	B	
W	46.5333	56.4667	51.5000
N	50.9733	64.1767	57.5750
	48.7533	60.3217	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	110.7188	1	110.7188	8.3209
Columns	401.5000	1	401.5000	30.1740
Interaction	8.0313	1	8.0313	0.6036
Subtotal	520.2500	3		
Within	106.4492	8	13.3062	
Total	626.6992	11		

Probability of F >= 8.3209 with 1 and 8 D.F. is 0.0305
Probability of F >= 30.1740 with 1 and 8 D.F. is 0.0000
Probability of F >= 0.6036 with 1 and 8 D.F. is 0.4596

Least Significant Difference p = 0.05 6.88

3c) Stellaria media 9 months.

Table of Mean Values

	N	W	
A	42.8333	19.4333	31.1333
B	27.1100	40.0000	33.5550
C	27.8000	47.4667	37.6333
	32.5811	35.6333	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	129.5195	2	64.7598	0.1207
Columns	41.9336	1	41.9336	0.0782
Interaction	1608.8047	2	804.4023	1.4997
Subtotal	1780.2578	5		
Within	6436.3594	12	536.3633	
Total	8216.6172	17		

Probability of F >= 0.1207 with 2 and 12 D.F. is 0.8873
Probability of F >= 0.0782 with 1 and 12 D.F. is 0.7845
Probability of F >= 1.4997 with 2 and 12 D.F. is 0.2622

3d) Stellaria media 9 months - Percentage of germinable seeds.

Table of Mean Values

	A	B	C	
N	68.1667	69.1667	74.7900	70.7078
W	86.1667	71.4000	74.8667	77.4778
	77.1667	70.2833	74.8283	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	206.2500	1	206.2500	1.2152
Columns	147.0625	2	73.5313	0.4332
Interaction	287.2500	2	143.6250	0.8462
Subtotal	640.5625	5		
Within	2036.7500	12	169.7292	
Total	2677.3125	17		

Probability of F >= 1.2152 with 1 and 12 D.F. is 0.2919
Probability of F >= 0.4332 with 2 and 12 D.F. is 0.6582
Probability of F >= 0.8462 with 2 and 12 D.F. is 0.4531

3e) Stellaria media 15 months burial.

Table of Mean Values

	A	B	C	
N	61.4000	33.8333	51.5000	48.9111
W	62.6333	32.2000	13.5667	36.1333
	62.0167	33.0167	32.5333	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	734.7070	1	734.7070	1.7932
Columns	3421.0177	2	1710.5059	4.1748
Interaction	1429.9258	2	714.9629	1.7450
Subtotal	5585.6445	5		
Within	4916.6484	12	409.7207	
Total	10502.2930	17		

Probability of F >= 1.7932 with 1 and 12 D.F. is 0.2054
Probability of F >= 4.1748 with 2 and 12 D.F. is 0.0420
Probability of F >= 1.7450 with 2 and 12 D.F. is 0.2162

3f) Stellaria media 15 months - Percentage of germinable seeds.

Table of Mean Values

	A	B	C	
N	67.0667	73.0333	75.9000	72.0000
W	83.2333	76.0333	77.2990	
	69.8333	78.1333	75.9667	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	125.8750	1	125.8750	1.1283
Columns	222.4375	2	111.2188	0.9970
Interaction	76.1875	2	18.0938	0.3415
Subtotal	424.5000	5		
Within	1338.6875	12	111.5573	
Total	1762.1875	17		

Probability of F >= 1.1283 with 1 and 12 D.F. is 0.3090
Probability of F >= 0.9970 with 2 and 12 D.F. is 0.3976
Probability of F >= 0.3415 with 2 and 12 D.F. is 0.7174

4a) Senecio vulgaris Fresh seeds.

Table of Mean Values

	A	B	C	
W	38.6467	2.7100	0.0000	13.7855
N	46.5333	0.000	14.0500	20.1944
	42.5900	1.3550	7.0250	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	184.8359	1	184.8359	28.9053
Columns	5994.6875	2	2997.3438	468.7354
Interaction	215.5820	2	107.7910	16.8567
Subtotal	6395.1055	5		
Within	76.7344	12	6.3945	
Total	6471.8398	17		

Probability of F >= 28.9053 with 1 and 12 D.F. is 0.0000
Probability of F >= 468.7354 with 2 and 12 D.F. is 0.0000
Probability of F >= 16.8398 with 2 and 12 D.F. is 0.0000

Least Significant Difference between nitrate and water p = 0.05 4.50

Duncan's MRT.

P = 0.01

All three environments significantly different.

4b) Senecio vulgaris 3½ months burial.

Table of Means

	A	B	C	
N	50.9750	24.0250	38.0750	37.6917
W	47.6000	24.2750	37.1750	36.3500
	49.2875	24.1500	37.6250	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	10.7969	1	10.7969	0.3028
Columns	2531.9531	2	1265.9766	35.5098
Interaction	13.7344	2	6.8672	0.1926
Subtotal	2556.4844	5		
Within	641.7266	18	35.6515	
Total	3198.2109	23		

Probability of F >= 0.3028 with 1 and 18 D.F. is 0.5889
Probability of F >= 35.5098 with 2 and 18 D.F. is 0.0000
Probability of F >= 0.1926 with 2 and 18 D.F. is 0.8265

Duncan's MRT.

P = 0.05

All three environments significantly different.

4c) Senecio vulgaris 9 months burial. Three-way ANOVA.

Table of Mean Values

		A	B	C	
Original Experiment	N	36.87	25.70	43.67	35.41
	W	53.30	15.27	33.60	34.72
		46.09	20.49	38.64	
Repeat Experiment	N	40.40	46.60	48.00	45.00
	W	61.13	31.75	34.75	42.54
		48.43	29.83	40.01	

Source	Sum of squares	D.F.	Mean square	F-ratio
1. Environments A, B and C	2167.095	2	1083.550	5.900
2. Nitrate or Water	30.345	1	30.400	0.170
3. Original or repeat experiment	779.272	1	779.300	4.244
Interactions				
1 x 2	2415.939	2	1207.950	6.580
1 x 3	519.434	2	259.700	1.414
2 x 3	8.051	1	8.050	1.044
1 x 2 x 3	21.859	2	10.930	1.060
Subtotal	5941.995	11		
Within	5508.628	30	183.620	
Total	11450.623	41		

Probability of F >= 5.900 with 2 and 30 D.F. is <0.01
Probability of F >= 4.244 with 1 and 30 D.F. is <0.05
Probability of F >= 6.580 with 2 and 30 D.F. is <0.01
Others non-significant.

Least Significant Difference between nitrate and water in the light when the original and repeat experiments were combined. p = 0.05 L.S.D. = 14.78

L.S.D. between nitrate and water in the dark when the original and repeat experiment values for both temperature regimes were combined. p = 0.05
L.S.D. = 14.78

Duncan's MRT. p = 0.05 for figures from the original experiment.

A
C
B

4d) Senecio vulgaris 15 months burial.

Table of Mean Values

		A	B	C	
	N	45.3667	25.6333	19.4667	30.1555
	W	20.1000	24.0000	26.3333	23.4778
		32.7333	24.8167	22.9000	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	200.6953	1	200.6953	0.5538
Columns	326.1172	2	163.0586	0.4500
Interaction	831.6445	2	415.8223	1.1475
Subtotal	1358.4570	5		
Within	4348.5273	12	362.3772	
Total	5706.9844	17		

Probability of F >= 0.5538 with 1 and 12 D.F. is 0.4711
Probability of F >= 0.4500 with 2 and 12 D.F. is 0.6480
Probability of F >= 1.1475 with 2 and 12 D.F. is 0.3499

4e) Senecio vulgaris 15 months - Percentage germinable seeds.

Table of Mean Values

	A	B	C	
N	78.4333	48.1333	75.3000	67.2889
W	81.9667	69.2000	57.0667	69.4111
	80.2000	58.6667	66.1833	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	20.2500	1	20.2500	0.0530
Columns	1433.2500	2	716.6250	1.8754
Interaction	1162.9375	2	581.4688	1.5217
Subtotal	2616.4375	5		
Within	4585.3750	12	382.1145	
Total	7201.8125	17		

Probability of F >= 0.0530 with 1 and 12 D.F. is 0.8218
Probability of F >= 1.8754 with 2 and 12 D.F. is 0.1956
Probability of F >= 1.5217 with 2 and 12 D.F. is 0.2576

4f) Senecio vulgaris t-tests to compare germination in different months.

Comparison

Dark germination
Fresh seeds and 9 months burial

$$t = \frac{29.55 - 10.77}{\sqrt{\frac{228.81 + 6.39}{24}}} = 6.00 \qquad p < 0.01$$

Dark germination
Fresh seeds and 15 months burial

$$t = \frac{23.86 - 10.77}{\sqrt{\frac{6.39 + 362.37}{24}}} = 3.34 \qquad p < 0.05$$

Dark germination
9 and 15 months burial

$$t = \frac{29.55 - 23.86}{\sqrt{\frac{228.81 + 362.37}{24}}} = 1.15 \qquad p > 0.05 \text{ non-sig.}$$

Light germination
Fresh seeds and 9 months burial

$$t = \frac{46.1 - 42.6}{\sqrt{\frac{6.39 + 228.81}{6}}} = 0.56 \qquad p > 0.05 \text{ non-sig.}$$

Appendix H: Analyses for Chapter 5.

Germination of a natural seedbank in controlled conditions.

a) Garden soil.

Two-way ANOVA and Duncan's MRT for:

i) Total germination

ii) Dicot. germination

iii) Monocot. germination

b) Calcareous soil.

Two-way ANOVA with blocks for total germination.

c) Acid heathland seedbank.

One-way ANOVA with blocks for total germination.

a)i) Garden soil-total germination.

Table of Mean Values

	Treatment.				
	A	B	C	D	
N	64.5000	45.7500	28.5000	23.0000	40.4375
W	62.7500	37.2500	32.2500	22.2500	38.6250
	63.6250	41.5000	30.3750	22.6250	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	26.2813	1	26.2813	0.1086
Columns	7632.3438	3	2544.1145	10.5169
Interaction	193.5938	3	51.1979	0.2116
Subtotal	7812.2188	7		
Within	5805.7500	24	241.9063	
Total	13617.9688	31		

Probability of F >= 0.1086 with 1 and 24 D.F. is 0.7446
Probability of F >= 10.5169 with 3 and 24 D.F. is 0.0000
Probability of F >= 0.2116 with 3 and 24 D.F. is 0.8873

Duncan's MRT.

P < 0.05

A
B
C
D

a) ii) Garden soil - Dicot germination.

Table of Mean Values

	Treatment.				
	A	B	C	D	
N	48.0000	31.5000	23.2500	21.2500	31.0000
W	43.0000	25.2500	27.7500	20.2500	29.0625
	45.5000	28.3750	25.5000	20.7500	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	30.0313	1	30.0313	0.1758
Columns	2798.5983	3	929.8645	5.5284
Interaction	140.5983	3	46.8648	0.2786
Subtotal	2960.2188	7		
Within	4036.7500	24	168.1979	
Total	6996.9688	31		

Probability of F >= 0.1758 with 1 and 24 D.F. is 0.6764
Probability of F >= 5.5284 with 3 and 24 D.F. is 0.0050
Probability of F >= 0.2786 with 3 and 24 D.F. is 0.8403

Duncan's MRT.

P < 0.05

A
B
C
D

a) iii) Garden soil - Monocot germination.

Table of Mean Values

	Treatment.				
	A	B	C	D	
N	16.5000	14.2400	5.2500	1.7500	9.4375
W	19.7500	12.0000	4.5000	2.0000	9.5625
	18.1250	13.250	4.8750	1.8750	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	0.1250	1	0.1250	0.0073
Columns	1336.5000	3	445.5000	26.0146
Interaction	32.3750	3	10.7917	0.6302
Subtotal	1369.0000	7		
Within	411.0000	24	17.1250	
Total	17800.0000	31		

Probability of F >= 0.0073 with 1 and 24 D.F. is 0.9326
Probability of F >= 26.0146 with 3 and 24 D.F. is 0.0000
Probability of F >= 0.6320 with 3 and 24 D.F. is 0.6027

Duncan's MRT.

P < 0.05

A
B
C
D

b) Calcareous soil.

Table of mean values

	Block (a = NO_3, b = H_2O)										
	a	b	a	b	a	b	a	b	a	b	
5/25°C Light	8.00	11.00	10.00	8.50	12.00	9.00	11.50	11.00	10.38	9.88	10.13
Environment. 8/18°C Light	13.50	4.50	13.00	8.00	8.50	9.00	12.50	10.50	11.88	8.00	9.94
8/18°C Dark	6.50	6.50	7.50	11.50	10.50	10.50	7.00	4.00	7.88	8.13	8.00
	9.33	7.33	10.17	9.33	10.33	9.50	10.33	8.50	10.05	8.67	9.36

Source	Sum of squares	D.F.	Mean Square	F-ratio
Blocks	51.73	3	17.24	2.52
Environment	41.54	2	20.77	3.04
Nitrate/Water	11.02	1	11.02	1.61
Environment x Nitrate/Water	17.80	2	8.90	1.30
Subtotal	122.09	8	6.93	
Within	266.40	39		
Total	388.48	47		

Probability of F > = 2.52 with 3 and 39 D.F. is <0.01
Probability of F > = 3.04 with 2 and 39 D.F. is <0.01
Probability of F > = 1.61 with 1 and 39 D.F. is >0.01
Probability of F > = 1.30 with 2 and 39 D.F. is >0.01

c) Acid heathland seedbank

Table of Mean Values

	Blocks				
N	311.5000	1060.0000	306.50000	946.5000	656.1250
W	359.5000	1108.0000	430.0000	1111.0000	752.1250
	335.5000	1084.0000	368.2500	1028.7500	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	36864.0000	1	36864.0000	8.4381
Columns	1993528.0000	3	664509.3125	152.1051
Interaction	10056.0000	3	3352.0000	0.7673
Subtotal	2040448.0000	7		
Within	34950.0000	8	4368.7500	
Total	2075398.0000	15		

Probability of F >= 8.4381 with 1 and 8 D.F. is 0.0197
Probability of F >= 152.1051 with 3 and 8 D.F. is 0.0000
Probability of F >= 0.7673 with 3 and 8 D.F. is 0.5437

Appendix I: Analyses for the 1981 Field experiment. Chapter 6.

1. Total germination; Two-way ANOVA using $\sqrt{x+0.5}$ transformed data for five treatments at monthly intervals between April and September.
2. Poa spp. germination; Two-way ANOVA using $\sqrt{x+0.8}$ transformed data for five treatments at monthly intervals between April and September.
3. Stellaria media emergence.
   i) April; One-way ANOVA and Student-Newman-Keuls' test.
   ii) May; One-way ANOVA on log(x+1) transformed data.
   iii) June; One-way ANOVA on untransformed data.
   iv) July; One-way ANOVA on log(x+1) transformed data.
   v) September; One-way ANOVA on log(x+1) transformed data.

1. Two-way ANOVA using $\sqrt{x+0.5}$ transformed data of total seedling emergence.

Table of Mean Values

Treatment	Month. Apr	May	Jun	Jul	Aug	Sept	
NO_3-flush	6.02	3.52	4.63	2.71	2.01	3.51	3.73
N-serve	5.95	3.49	4.71	2.03	1.97	3.46	3.60
Water	5.35	3.41	4.54	2.36	1.84	2.89	3.40
No Treatment	5.74	3.69	4.37	2.25	1.71	3.59	3.56
NO_3-monthly	6.27	3.35	4.59	2.18	1.78	3.51	3.61
	5.87	3.53	4.57	2.31	1.86	3.39	3.58

Correction Factor = 5770.60

	Sum of squares	D.F.	Mean square	F-ratio
Mean	830.32	29	28.63	61.39
Columns	811.47	5	162.29	349.97
Rows	7.59	4	1.90	4.07
Interaction	11.26	20	0.56	1.20
Within	195.89	420	0.47	
Total	1026.21	449		

Probability of F >= 349.97 with 5 and 420 D.F. is <0.001
Probability of F >= 4.07 with 4 and 420 D.F. is <0.01
Probability of F >= 1.20 with 20 and 420 D.F. is >0.05

Duncan's MRT at p < 0.05

	Mean
NO_3-Flush	3.733
NO_3-Every month	3.617
N-Serve	3.602
No Treatment	3.558
Water	3.398

2. Two-way ANOVA using $\sqrt{x+0.5}$ transformed data of seedling emergence for Poa spp.

Table of Mean Values

Treatment	Month. Apr	May	Jun	Jul	Aug	Sept	
NO_3-flush	2.68	2.52	2.53	2.10	1.48	2.36	2.28
N-Serve	2.35	2.55	2.49	1.49	1.44	2.09	2.07
Water	2.22	2.37	2.46	1.87	1.51	1.73	2.03
No Treatment	2.11	2.52	2.39	1.58	1.40	2.23	2.04
NO_3-monthly	2.45	2.49	2.55	1.55	1.39	2.15	2.10
	2.36	2.49	2.49	1.72	1.44	2.11	2.10

Correction Factor = 1987.86

	Sum of squares	D.F.	Mean square	F-ratio
Mean	81.79	29	2.82	8.37
Columns	70.97	5	14.19	42.12
Rows	3.74	4	0.93	2.77
Interaction	7.09	20	0.35	1.05
Within	141.54	420	0.34	
Total	223.33	449		

Probability of F >= 42.12 with 5 and 420 D.F. is <0.01
Probability of F >= 2.77 with 4 and 420 D.F. is <0.05
Probability of F >= 1.05 with 20 and 420 D.F. is >0.05

Duncan's MRT at p < 0.05

NO_3-Flush	2.278
NO_3-Every month	2.096
N-Serve	2.068
No Treatment	2.039
Water	2.020

3i) Stellaria media April emergence without transformation.

Analysis of Variance Table

Source	SS	DF	MS
Grand Total	1951.0000	75	
Grand Mean	26.0133	1	
Treatments	606.5820	4	151.6455
Error	4196.4063	70	59.9487
Total	4802.9883	74	

F = 2.530 on [4, 70] degrees of freedom.

Probability of F greater than or equal to 2.530 with [4, 70] degrees of freedom is 0.048

Student-Newman-Keuls' test

	Mean	Number of replicates	p < 0.05
1 NO_3-Flush and NO_3-Every	28.70	30	1
2 N-Serve	26.87	15	2]
3 No Treatment	25.27	15	3]
4 Water	21.20	15	4]

Least significant range between 1 and 4 = $3.73 \times \sqrt{59.95} \times \sqrt{\frac{30 + 15}{2 \times 30 \times 15}} = 6.46$

3ii) Stellaria media May emergence with log(x+1) transformed data.

	SS	D.F.	MS	F-ratio
Treatment	2.49	4	0.6225	2.31
Within	18.86	70	0.2964	
Total	21.35	74		

Probability of F >= 2.31 with 4 and 70 D.F. is >0.05

3iii) Stellaria media June emergence with untransformed data.

	SS	D.F.	MS	F-ratio
Treatment	54.39	4	13.5986	0.55
Within	1740.27	70	24.8610	
Total	1794.69	74		

Probability of F >= 0.55 with 4 and 70 D.F. is >0.05

3iv) Stellaria media July emergence with log(x+1) transformed data.

	SS	D.F.	MS	F-ratio
Treatment	1.02	4	0.2538	0.75
Within	23.67	70	0.3382	
Total	24.69	74		

Probability of F >= 0.75 with 4 and 70 D.F. is >0.05

3v) Stellaria media September emergence with log(x+1) transformed data.

	SS	D.F.	MS	F-ratio
Treatment	1.88	4	0.4692	1.13
Within	28.88	70	0.4692	1.13
Total	30.76	74		

Probability of F >= 1.13 with 4 and 70 D.F. is >0.05.

Appendix J: Analyses for the 1982 Field experiment, Garden soil. Chapter 6.

1. Total germination; Two-way ANOVA using log(x+1) transformed data.
2. *Senecio vulgaris* germination; Two-way ANOVA using untransformed data.
3. *Cardamine hirsuta* germination; Two-way ANOVA using log(x+1) transformed data.
4. *Poa* spp. ; Two-way ANOVA using untransformed data.

1. Two-way ANOVA using log(x+1) transformed data of total seedling emergence.

Table of Mean Values

Treatment	Monitoring. 1	2	3	
Water	6.21	4.22	4.50	4.98
No Treatment	6.42	5.23	5.05	5.57
NO_3-flush	6.15	4.21	4.75	5.03
NO_3-every	6.14	4.21	4.75	4.91
	6.23	4.44	4.70	5.12

Correction Factor = 1260.14

	Sum of squares	D.F.	Mean square	F-ratio
Mean	34.30	11	3.12	41.80
Columns	29.92	2	14.96	200.54
Rows	3.26	3	1.09	14.57
Interaction	1.12	6	0.19	2.50
Within	2.69	36	0.075	
Total	36.99	47		

Probability of F >= 200.54 with 2 and 36 D.F. is <0.001
Probability of F >= 14.57 with 3 and 36 D.F. is <0.01
Probability of F >= 2.50 with 6 and 36 D.F. is <0.05

Overall Duncan's MRT at $p < 0.05$

No Treatment	5.56
NO_3-flush	5.03
Water	4.97
NO_3-every	4.91

Student-Newman-Keul's test for monitoring 1., with the NO_3-flush and NO_3-every treatments combined.

	Mean	Number of replicates
No Treatment	6.43	4
Water	6.21	4
NO_3-flush/ NO_3-every	6.14	8

Individual Duncan's MRTs for monitorings 2. and 3. at $p < 0.05$.

2.

No Treatment	5.23
Water	4.22
NO_3-flush	4.21
NO_3-every	4.12

3.

No Treatment	5.05
NO_3-flush	4.75
Water	4.50
NO_3-every	4.49

2. Two-way ANOVA using untransformed data of Senecio vulgaris emergence.

Table of Mean Values

Treatment	Monitoring. 1	2	3	
Water	115.0000	23.0000	15.5000	51.1667
No Treatment	180.0000	102.2500	33.0000	105.0833
NO_3-flush	125.5000	20.7500	36.2500	60.8333
NO_3-every	133.5000	21.2500	15.2500	56.6667
	138.5000	41.8125	25.0000	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	22050.9375	3	7350.3125	8.3514
Columns	120071.3750	2	60035.6875	68.2121
Interaction	8818.8125	6	1469.8020	1.6700
Subtotal	150941.1250	11		
Within	31684.7500	36	880.1318	
Total	182625.8750	47		

Probability of F >= 8.3514 with 3 and 36 D.F. is 0.0000
Probability of F >= 68.2121 with 2 and 36 D.F. is 0.0000
Probability of F >= 1.6700 with 6 and 36 D.F. is 0.1568

Duncan's MRT at $p < 0.05$

No Treatment	105.08
NO_3-flush	60.83
NO_3-every	56.67
Water	51.17

3. Two-way ANOVA using log(x+1) transformed data of Cardamine hirsuta emergence.

Table of Means

Treatment	Monitoring. 1	2	3	
Water	5.31	2.81	2.83	3.65
No Treatment	5.36	3.67	3.87	4.30
NO_3-flush	5.08	2.69	3.03	3.60
NO_3-every	5.14	2.90	3.05	3.70
	5.22	3.02	3.20	3.81

Correction Factor = 697.38

	Sum of squares	D.F.	Mean square	F-ratio
Mean	53.15	11	4.83	34.76
Columns	48.03	2	24.01	172.75
Rows	3.87	3	1.29	9.29
Interaction	1.25	6	0.21	1.50
Within	5.00	36	0.139	
Total	58.15	47		

Probability of F >= 172.75 with 2 and 26 D.F. is <0.01
Probability of F >= 9.29 with 3 and 36 D.F. is >0.01
Probability of F >= 1.50 with 6 and 36 D.F. is <0.05

Duncan's MRT at p < 0.05

No Treatment	4.30
NO_3-every	3.70
Water	3.65
NO_3-flush	3 .60

4. Two-way ANOVA using untransformed data of Poa spp. emergence.

Table of Mean Values

Treatment	Monitoring. 1	2	3	
Water	30.7500	12.2500	9.0000	30.6667
No Treatment	69.5000	22.000	8.7500	33.4167
NO_3-flush	81.5000	13.2500	9.7500	34.8333
NO_3-every	70.5000	13.2500	5.7500	29.8333
	73.0625	15.1875	8.3125	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	196.3945	3	65.4648	0.7967
Columns	40476.5000	2	20238.2500	246.2865
Interaction	476.1680	6	79.0280	0.9617
Subtotal	41147.0625	11		
Within	2958.2500	36	82.1736	
Total	44105.3125	47		

Probability of F >= 0.7967 with 3 and 36 D.F. is 0.5038
Probability of F >= 246.2865 with 2 and 36 D.F. is 0.0000
Probability of F >= 0.9617 with 6 and 36 D.F. is 0.4646

Duncan's MRT - No significant differences.

Appendix K: Analyses for the 1982 Field experiment, Calcareous soil. Chapter 6.

1. Total germination excluding seedlings of Cardamine hirsuta, Senecio vulgaris and Stellaria media, which were considered to be contaminants: Two-way ANOVA on untransformed data.

2. Germination of Holcus lanatus for the first monitoring alone: One-way ANOVA and Duncan's MRT on untransformed data.

1. Total germination excluding ruderal contaminants.

Table of Mean Values

Treatment	Monitoring 1	2	3	
Water	250.7500	91.7500	42.5000	128.3333
No treatment	231.7500	75.5000	55.0000	120.75000
NO_3-flush	233.7500	110.5000	51.7500	132.0000
NO_3-every	254.2500	127.0000	52.5000	144.5833
	242.6250	101.1875	50.4375	

Source	Sum of squares	D.F.	Mean square	F-ratio
Rows	3563.7500	3	1187.9165	2.1966
Columns	317418.7500	2	158709.3750	293.4685
Interaction	4396.2500	6	732.7083	1.3548
Subtotal	325378.7500	11		
Within	19469.0000	36	540.8054	
Total	344847.7500	47		

Probability of F >= 2.1966 with 3 and 36 D.F. is 0.1053
Probability of F >= 293.4685 with 2 and 36 D.F. is 0.0000
Probability of F >= 1.3548 with 6 and 36 D.F. is 0.2591

2. Holcus lanatus emergence in first monitoring alone.

Analysis of Variance Table

Source	SS	DF	MS
Grand Total	668.0000	16	
Grand Mean	41.7500	1	
Treatments	1459.0000	3	486.3333
Error	884.0000	12	73.6667
Total	2343.0000	15	

F = 6.602 on [3, 12] degree of freedom.

Probability of F greater than or equal to 6.602
with [3, 12] degrees of freedom is 0.007.

Duncan's MRT

P < 0.05

NO_3-every	49.5
Water	47.5
NO_3-flush	44.5
No Treatment	25.5

BIBLIOGRAPHY

Andersen, A.M. (1932). The effect of removing the glumes on the germination of seeds of Poa compressa. (Abs) Am. J. Bot. **19**, 835-6.

Barton, L.V. (1962). The germination of weed seeds. Weeds **10**, 174-182.

Baskin, J.M. and Baskin, C.C. (1979). Promotion of germination of Stellaria media seeds by light from a green safe lamp. New Phytol. **82**, 381-383.

Baskin, J.M. and Baskin, C.C. (1981). Seasonal changes in germination responses of buried seeds of Verbascum thapsus and V. blattaria and ecological implications. Can. J. Bot. **59**, 1769-1775.

Benjamin, S.K. (1974). The effects of environment on the germination of Chenopodium album, Capsella bursa-pastoris and Poa annua. Ph.D. Thesis, University of Reading.

Biswas, P.K., Bonamy, P.A. and Paul, K.B. (1972). Germination promotion of Loblolly Pine and Bald Cypress seeds by stratification and chemical treatments. Physiologia Plant. **27**, 71-76.

Black, J.N. (1956). The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (Trifolium subterraneum L.). Aust. J. agric. Res. **7**, 98-109.

Bogner, W. (1968). Experimentelle Prüfung von Waldbodenpflanzen auf ihre Ansprüche an die Form der Stickstoff-Ernahrung. Mitt. Ver. forstl. Standortskunde u. Forstpflanzenzuchtung, **18**, 3-45.

Bormann, F.H., Likens, G.E., Fisher, D.W. and Pierce, R.S. (1968). Nutrient loss accelerated by clear-cutting of a forest ecosystem. Science, N.Y. **159**, 882-884.

Bostock, S.J. (1976). The life history strategies of selected perennial Compositae of disturbed ground. Ph.D. Thesis. University of Manchester.

Bostock, S.J. (1978). Seed germination strategies of five perennial weeds. Oecologia (Berlin), **36**, 113-126.

Burns, I.G. (1977). Nitrate movement in soil and its agricultural significance. Outl. Ag. **9**, (3), 144-8.

Cain, J.C. (1952). A comparison of ammonium and nitrate nitrogen for blueberries. Proc. Am. Soc. Hort. Sci. **59**, 161.

Chancellor, R.J. (1978). The identification of weed seedlings of farm and garden. Blackwell Scientific Publications.

Chancellor, R.J. (1981). The manipulation of weed behaviour for control purposes. Phil. Trans. R. Soc. Lond. Ser. B **295**, 103-110.

Chancellor, R.J. (1982). Dormancy in weed seeds. Outl. Ag. **11**, (2), 87-93.

Chavagnat, A. and Jeudy, B. (1981). Étude de la germination des semences de Primula obconica. Seed Sci. and Technol. **9**, 577-586.

Clapham, A.R., Tutin, T.G. & Warburg, E.F. (1981). Excursion Flora of the British Isles. 3rd Ed. Cambridge University Press, Cambridge.

Cook, R. (1980). The Biology of Seeds in the Soil. In: Demography and Evolution in Plant Pops. - Bot. Monogs. Vol. 15. pp. 107-129, Blackwell. Ed. Solbrig O.T.

Courtney, A.D. (1968). Seed dormancy and field emergence in Polygonum aviculare L. J. appl. Ecol., 5, 675-684.

Davy, A.J. and Taylor, K. (1974). Water characteristics of contrasting soils in the Chiltern Hills and their significance for Deschampsia caespitosa (L.) Beauv. J. Ecol. **62**, 367-78.

Dey, B.B. and Choudhuri, M.A. (1982). Seed germination as affected by plant age, growth and development stages of Ocimum sanctum Seed Sci. and Technol. **10**, 243-255.

Eagle, D.J. (1961). Determination of the nitrogen status of soils in the West Midland. J. Sci. Fd. Agric. **12**, 712-727.

Ellenberg, H. (1964). Stickstoff als Standortsfaktor. Ber. dt. bot. Ges. **77**, 82-92.

Ellenberg, H. (1977). Stickstoff als Standorfaktor, insbesondere fur mitteleuropäische Pflanzengesellschaften. Oecol. Plant. **12**, 1-22.

Engelhardt, M. Vincente, M. and Silberschmidt, K. (1962). The stimulating effect of light and potassium nitrate on the germination of seeds of Amaranthus hybridus L. Rev. Brasil. Biol. **22**, 1-7.

Evans, R.A. and Young, J.A. (1975). Enhancing germination of dormant seeds of Downy Brome. Weed Sci. **23**, (5), 354-7.

Evenari, M. (1965). Light and seed dormancy. Encyclopedia of Plant Physiology, **15**, (Ed. by W. Ruhland), 804-847. Springer, Berlin, Heidleberg, New York.

Fawcett, R.S. and Slife, F.W. (1978). Effects of field applications of nitrate on weed seed germination and dormancy. Weed Sci. **26**, 594-596.

Fay, P.K. (1975). The effect of germination stimulators on wild oat (Avena fatua L.) emergence in the field. Proc. N. Cent. Weed Control Conf. **30**, 110-111.

Frankland, B. (1961). Effects of GA, kinetin, and other substances on seed dormancy. Nature, Lond. **192**, 678-679.

Freijsen, A.H.J., Troelstra, S.R., and van Kats, M.J. (1980). The effect of soil nitrate on the germination of Cynoglossum officinale L. (Boraginaceae) and its ecological significance. Acta Oecol. Ecol. Plant. I, (15), no.1, 71-9.

Froud-Williams, R.J., Drennan, D.S.H., and Chancellor, R.J. (1984). The influence of burial and dry-storage upon cyclic changes in dormancy, germination and response to light in seeds of various arable weeds. New Phytol. **96**, 478-481.

Fuerst, E.P., Upadhyaya, M.K., Simpson, G.M., Naylor, J.M. and Adkins, S.N. (1983). A study of the relationship between seed dormancy and pentose phosphate pathway activity in Avena fatua Can. J. Bot. **61**, (3), 667-670.

Gasser, J.K.R. (1970). Nitrification inhibitors - their occurrence, production and effects of their use on crop yields and compostion. Soils and Ferts. **33**, 547-554.

Gassner, G. (1951a). Einige neue Fälle von keimungauslosender Wirkung der Stickstoffverbindungen auf lichtempfindliche Samen. Ber. dt. bot. Ges. **33**, 217-232. (Original not seen; cited in M. Evenari. 1965. Light and seed dormancy 804-847. In W. Ruhland (Ed.). Encyclopedia of Plant Physiology. Springer-Verlag, Berlin. Vol. 15).

Gassner, G. (1951b). Über die keimungsauslösende Wirkung der Stickstoffsalze auf lichtempfindliche Samen. Jb. Wiss. Bot. 55, 259-342. (Original not seen; cited in M. Evenari. 1965. Light and seed dormancy. 804-847. In W. Ruhland (Ed.). Encyclopedia of Plant Physiology. Springer-Verlag, Berlin. Vol. 15).

Gigon, A. and Rorison, I.H. (1972). The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. J. Ecol. **60**, 93-102.

Griedanus, T., Peterson, L.A., Schrader, L.E. and Dana, M.N. (1972). Essentiality of ammonium for cranberry nutrition. J. Am. Soc. Hort. Sci. **97**, 272.

Grime, J.P. (1979). Plant Strategies and Vegetation Processes. Wiley, Chichester.

Grime, J.P., Mason, G., Curtis, A.V., Rodman, J., Band, S.R., Mowforth, M.A.G., Neal, A.M. and Shaw, S. (1981). A comparative study of germination characteristics in a local flora. J. Ecol. **69**, 1017-1059.

Hanf, M. (1971). Weeds and their seedlings. BASF.

Harmsen, G.W. and van Schreven, D.A. (1955). The mineralization of organic nitrogen in the soil. Adv. Agron. **7**, 299-398.

Harper, J.L. (1955). The influence of the environment on seed and seedling mortality. Ann. appl. Biol., **43**, 696-708.

Harper, J.L. (1957). The ecological significance of dormancy and its importance in weed control. Proc. 4th Int. Congr. Crop Bot., **1**, 415-420.

Harper, J.L., Williams, J.T. and Sagar, G.R. (1965). The behaviour of seeds in soil. 1. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. J. Ecol., **53**, 273-286.

Havill, D.C., Lee, J.A. and De-Felice, J. ((1977). Some factor limiting nitrate utilization in acidic and calcareous grasslands. New Phytol. **78**, 649-659.

Hay, J.R. and Cumming, B.G. (1959). A method for inducing dormancy in wild oats (Avena fatua) Weeds **7**, 34-40.

Hendricks, S.B. and Taylorson, R.B. (1972). Promotion of seed germination by nitrates and cyanides. Nature, Lond. **237**, 169-70.

Hendricks, S.B. and Taylorson, R.B. (1974). Promotion of seed germination by nitrate, nitrite, hydroxylamine, and ammonium salts. Pl. Physiol. **54**, 304-309.

Henson, I.E. (1970). The effects of light, potassium nitrate and temperature on the germination of Chenopodium album. Weed Res. **10**, 27-39.

Hilton, J.R. (1983). The influence of light on the germination of Senecio vulgaris L. New Phytol. **94**, 29-37.

Karssen, C.M. (1980-1a). Environmental conditions and endogenous mechanisms involved in secondary dormancy of seeds - review. Israel J. Bot. **29**. 45-64.

Karssen, C.M. (1980-1b). Patterns of change in dormancy during burial of seeds in soil. Israel J. Bot. **29**, 65-73.

Kurkin, K.A. (1977). Systems Approach to studies of nitrate regime in grassland biogeocenoses. Oecol. Plant. **12**(1), 23-31.

Larsen, A.L., Mongillion, D.P. and Schroeder, E.M. (1973). Germination of dormant and non dormant rescue grass seed on the thermogradient plate. Agronomy **65**, 56-59.

Lawson, H.M., Waister, P.D. and Stephens, R.J. (1974). Patterns of emergence of several important weed species. British Crop Protection Council Monograph No. 9, 121-135.

Leach, L.D. (1947). Growth rates of host and pathogen as factors determining the severity of pre-emergence damping off. J. agric. Res. **75**, 161-179.

Lehmann, E. (1909). Zur Keimungsphysiologie und -biologie von Ranunculus scleratus L. und einigen anderen Samen. Ber. dt. bot. Ges. **27**, 476-494. (Original not seen; cited in M. Evenari. 1965. Light and seed dormancy. 804-847. In W. Ruhland (Ed.). Encyclopedia of Plant Physiology. Springer-Verlag, Berlin. Vol. 15).

Lugo, H.L. (1955). The effect of nitrogen on the germination of Vanilla planifolia. Am. J. Bot. **42**, 679-681.

Mason, G. (1976). An investigation of the effect of temperature upon the germination and growth of native species using temperature-gradient techniques. Ph.D. Thesis, University of Sheffield.

Mayser, R. (1954). Ökologische Untersuchungen uber die Stickstoffan-spruche der Ruderalpflanzen. Unpublished PhD dissertation, Stuttgart, Germany. (Original not seen; cited in H. Walter, 1960. Grundlagen der Pflanzen verbreitung. Einfuhrung in die Phytologie Band III, I Teil: Standortslehre. Eugen Ulmer, Stuttgart).

Metson, A.J. (1956). Methods of chemical analysis for soil survey samples. Rep. Dep. scient. Ind. Res. N.Z., 139-45.

Moursi, M.A., Rizk, T.Y. and El-Deepah, H.R. (1977). Weed seed germination responses to some chemical treatments. Egyptian J. of Agron. **2**, 197-209.

Murdoch, A.J. (1982). Factors influencing the depletion of annual weed seeds in the soil. Ph.D. Thesis, Reading University.

Murdoch, A.J., and Roberts, E.H. (1982). Biological and financial criteria of long-term control strategies for annual weeds. Proc. British Crop Protection Conference - Weeds. 741-8.

Naylor, R.E.L. and Abdalla, A.F. (1982). Variation in germination behaviour. Seed Sci. and Technol. **10**, 67-76.

Nelson, A. (1927). The Germination of Poa spp. Ann. appl. Biol. **14**, 157-74.

Ogawara, K. and Ono, K. (1955). Effects of various nitrogen compounds and respiratory intermediates on the germination of light-favoured Tobacco seeds. Bull. Sch. Ed. Okayama Univ. **1**, 97-104.

Petersen, D.L. and Bazzaz, F.A. (1978). Life cycle characteristics of Aster pilosa in early successional habitats. Ecology, **59**, 1005-1013.

Popay, A.I. (1968). The ecology of annual weeds in relation to germination behaviour. Ph.D. Thesis, Univ. of Manchester.

Popay, A.I. and Roberts, E.H. (1970a). Factors involved in the dormancy and germination of Capsella bursa-pastoris (L.) Medik. and Senecio vulgaris L.. J. Ecol. **58**, 103-122.

Popay, A.I., Roberts, E.H. (1970b). Ecology of Capsella bursa-pastoris (L.) Medik and Senecio vulgaris L. in relation to germination J. Ecol., **58**, 123-139.

Rice, E.L. and Pancholy, S.K. (1972). Inhibition of nitrification by climax vegetation. Am. J. Bot. **59**, 1033-1040.

Roberts, E.H. (1963). The effects of inorganic ions on dormancy in rice seed. Physiologia Pl. **16**, 732-744.

Roberts, E.H. (1972). Dormancy: a factor affecting seed survival in the soil. In: E.H. Roberts, ed., Viability of seeds. Chapman and Hall, London, 321-359.

Roberts, E.H. (1973). Oxidative processes and the control of seed germination. Seed Ecology, Proceedings of the 19th Easter School in Agricultural Science, University of Nottingham. (Ed. by W. Heydecker) 189-218. Butterworths, London.

Roberts, E.H. and Benjamin, S.K. (1979). The interaction of light, nitrate and alternating temperature on the germination of Chenopodium album, Capsella bursa-pastoris, and Poa annua before and after chilling. Seed Sci. and Technol. **7**, 379-392.

Roberts, E.H. and Totterdell, S. Seed dormancy in Rumex spp. in response to environmental factors. Plant Cell Environ. **4**, 97-106.

Roberts, H.A. (1970). Viable weed seeds in cultivated soils. Rep. natn. Veg. Res. Stn. (1969). 25-38.

Roberts, H.A. (1981). Seebanks in soils. Ad. appl. Biol. **6**, 1-55.

Roberts, H.A. Ed. (1982). Weed Control Handbook: Volume 1. Principles. 7th Ed. Blackwell Scientific Publications.

Roberts, H.A. and Dawkins, P.A. (1967) Effect of cultivation on the numbers of viable weed seeds in soil. Weed. Res. **7**, 290-301.

Roberts, H.A. and Feast, P.M. (1970). Seasonal distribution of emergence in some annual weeds. Expl. Hort. **21**, 36-41.

Roberts, H.A. and Lockett, P.M. (1975). Germination of buried and dry-stored seeds of Stellaria media. Weed Res. **15**, 199-204.

Roberts, H.A. and Lockett, P.M. (1978a). Seed dormancy and periodicity of seedling emergence in Veronica hederifolia L. Weed Res. **18**, 41-48.

Roberts, H.A. and Lockett, P.M. (1978b). Seed dormancy and field emergence in Solanum nigrum L.. Weed Res. **18**, 231-241.

Roberts, H.A. and Neilson, J.E. (1982). Seasonal changes in the temperature requirements for germination of buried seeds of Aphanes arvensis L. New Phytol. **92**, 159-166.

Roberts, H.A. and Potter, M.E. (1980). Emergence patterns of weed seedlings in relation to cultivation and rainfall. Weed Res. **20**, 377-386.

Roberts, H.A. and Stokes, F.G. (1966). Studies on the weeds of vegetable crops. VI. Seed populations of soil under commercial cropping. J. appl. Ecol. **3**, 181-190.

Runge, M. (1974). - Die Stickstoff-Mineralisation im Boden eines Sauerhumus-Buchenwaldes. I. Mineralstickstoff-Gehalt und Netto-Mineralisation. II. Die Nitratproduktion. Oecol. Plant., **9**, 201-218 u. 219-230.

Russell, E.J. (1973). Soil conditions and plant growth. 10th ed. Longmans Group Limited .

Schimpf, D.J. (1977). Soil Inorganic Nitrogen as an environmental trigger for weed seed germination. Ph.D. Thesis Utah State University.

Schimpf, D.J. and Palmblad, I.G. (1980). Germination response of weed seeds to soil nitrate and ammonium with and without simulated overwintering. Weed Sci. **28**, 190-193.

Schonbeck, M.W. and Egley, G.H. (1980). Redroot pigweed (Amaranthus retroflexus) seed germination responses to afterripening, temperature, ethylene, and some other environmental factors. Weed Sci. **28**, 543-8.

Schwendiman, A. and Shands, H.L. (1943). Delayed germination or seed dormancy in Vicland Oats. J. Amer. Soc. Agron. **35**, 681-688.

Scurfield, G. and Boswell, J.G. (1953). The microbiology of acid soils. III. The analysis of soil profiles under Deschampsia flexuosa (L). Trin. and Holcus mollis (L). New Phytol. **52**, 178-185.

Seifert, J. (1962). The effects of winter on the number of bacteria and nitrification power of soils. II. Acta Univ. Carol. Biol. Suppl. 41-9.

Sexsmith, J.J. and Pittman. U.J. (1963). Effect of nitrogen fertilizers on germination and stand of wild oats. Weeds **11**, 99-101.

Sinyagin, I.I. and Teper, E.N. (1967). The effect of fertilizers on the germination of weed seeds. Doklady Vsesoyuznoi Akademii Sel'skokhozyaistrennykh Nauk 1967. (1):2-4. Original not seen; cited in Schimpf, D.J. 1977).

Smith, H. (1975). Phytochrome and Photomorphogenesis. McGraw Hill, London.

Sokal, R.R. and Rohlf, F.J. (1969). Biometry, W.H. Freeman and Co.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics: A biometrical approach. 2nd Ed. McGraw-Hill.

Steinbauer, G.P. and Grigsby, B. (1957). Interaction of temperature, light and moistening agent in the germination of weed seeds. Weeds. 5, 175-182.

Stoller, E.W. and Wax, L.M. (1974). Dormancy changes and fate of some annual weed seeds in the soil. Weed Sci. **22**, 151-155.

Srivastava, H.S., Oaks, A. and Bakyta, I.L. (1976). The effect of nitrate on early seedling growth in Zea mays. Can. J. Bot. **54**, (2) 923-929.

Taylor, A.A., De-Felice, J. and Havill, D.C. (1982). Seasonal availability and utilization of nitrate in an acidic and calcareous soil. New Phytol. **92** 141-152.

Taylorson, R.B. (1970). Changes in dormancy and viability of weed seeds in soils. Weed Sci. **18**, 265-269.

Taylorson, R.B. and Hendricks, S.B. (1969). Action of phytochrome during prechilling of Amaranthus retroflexus L. seeds. Pl. Physiol. **44**, 821-825.

Thompson, K. (1977). An ecological investigation of germination responses to diurnal fluctuations in temperature. Ph.D. Thesis, University of Sheffield.

Thompson, K. and Grime, J.P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. J. Ecol. **67**, 893-921.

Thompson, K. and Grime, J.P. (1983). A comparative study of germination responses to diurnally-fluctating temperatures. J. appl. Ecol. **20**, 141-156.

Thurston, J.M. (1959). Dormancy in weed seeds. The biology of weeds. Brit. Ecol. Soc. Symp. 1959, edited by J.L. Harper.

Toole, V.K. (1941). Factors Affecting the Germination of Various Dropseed Grasses (Sporobolus Spp.). J. agric. Res. **62**, 691-715.

Toole, V.K. (1973). Effects of light, temperature and their interactions on the germination of seeds. Seed Sci. and Technol. **1**, 339-396.

Toole, E.H., Hendricks, S.B., Borthwick, H.A. and Toole, V.K. (1956). Physiology of seed germination. A. Rev. Pl. Physiol. **7**, 299-324.

Totterdell, S. and Roberts, E.H. (1980). Characteristics of alternating temperatures which stimulate loss of dormancy in seeds of Rumex obtusifolius and Rumex crispus. Plant Cell Environ. **3**, 3-12.

Townsend, L.R. (1966). Effect of nitrate and ammonium nitrogen on the growth of the low bush blueberry. Can. J. Pl. Sci. **46**, 209.

Vegis, A. (1964). Dormancy in Higher Plants. A. Rev. Pl. Physiol. **15**, 185-224.

Villiers, T.A. (1973). Ageing and Longevity of Seeds in Field Conditions. 268-288 in 'Seed Ecology' ed. W. Heydecker, Butterworths.

Vincent, E.M. (1974). The ecology of some weed species in relation to their germination behaviour. Ph.D. Thesis University of Reading.

Vincent, E.M. and Roberts, E.H. (1977). The interaction of light, nitrate and alternating temperature in promoting the germination of dormant seeds of common weed species. Seed Sci. and Technol. **5**, 659-670.

Vincent, E.M. and Roberts, E.H. (1979). The influence of chilling, light and nitrate on the germination of dormant seeds of common weed species. Seed Sci. and Technol. **7**, 3-14.

Walter, H. (1963). Uber die Stickstoffanspruche (die Nitrophilie) der Ruderalplanzen. Mitt. flor.-soz. ArbGemeinsch. N.F., **10**, 56-69.

Watkins, F.B. (1966). Effect of nitrogen fertiliser on the emergence of wild oat (Avena ludoviciana). Queensland J. agric. Sci. **27** 49-53.

Watkins, F.B. (1971). Effect of annual dressings of nitrogen fertilizer on wild oat infestations. Weed Res. **11**, 292-301.

Wells, G.J. (1974). The biology of Poa annua and its significance in grassland. Herb. Abstr. **44**, 385-391.

Wesson, G. and Wareing, P.F. (1967). Light requirements of buried seeds. Nature, Lond. **213** 600.

Wesson, G. and Wareing, P.F. (1969). The role of light in the germination of naturally occurring populations of buried weed seeds. J. exp. Bot. **20**, 402.

Williams, E.D. (1983a). Effect of temperature, light, nitrate and pre-chilling on seed germination of grassland plants. Ann. appl. Biol. **103** 161-172.

Williams, E.D. (1983b). Effect of temperature fluctuation, red and far-red light and nitrate on seed germination of five grasses. J. appl. Ecol. **20**, (3) 923-935.

Williams, J.T. (1968). The nitrogen relations and other ecological investigations on wet fertilized meadows. Veröff. geobot. Inst., Zürich. **41**, 70-193.

Williams, J.T. (1969). Mineral nitrogen in British grassland soils. I. Seasonal patterns in simple models. Oecol. Plant. **4**, 307-320.

Williams, J.T. and Harper, J.L. (1965). Seed polymorphism and germination. 1. The influence of nitrates and low temperatures on the germination of Chenopodium album. Weed Res. 5, 141-150.

Woolley, J.T. and Stoller, E.W. (1978). Light penetration and light-induced seed germination in soil. Pl. Physiol. **61**, 597-600.