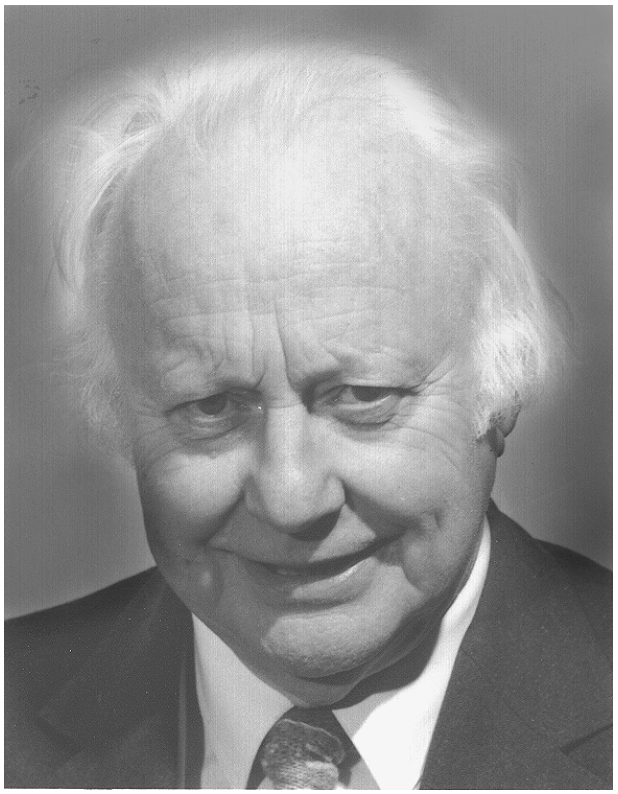
PERSONAL VIEW

A search for pattern and form

Eric H. Roberts
Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT, UK



Eric Roberts

Abstract

Seed science is a microcosm of biology which deals with a wide range of hierarchical levels of organization — from molecular biology to population ecology. Information at every level accumulates at an alarming rate. Making sense of it all calls for generalizations. Those which enable predictions to be made for circumstances different from those in which the observations were first made are particularly useful. Many scientists approach these problems by searching, consciously or unconsciously, for patterns or forms. Patterns for this purpose are defined here as discontinuities with some measure of repetition, whereas forms are thought of as continuous shapes in two or more dimensions; both can be visualized as spatial structures (most people have difficulty in conceptualising more than three or, at most, four dimensions). If one considers the whole range of hierarchical organization in biology, from molecules to communities, the recognition of the patterns and structures of processes at one hierarchical level of organization may assist in understanding the processes at adjacent levels, but such knowledge may not always be helpful in explaining processes at more remote hierarchical levels where different rules and mechanisms may predominate. Arbitrary curve fitting sometimes has a role in the recognition of the form of processes, but it is usually better to try and discover relationships where the coefficients have some biological meaning. Not only can a search for patterns and forms of processes help in the interpretation of data and the development of new ideas, but it can also sometimes help in the design of experiments. This personal view deals with some examples taken from seed research in the hierarchical levels in which I have been involved — usually somewhere between molecules and ecology.

Keywords: chromosome aberrations, seed dormancy, flowering, photoperiodism, pollination, quantitative models, seed viability, seed longevity

*Correspondence
Fax: (0118) 935-2421
Email: roberts@fal.u-net.com

Introduction

When I was invited to contribute a personal view, I wondered whether I had one which would be worth revealing. Thinking about this led me to discover that perhaps there was a theme running through my work which I had not properly recognised before, and one on which it may be worth commenting. Most of the

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

time, it seems, I have been searching for patterns or forms. I think many scientists work like this; but the fact that they do is often obscured by the editorial conventions of scientific journals (which, I understand, are relaxed for this self-indulgent piece). Further, this search for pattern or form is not unique to science: it seems to run through much of scholarship and the arts. Had I recognised earlier that this is what many of us do — sometimes unconsciously — then perhaps I might have made better use of this approach in developing my own abilities and those of my students.

Early influences

I was born in 1930 and from an early age I believe I had an interest in patterns and shapes: for as long as I can remember I was interested in the plastic arts. The biological aspects of this interest in form were encouraged when, immediately after leaving Lucton School in Herefordshire (where art was not taught), I worked for a year (1947–8) as a junior laboratory technician for the late Professor Eric Ashby (later Sir Eric and then Lord Ashby), one of the wisest and most sympathetic men I have known, and at that time Head of Botany at Manchester University. Eric Ashby was then primarily interested in experimental morphology — particularly as applied to the changes in leaf shape that occur with physiological age. I used to measure these changes on innumerable blueprints of leaves, and I also drew the illustrations of these and other diagrams for his papers and those of other staff in the department at that time. That year I also attended life-drawing sessions at the Mid-day Studio in Manchester and exhibited a small sculpture at the Manchester Academy of Fine Art and a couple of oil paintings at the annual show at Salford City Art Gallery (fortunately this exhibitionist tendency has not so far re-emerged). Typical Saturdays were spent visiting the various galleries in Manchester in the morning and playing rugby for Sale Football Club 'A' in the afternoon.

Following this exciting and formative year, at Eric Ashby's suggestion, I read botany at Manchester and was formally introduced by him to plant physiology in the lectures he gave to freshers. More advanced physiology was subsequently dealt with by the late Herbert Street and Philip Wareing — the former dealing with metabolic physiology and the latter with developmental physiology. Another influence at that time was Claude Wardlaw, Professor of Cryptogamic Botany, who had originally been a plant pathologist in the West Indies, but who by then had become an experimental morphologist who studied development as determined by the shoot apex, especially in ferns. After graduation I became a PhD student in Street's tissue culture laboratory and worked on techniques for maintaining individual root meristems in culture in perpetuity. Street was an enormous enthusiast. Innumerable research students passed through his care, all of whom, I think, had a very great affection for him. And so did I, though I did not always agree with his philosophy. He was a great believer in working at the bench and not 'wasting' time in the library. He also could not see the point of either vacations (illness was the only justification) or recreation, and so he could not understand why I should spend Wednesday afternoons 'wrestling' (I was in fact practising judo).

After Manchester I went to Cambridge for a year where I learned something of agriculture, plant breeding and a little more genetics. Some of the genetics was taught by that giant R. A. Fisher. I did not understand much of what he said, but I felt privileged to sit at his feet. The Cambridge interlude was a preparation for going to a small research institute in Sierra Leone known as the West African Rice Research Station. I spent eight years there (1955–1963), first as a plant breeder and subsequently as a crop physiologist. And it was out of the practical problems of breeding rice that I developed an interest in seed physiology and photoperiodism. In essence these problems were concerned with the timing of seed production, the genetic purity of that seed, the removal of dormancy from it in order to shorten the duration of breeding programmes by growing two or more generations a year, and the safe storage of genetic stocks of seed in a climate where viability could be lost very quickly.

After eight years in Sierral Leone, I returned to the UK and, almost by accident, became an academic back in Manchester (as Lecturer in Horticulture), where Claude Wardlaw was then Head. Shortly after my return to Manchester, Wardlaw took me to lunch to explain his system of management which, in essence, was to assume everyone was a gentleman [or a lady, one should now add] — a precept I have always tried to follow, with good but not infallible results. He also explained that he had a very fine staff but none, he feared, had known what it was to have had to crawl the last 200 meters back home on their hands and knees. I could tell that he assumed that I, having spent time in the tropics, would have had such experiences and that, he considered, was a plus on my CV. I had neither the heart nor courage enough to disappoint him merely for the sake of historical accuracy.

Five years later (1968), after spending an enlightening six-month sabbatical in Jack Hanson's laboratory at the University of Illinois, I moved to the University of Reading as Professor of Crop Production, a post I retired from in 1995. Throughout this academic period I kept the interest in seeds which I had developed out of practical necessity in Sierra Leone. I suppose all along I had, often unconsciously, been searching for patterns and forms, a pursuit which I will try and illustrate later. In this I have been encouraged by many research students and post-doctoral fellows who contributed a great deal to the

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

ideas outlined here, and whom I thank at the end of this article.

Concepts of pattern and form and their relevance

Before moving on to the examples which illustrate my theme, I need to clarify some terms. Since the words pattern and form can be used in several ways, I should explain how they are used here. The word pattern is often used to describe a repeating motif in two dimensions. I use it here in a similar way, but it need not be restricted to two dimensions, nor need the motifs of a pattern repeat exactly — there may be recognizable developments along one or more axes. One essential element in the notion of pattern as used here is an element of discontinuity. The recognition of the discontinuities is often a first step to recognising the pattern. Such patterns of discontinuity are often fundamental in the development of science, e.g. in the discovery of the periodic table in chemistry, or the development of the concept of family, genus and species in evolution and other aspects of biology.

The word form is used here to describe a continuous shape in two or more dimensions. In one sense any shape could be a form, but the existence of words like shapeless or amorphous imply that the word form often means something more organized. In the plastic arts Roger Fry (1920) in his book *Vision and Design* developed the idea of 'significant form' — a term invented by Clive Bell who had claimed it was at the root of all aesthetic experience. The term later fell out of favour because the arguments for it , it was said, were circular. However, it does express the view that some forms have more aesthetic significance than others. But for my purpose here I sought something relevant to science and more easily defined. This led me to recognise that many forms which turn out to be useful in science can be described by a few, relatively simple, parameters. Further — and here is a meeting ground with art — such forms also tend to appear strong, satisfying, and even beautiful. Examples of how apparently complicated and rather beautiful forms or patterns can be described by a few parameters are shown in Fig. 1.

So why should we be looking for such patterns and forms in science? I remember reading somewhere that 95% of all the scientists the world has known are currently living — a statistic which helps to explain the frighteningly rapid and accelerating growth of scientific information. This information explosion could be dispiriting. It is only made tolerable by theories which explain how some of the facts fit together and which enable predictions to be made about other facts, events, properties and behaviour in different circumstances. The patterns or forms which lead to the theories typically employ only a few parameters — certainly fewer than the number of facts they explain or predict

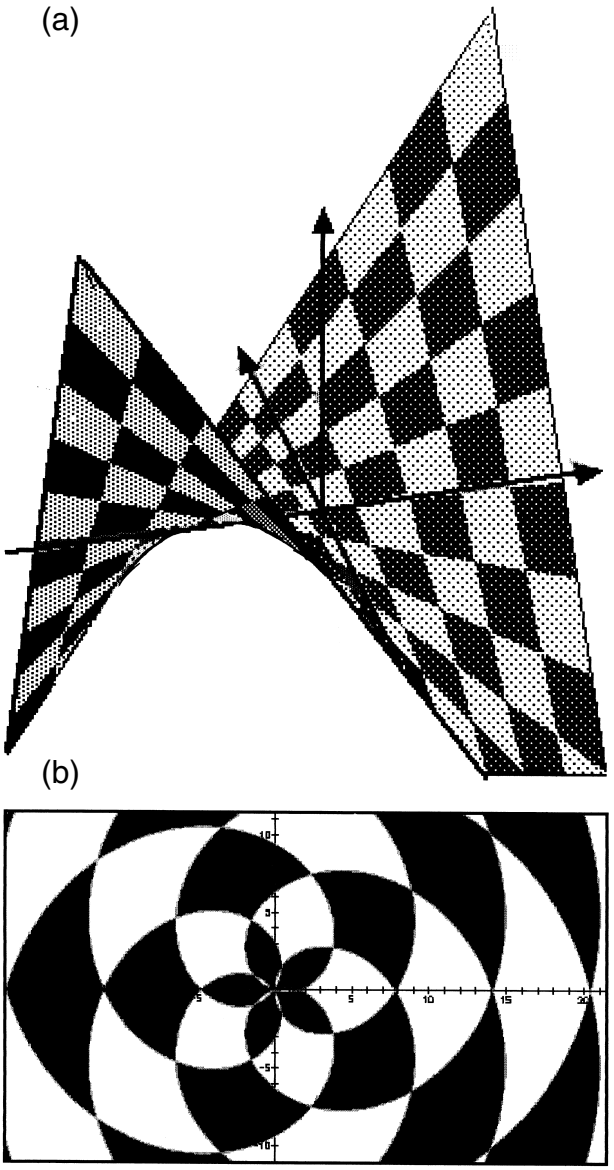


Figure 1. Apparently complicated forms and patterns often have simple solutions: (a) Graph of $z = xy$, a three-dimensional form which looks complicated but is one of the simplest to describe (reminiscent, perhaps, of the brim of the helmet designed by Michelangelo for the Swiss Papal Guard); (b) Graphical representation of the pattern cos5Θ < sin*r* (reminiscent of a phyllotactic diagram).

(This is fortunate for people with a poor memory, such as I, for it means we do not have to remember as much.). Thus, while gathering observations is essential to science, it complicates our view of the world; whereas pattern and form recognition can help to simplify it and enable us to make better use of the information. Such recognition can also transform apparently meaningless information into meaningful

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

knowledge. I think the process relates to intelligence: Barlow (1983) pointed out 'A satisfactory definition of intelligence has never been found . . . but theory and practice of information handling have clarified what it does for us: it enables us to guess better, and the *discovery of unexpected orderliness* [my italics] is the chief means of doing this.' In other words, he said, 'it is the capacity to guess right by discovering new order.'

The complexity of biology, which pattern or form recognition seeks to simplify, is structured at different hierarchical levels. Usually we consider that molecular biology lies at the base (or, depending on one's point of view, the head) and provides the blueprint of everything else — the other hierarchical levels, in order, being organelles, cells, tissues, organs, organisms, populations and communities. But while knowing its genetic code may predict some of the properties and behaviour of an organism, there will be much it cannot explain. The trick is to discover the simplifying rules or theories which help to explain what happens at the hierarchical level of interest. Such knowledge may also be relevant to understanding other levels, but less so as they become more remote. For example, it is not difficult to appreciate that a quantitative understanding of inter-specific plant competition and the related concept of optimum economic plant population density — of fundamental importance in agronomy — is unlikely to be helped directly by molecular biology. By a similar token it would not be helpful for molecular biologists to concern themselves with hadrons — indeed, some may not even know what they are. If so, they need not worry about it. Or, moving in the opposite direction, there is not much in ecology to illuminate molecular biology, but it may well assist the understanding of evolutionary pressures and physiological adaptations. Although much of this is obvious, unfortunately it is not always clear to all those who fund science.

In this article examples of pattern recognition will be chosen from those levels of organization in seed biology which deal mostly with whole organisms and populations. The examples I have chosen will be taken more or less in order of developmental sequence from seed production and on through seed dormancy to loss of viability. However, loss of seed viability will be taken before some of the deteriorative events which precede it, as I think the link between the two is easier to describe in that way.

Examples of pattern and form recognition in seed research

The timing of seed production

The West African Rice Research Station was situated in Sierra Leone but was meant to do work relevant also to all other British West African countries at that time — Gambia, Ghana and Nigeria. These territories included a wide range of latitudes, growing seasons, and therefore daylengths. Responses to temperature and especially photoperiod are crucial, as we now know (Roberts *et al*., 1993, 1997a, 1997b), in the adaptation of crops to their environment because the timing of ripening and harvest is crucial. We were beginning to sense this. It seemed important to me that we should understand more about the quantitative effects of photoperiodism in adaptation, and so I designed and built a suite of ten growth cabinets (Roberts, 1962a) to study this and the interaction of photoperiod with temperature. The work gave some quantitative insight to the problems (Roberts and Carpenter, 1962; Roberts and Carpenter, 1965). And much later at Reading, with other colleagues, I returned to this topic in rice (Collinson *et al*., 1992; Summerfield *et al*., 1992). But this early work in Sierra Leone also gave me a more general interest in photoperiodism, one I was glad to indulge when, in 1973, I became Director of the Plant Environment Laboratory at Reading.

This new responsibility led to a productive scientific partnership with Rod Summerfield (who was responsible for managing the enterprise) and others. Through a series of grants from the government (Overseas Development Administration), we were able to work on the photothermal flowering responses of a sequence of crops, especially grain legumes and cereals. From this experience I became aware of a geographical pattern of crop photothermal responses in relation to their geographical origins, from which it was possible to conclude something which seems obvious on reflection, but I have not seen previously stated: long-day plants have arisen in Mediterranean or temperate climates, whereas short-day plants (with a few explicable exceptions) have arisen in the tropics (Roberts 1991; Roberts *et al*., 1997a, 1997b). This pattern can be understood if it is assumed that generally plants have been selected to produce seeds towards the end of a favourable growing season, when sufficient photosynthate has accumulated to produce a large crop of propagules, and in a period which avoids inclement conditions which would jeopardise the quality of the seeds. In addition to vernalization (a specific cool-temperature response common to many variants of long-day plants which permits a subsequent response to long days), there is also a basic temperature effect on flowering — dealt with later — which is apparently universal in short-day, long-day and day-neutral plants.

At Reading we resolved the apparently complex effects of photoperiod and the basic temperature response into a relatively simple quantitative model (Roberts *et al*., 1993). This allows the time of flowering

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

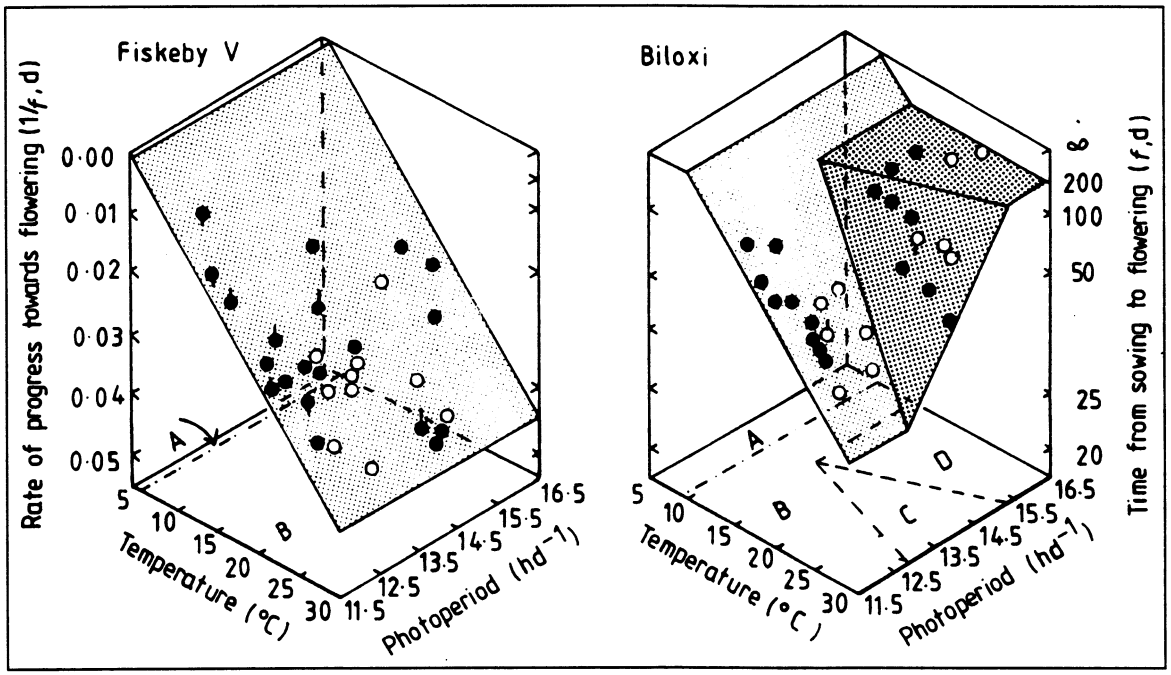


Figure 2. Effects of photoperiod and temperature on the rate of progress from sowing towards first flowering (*left vertical scale*); transformed to days to flower (*right vertical scale*) in soyabean cultivars 'Fiskeby V' (photoperiod-insensitive) and 'Biloxi' (photoperiod-sensitive). Results from six contrasting sites in Australia from 1986 to 1988 (●) and one in Australia and two in Taiwan in 1989–90 (○). Vertical lines indicate deviations of observations from fitted planes. Projection of the boundaries between response planes to the base (*broken lines*) show four environmental domains where progress towards flowering is *A* impossible (too cool), *B* solely temperature-dependent, *C* dependent on both photoperiod and temperature, and *D* maximally delayed by photoperiod and unresponsive to variation in either photoperiod or temperature. Response in domain *B* is defined by Equation (1), in domain *C* by Equation (2) and in domain *D* by Equation (3). From Summerfield *et al*. (1993).

of any genotype to be predicted in almost any climate from relatively few observations; it can also be used for screening germplasm collections (Roberts *et al*., 1996) or facilitating the genetic analysis of photoperiod-sensitivity genes and any epistatic interactions between them (Upadhyay et *al*., 1994).

The key to the perception of the relevant form in this case was the recognition that the time taken to complete a journey depends on the rate of progress, and not *vice versa*. In a motor car the rate of progress can be measured directly by looking at the speedometer. In the case of progress towards flowering, however, there is no instrument which can serve this purpose; one can only measure rate indirectly and retrospectively. In this case we can learn from enzymologists who cannot measure rates of enzyme reactions directly, but do so retrospectively by taking the reciprocal of the time taken to complete them. Thus, by analogy, we transformed the times taken to flower to rates of progress towards flowering by taking their reciprocals. Several benefits emerged when we followed this approach, chief amongst which were that (1) responses become linear, (2) over wide ranges of conditions (the limits to which can be defined) interactions between photoperiod and temperature disappear, and (3) the separate effects of genes controlling photoperiod and temperature responses can then be analysed independently. (Without transformation, effects of temperature and photoperiod *apparently* interact and consequently proper genetic analysis of the controlling genes is prevented.) Moreover, the value of the rate-of-development coefficients controlled by these genes turned out to be independent of climate — they are true genotype descriptors — and so, once determined, *could be applied any time, anywhere*, thus describing the phenotypic response in any environment. All this was almost too much to have hoped for. The model did not depend on any molecular or biochemical theory of photoperiodism, nor could it because unfortunately, in spite of much effort since photoperiodism was discovered 80 years ago, there is so far no such satisfactory theory.

The response of cultivars of soyabean to photoperiod and temperature (Summerfield *et al*., 1993; Roberts *et al*., 1996) (Fig. 2) illustrates the form of

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

the model which is described by three simple equations defining three intersecting planes — rather like roofs. In all cases there is a fundamental mono-pitch roof — the temperature response (for temperatures which do not exceed the optimum). Then, in the case of photoperiod-sensitive plants, there is also a flat-topped gable (the photoperiod response) which emerges at right-angles from the mono-pitch and describes the delays to flowering caused by inhibitory photoperiods (i.e. in daylengths greater than the critical photoperiod).

The mathematical description is as follows. In photoperiod-insensitive plants, or in short-day plants in photoperiods less than a critical photoperiod (see later) the time taken to flower in days (f) is given by

$$1/f = a + bT \quad (1)$$

where T is mean temperature (°C) and a and b are genotypically determined constants. Above the critical photoperiod both photoperiod and temperature affect the rate of progress ($1/f$) but do not interact and, under these conditions, the rate is given by

$$1/f = a' + b'T + c'P \quad (2)$$

where P is daylength (h) and a' b' *and* c' are genotypically determined constants. The maximum delay caused by increase in daylength occurs at the ceiling photoperiod (P_{ce}) in excess of which there is no further delay in flowering and where the time taken to flower is affected by variation in neither daylength nor temperature. Accordingly this plane is described simply by

$$1/f = d' \quad (3)$$

where d' is a genotypically determined constant. A little algebra shows that the critical photoperiod (P_c) and ceiling photoperiod (P_{ce}) may also be described in terms of these genotypically determined constants and are given, respectively, by

$$P_c = [a + a' + T(b - b')]/c' \quad (4)$$

and

$$P_{ce} = [d' - (a + b'T)]/c' \quad (5)$$

With only minor changes, the pattern described by this family of equations also describes the responses of long-day plants, and so far they have been shown to apply to all 19 species in which they have been tested (Summerfield *et al*., 1997). Thus once the six genotypic constants (a, b, a', b', c', d') have been determined from observations in a few contrasting environments, it is possible to predict the time taken to flower in any other environment (Roberts *et al*., 1996), and this can form the basis on which the time of seed maturation is calculated. The recognition of the forms described by these equations enables a few appropriate natural environments to be identified which are suitable for screening germplasm in order to estimate the value of the coefficients which predict how the accessions would respond in any other environment (Roberts *et al*. 1996, 1997a, 1997b).

Hypothetical flowering promoters and inhibitors are emphasised in the photoperiod literature. The forms described here say little about the molecular or biochemical mechanisms involved. They do, however, emphasise the importance of inhibitors, since the main behavioural difference between photoperiod-insensitive and photoperiod-sensitive cultivars in this short-day plant, *Glycine max*, is the delay in flowering caused by long days. This can be seen by comparing a typical photoperiod-sensitive cultivar (Biloxi) with an insensitive one (Fiskeby V) in Fig. 2. Thus if promoters, and especially inhibitors, are to be sought, these forms indicate the most promising environmental conditions in which to seek them.

Cross-pollination conundrum

One aspect of seed quality is genetic purity. In this context it is important for plant breeders and seed producers to know what precautions they need to take to prevent unwanted cross-pollination. Although it was well-known that rice is mainly self-pollinating, it was also known that some cross-pollination can take place. Whether the amount varies between climates was not clear, but at that time no investigation had been carried out in Africa. It seemed to me when I first became involved in the breeding programme in Sierra Leone that we ought to know more about the frequency of cross-pollination so that we could either be reassured or take more stringent precautions to prevent it.

Experimental designs to determine the amount of cross-pollination normally depend on growing two similar cultivars closely together, where one of them has an easily identifiable character determined by a dominant allele or homozygous recessive alleles. But then comes the question: in what pattern should the plants be grown? It occurred to me that it would be most convenient if one were to adopt a pattern in which each plant containing the homozygous dominant alleles was surrounded, at any given distance, by an equal number of plants of its own kind and of plants containing the double recessive alleles and *vice versa*. Then if one grew the progeny of those plants showing the recessive character, the number of these which expressed the dominant phenotype would estimate half the number of cross-pollinations which had occurred in the plot. Doubling this number and expressing it as a percentage of the total number of progeny would estimate the total amount of cross-pollination taking place within the plot. But was there a pattern which would meet these criteria?

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

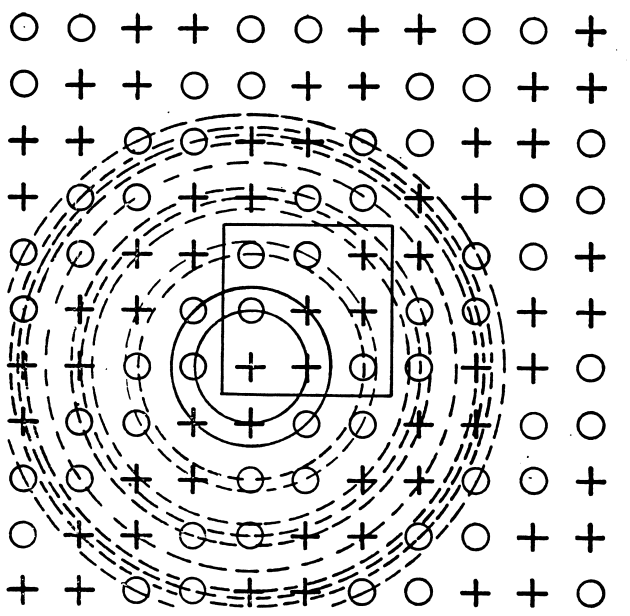


Figure 3. Planting diagram of a cross-pollination experiment. Each small circle represents a plant of a cultivar homozygous for the dominant allele (coloured apiculus) and each cross represents a plant of a cultivar homozygous for the recessive allele. Concentric circles have been described round one of the 'recessive' plants to demonstrate those plants which are equidistant from it for the 12 innermost concentric circles of plants. In the immediate neighbourhood of any plant (solid concentric circles) there is an equal number of plants of each cultivar equidistant from it. From Roberts *et al*. (1961).

Exploring possibilities with a sequence of doodles, rather like giant noughts-and-crosses (or tick-tack-toe) games, suggested a pattern which came close to the ideal (Fig. 3) (can you think of a better one?). For the two innermost concentric circles of neighbours, the ideal is in fact achieved. Further away the pattern deviates slightly from the ideal, but this is probably not important since, by analogy with spore dispersal studies, the probability of cross-pollination would be inversely proportional to the square of the distance. Experiments based on this design in both Sierra Leone and what is now the Republic of Guinée showed that percentage cross-pollination, under growing conditions which maximise it, gave one per cent or less cross-pollination; and so the precautions we needed to take to prevent unwanted crossing did not have to be very stringent (Roberts *et al*., 1961).

Seed dormancy

It was this early experience as a rice breeder which also led to an interest in seed dormancy. We were trying to devise techniques which would enable us to hasten the production of finished cultivars from an initial hybridization — a process which normally took about ten years. Each generation, one per year, was grown in its natural season. No selection is possible in F_1, and the very large amount of segregation in F_2 and F_3 suggests that, apart from the removal of completely ill-adapted phenotypes, no serious selection for the target environments is feasible until F_4 and beyond.

It is not until about F_6 or F_7 that segregation is sufficiently reduced to allow three years of field trials in different environments to identify improved pure lines for release. Since no selection was feasible in the first few generations, we argued that we should try and get through these as rapidly as possible, and any environment would do. To do this required solutions to two main problems: first a system for decreasing crop duration (time from sowing to harvest); secondly a method for removing seed dormancy. Clearly in both cases physiological rather than genetical solutions were required, otherwise one would end up with very short-duration cultivars liable to sprout in the panicle.

Enough was already known about photoperiodism in rice to devise a method for reducing crop durations satisfactorily, and we had contributed a little to the understanding of the quantitative relationship between daylength and flowering in this species (Roberts and Carpenter, 1962) — a glimmer of what later influenced what was described in the previous section; and so we grew the early generations in artificially shortened days (by wheeling plants grown in hydroponics in and out of a dark shed). The second problem of physiologically removing seed dormancy was solved by storing seeds at 47°C for 7 days immediately after harvest (Carpenter and Roberts, 1962). This solution arose out of the perception of the form in which temperature affects populations of dry seeds (which is quite different from the the way it affects moist seeds) (Roberts, 1962b). The key to this was the simple observation that when seeds are stored under constant environmental conditions and germination tested at intervals, the resulting sigmoid curves which show the progress of germination capability of the seed population are in fact cumulative normal distributions or ogives; in other words the individual dormancy periods of seeds in a population are normally distributed (Roberts, 1961a). This somewhat trite observation nevertheless not only led to a profitable approach to some aspects of seed dormancy, but also to a better insight to viability problems (see later).

Figure 4 shows the loss of dormancy with time when rice seeds of one cultivar are stored at five different temperatures. Curves were fitted to the results by probit analysis, i.e. on the assumption that the individual seed dormancy periods were normally distributed. From results such as these on six cultivars

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

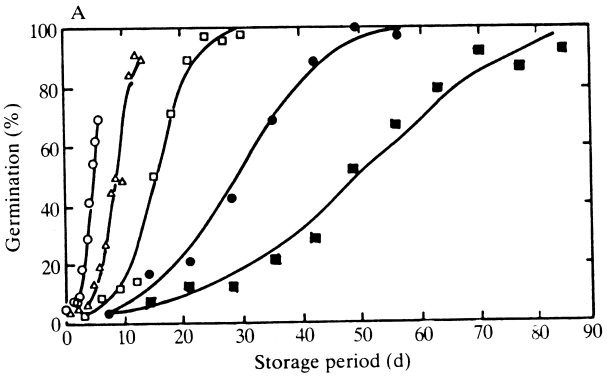


Figure 4. Progress of germination capability of seeds of rice, *Oryza sativa* cv Lead 35 when stored at different temperatures and germinated at 32°C. Storage temperatures: 27°C (■), 32°C (●), 37°C (□), 42°C (△), 47° (○). From Roberts (1965).

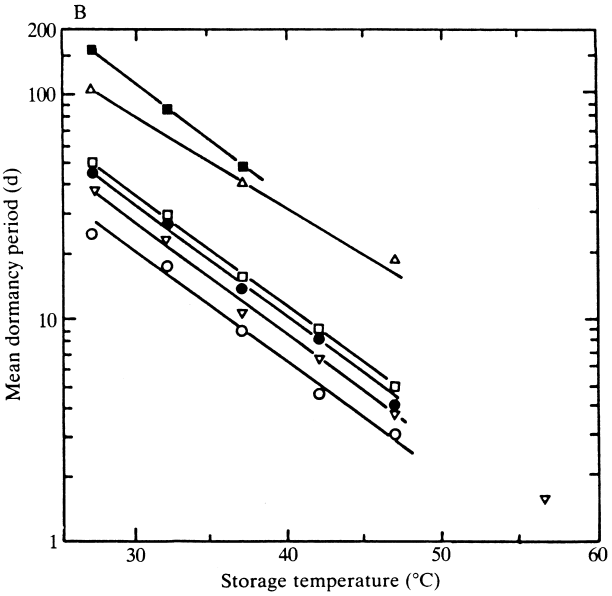


Figure 5. The effect of storage temperature on mean dormancy period of seeds in cultivars of the two domesticated species of rice. *Oryza glaberrima* cvs: Masalacci A4 (■), Bayawuri (△). *Oryza sativa* cvs: Lead 35 (□), India Pa Lil (●), Nam Dawk Nai (▽), Mas 2401 (○). Germination tests at 32°C. From Roberts (1965).

(Roberts, 1965), mean dormancy periods for each temperature were estimated (roughly equivalent to the time taken for half the seeds to lose dormancy), and these results are plotted in Fig. 5. With one exception (cv. Bayawuri where there were some experimental difficulties), a common slope could be adopted for all other linear regressions. This means that the Q_{10} for all cultivars is the same (in this case 3.38). The value is higher than for most enzyme reactions and, uncharacteristically for such reactions, does not vary with temperature over this relatively wide range. The relatively large Q_{10} is more typical of an uncatalysed chemical reaction, but we do not know which. Nevertheless, though we do not know the mechanism, the common Q_{10} suggests (not unexpectedly) that it is the same for all cultivars — in spite of the very big differences in mean dormancy periods at near-ambient temperature (27°C), which varied between cultivars from 12 to 160 days (Fig. 5). However, because of the semi-logarithmic relationship it emerged that, for *Oryza sativa* cultivars at least, the mean dormancy periods and also the inter-cultivar variation in them, were relatively small at 47°C — from about 3 to 5 days (see *Oryza sativa* cultivars in Fig. 5). Thus a common treatment of 7 days at 47°C was likely to be suitable for all *sativa* material, and we used this successfully as a practical technique. Temperatures greater than about 47°C would have been dangerous because under these conditions significant loss of viability could have occurred before completing the dormancy-removal treatment (see the section on viability later).

Interactions between dormancy-removal agents

The less intensively domesticated West African *Oryza glaberrima* varieties were little used in breeding programmes at that time (the 1950s), but currently there has been more interest in them, and some cultivars derived from *sativa* × *glaberrima* crosses are very promising in West Africa. Although the form (equation) relating dormancy to the environment in this species is very similar to that of *O. sativa* (Fig. 5), dormancy is generally much more pronounced (larger values of the intercept constant) in the *glaberrimas*. Consequently, a longer warm-temperature treatment would be needed to remove dormancy; but such a treatment is not satisfactory because viability begins to fall before dormancy is lost. Much later in Reading, we investigated alternative dormancy-releasing treatments which would be suitable for this species as well as the *sativas*. The alternative depended on exploiting positive interactions between dormancy-breaking treatments (which, as will be discussed later, we now know are very common): it involved a combination of pretreatment in 0.1M HNO_3 for 24 h followed by further 24 h in 0.25M H_2O_2 (both at 20°C), and then germinating in an alternating temperature regime (16 h at 34°C/8h at 11°C) in a medium of 0.01M 2-mercaptoethanol (Ellis *et al.*, 1983).

This is an example of a very complex interaction involving five different agents — acidity (H^+ ions), NO_3^- ions, peroxide, alternating temperature and a sulphydryl compound, 2-mercaptoethanol (which is more effective than any other -SH compound we have

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

Table 1. Percentage seed germination of three weed species after 4 weeks in the presence or absence of diffuse daylight, alternating temperatures (8h at 25°C/16h at 15°C), and 10^{-2}M KNO_3. Data from Vincent and Roberts (1977)

Dark				Light			
const. temp.		alternating temp.		const. temp.		alternating temp.	
water	nitrate	water	nitrate	water	nitrate	water	nitrate
Chenopodium polyspermum							
0	0	0	0	1	26	0	41
Rumex crispus							
0	10	0	40	0	100	0	100
Stellaria media							
0	6	0	45	54	54	100	98

tried). We were led to develop this complex treatment because earlier work on the seed ecology of weed species had shown how frequent and important are positive interactions between normal environmental factors which affect dormancy: they are more often the rule than the exception (Roberts, 1973b; Vincent and Roberts, 1977; Roberts *et al.*, 1987).

We studied the seed dormancy of annual weeds because their opportunistic behaviour, which is typical of plants which colonize disturbed habitats, seems to depend very largely on their germination characteristics. In particular they have the ability to remain dormant, often for decades, when buried; but they tend to germinate rapidly when exposed by soil disturbance, especially in certain seasons. It is this opportunistic behaviour which makes them so difficult to control. Accordingly, we have studied four of the environmental factors which, the evidence suggested, are mainly responsible for enabling small seeds to detect and respond to their proximity to the soil surface and also to respond to appropriate seasonal signals by losing their dormancy. The main responses involve sensitivity to stratification (cool temperatures on moist seeds), alternating temperatures, light, and nitrate ions (Roberts, 1981; Roberts *et al.*, 1987).

The possibility of interactions has not always been given the attention it deserves. There are a number of reasons for this. Laboratory scientists like to keep things simple and often prefer to investigate one thing at a time, keeping everything else constant; indeed I was encouraged to do this as an undergraduate. Field agronomists are more used to factorial designs which expose interactions, but large interactions are not all that common in the sorts of things which they tend to investigate. Failure to recognise the possibility of interactions in seed dormancy has led to some misleading reports; e.g. some light-sensitive seeds which were reported to be insensitive to light before burial and develop sensitivity during burial (e.g. Wesson and Wareing, 1969) were, in fact, probably sensitive before burial. They were probably recorded as insensitive to light because it was not recognised that the expression of light sensitivity in many species is often dependent on simultaneous exposure of the seeds to alternating temperatures (e.g. Vincent and Roberts,1977; Roberts and Totterdell, 1981). In Wesson and Wareing's laboratory constant temperatures had been used but, in the field experiments reported, the seeds would also have automatically been exposed to ambient temperature alternations when a layer of soil had been removed to expose the seed to light.

Most of us have some difficulty in thinking clearly about interactions and, as politicians say, this may be partly a matter of presentation rather than substance. When politicians emphasise that public misunderstanding is a matter of presentation we should immediately become sceptical; but when dealing with interactions in biology, we really do need all the presentational help we can get. Tables showing the results of factorial experiments tend to induce headaches. Table 1 is a relatively simple example, but it helps to illustrate the point. It shows the results from 2^3 factorial experiments on three species taken from an investigation on the effects of the presence or absence of light, alternating temperatures, and potassium nitrate on removing seed dormancy (Vincent and Roberts, 1977). Most people, I believe, find it difficult to discern patterns from tables such as this, and so for a while we used Richards diagrams (Richards, 1941) to think about and report the results of this type of experiment. Figure 6a shows the results for *Stellaria media* taken from Table 1 expressed as a Richards diagram. In this format the results (% germination in this case) are plotted against the number of potentially promotory factors in each of the treatment combinations, viz. 0, 1, 2, 3 in this type of experiment. These diagrams result in a series of quadrilaterals, which are constrained to parallelograms if there are no interactions. Departures from parallelograms indicate interactions. A distortion resulting in a beak on the right which points upwards indicates a positive interaction, whereas one pointing downwards

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

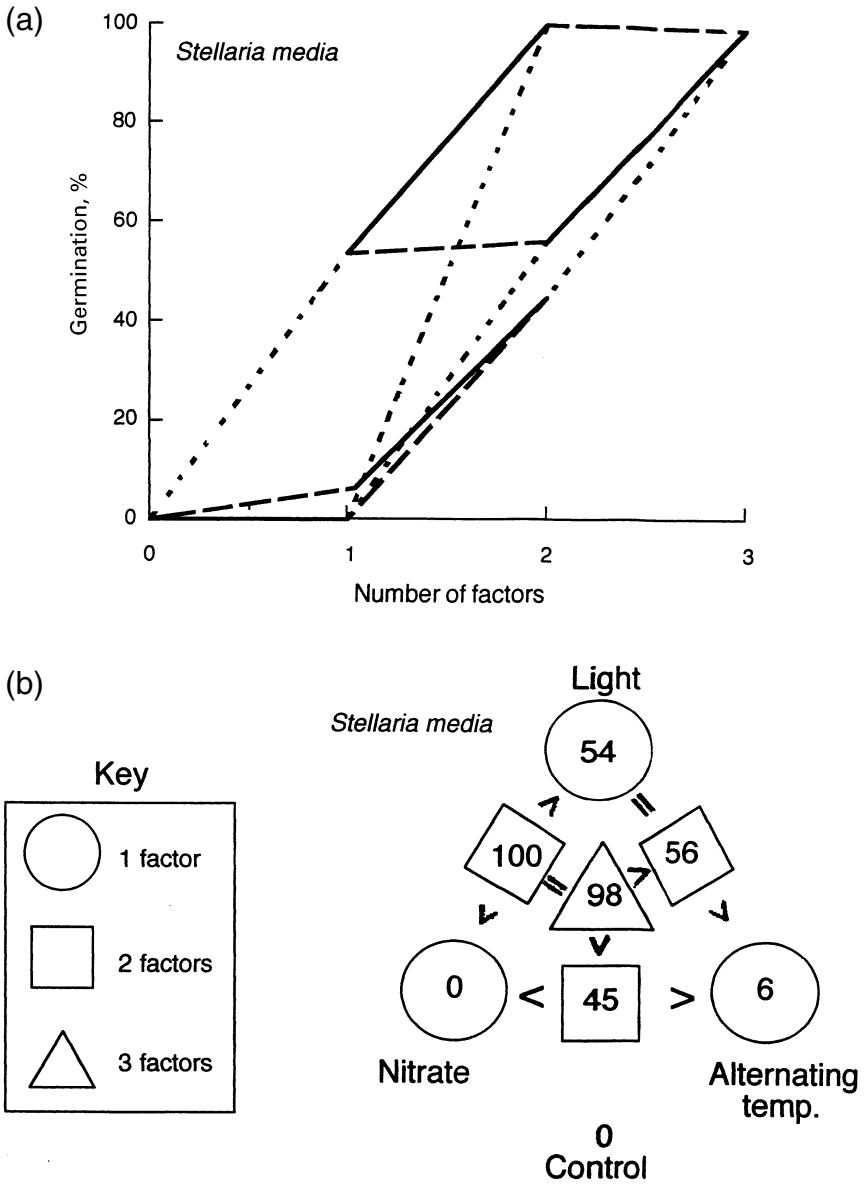
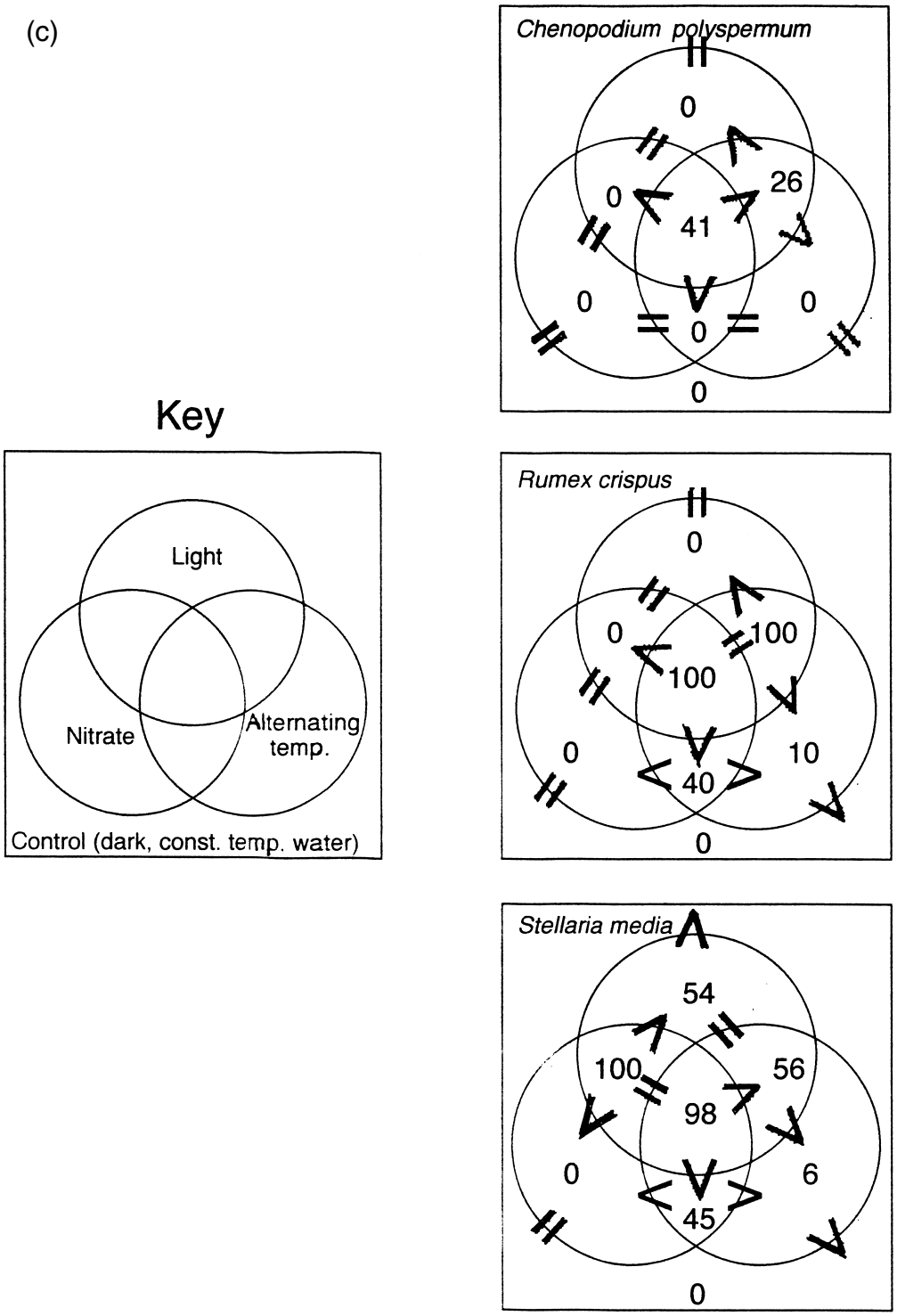


Figure 6. Different ways of presenting the results of 2^3 factorial experiments shown in Table 1. (a) Richards diagram for the germination of *Stellaria media* in which the control (no potentially stimulatory factors) (0 on the *x*-axis) is considered to be a constant temperature applied to the seed imbibed in water in the dark. The three results of adding light (·····), or alternating temperatures (------), or nitrate (——) to the control are shown above 1 factor on the *x*-axis. Further additions lead to the three results shown above the 2-factor position on the *x*-axis, and there is single result above the 3-factor position. (b) Pawn-broker sign diagrams of the same results for *Stellaria media* in which the percentage germination of the control is shown beneath the sign, the germination of the three single-factor treatments in the three balls, the three 2-factor results in rectangles between the respective balls, and the single 3-factor result in the central triangle. Significant differences are indicated by > whereas identical or non-significant differences are indicated by =. (c) Venn diagrams for experiments on *Chenopodium polyspermum, Rumex crispus* and the same results for *Stellaria media* as in (a) and (b) in which the results are shown as sets, with the control treatment outside all sets. Significance indicated as in (b). Original data from Vincent and Roberts (1977).

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press



indicates a negative interaction. Thus, for example, in Fig. 6a in *Stellaria media* there is clearly a first-order positive interaction between nitrate and alternating temperature, and between nitrate and light. Note that *none* of the three potentially stimulatory factors had any effect on its own.

For the most simple of factorial experiments, 2^2, Richards diagrams are tolerably clear to all, and they have recently been used effectively to illustrate changes with time in the dormancy status of *Avena fatua* seeds during burial when subsequently exposed to light and/or nitrate (Murdoch, 1998).

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

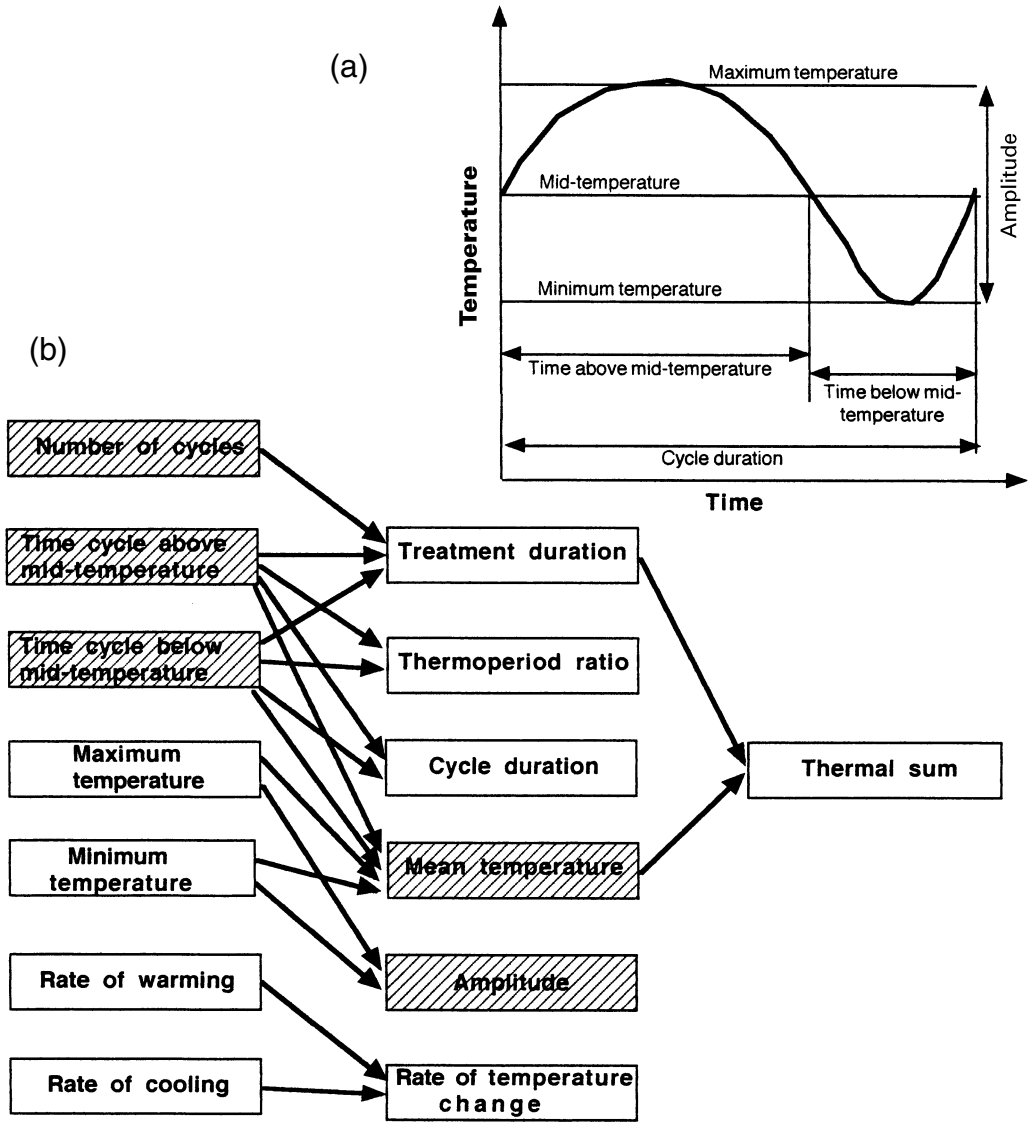


Figure 7. (a) A stylised diurnal temperature cycle illustrating some of its characteristics. (b) Characteristics of temperature cycles which could conceivably affect their dormancy-removal efficacy; those thought to be most important, based on experiments of the type illustrated in Figs. 8 and 9, are hatched. Note the dependencies amongst them, and that it is not possible to change the value of any of the characteristics without confounding it with a change in at least one other. Modified from Murdoch *et al.* (1989).

Although Richards diagrams are ingenious, those unfamiliar with them often need some time before they grasp how a 2^3 diagram can be interpreted, and more complicated designs (e.g. Heath, 1970) result in fiendishly daunting webs. Further, when some treatment combinations in a factorial experiment happen to produce identical values, the resulting overlapping lines are difficult to represent, and the diagrams become particularly difficult to interpret. Therefore, for a while I reverted to a different convention — a 'pawnbroker sign' device — which I had earlier developed for displaying the results of 2^3 factorial experiments (Roberts, 1973b) (Fig. 6b). It was only much later that it dawned on me, having read an article by Edwards (1989), that the obvious device to use for displaying and understanding the results of this type of experiment is a Venn diagram. This is probably the least complicated solution, and the meaning is more immediately apparent to everyone. Compare Fig. 6c with Table 1: clearly the existence of first and second order interactions are much more obvious in the Venn diagrams. I wonder

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

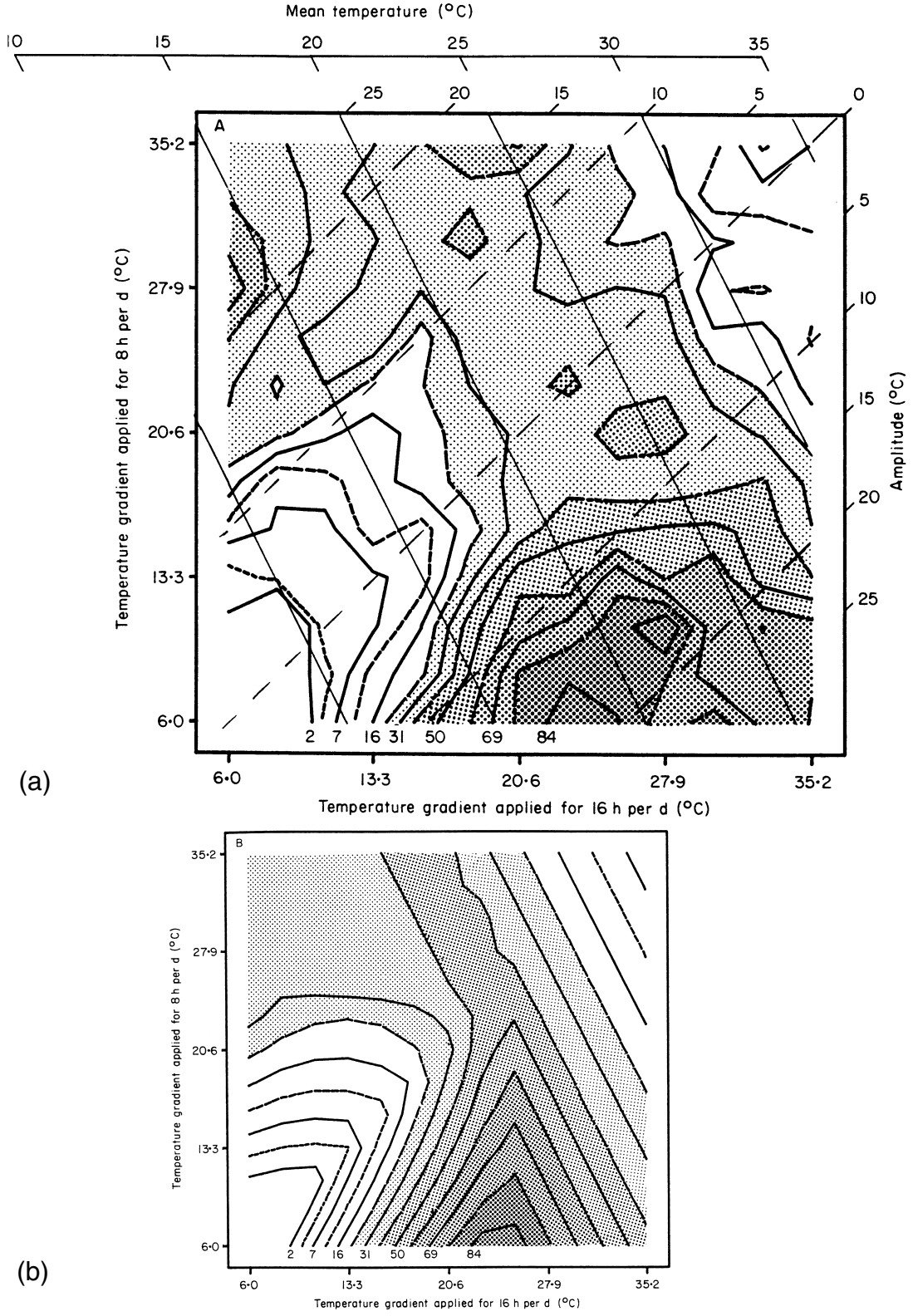


Figure 8. (a) A map showing the germination percentage of seeds of *Chenopodium album* after 24 days on a double-vector temperature-gradient plate. (b) Values fitted according to the model described in the text and illustrated in Fig. 9. From Murdoch *et al*. (1989).

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

how I could have overlooked such an obvious device. Other recent examples of Venn diagrams in seed research can be found in Carmona and Murdoch (1995).

Stimulatory characteristics of alternating temperatures

The examples of interactions given above included only one alternating-temperature regime, but clearly some alternating-temperature treatments are more effective than others. Investigating the characteristics which make a regime more or less effective is, however, not a trivial matter. Alternating temperatures are difficult to investigate because they have a number of different characteristics which might conceivably affect their dormancy-releasing properties. We have pointed out that there are 13 or 14 such characteristics, even if one does not venture outside 24 h cycles (Roberts *et al.*, 1987; Murdoch *et al.*, 1989). Figure 7 is an updated diagram which illustrates these and makes clear that it is not possible to change any one of these characteristics without changing at least one other. Try it out if you are sceptical. In other words confounding of treatments is inevitable. How then does one make progress?

In view of the inevitable confounding, it seemed to me that the only way forward was to carry out experiments involving a large number of treatment combinations and then adopt a modelling approach, i.e. attempt to discover minimal-parameter models which would fit a wide range of treatment combinations. It was this approach which, in spite of the confounding problems, enabled certain characteristics of alternating-temperature treatments to be identified which are more important in determining regime efficacy, and these are hatched in Fig. 7.

However before reaching these conclusions, it was necessary first to develop some apparatus which could produce the large number of environments required. At the time I was aware of single-gradient temperature plates which could produce a range of constant temperatures. Thinking about these led me to the concept of a square plate in which a gradient could be established in one direction for part of the day and another at right-angles to it for the rest of the day, and so we produced a prototype. I did not realise we were not quite as original as I had thought, because an apparatus based on exactly this concept had already been developed by Larsen and Skaags (1969). Our prototype worked quite well and, flushed with our success, my colleague Alistair Murdoch did some proper engineering, and we produced an apparatus which was subsequently commercially developed by Grant Instruments, Cambridge (and which is still available to order). A description and illustration of the pre-commercial version appears in Murdoch *et al.* (1989). An example of some results from this article is shown in Fig. 8.

One useful way of displaying the results from a double-vector thermo-gradient plate such as this is to construct a map of the plate showing isopleths of equal germination (Fig. 8). I have already touched upon the idea that the distribution of the individual dormancy periods is normal with respect to storage period. It is also normal with respect to variation in many other factors. Accordingly, the isopleths of percentage germination shown in Fig. 8 are shown at unequal percentage intervals which relate to equal sub-divisions of probits or normal equivalent deviates. This procedure tends to linearize the gradients and clarify the presentation and interpretation of the resulting 'contour' map. While a typical map from this type of experiment shows a complicated 'topography', it is possible to interpret the shapes and determine which characteristics dominate the topographical responses by superimposing several different scales upon the map — viz. temperature applied for the shorter period in the diurnal cycle (ordinate), temperature applied for the longer period (abscissa), amplitude (forward diagonal lines), and mean temperature (backward diagonal lines) (Fig. 8). Examination of the orientation of the germination isopleths in relation to these scales for experiments on several species makes it evident that the extent to which germination is stimulated depends primarily on (1) amplitude, (2) the ratio between the period at the upper and lower temperatures in the cycle (sometimes known as thermoperiod or periodic time), and (3) mean temperature. Accordingly, an empirical model was developed which was based on these observations.

Essentially the model recognised that, after transformation to probits (or normal deviates) germination in *Chenopodium album* increased approximately linearly with increase in constant temperature up to about 25°C (Fig. 9). As temperature increased above this break point, germination decreased linearly with increase in mean temperature, and at these supra-optimal temperatures most of the variation is explained by mean temperature alone, i.e. there is no detectable effect of alternating temperatures. But below the break point there is a large effect of diurnal amplitude, and the effect of amplitude increases linearly with a decrease in mean temperature. Within this lower range of mean temperature, there is also a clear effect of thermoperiod. Symptomatic of this is the pattern of fitted lines showing the effect of amplitude which are further apart in Fig. 9(b) as compared with Fig. 9(a).

This model appears to apply to at least several unrelated species. The mathematical description is given in Murdoch et *al.* (1989), but I will not go into it here because we consider it to be an interim solution

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

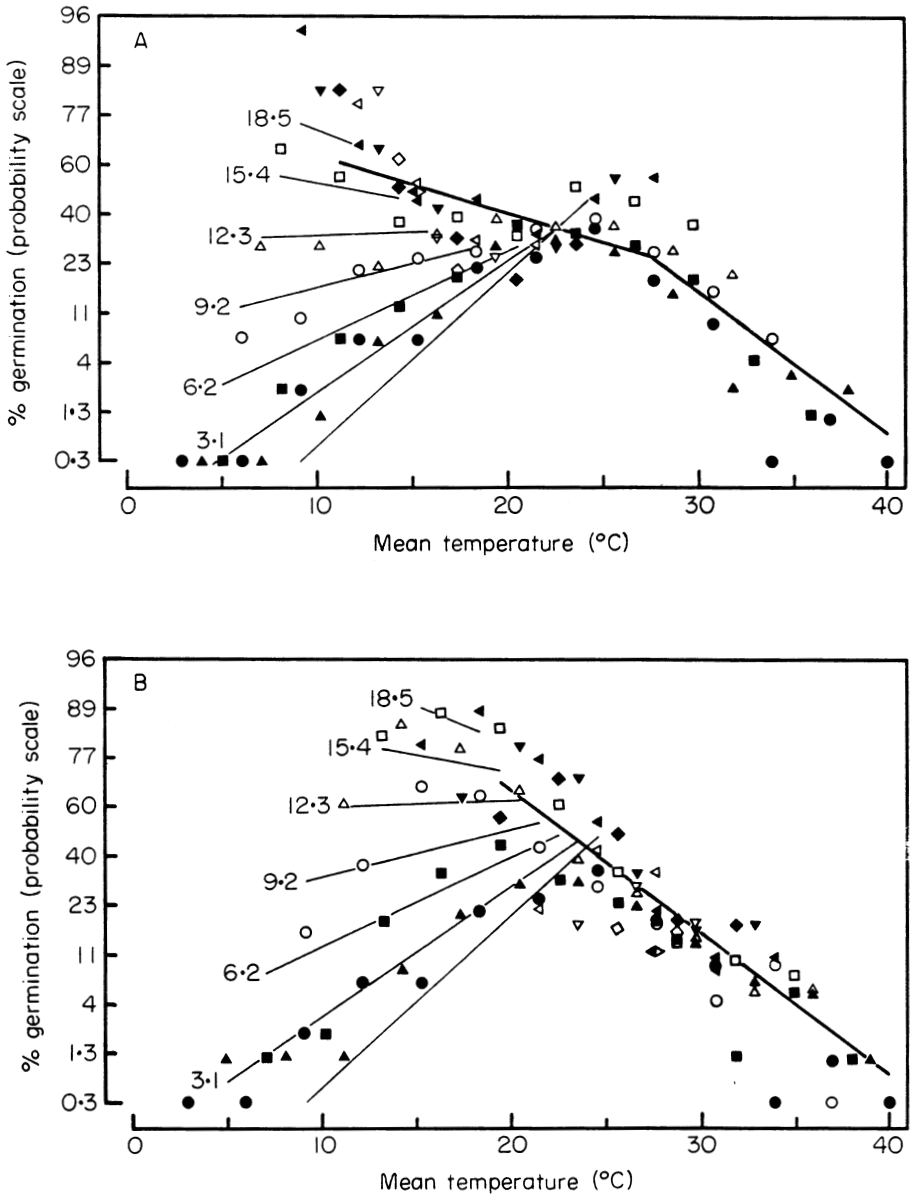


Figure 9. Germination percentage of seeds of *Chenopodium album* after 24 days as a function of mean temperature and amplitude (A) in a thermoperiod of 8h warmer temperature/16h cooler temperature and (B) in a thermoperiod of 16h warmer temperature/8h cooler temperature. Note the advantage of (B) especially at wide amplitude. Temperature amplitudes: (●) 0°C (nominally constant temperature); (▲) 3.1°C; ;(■) 6.2°C; (○) 9.2°C; (△) 12.3°C; (□) 15.4°C; (◀) 18.5°C; (▼) 21.6°C; (◆) 24.7°C; (◁) 27.8°C; (▽) 30.8°C; (◇) 33.9°C; (◐) 37.0°C. From Murdoch *et al*. (1989).

on the way towards what we hope will be a simpler version which can be stated in terms which relate more understandably to the underlying physiology. It needs further work. The hypothesis, which could possibly explain the apparently rather complicated responses to alternating temperatures, may be encapsulated in five statements: (1) Each seed needs to experience within the cycle, at least transitorily, a *critical minimum* temperature; (2) this critical minimum temperature varies amongst the seeds of a population and is normally distributed; (3) each seed is inhibited from germination above a *critical mean* temperature; (4) this critical mean temperature is normally distributed among the seed population; and (5) both

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

distributions are independent of each other. But this idea remains to be tested.

The pentose phosphate pathway

One of the difficulties of understanding seed dormancy is that so many diverse treatments tend to remove it. Some scientists have tended to concentrate on one or just a few of these, and then developed an hypothesis to explain the responses. Consequently, many different dormancy hypotheses have been developed and, as a result, the idea emerged that there may be many different dormancy mechanisms. Whilst this may turn out to be true, I was more impressed from a literature search that, when the wide range of dormancy-removal agents which have been explored in a many species are considered, the evidence suggests that most species show at least some response to most if not all of them, though the efficacy of the different agents varies to some extent amongst species. Further, it seemed to me that any satisfactory dormancy hypothesis should be capable of explaining all the responses, not just a few of them. So the search was for some fundamental biochemical mechanism which would allow an otherwise meaningless array of responses to be understood.

In a preliminary survey I constructed a matrix — albeit incomplete — showing the responses of 18 species from eight families to up to 26 dormancy-removal treatments (Roberts, 1973b). Later we produced a similar table for the responses of 137 species of the Gramineae to 23 treatments (Roberts and Smith, 1977). These surveys confirmed what we had suspected — that the patterns of response are often very similar across a wide range of species, and thus it seemed it was worth searching for an underlying mechanism which may be common to many, if not all. But this suspicion, and the research leading to it, had begun much earlier in Sierra Leone.

During the course of trying to discover a practical technique for removing dormancy, I had exposed rice seeds to most of the agents and treatments which had been reported to be efficacious in one species or another (Roberts 1961d, 1962b, 1963a, 1963b, 1964a, 1964b, 1969). From the pattern of responses, it occurred to me that some oxidation process could be involved. This could explain the common dormancy-removal property of several apparently diverse agents, e.g. oxygen, hydrogen peroxide, and other electron acceptors — nitrates, nitrites (but not ammonium ions, urea or amino acids) and methylene blue — removing covering structures, low temperature in moist seeds (perhaps because of the greater solubility of oxygen) and warm temperatures in dry seeds (perhaps because of an enhanced rate of the oxidation reaction).

An obvious oxidation reaction to consider was conventional respiration and, in order to test this hypothesis, I also investigated the effect of a number of respiratory inhibitors (Roberts, 1964a). Amongst these were those that affect cytochrome oxidase, viz. carbon monoxide, potassium cyanide, sodium azide, hydroxylamine, and hydrogen sulphide. Much to my surprise, instead of increasing dormancy, these agents all tended to remove it; KCN and NaN_3 were particularly effective. Therefore, I concluded that there must be some other oxidising reaction involved in the loss of dormancy and that conventional respiration could be competing with it (Roberts, 1964b), since within the seed the oxygen potential is very low when the covering structures are intact, and cytochrome oxidase has an extraordinarily high affinity for oxygen.

On return from Sierra Leone in 1963, my research student Wendy Major (now Bridle) and I followed up these ideas in Manchester using barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) as well as rice (Major and Roberts, 1968a, 1968b). We found that barley and oats responded in a similar fashion to rice, although some respiratory inhibitors of glycolysis and the Krebs cycle in addition to the terminal oxidase inhibitors also showed slight activity. We also made the surprising discovery that, during the initial stages of imbibition, the rate of oxygen uptake is much greater in dormant as compared with non-dormant barley seeds, in spite of the fact they are not destined to germinate. Further, both KCN and CO inhibited the oxygen uptake of dormant seeds to a much greater extent than non-dormant seeds. This and other evidence led to the idea that two respiratory pathways operate during the early imbibition of seeds, one being conventional respiration in which the passage of electrons is finally transferred to oxygen through cytochrome oxidase (sensitive to inhibition by KCN, CO, NaN_3, etc) and another unknown process (Roberts, 1964b). It was the late Brian Truelove (then at Manchester but subsequently Auburn, Alabama) who suggested the possibility that the unknown process could be the pentose phosphate pathway (PPP), operating via an unknown but cyanide-insensitive terminal oxidase.

In order to test this hypothesis, another research student, Roger Smith (now Head of the Kew gene bank at Ardingly) who had moved with me from Manchester to Reading in 1968, investigated the C6/C1 ratios of dormant and non-dormant barley seeds during the early stages of imbibition. This is the technique in which glucose-6-^{14}C and glucose-1- ^{14}C are fed separately to two similar samples of seed, and the C6/C1 ratio of the $^{14}CO_2$ evolved is then determined. Conventional respiration should result in a ratio of unity, whereas anything less indicates some activity of the PPP. The interpretation depends on the fact that in glycolysis the glucose molecule is split into two 3-carbon units and both the carbon-1 and carbon-6

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

atoms end up in the methyl group of pyruvate and are consequently decarboxylated in an identical fashion. If glucose is catabolised via the PPP, then the carbon-1 atom of the original molecule is decarboxylated first. There are criticisms of the technique, especially if too much reliance is placed on the actual quantitative values but, nevertheless, it may be assumed that the lower the ratio the greater is the activity of the PPP. These experiments indicated that non-dormant seeds have very high levels of PPP activity during early stages of imbibition, and that conventional respiration is relatively much more active in dormant than non-dormant seeds.

The experiments also included an examination of the effects of two dormancy-removal agents which could also be expected to increase the PPP activity, as had already been established in the literature for other tissues, KCN and $NaNO_2$. Nitrite is thought to be stimulatory because nitrite reductase operates through NADP — the coenzyme also utilized by the dehydrogenases of the PPP. In addition, we also investigated the effects of three dormancy-removal agents in which the effect on the PPP could not easily be predicted from their known effects on metabolism but, if the dormancy hypothesis were correct, should stimulate the PPP by virtue of their dormancy-removal activity. Of these, gibberellic acid was included because of its well-known activity in a wide variety of seeds, and the two isomers, D-threo-chloramphenicol and L-threo-chloramphenicol, were included because both had been shown by Black and Richardson (1965, 1967) to have dormancy-removal activity in lettuce. While both the D and L forms inhibit oxidation and phosphorylation in mitochondria — the latter isomer more than the former — only the D isomer has antibiotic activity and is able to inhibit protein synthesis at low concentrations. We found that all these dormancy-removal agents had little effect on the C-6/C-1 ratios of non-dormant seeds but markedly decreased the ratios in dormant seeds.

Subsequent work (Roberts and Smith, 1977) showed that in several cultivars of barley C6/C1 ratios of between 0.3 and 0.4 are common in dormant seeds during the first few hours of imbibition, whereas non-dormant seeds typically show values of between 0.1 and 0.2. It was confirmed that the following dormancy-removal treatments clearly decreased the ratio: KCN, both chloramphenicols, $NaNO_2$, 2-mercaptoethanol, dithiothreitol, GA_3, imbibition at 15°C as compared with 25°C, and dry 'after-ripening' of seeds. The lowest ratio obtained 0.04, was produced by KCN at 10^{-2}M (possibly a world record low ?).

The overall hypothesis can be seen best in terms of a metabolic-pathway map (Fig. 10). Several researchers have subsequently tested a number of aspects of this hypothesis — some publications supporting it but others finding no support. The literature is too voluminous to review here, but I believe there may still be something here worth salvaging and that further work might still be worthwhile. Although the structure epitomised in Fig. 10 may yet crumble, I do not know yet of any other hypothesis which is capable of 'explaining' so many of the diverse dormancy responses.

Classification of seed storage behaviour

After description and cataloguing, I believe that pattern recognition, as mentioned in the introduction, is an essential phase in the development of any science. Taxonomic classification was one of the examples cited. Some biologists think it was an essential preliminary to the development of the theory of evolution. Kenneth Dormer, who valiantly tried to teach me taxonomy as an undergraduate at Manchester, took an extreme but considered view on the matter, and held that, on their own, the patterns displayed by taxonomic classification systems were evidence enough for the theory of evolution.

On a more modest scale, but not an entirely unrelated topic, I now turn to the classification of seeds with respect to their storage characteristics. In the first half of this century, it was recognised that there is considerable variation in the ability of different species of seeds to survive, and there is some variation in how individual seeds respond to the deteriorative pressures of the environment; but it was not obvious what pattern, if any, there might be in this variation.

In the next section I shall describe how I first came to work on seed storage and longevity in the late 1950s in Sierra Leone. But it was almost 20 years later that increased familiarity with the literature made me realise that there were at least two categories of seed storage behaviour (we now believe at least three). However, I think the next section will be clearer if I take advantage of hindsight and clarify first what what we know about the major categories of seed storage behaviour, even if it is taken out of its historical sequence.

In the early 1970s further study of the literature led me to suggest that two distinct types of storage behaviour exist, which I termed orthodox and recalcitrant (Roberts, 1973a). The term orthodox was used because seeds of such species all appeared to obey very clear laws which determined their response to the environment: in particular, longevity was increased in a quantitatively predictable manner with a decrease in storage temperature and also with a decrease in moisture content over a wide range (this will be elaborated further in the next section). This group is particularly important since it seems to include all arable crops and forage species (Ellis *et al.*,

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

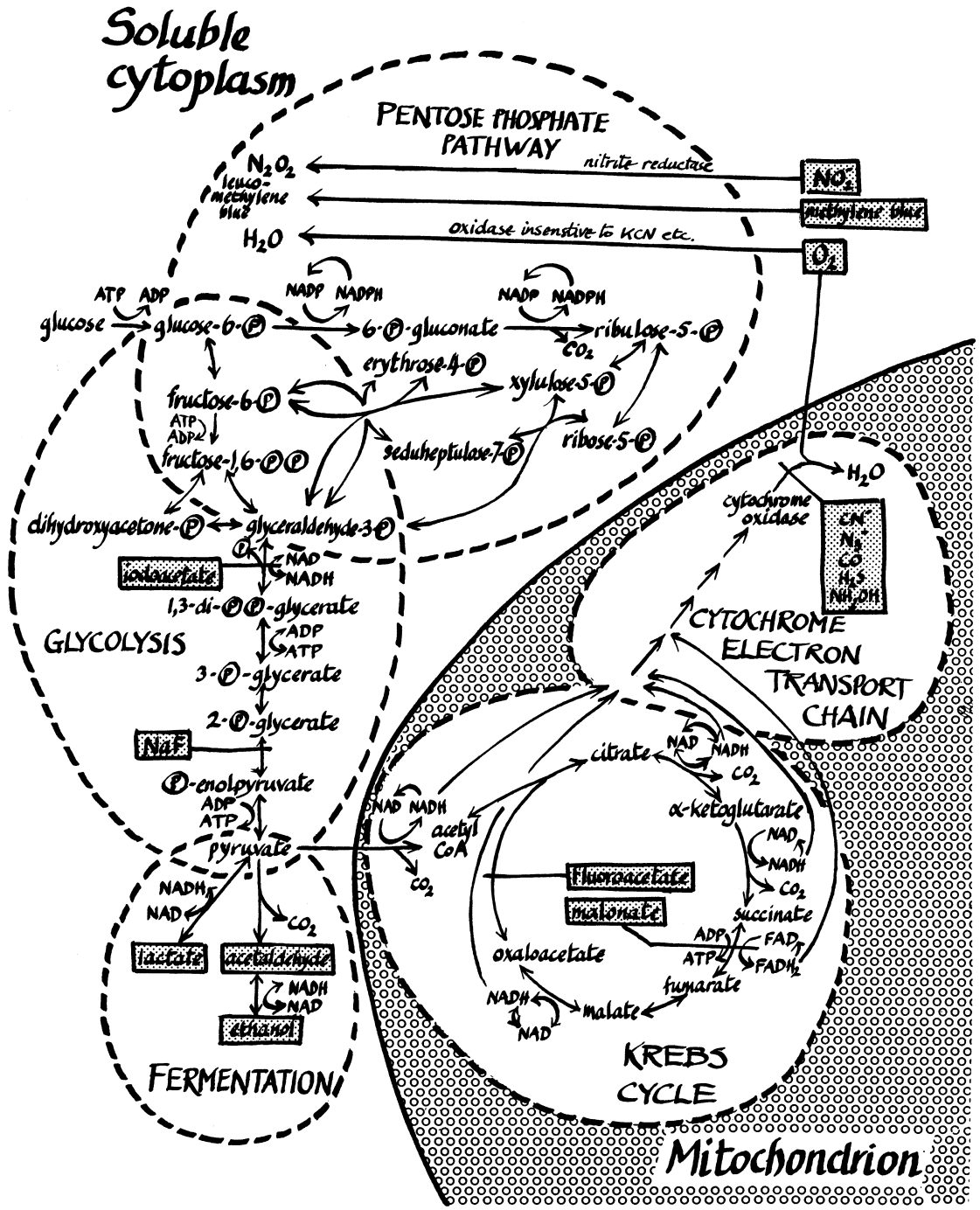


Figure 10. Active substances (shown in stippled rectangles) affecting the postulated competition for oxygen between conventional respiration (glycolysis, Krebs cycle, cytochrome electron transport chain) and the oxidative pentose phosphate pathway (PPP) in low oxygen tensions experienced by imbibing seeds. It is postulated the PPP activity in these circumstance is increased by increasing the oxygen availability (e.g. by removal of covering structures, increasing the partial pressure of oxygen, or applying hydrogen peroxide), providing alternative electron acceptors for the PPP (nitrite or methylene blue), inhibiting cytochrome oxidase (CN^-, N_3^-, CO, H_2S, NH_2OH) or, less effectively, by inhibitors of glycolysis, or the Krebs cycle. All these substances (shown in stippled rectangles) have been shown to have dormancy-releasing activity by acting, it is suggested, at the metabolic sites indicated. Other subtances which release dormancy also increase the relative activity of the PPP, e.g. GA and sulphydryl compounds; they do not appear on this diagram since the mechanisms are obscure. From Roberts (1973).

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

1985). But there were seeds of a number aquatic plants and some, but not all woody species which, it was clear, did not obey these rules. This second group survived best under very moist conditions; but, even under these optimum conditions, they retained viability only for relatively short periods — typically a few weeks or months or, at most, a few years. The quantitative relationship between longevity and storage conditions in these seeds (i.e. the rules governing their behaviour) was not and is still not clear. Resulting from this and because they are difficult to handle, I called them recalcitrant (Roberts, 1973a). Norman Simmonds has chided me more than once for introducing these terms on the grounds that they are anthropocentric. However, it seems to me that anthropocentric terms are only a potential problem if they are used to 'explain' biological behaviour, and clearly these terms are not used in this way. Further, I followed a well established precedent of using the term recalcitrant to describe the behaviour of non-human objects. So, for example, no less an authority than the *Oxford English Dictionary* (complete edition) makes it clear that the word recalcitrant has been used to describe the behaviour of animals and things as well as people, and in support quotes one of the earlier uses of the word in the *Cornhill Magazine* (1866): 'a recalcitrant pin falling from its rightful place.' But in any case the terms orthodox and recalcitrant now seem to be established in the literature.

More recently we have recognised a third category which we termed — rather weakly — intermediate (Ellis *et al*., 1990a, 1990b, 1991a, 1991b). We sought a more meaningful word, or at least a more colourful one, but our imagination failed us. The seeds in this category have something in common with the other two, but with some important distinctions from both. Unlike recalcitrant seeds intermediate ones can be partially dried with some advantage to longevity, but drying below some relatively high value, 12% moisture content in some species, is damaging. Even when dried to their optimum value, which is low enough to assume there is no significant metabolism, intermediate seeds of tropical origin cannot be cooled below about 15ºC without damage. This is understandable in moist tropical recalcitrant seeds in which all the plant tissues are susceptible to chilling injury, but it is more of a mystery why relatively dry seeds should be sensitive to chilling.

Although there is some controversy with regard to the lower limits of beneficial seed drying (e.g. Walters, 1998), we believe the best evidence so far suggests that seeds can be dried to moisture contents in equilibrium at ambient temperatures with relative humidities of about 10% for orthodox seeds (evidence briefly summarised by Ellis, 1998), 40–50% for intermediate seeds, and 90% for recalcitrant seeds (Roberts and Ellis, 1989; Ellis and Hong, 1995).

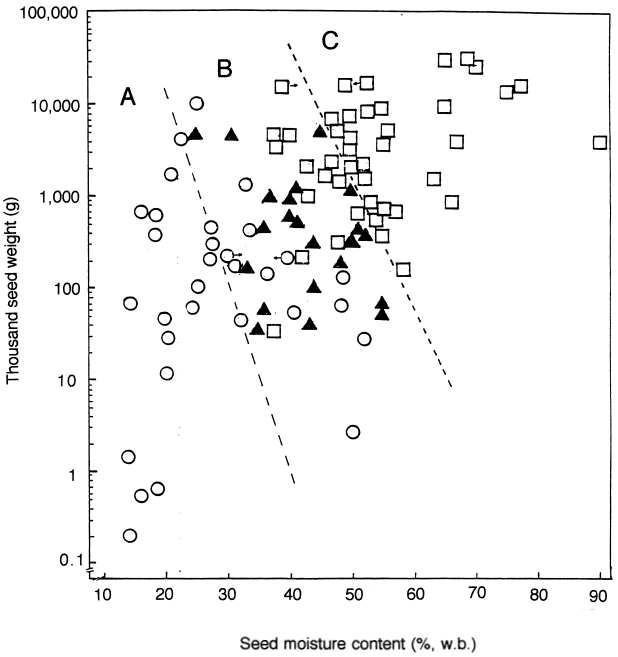


Figure 11. Seed storage behaviour of 94 contrasting species in relation to thousand seed weight and moisture content (%, wet basis) at harvest or natural shedding. Individual species are shown as: (○) orthodox; (□) recalcitrant; (▲) intermediate. The results define three areas: region A, seeds are probably orthodox; region B, seeds could be orthodox, intermediate, or recalcitrant; region C, seeds are probably recalcitrant. Modified slightly from Hong and Ellis (1996a) where details of the individual species may also be found.

It would be convenient if the three types of seed behaviour were associated with another easily identifiable characteristic. Unfortunately, so far no such single criterion has been found. However, it does seem to be possible to make an intelligent guess, based upon multiple criteria, for seeds whose behaviour is unknown (Hong and Ellis 1996a, 1996b). The two most important criteria are size (thousand seed weight) and seed moisture content at maturity or shedding (Fig. 11 shows the pattern), but taxonomy and morphology can also give additional clues. In general species in the Chenopodiaceae, Labiatae, Solanaceae, and Pinaceae show orthodox behaviour while species in Rhizophoraceae (in which vivipary predominates) are recalcitrant. Most species of the Leguminoceae, Gramineae, Cucurbitaceae, Crucifereae and Rosaceae also show orthodox behaviour, but with several notable exceptions. But behaviour can sometimes differ between species even within a genus, e.g. within *Araucaria* (Tomsett, 1984a, 1984b) and *Acer* (Hong and Ellis, 1990). Orthodox seeds are typical of species which produce achenes, many-seeded berries, many-seeded dehiscent capsules, many-seeded dry pods

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

(but not arrilate), many-seeded follicles, schizocarps and utricles, and most species which produce siliquas and caryopses. Hong and Ellis (1996a,1996b) go into further detail.

The prediction of longevity in orthodox seeds

The classification of seed storage behaviour arose out of the discovery that there was this large category of seeds with orthodox behaviour in which it is possible to predict percentage viability after any storage period within a wide range of temperatures and moisture contents. The original work started in Sierra Leone, which had the unenviable reputation as 'The Whiteman's Grave' because of the prevalence of yellow fever and malaria transmitted by mosquitoes which flourished in its hot and humid climate. Those climatic conditions are also bad for seed viability. And so in 1955, when I first arrived at the small rice research station at Rokupr at the edge of the mangrove swamps on the Great Scarcies River, I found that we were having to replant the germplasm collection every year in order to maintain viable stocks. In view of the number of stages in the process (sowing, transplanting, roguing, harvesting, threshing, drying, purity monitoring, and storing) and the precautions which had to be taken during each stage to prevent mixing, the cost and effort were considerable. Consequently I became interested in seed storage in order to devise a cheap and reliable method for extending seed longevity and thus reducing these problems.

I had the advantage of being entirely new to seed science and, although isolated from others in the field, I was able to examine a lot of literature (copies kindly provided by the Commonwealth Agricultural Bureaux, now CAB International) with no preconceived notions. Plotting the results of previous research published by others on temperate cereals and, to a lesser extent, on rice, I discovered a form which could be described by three linked equations or statements: (1) under constant storage conditions the distribution of individual seed lifespans is normally distributed, i.e. survival curves are linear when plotted on a probability scale; (2) the spread of the distribution (σ) is linearly related to the mean viability period; and (3) the log of the mean viability period is linearly related to moisture content and independently also to temperature (Roberts, 1960, 1961b). These conclusions were confirmed in rice in an experiment specifically designed to test these propositions (Roberts, 1961c). Figure 12 illustrates the first two statements which have now been confirmed on innumerable species. The third statement remains approximately true over relatively narrow ranges of conditions but, as became evident, required modification to take into account different relationships which are exposed when dealing with a very wide range of environments (Roberts and Ellis, 1977). Another problem was that, although within these narrow limits they satisfactorily estimated the survival of good quality seed lots, they could not take into account the initial quality of the seed which affects subsequent longevity, and therefore they could not be used to predict the behaviour of poor-quality seed lots. Both problems were solved twenty years later at Reading through the work of one of my research students and subsequent research fellow, Richard Ellis (now Professor of Crop Physiology).

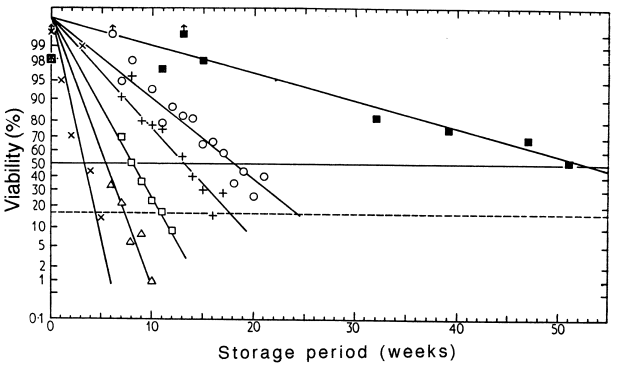


Figure 12. Percentage germination of wheat (cv Atle) plotted on a probability scale against storage period. Since a value of 100% does not appear on a probability scale, where such a value occurred it is represented by a point at 99.5% and an arrow pointing upwards. Storage conditions: 25°C in combination with 22.5% moisture content (mc) (×), 20.5% mc (△), and 18.6% mc (□); and 15°C in combination with (○) 20.6% mc and (■) 17.1% mc. The time taken for viability to fall to 50% (solid horizontal line), known as the p_{50} value, is approximately the same as the mean viability period p. The time taken for viability to drop from p_{50} to 15.9% germination (broken horizontal line) is equal to σ, the standard deviation of the distribution of viability periods. The geometry shows that $p \propto \sigma$. From Roberts (1960) replotted from original raw data kindly provided by M. B. Hyde.

The key to the solution of the first problem resulted from carrying out experiments on barley over a very wide range of conditions. It was then possible to improve the original equations by discerning that the relationship of longevity with moisture content is logarithmic (rather than semi-logarithmic as originally described), and that the supposed semi-logarithmic relation with temperature is much improved by the introducing a quadratic term which bends the log-linear relation slightly. But the really good idea which occurred to Ellis not only provided the means of taking into account the initial seed quality, but also had other far-reaching consequences. This was the the idea of using the standard deviation (σ) of the distribution of seed lifespans as a measure of loss of viability, rather than the mean viability period. The notion of using a

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

measure of variation in seed longevity as a measure of the overall longevity of the population may seem odd at first sight; it seems less odd, however, if one recognises that σ may also be thought of as the time for viability to drop through various definable amounts of viability, e.g. from 97.7% to 84.1%, or from 84.1% to 50%, or from 50% to 15.9%. The main value of this approach is that, unlike the mean viability period (the previously used criterion for longevity), σ is not affected by the initial quality of the seed lot (Ellis and Roberts, 1980a, 1980b). With some exceptions (e.g. Hay *et al.*,1997) it is generally found that survival curves of different seed lots of the same species stored under the same storage conditions show survival curves of similar slope, i.e. they have an identical value of σ. The results of an experiment which demonstrated this in different seed lots artificially produced by different pre-treatments are shown in Fig. 13. It then follows that, since linear survival curves are defined solely by their intercept and slope constants, if different seed lots of the same species have different mean viability periods when stored under the same conditions, this can only be explained in terms of different intercept values. Thus seed quality affects the intercept constant whereas the environment only affects the slope constant. These concepts are illustrated in Fig. 14. An important corollary of this is that the intercept value of survival curves determined by probit analysis also provides one of the best measures of seed vigour which correlates well with rate (sometimes erroneously termed speed) of germination, field emergence (Ellis and Roberts, 1980c, 1981; Khah *et al.*,1986) and in certain circumstances with yield (Khah *et al.*, 1989).

This research gave rise to what I think could now be called the general orthodox seed viability equation since it has been found to apply to work done in several independent laboratories on a wide range of species representing four of the ten super-orders of flowering plants and also to plants of different life form, habitat and seed characteristics (Dickie *et al.*, 1990). This equation predicts percentage seed viability for a seed lot of any quality after any time under a very wide range of storage temperatures and seed moisture contents. It takes the form

$$v = K_i - p/10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2} \qquad (6)$$

where v is probit percentage viability after p days in storage at m% moisture content (f.wt) and t °C; K_i is a constant specific to the seed lot, and K_E, C_W, C_H and C_Q are species viability constants.

Equation (6) looks complicated, and it is not easy to assimilate immediately what it means. But it is not difficult to use, and it can be transformed into nomographs for doing a number of different types of quick calculation (e.g. Ellis and Roberts, 1980a, 1980b). The principles of it, however, are easier to grasp by considering how it was derived. It combines the simple equation for survival curves in their linear form

$$v = K_i - p/\sigma \qquad (7)$$

with the power term of equation (6), which determines the slope of this curve and may be written as

$$\log_{10}\sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \qquad (8)$$

Equation (7) is represented in Figs 12, 13 and 14 and Equation (8) in Fig. 15.

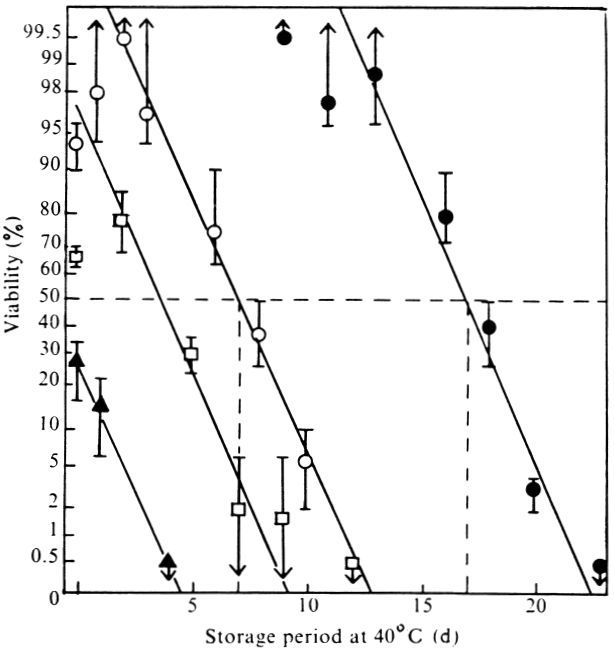


Figure 13. Survival curves of barley seed (cv. Proctor) at 15.4% moisture content in hermetic storage at 40°C after 0 (●), 3 (○), 4 (□), and 5 (▲) days previous storage at 50°C. Percentage viability (mean of five replicates of 50 seeds each) is plotted on a probability scale. The vertical bars represent the extreme values of the replicates. Since 100% and 0% cannot be shown on a probability scale such values are represented by arrows pointing upwards at 99.5% or downwards at 0.5%. The different periods of preliminary storage at 50°C represent different seed lots of different initial quality. Note, amongst other features, that 3 days of preliminary storage had no significant effect on initial percentage viability, but decreased the mean viability period from 17 to 7 days. From Roberts and Ellis (1977).

Chromosome damage associated with seed deterioration

Seed viability is a binary business: either a seed is dead or alive. The previous section dealt with

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

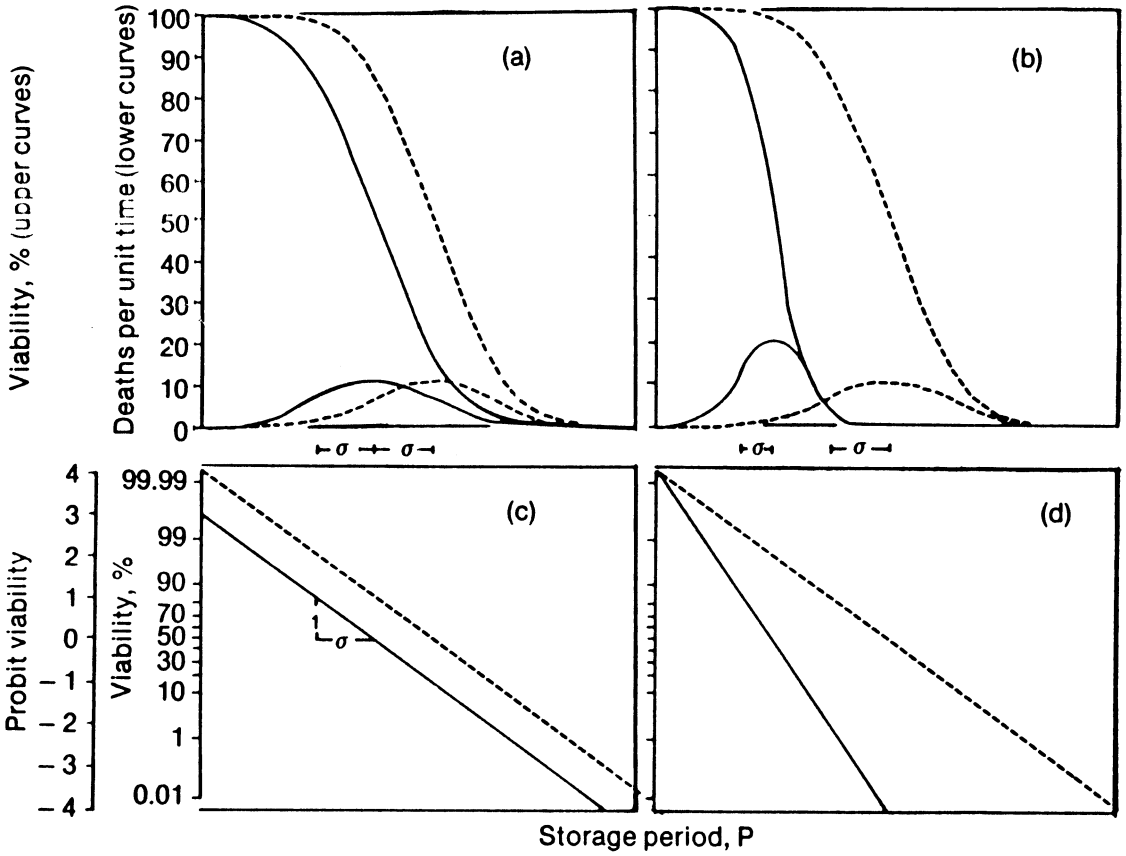


Figure 14. Graphs (a) and (b) show typical survival curves which are cumulative normal distributions of negative slope under conditions when the temperature and moisture content remain constant during storage. The frequency distribution of the individual life-spans which give rise to these survival curves are also shown. The standard deviation, σ, is indicated below each curve. Graphs (c) and (d) show the same survival curves as (a) and (b), respectively, when percentage viability is transformed to probits (described by equation (7)), and the slope, $1/\sigma$, is indicated on one of the curves in (c). Graphs (a) and (c) show two survival curves representing two different seed lots of the same species stored under identical conditions which, therefore, have identical slopes but where the the seed lot constant (the intercept, K_i) of one seed lot (------) has a greater value than the other seed lot (———). Graphs (b) and (d) show two survival curves of the same seed lot (therefore having the same initial viability and therefore the same K_i value) stored in two different environments so that the slope, $1/\sigma$, in the less deleterious environment (------) has half the value of the more deleterious environment (———). From Roberts (1986).

predicting when the final catastrophes would occur for different proportions of seeds. Although one cannot predict this event in a single seed, it is possible to be quite accurate about populations (seed lots). In this sense we can be like actuaries — except we deal with seeds rather than people.

However, though a death is considered as a sudden event, before this final catastrophe, as with ageing in people (of which I am well qualified to speak), each seed goes through a gradual process of deterioration, of which there are innumerable symptoms (Ellis and Roberts, 1980c ; Roberts and Ellis, 1982; Roberts, 1986). Included as part of the sub-cellular syndrome is damage to the chromosomes, some of which appears as gross chromosomal abnormalities, particularly in the first cell divisions following the onset of germination, and some as gene mutations segregating in the second and subsequent generations (Roberts, 1988).

The accumulation of nuclear damage in seeds was reported in the 1930s and was originally thought to be a function of chronological age, but by the second half of the decade it was recognised that it was probably a function of temperature, moisture content, and time (Roberts, 1988). The topic had then been lost sight of for about 30 years until I wondered whether there was an association between this type of damage and loss of viability, because the same factors affected both. From

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

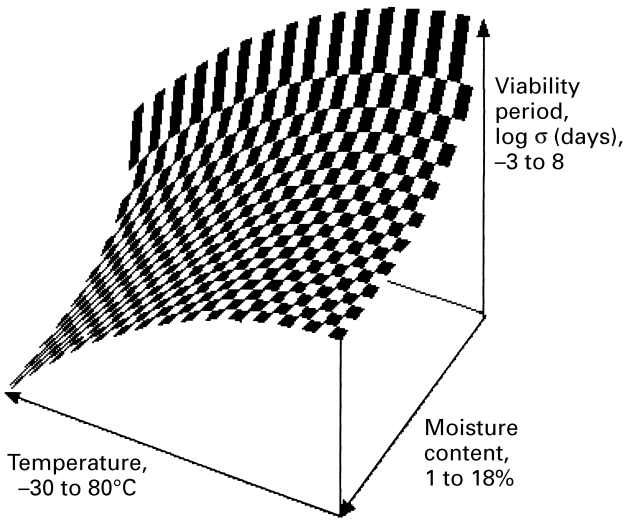


Figure 15. Graphical representation of Equation (8), i.e. the relation between moisture content, temperature and viability period expressed as the standard deviation of individual periods of longevity (e.g. the time taken for viability to fall from 97.7% to 84.1%, or from 84.1% to 50%), based on the constants (Dickie *et al.*, 1990) calculated for soyabean. Note that the relationship between moisture content and longevity is concave with respect to the origin of the graph, whereas the relationship with temperature is convex. This means that, while there is advantage in reducing the value of both factors, for each successive 1% drop in moisture content the improvement gets relatively greater, whereas for each 1°C successive drop in temperature the improvement becomes relatively smaller. This is why it is particularly important to dry orthodox seeds to moisture contents in equilibrium with a relative humidity of about 10–12% at ambient temperature (the lower limit for the viability equation), but there is probably little point in reducing temperatures much below –20°C. With this combination we are talking about very long storage periods; and so, if I am wrong, I will probably no longer be available to be told so, and furthermore you may no longer be available to tell me.

experiments on barley, faba beans and peas carried out at moisture contents between about 11% and 18% and at temperatures between 25°C and 45°C, we concluded that indeed there was (Roberts *et al.*, 1967: Abdalla and Roberts, 1968). Further, the results suggested that, irrespective of the storage conditions (except the most extreme), or how rapidly the seeds had lost viability, within a species the relationship between loss of viability and the frequency of gross chromosome abnormalities was always the same; in other words a knowledge of percentage viability was sufficient to estimate the amount of chromosome damage in the surviving seed population. These conclusions were subsequently confirmed in barley by Murata *et al.* (1979, 1981).

Almost twenty years later, in the mid 1980s, we returned to the problem. This was because I had been trying to persuade the, by now, growing community of those concerned with the conservation of plant genetic resources that, in designing protocols for the storage and regeneration of seed accessions in gene banks, we needed not only to be concerned with seed viability but also with the genetic condition of the surviving seeds. More work was needed, especially since at that time no research had been undertaken on the accumulation of nuclear damage in very dry seeds which, for good reason, is the way seeds are stored for long-term genetic conservation. Accordingly, we examined the problem further over a much wider range of conditions and showed that, with reduction of seed moisture content to levels lower than are typically used in conventional seed storage, the relationship changes so that, for a given loss of viability, there is a much greater accumulation of damage in the surviving seeds (Rao *et al.*, 1987). The relationship between moisture content, loss of viability and the percentage of aberrant cells in the surviving seeds originally appeared very complex (Roberts, 1988) (Fig 16a). It was only when we considered that, as with loss of seed viability, the accumulation of chromosome damage, too, might involve a cumulative normal distribution that a tolerably clear picture emerged. So when probability scales were used to plot both the percentage of viable seeds and the proportion of aberrant cells in those seeds, the relationship between the two turned out to be relatively simple (Fig. 16b) (Rao *et al.*, 1987).

The very high numbers of cells containing chromosome aberrations which appeared with loss of viability in the very dry seed was disconcerting, because although gross chromosome damage is mostly lost — but not entirely — during plant growth by diplontic selection, some does emerge again at meiosis during gametogenesis (Rao and Roberts, 1989). Further, there is a correlation between visible chromosome aberrations and the amount of initially invisible genetic damage, most of which behaves as recessive mutations and can be detected by phenotypic segregation in the second and subsequent generations in plants grown from seed lots which have suffered significant loss of viability (Abdalla and Roberts, 1969; Roberts, 1988).

Although the *amount* of chromosome damage which can accumulate in dry seeds is very much more than in more moist seeds, the *rate* of accumulation of that damage is much slower. In fact it turns out that the accumulation of chromosome damage with time follows a very similar pattern to the loss of viability, and its relationship with seed moisture content and temperature can be described by an equation very similar to equation (6) (Rao *et al.*, 1988).

Both equations were applied to data from a storage experiment on lettuce seed and the results are shown

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

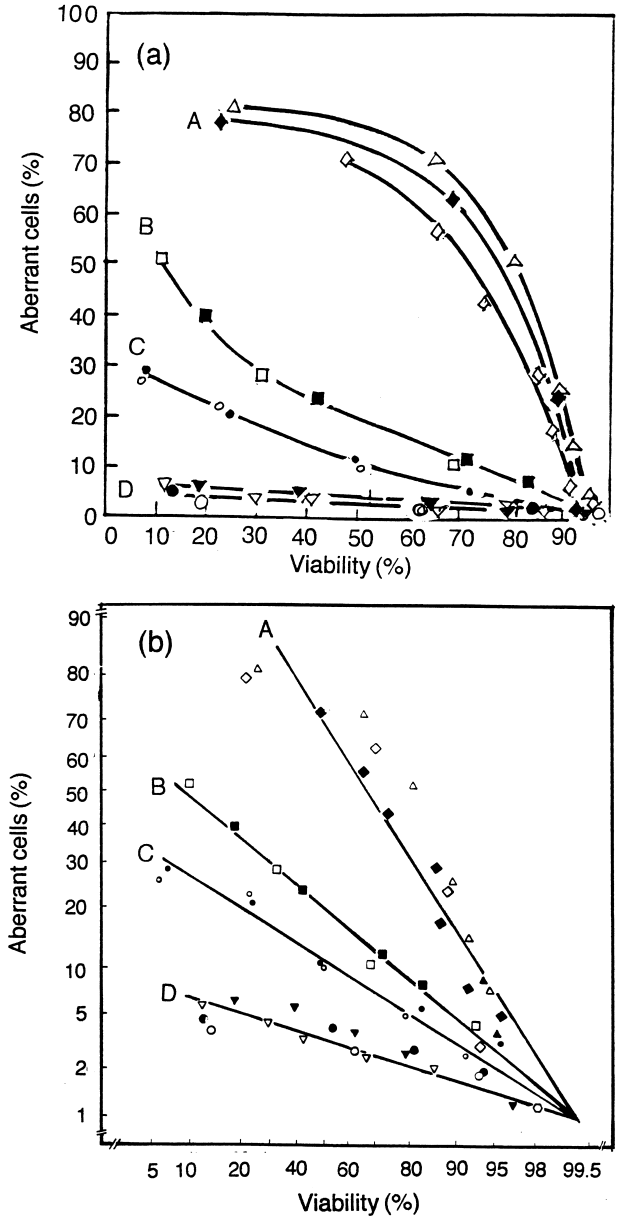


Figure 16. Relations between the frequency of aberrant anaphase cells in surviving seeds of lettuce after storage for various periods under different conditions. (a) Data as originally presented in which the percentages of aberrant cells in the first mitoses were plotted against percentage viability (from Roberts, 1988). (b) An augmented data set was subsequently plotted (but reached publication earlier: Rao *et al*., 1987) using probability scales for both axes. Note that the different relationships A, B, C and D are a function of moisture content. A: 3.3 % at 50°C (◆), 3.3% at 60°C (◇), 5.5% at 30°C (▲), 5.5% at 50°C (△). B: 8.1% at 35°C (■), 8.1% at 40°C (□). C: 9.8% at 35°C (●), 9.8% at 40°C (○). D: 13.0% at 35°C (▼), 13.0% at 40°C (▽), 18.1% at 30°C (●), 18.1% at 40° (○). Control (no storage) (○). From Rao *et al.* (1987).

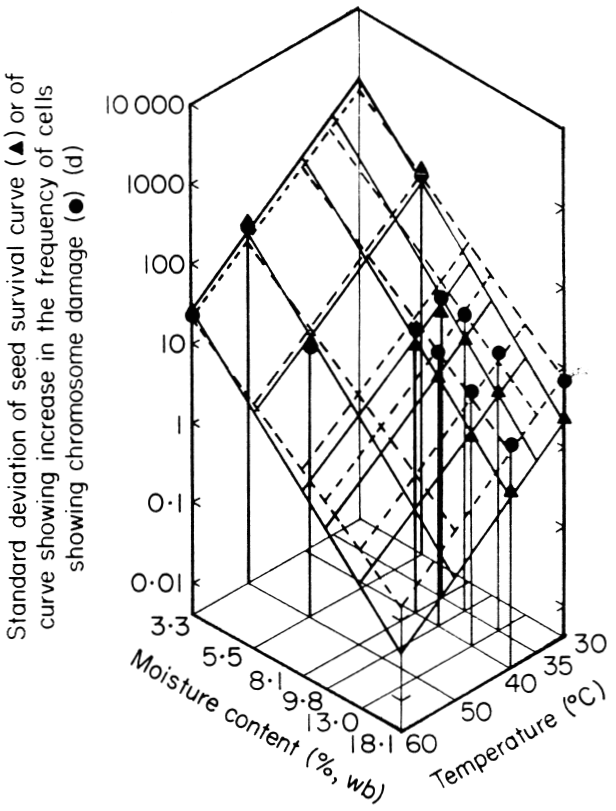


Figure 17. The effect of seed moisture content and temperature during storage on the standard deviations of distribution of seed longevity (▲) and of cells becoming aberrant (i.e. accumulating one or more chromosome aberrations expressed during the first mitoses of germination) (●) for a seed lot of lettuce originally showing 98% viability and 1.0% aberrant cells. The fitted planes are those described by equation (6) for loss of viability (solid lines) and a similar equation for accumulation of chromosome aberrations (broken lines). From Rao *et al.* (1988).

in Fig. 17. From this it can be seen that the pattern of accumulation of chromosome aberrations is indeed very similar to that for loss of seed viability. However, the moisture-content coefficient for aberrations is a little smaller than the corresponding coefficient for loss of viability, and this accounts for the fact that more chromosome damage can accumulate in dry seeds before they die than in more moist seeds. But since the rate of accumulation of aberrations as well as the loss of viability is slower in dry seeds, it is still a better policy to store seeds at low moisture content for genetic conservation. However, these relationships also emphasise the original advice given for the the genetic conservation of species producing orthodox seeds (Cromarty *et al*., 1982): in addition to drying the seeds to 5±1% moisture content and storing

https://doi.org/10.1017/S0960258599000203 Published online by Cambridge University Press

hermetically at ≤ –18°C, it was suggested that accessions should be regenerated *before* viability has fallen very far.

Conclusions

A few points emerge from the experiences touched on here:

(1) Thinking about patterns can sometimes be helpful when designing experiments (e.g. Figs 3, 7, 8(a) and 10).
(2) Choosing appropriate modes of presentation can sometimes help to expose patterns or forms inherent in data which are not otherwise immediately obvious (e.g. Figs 6, 8–13, 16 and 17).
(3) Perception is often made easier by transformations which reshape data into straight lines or planes; such simplifications have the additional advantage that they reduce the number of points required to define a line or a plane — theoretically two for a line and three for a plane, although statistics demands a few more (e.g. Figs 2, 5, 9, 12, 13, and 17). Detection of linear relationships simplifies equations and decreases the number of coefficients needed to describe relationships.
(4) It is not often very useful to fit entirely arbitrary models to data: it is usually much more productive to seek solutions in which the coefficients have some biological meaning. If they are stable across environments, so much the better, for then the solution will have predictive value. Equations (1), (2), (3), and their derivatives (4) and (5), also (6), (7), (8) and (9), together with those inherent in Figs 4 and 9 represent models of this type. As Alexander Isaakovich Kitaigorodskii said in a lecture in Amsterdam (1975): 'A first-rate theory predicts; a second-rate theory forbids; and a third-rate theory explains after the event.'

Acknowledgements

Many people have contributed to the ideas discussed here. In particular I remember discussions with those who were graduate students or research fellows at the time when most of the ideas were being developed, some of whom went on to become valued colleagues. Therefore, I should like to record my gratitude to Farouk Abdalla, 'S.K.' Banerjee, Sampath Benjamin, Wendy Bridle (née Major), Ricardo Carmona, Sarah Covell-Barret, Peter Craufurd, Anna Dourado, Richard Ellis, Sala Gaber, Mahteme Giorgis, Clara Goedert, Paul Hadley, Tran Dang Hong, Ahmed Ibrahim, Massoud Khah, Martin King, Philippe Koole, Warren Kuo, Ann Minchin, Alistair Murdoch, Sam Olosuyi, Kwabema Osei-Bonsu, Ian Popay, Aiming Qi, Nanduri Kamaswara Rao, Luigi Russi, Roger Smith, Usep Soetisma, Tjeerd Stomph, Rod Summerfield, Sue Totterdell, Ambika Upadyay, Elizabeth Vincent, Tim Wheeler and Mehari Zewdie. I would also like to pay tribute to the late Claude Wardlaw who encouraged me in an interest in form through experimental morphology and to those, sadly all now dead, who originally taught me plant physiology — Lord Eric Ashby, Herbert Street and Philip Wareing. I am most grateful to Marc Cohn for helpful suggestions on the first draft. I have always taken for granted the encouragement and constructive criticism of my wife, Dorothy, but for once I should also like to thank her, too.

References

Abdalla, F.H. and Roberts, E.H. (1968) The effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans, and peas during storage. *Annals of Botany* **32**, 119–136.

Abdalla, F.H. and Roberts, E.H. (1969) The effects of temperature and moisture on the induction of genetic changes in seeds of barley, broad beans and peas during storage. *Annals of Botany* **33**, 153–167.

Barlow, H.B. (1983) Intelligence, guesswork, language. *Nature* **304**, 207–209.

Black, M. and Richardson, M. (1965) Promotion of germination in light requiring seed by chloramphenicol. *Nature* **208**, 1114–1115.

Black, M. and Richardson, M. (1967) Germination of lettuce seed induced by inhibitors of protein synthesis. *Planta* **73**, 344–356.

Carmona, R. and Murdoch, A.H. (1995) Interactions of temperature and dormancy-relieving compounds on the germination of weed seeds. *Seed Science Research* **5**, 227–236.

Carpenter, A.J. and Roberts, E.H. (1962) Some useful techniques in speeding up rice-breeding programmes. *Empire Journal of Experimental Agriculture* **30**, 127–131.

Collinson, S.T., Ellis, R.H., Summerfield, R.J. and Roberts, E.H. (1992) Durations of the photoperiod-sensitive and photoperiod-insensitive phases of development to flowering in four cultivars of rice (*Oryza sativa* L.). *Annals of Botany* **70**, 339–346.

Cromarty, A.S., Ellis, R.H. and Roberts, E.H. (1982). *The design of seed storage facilities for genetic conservation.* Rome, Italy, International Board for Plant Genetic Resouces.

Dickie, J.B., Ellis, R.H., Kraak, H.L., Ryder, K. and Tompsett, P.B. (1990) Temperature and seed storage longevity. *Annals of Botany* **65**, 197–204.

Edwards, A. (1989) Venn diagrams for many sets. *New Scientist* **121**, 51–56.

Ellis, R.H. (1998) Longevity of seeds stored hermetically at low moisture contents. *Seed Science Research* **8** (Supplement 1), 9–10.

Ellis, R.H. and Hong, T.D. (1995) Seed quality: seed development and storage, pp 80–92 *in* Yappa, A.C. (Ed) *Proceedings of the international symposium on recent advances tropical seed technology and planting stock production.* Thailand, Haad Yai, ASEAN Forest Tree Seed Centre.

Ellis, R.H. and Roberts, E.H. (1980a) Improved equations for the prediction of seed longevity. *Annals of Botany* **45**, 13-30.

Ellis, R.H. and Roberts, E.H. (1980b) The influence of temperature and moisture content on seed viability period in barley (*Hordeum distichum* L.). *Annals of Botany* **45**, 31–37.

Ellis, R.H. and Roberts, E.H. (1980c) Towards a rational basis for testing seed quality. pp 605–635 *in* Hebblethwaite, P.D. (Ed) *Seed production*. London, Butterworth.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1985) *Handbook of seed technology for genebanks.* Rome, Italy, International Board for Plant Genetic Resources.

Ellis, R.H. and Roberts, E.H. (1981) The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* **9**, 373–409.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1983) Procedures for the safe removal of dormancy from rice seed. *Seed Science and Technology* **11**, 77–112.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1990a) An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany* **41**, 1167–1174.

Ellis, R.H., Hong, T.D., Roberts, E.H. and Soetisna, U. (1990b) Seed storage behaviour in *Elaeis guineensis. Seed Science Research* **1**, 99–104.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1991a) An intermediate category of seed storage behaviour. II. Effects of provenance, immaturity, and imbibition on desiccation-tolerance in coffee. *Journal of Experimental Botany* **42**, 653–657.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1991b) Effects of storage temperature and moisture on the germination of papaya seeds. *Seed Science Research* **1**, 69–72.

Fry, R. (1920) *Vision and design.* London, Chatto & Windus.

Hay, F.R., Probert, R.J. and Smith, R.D. (1997) The effect of maturity on the moisture relations of seed longevity in foxglove (*Digitalis purpurea* L.). *Seed Science and Technology* **7**, 341–349.

Heath, O.V.S. (1970) *Investigation by experiment*. London, Edward Arnold.

Hong, T.D. and Ellis, R.H. (1990) A comparison of maturation drying, germination, and desiccation tolerance between developing seeds of *Acer pseudoplatanus* L. and *Acer platanoides* L. *New Phytologist* **116,** 589–596.

Hong, T.D. and Ellis, R.H. (1996a) *A protocol to determine seed storage behaviour.* Rome, International Plant Genetic Resources Institute.

Hong, T.D. and Ellis, R.H. (1996b) *Ex situ* biodiversity conservation by seed storage: multiple-criteria keys to estimate seed storage behaviour. *Seed Science and Technology* **25**, 157–161.

Khah, E.M., Ellis, R.H. and Roberts, E.H. (1986) Effects of laboratory germination, soil temperature and moisture content on the emergence of spring wheat. *Journal of Agricultural Science, Cambridge* **107**, 431–438.

Khah, E.M., Roberts, E.H. and Ellis, R.H. (1989) Effects of seed ageing on the growth and yield of spring wheat at different plant-population densities. *Field Crops Research* **20**, 175–190.

Larsen, A.L. and Skaags, D.P. (1969). Crambe seed germination response on a thermogradient plate. *Proceedings of the Association of Official Seed Analysts* **59**, 44–50.

Major, W. and Roberts, E.H. (1968a) Dormancy in cereal seeds. I. The effects of oxygen and respiratory inhibitors. *Journal of Experimental Botany* **19**, 77–89.

Major, W. and Roberts, E.H. (1968b) Dormancy in cereal seeds. II. The nature of the gaseous exchange in imbibed barley and rice seeds. *Journal of Experimental Botany* **19**, 90–101.

Murata, M., Roos, E.E. and Tsuchiya, T. (1979) Relationship between loss of germinability and the occurrence of chromosomal aberrations in artificially aged seeds of barley. *Barley Genetics Newsletter* **9**, 65–67.

Murata, M., Roos, E.E. and Tsuchiya, T. (1981) Chromosome damage induced by artificial aging in barley. I. Germinability and frequency of aberrant anaphases at first mitoses. *Canadian Journal of Genetics and Cytology* **23**, 267–280.

Murdoch, A.J. (1998) Dormancy cycles of weed seeds in soil. *Aspects of Applied Biology* **51**, 119–126.

Murdoch, A.J., Roberts, E.H. and Goedert, C.O. (1989) A model for germination responses to alternating temperatures. *Annals of Botany* **63**, 97–111.

Rao, N.K. and Roberts, E.H. (1989) Seed ageing and meiotic chromosomal abnormalities in lettuce. *Cytologia* **54**, 373–379.

Rao, N.K., Roberts, E.H. and Ellis, R.H. (1987) Loss of viability in lettuce seeds and the accumulation of chromosome damage under different storage conditions. *Annals of Botany* **60**, 85–96.

Rao, N.K., Roberts, E.H. and Ellis, R.H. (1988) A comparison of the quantitative effects of seed moisture content and temperature on the accumulation of chromosome damage and loss of seed viability in lettuce. *Annals of Botany* **62**, 245–248.

Richards, F.J. (1941) The diagrammatic representation of the results of the physiological and other experiments designed factorially. *Annals of Botany* **5**, 249–261.

Roberts, E.H. (1960) The viability of cereal seed in relation to temperature and moisture. *Annals of Botany* **24**, 12–31.

Roberts, E.H. (1961a) Dormancy in rice seed. I. Distribution of dormancy periods. *Journal of Experimental Botany* **12**, 319–329.

Roberts, E.H. (1961b) Viability of cereal seed for brief and extended periods. *Annals of Botany* **25**, 373–380.

Roberts, E.H. (1961c). The viability of rice seed in relation to temperature, moisture content, and gaseous environment. *Annals of Botany* **25**, 381–390.

Roberts, E.H. (1961d) Dormancy in rice seed. II. Influence of covering structures. *Journal of Experimental Botany* **12**, 430–445.

Roberts, E.H. (1962a) Design of some inexpensive growth chambers for growing rice plants to maturity under controlled light and temperature in the tropics. *Journal of Agricultural Engineering Research* **7**, 316–319.

Roberts, E.H. (1962b) Dormancy in rice seed. III. The influence of temperature, moisture, and gaseous environment. *Journal of Experimental Botany* **13**, 75–94.

Roberts, E.H. (1963a). The effects of inorganic ions on dormancy in rice seed. *Physiologia Plantarum* **16**, 732–744.

Roberts, E.H. (1963b) The effects of some organic growth substances and organic nutrients in dormancy of rice seed. *Physiologia Plantarum* **16**, 745–755.

Roberts, E.H. (1964a) The distribution of oxidation-reduction enzymes and the effects of respiratory inhibitors and oxidising agents on dormancy in rice seed. *Physiologia Plantarum* **17**, 14–29.

Roberts, E.H. (1964b) A survey of the effects of chemical treatments on dormancy in rice seed. *Physiologia Plantarum* **17**, 30–43.

Roberts, E.H. (1965) Dormancy in rice seed. IV. Varietal responses to storage and germination temperatures. *Journal of Experimental Botany* **16**, 341–349.

Roberts, E.H. (1969) Seed dormancy and oxidation processes. *Symposium of the Society of Experimental Biology* **23**, 161–192.

Roberts, E.H. (1973a) Predicting the storage life of seeds. *Seed Science and Technology* **1**, 499–514.

Roberts, E.H. (1973b) Oxidative processes and the control of seed germination. pp 189–218 *in* Heydecker, W. (Ed) *Seed ecology*. London, Butterworths.

Roberts, E.H. (1981) The interaction of environmental factors controlling loss of dormancy in seeds. *Annals of Applied Biology* **98**, 552–555.

Roberts, E.H. (1986) Quantifying seed deterioration. pp 101–123 *in* McDonald Jr, M.B.; Nelson, C.J. (Eds) *Physiology of seed deterioration*. Madison, USA, Crop Science Society of America.

Roberts, E.H. (1988). Seed aging: The genome and its expression. pp 465–498 *in* Noodén, L.D.; Leopold, A.C. (Eds) *Senescence and aging in plants*. New York, Academic Press.

Roberts, E.H. (1991) How do crops know when to flower? The importance of daylength and temperature. *Biological Sciences Review* **3(4)**, 2–7.

Roberts, E.H. and Carpenter, A.J. (1962) Flowering response of rice to different photoperiods of uniform daily amounts of light radiation. *Nature* **196**, 1077–1078.

Roberts, E.H. and Carpenter, A.J. (1965) The interaction of photoperiod and temperature on the flowering response of rice. *Annals of Botany* **29**, 359–364.

Roberts, E.H. and Ellis, R.H. (1977) Prediction of seed longevity at sub-zero temperatures and genetic resources conservation. *Nature* **268**, 431–433.

Roberts, E.H. and Ellis, R.H. (1982) Physiological, ultrastructural and metabolic aspects of seed viability. pp 465–485 *in* Khan, A.A. (Ed) *The physiology and biochemistry of seed development, dormancy and germination*. Amsterdam, Elsevier Biomedical Press.

Roberts, E.H. and Ellis, R.H. (1989) Water and seed survival. *Annals of Botany* **63**, 39–52.

Roberts, E.H. and Smith, R.D. (1977) Dormancy and the pentose phosphate pathway. pp 385–411 *in* Khan, A.A. (Ed) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, Netherlands, North Holland Publishing Co.

Roberts, E.H., Craufurd, R.Q. and Le Cochec, F. (1961) Estimation of percentage natural cross-pollination: experiments on rice. *Nature* **190**, 1084–1085.

Roberts, E.H., Abdalla, F.H. and Owen, R.J. (1967) Nuclear damage and the ageing of seeds. *Symposium of the Society of Experimental Biology* **21**, 65–100.

Roberts, E.H. and Totterdell, S. (1981) Seed dormancy in *Rumex* species in response to environmental factors. *Plant, Cell and Environment* **4**, 97–106.

Roberts, E.H., Murdoch, A.J. and Ellis,. R.H. (1987) The interaction of environmental factors on seed dormancy. pp 687–694 *in Proceedings of the British crop protection conference, weeds.* Thornton Heath, Surrey, British Crop Protection Council Publications.

Roberts, E., Summerfield, R., Ellis, R. and Qi, A. (1993) Adaptation of flowering in crops to climate. *Outlook on Agriculture* **22**, 105–110.

Roberts, E.H., Qi, A., Ellis, R.H., Summerfield, R.J., Lawn, R.J. and Shanmugasundaram, S. (1996) Use of field observations to characterize genotypic flowering responses to photoperiod and temperature: a soya-bean exemplar. *Theoretical and Applied Genetics* **93**, 519–533

Roberts, E.H., Summerfield, R.J. and Ellis, R.H. (1997a) Reproductive development and crop adaptation. *Journal of Biological Education* **31(2)**, 97–105.

Roberts, E.H., Summerfield, R.J., Ellis, R.H., Craufurd, P.Q. and Wheeler, T.R. (1997b) The induction of flowering. pp 69–99 *in* Wien, H.C. (Ed) *The physiology of vegetable crops*. Wallingford, UK, CAB International.

Summerfield, R.J. Collinson, S.T., Ellis, R.H., Roberts, E.H. and Penning de Vries, F.W.T. (1992) Photothermal responses of flowering in rice (*Oryza sativa*). *Annals of Botany* **69**, 101–112.

Summerfield, R.J., Lawn, R.J., Qi, A., Ellis, R.H., Roberts, E.H., Chay, P.M., Brouwer, J.B., Rose, J.L., Shanmugasundaram, S., Yeates, S.J. and Sandover, S. (1993) Towards the reliable prediction of time to flowering in six annual crops. II. Soyabean (*Glycine max*). *Experimental Agriculture* **29**, 253–289.

Summerfield, R.J., Ellis, R.H., Craufurd. P.Q., Aiming, Q., Roberts, E.H. and Wheeler, T.R. (1997) Environmental and genetic regulation of flowering of tropical annual crops. *Euphytica* **96**, 83–91.

Tompsett, P.B. (1984a) Desiccation studies in relation to storage of *Araucaria* seed. *Annals of Applied Biology* **105**, 581–586.

Tompsett, P.B. (1984b) The effect of moisture content and temperature on the seed storage life of *Araucaria columnaris. Seed Science and Technology* **12**, 801–816.

Upadhyay, A.P., Ellis, R.H., Summerfield, R.J., Roberts, E.H. and Qi. A.(1994) Characterization of photothermal flowering responses in maturity isolines of soyabean [*Glycine max* (L.) Merrill] cv Clark. *Annals of Botany* **74**, 87–96.

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 Science and Technology* **5**, 659–670.

Walters, C. (1998) Ultra-dry technology: perspective from the National Seed Storage Laboratory, USA. *Seed Science Research* **8** (Supplement 1), 11–14.

Wesson, G. and Wareing, P.F. (1969) The induction of light sensitivity in weed seeds by burial. *Journal of Experimental Botany* **20**, 414–425.

Received 21 October 1998,
accepted 1 March 1999