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# DORMANCY IN MALTING BARLEY: STUDIES ON DRYING, STORAGE, BIOCHEMISTRY AND PHYSIOLOGY

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# DORMANCY IN MALTING BARLEY: STUDIES ON DRYING, STORAGE, BIOCHEMISTRY AND PHYSIOLOGY

by

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## ABSTRACT

The project report describes an investigation into the problem of dormancy in malting barley through a combination of three approaches. The influence of thermal processing, microbes on grain and biochemical changes in the grain are each examined.

### Thermal processing

Samples of a batch of deeply dormant Triumph barley were dried to 12% moisture content at various rates, by varying the temperature of the drying air (27°, 38°, 48°) and its relative humidity. Drying times were in the range 4 - 24 h. Batches were then stored in sealed containers at 15°, 25° and 38°C. At intervals sub-samples were taken and their germinabilities were assessed. It was apparent that two independent processes were operating: (1) post-harvest maturation, causing a decline in dormancy, and (2) progressive loss of viability, preceded by a loss in vigour. Dormancy declined faster at higher storage temperatures, but the rate of decline was independent of the drying regime that had been used. At 15°C the rate of recovery from dormancy for one of the barleys was so slow that even after a years storage the grain was not fit to malt. Loss of viability was more rapid at higher temperatures.

Other samples of Triumph barley were dried to various moisture contents, between 9.4 and 14.5% and were stored at 27° and 38°C. The initial rate of recovery from dormancy was still temperature dependent, but was independent of the moisture content of the grain. Grain samples dried to 9.4 and 10.3% moisture and stored at 38°C achieved germinations close to the viability value in both the 1 ml (agar) and 3 ml (agar) tests and showed no loss of viability in 30 weeks storage. On the other hand, grain held at 11% moisture deteriorated after 12 weeks and that stored at 13% and 14.5% moisture deteriorated after 3 weeks. The germination of samples dried to 9.4% moisture and stored at 38°C for 15 weeks or more (1 ml and 3 ml agar tests) was so good that it exceeded any value likely to be achieved with ambient storage. Since low moisture contents protect against loss of viability but do not impair break of dormancy, they offer the opportunity for higher temperature treatments. Further work is needed in this area.

Experiments carried out on other grain samples, including other samples of Triumph (12% moisture content), confirmed the initial conclusions based on experiments using the first sample of Triumph. However, in probit germination/storage time curves the results of the first trials tended to give two-stage, 'dog-leg' curves, which were much less apparent in the other data. Consequently, the initial data were not used in developing the probit description of dormancy. Data was stored on a computer data base, and used to develop a model of dormancy, based on probit analysis of the results combined with the data of other workers on the viability of barley. The model allows a computer simulation of the 'life history' of a batch of stored barley and permits the prediction of the rate of recovery from dormancy of samples of Triumph barley, stored under different conditions. At higher storage temperatures, the rate of emergence from dormancy fell below that predicted by probit analysis for germination values over about 80%. This may be associated with a loss of grain vigour, when warm storing grain at 12% moisture content.

The development of the probit model of dormancy employed 9 x 100 seed counts which reduced the standard deviation of the data. This enabled a better quantitative comparison of the rate of break of dormancy for Triumph barleys from two sites and two seasons. In the temperature range, 15-38°C, the effect of site and season was not distinguishable.

In the course of the experiments, grain was cooled and frozen after harvest and subsequent to warm storage for transportation. No effect on germinability or rate of break of dormancy was observed.

**Microbes on grain**

Various studies have shown that microbes depress the germination and vigour of barley. It was demonstrated that radioactive glucose was metabolised by microbes in the surface layers of the grains, and that the sugar did not reach the living tissues of the grain itself. Microbes on the grain can reduce triphenyl tetrazolium chloride to a red formazan, which can be quantified. This observation was used to develop a technique for quantifying the microbial populations. The technique lacked sensitivity, but showed that the microbes increased, especially during warm and aerated steeping and that growth was checked by dilute acid.

Measurements of the oxygen uptake rates of strips of husk and the water in which the husks are suspended confirmed the French reports that husk 'respiration' is initially lower in samples taken from mature grains than in samples taken from dormant grains. It was shown that the respiration was due to microbes which proliferated on the husk and in the surrounding water, supported by nutrients leached from the husk. Anaerobic and acidic steeping conditions check microbial multiplication.

**Sulphur containing substances**

Total thiols were determined in extracts of barley embryos using DTNB, (5,5'-dithiobis(2-nitrobenzoic acid)). Thiols were separated from crude barley extracts using binding to agarose substituted with p-hydroxymercuribenzoate (p-HMB agarose). Disulphides were recovered, as thiols, by trapping them in the effluent from the first p-HMB agarose column, on an ion exchange resin. After elution they were reduced with DTT and derivatised with DTNB and the derivatives were separated by h.p.l.c., being quantified by their absorbance at 330 nm. The major thiols in barley embryos were cysteine and glutathione, but traces of γ-glutamylcysteine and cysteinylglycine were detected. The major disulphides were cystine and oxidised glutathione.

The changes in cysteine, cystine, glutathione and oxidised glutathione were determined in the embryos of mature and dormant samples of barley grown under malting conditions. The most striking changes were the declines in glutathione and oxidised glutathione early in the malting process and the rise in the latter as visible germination took place. No significant differences were found between the levels of the thiols and disulphides in dormant and mature grains.

When grains were steeped in hydrogen peroxide, as in the germinative capacity test, glutathione levels were slightly lower in the dormant than in the mature grain, but the cysteine and disulphide levels were essentially the same. When dormant and mature grains were steeped in a solution of 2,3-dimercaptopropanol (BAL) the germination of the mature grain was retarded, but that of the dormant grain was enhanced. The thiol and disulphide levels were the same in the dormant and mature grains. However, in both cases, the apparent levels of cysteine were greatly enhanced. This could not be confirmed by analysis of the amino-acids

in extracts of the barley grains, and may have been due to the DTNB derivative of a metabolite of BAL co-chromatographing with DTNB-cysteine on h.p.l.c. Subsequent experiments showed that BAL appeared to be metabolised by barley grains, but attempts to characterise the substance, by mass spectrometry, have not been successful. The DTNB derivative of BAL does not interfere with the h.p.l.c. assay of the other thiols.

The fall in glutathione and oxidised glutathione, which occurs in the early stages of germination, is due - at least in part - to the transfer of these substances to the starchy endosperm. The thiols initially present in the 'non-embryo' part of the grain appear to be confined to the aleurone layer.

Dormant and mature samples of barley were steeped, with or without BAL, and were germinated for 3 or 5 days before kilning. The malt samples so obtained were kindly analysed by Pauls Malt Ltd. The BAL steep retarded malting in the mature grain, as reflected in the hot water extract, the diastatic power, the soluble nitrogen ratio and the wort viscosity. The dormant barley, steeped with BAL, was less advanced than the water-steeped control after 3 days germination but after 5 days germination the BAL-treated samples were more advanced. However, the quality of the malts prepared from dormant barley, steeped in BAL, did not approach those prepared from the water-steeped, mature barley.

# 1. INTRODUCTION

Dormancy is expensive and inconvenient since it prevents newly harvested grain being malted immediately. Consequently it is necessary to have 'carry over' stocks from the previous season's harvest that have had time to mature. Similarly, it is expensive to British farmers since it makes their grain less attractive to buyers from foreign malting companies. Dormancy is known to vary in intensity with variety and the details of the growing and ripening season. Triumph is notorious for developing intense dormancy, and it is apt to be particularly dormant when grown in the north of England or in Scotland. The variety Doublet is also recognized as being prone to dormancy.

Warm drying and warm storage (30°-40°C) have long been known to accelerate the decline in dormancy (post-harvest ripening or maturation) and warm storage also accelerates grain deterioration (Pollock, 1962; Briggs, 1978). When this project was planned it was not clear what combinations of time, grain temperature and grain moisture content were 'safe' and 'best', nor was it possible to forecast the rate of disappearance of dormancy under various conditions.

The collaborative project was conducted by the groups at Birmingham and Newcastle. The project had two main objectives:-

1. To optimise the conditions needed in drying and warm storage to maximise the rate of recovery from dormancy in malting barley. Consequent on this, the data would be presented in a form to guide farmers and maltsters in choosing storage conditions. At the start of this project little or no relevant 'quantitative' data were available.

2. To attempt to identify factors which regulate the decline of dormancy in stored grain.

Many other workers have considered the effects of warm treatments on the break of dormancy and grain deterioration (Hiltner, 1901; Gordon, 1968; Narziss *et al.* 1980a and b; Briggs *et al.* 1981; Palmer and Taylor, 1983). It was believed that drying and warming (during grain drying) and extended warm storage at a low moisture content were all involved in reducing

dormancy. In fact, close inspection of published data could not distinguish between the possible influences of various factors, including loss of vigour and viability, and, further, little account was taken of the influence of microbes remaining on the grain surface and proliferating at grain moisture contents exceeding around 12.5%. Hiltner (1901) found that treatment in an airflow at 40°C up to 10 days, followed by storage at room temperature for 2-3 weeks, broke dormancy in his barleys. However, many samples of dormant Triumph are not fully matured by this treatment. In addition, it appears that many, supposedly mature, barleys malt better after a warm treatment (Briggs, unpublished) so suitable warm treatments may improve barley vigour.

The work of Gordon (1968) attempts to quantify loss of dormancy by means of an Arrhenius relationship with temperature. In his work on rice seeds, Roberts (1961) first proposed a normally distributed dormancy period. The normal distribution has been more extensively used to describe viability through probit analysis (Ellis and Roberts, 1980a; Ellis and Roberts, 1981). Their work is particularly relevant, since the probit description of viability was tested with barley seeds (Ellis and Roberts, 1980b). The work conducted here builds on these earlier experiments to quantify germination changes in seeds.

It is our view that dormancy is regulated by two groups of factors - those exterior to the grain proper - the microbes and the film of surface water, as examples, and those within the grain. Understanding the influence of microbes would be greatly facilitated if it were possible to quantify the amount of metabolising/respiring microbial material on the grain. 'Plating' techniques cannot supply the data needed since (a) they are inevitably selective for the species they reveal, and (b) they will detect quiescent spores as well as vegetative, actively growing microbes, both single bacteria and large fungal mycelia.

We therefore, as a secondary objective, attempted to find a suitable method of quantification of the actively metabolising microbes present on the surfaces of grains.

Essentially nothing is known about the endogenous factors which regulate dormancy within barley grains. The report by Belderok (1968) that embryos from mature barley grain released glutathione and cysteine while embryos from immature grain did not and that there was a

corelation between the levels of the total thiols after a 24 h soak and the percentage of grains germinated after 3 days appeared to us to be highly significant. Furthermore, the observations of Pollock and Kirsop (1956), confirmed by Doran and Briggs (1993a), that soaking grains in solutions of various thiols overcame dormancy also indicated that thiols are significant in regulating dormancy. We decided, therefore, to investigate thiols and disulphides (oxidised thiols) in dormant and non-dormant grains using modern, well-tested methods of analysis.

# 2. MATERIALS AND METHODS

## 2.1 Barley samples

Initially ten undried samples of barley of the 1988 crop were collected at Newcastle (Table 2.1). First attempts to establish the viabilities of these samples were unsuccessful because of their extreme dormancy. In all cases the 1 ml and 3 ml (agar) test results were low, as was hoped for, but according to the standard Institute of Brewing hydrogen peroxide test viabilities were only 32-73% (Table 2.2). Kiln drying (40°C/3 days) followed by a hydrogen peroxide test, in which the reagent was supplemented with gibberellic acid, gave higher results. However, the highest results, which we now believe to be realistic, were achieved with samples (a) that had been decorticated with sulphuric acid and then germinated on paper wetted with a solution of gibberellic acid, or (b) had been kiln dried (40°C/3 days) and then stored warm for an extended period (40°C/25 days) followed by a week at room temperature (ca 20°C) and were then subjected to a standard hydrogen peroxide test (Table 2.2).

Initial experiments were made with a batch of Triumph barley (Table 2.1), grown at Acklington and designated Ack-Tr-88.

Samples of barleys were also collected in 1989, 1990 and 1991 (Tables 2.3, 2.4 and 2.5). A typical sample size was 25 kg. The 1989 samples were non-dormant. Many of the 1990 and 1991 barleys were dormant, and were initially tested using the Institute of Brewing, 4 ml (paper) germination test (The Institute of Brewing Analysis Committee, 1986). In the earlier part of the work all barleys were obtained from trial sites. In the 1990 and 1991 harvests samples were taken from commercial maltings. These are readily identified by the places of origin, Pencaitland and Kirkaldy.

All barley samples were well mixed and sampled using a sample-divider. Sub-samples were double wrapped and were stored at -18°C, to maintain their state of dormancy. This has proved to be a very satisfactory and reliable technique and confirms the experience of Doran and Briggs (1992a and b). The use of frozen storage is shown (Section 2.4.1) not to affect subsequent dormancy changes as compared with barley at harvest.

## 2.2 Drying and storage

Sub-samples of around 2 kg were dried in thin layers (~ 20 mm). These conditions were used to obtain uniform drying of the samples. The temperature of the airstream was controlled to $\pm$ 0.1 °C and the relative humidity, which could be varied to alter the drying rate, was controlled in a counterflow saturating column with a water inlet temperature again controlled to $\pm$ 0.1 °C. The grain temperature could be remotely sensed using an infra-red pyrometer.

The sample being dried was continuously weighed during this process to permit the treatment to be stopped when the grain reached a chosen moisture content, usually 12%. Moisture content was determined according to the Institute of Brewing Analysis Committee (1986) and is presented on a wet basis (fresh weight) throughout. The data from the drying apparatus was continuously logged on a computer. The drying chamber (Fig. 2.1) and the ancillary apparatus was housed in a dedicated laboratory (Bala and Woods, 1984; 1991; 1992). A range of experiments was performed to establish the conditions required to minimise variations in temperature and drying rate and to establish the accuracy of temperature, humidity and weight measurements, particularly in relation to aerodynamic error (Woods and Favier, 1993).

In the first trial, samples were dried at 27°, 38° and 48°C, with the air adjusted to various humidities so that the drying times were varied in the range 4-24 h (Table 2.6). The dried samples were placed in sealed containers, then stored at either 15°, 25° or 38°C. At intervals, initially three weeks apart, sub-samples were taken, cooled and were dispatched from Newcastle to Birmingham in heavily insulated containers. Transit time was generally 24 h. The cooling of samples was shown not to affect subsequent dormancy behaviour (Section 2.4.2). The samples were stored cool until their germinabilities were determined, usually within one week.

Records were maintained at Birmingham and added to the computer data-base at Newcastle. A flow diagram illustrating these stages for the initial experiments is given in Fig. 2.2.

## 2.3 Germination testing

The germination tests at Birmingham were performed using the 1 ml and 3 ml agar plate tests (Doran and Briggs, 1992a). Usually the cumulative percentage germination was determined on days 1, 2, 3 and 4, using 3 x 100 seeds. In the latter half of the project, germination tests were performed at Newcastle using the I.o.B. 4 ml (paper) test, particularly for the examination of the probit hypothesis for dormancy (Section 4). The number of seeds counted was increased to 9 x 100 seeds during the work on probit analysis. Data is presented in terms of means and standard deviations indicated by error bars.

Unless otherwise stated, the results presented in this report are the cumulative germination counts at 3 days.

## 2.4 Control Experiments on Cooling and Freezing

### 2.4.1 *Frozen storage before drying and warm storage*

All of the barleys used in this work were bagged and transported to the laboratory as soon after harvest as possible. As it was not practicable to carry out experiments with all of the samples at once, it was necessary to store each in a manner which maintained the level of dormancy and viability. Noll and Czarnecki (1979) reported that it was possible to preserve dormancy in wheat by storing the grain at -15°C in a commercial deep-freezer. Mares (1983) looked at the effect of moisture content on the germinability of dormant wheat after three months at -15°C. He found that below 22% moisture content, the germinability was unaffected. Both reports comment that this method of storage enables large quantities of material to be screened for low germinability at harvest ripeness. Doran and Briggs (1992a and b), stored a number of barley samples for up to 3 years, at -18°C, without deterioration.

In order to assess the effect of chilling to 4°C followed by storage at -18°C on the germinability of dormant barley, a controlled experiment was carried out. Upon arrival at the laboratory lots were tested for germinability using the 4 ml test (Institute of Brewing Analysis Committee, 1986). All lots were stored at 4°C for three days while the level of dormancy

was determined. Where possible, samples were taken immediately, dried to 12% moisture content and stored at 27°C and 38°C. Alternatively, each lot was kept at 4°C for a further two to fourteen days before samples were taken for drying and storage. The germination curve during storage was determined for each sample/storage temperature by testing at regular intervals using the 4 ml test. The remainder of the bulk was then floor dried, where necessary, in ambient air to 20% moisture content and packed into either 5 kg or 2 kg heavy gauge polythene bags and stored at -18°C. Samples were taken from the bulk after eight months storage at -18°C and tumbled in a drum at ambient temperature (~ 20°C) for 16 h. They were then dried to 12% moisture content and stored at the same temperatures as the unfrozen samples. Each germination curve was determined in a similar manner to the unfrozen samples apart from an increase from 300 to 900 seed tests and shorter intervals between sampling in some cases.

Bulk storage freezing control experiments were carried out for most of the dormant Triumph barleys from the 1990 harvest. The germination curves for four 1990 barleys in warm storage, frozen and unfrozen before drying are shown in Figs. 2.3 - 2.6. These curves illustrate the lack of any significant pre-drying freezing effect. Fig. 2.6 shows a possible chilling effect on a barley which was stored at 4°C for fourteen days at 18.1% moisture content before freezing. The initial germinability is reduced by about 15% from the value for the sample which underwent minimal cold treatment. This may be evidence of an induced secondary dormancy due to chilling. The effect of low temperature and high moisture (> 18%) on induction of dormancy in wheat and barley before harvest is well documented (Belderok and Habekotte, 1979; Black *et al.*, 1987). Although the initial germinability is reduced, the rate of break of dormancy has not been affected. These results demonstrate that storage at -18°C is a suitable method for storage of undried barley for maintenance of germinability. Any increase in dormancy due to chilling, as long as the later rate of loss is not affected, is an advantage in this work as it extends the range of the germination curve.

### 2.4.2 *Freezing of subsamples from warm storage*

The determination of germination curves for dried barleys stored at temperatures above 20°C requires sampling from storage at periods of three days or less in order to provide sufficient

data points to accurately characterise the curve. The maximum number of germination tests which can be carried out by one analyst limits the number of sample/storage temperature combinations at high sampling frequencies. In order to increase the flexibility of the laboratory germination schedule, it was necessary to find a method of storing subsamples until time was available for testing them. The requirement of the storage regime is maintenance of the germinability. Previous experience with frozen storage of bulk samples made this method the obvious choice. However, whereas the bulk samples were chilled to -18°C from an average ambient temperature of 20°C, the subsamples from 27°C and 38°C storage experience a greater temperature shock.

In order to assess the effect of freezing a control experiment was carried out. Two different barley samples were dried and stored at 27°C and 38°C. When each was subsampled during the storage period a replicate sample was also removed. The first subsample was tested immediately while the replicate (double wrapped in polythene bags) was first cooled for between 3 and 24 h at 5°C and then stored at -18°C. The replicates were taken from the freezer after 4 weeks, thawed at ambient temperature for 3 h and then tested in the usual manner. The curves for the frozen and unfrozen samples at each storage temperature are shown in Figs. 2.7 and 2.8. There is no significant difference between the curves. The verification of this chilling technique enabled more frequent sampling of barleys in high temperature storage which gave more accurate germination curves.

# 3. THERMAL PROCESSING RESULTS

## 3.1 Initial drying and storage studies

Barley sample Ack-Tr-88 was exceedingly dormant, germinating only 5-10% in the 1 ml (agar) test and 0-4% in the 3 ml (agar) test before heat treatments and storage. These values altered little or not at all during at least 2 years storage at -18°C. The maximum observed viability of this sample was 97.5% (Table 2.2).

Samples were dried at 27°C, 38°C and 48°C, to moisture contents of 12%, and were stored at 15°C, 27°C and 38°C. Thus nine temperature combinations were investigated (Table 2.6). In addition, the drying rate was varied for each combination by means of drying air humidity. Initially samples were taken every three weeks but, because the grain was so intensely dormant and slow to mature during storage, sampling times had to be less frequent to retain an adequate stock to investigate. Because of the extremely slow maturation processes, experiments took very much longer to complete than had been envisaged at the planning stage.

As expected, germination initially increased with storage time, and this occurred more rapidly at higher temperatures (Figs. 3.1-3.8). However, recovery from dormancy was so slow that, even after one years storage, grain held at 15°C was not fit to malt.

Despite expectations to the contrary, the *rate* of drying had no detectable effect on the rate of recovery from dormancy. Possibly this would not be the case with a slightly dormant sample that recovered quickly, where the drying period might make up an appreciable proportion of a warm treatment period. This surprising conclusion was drawn from a direct inspection of the graphs (Figs. 3.1-3.8). A further mathematical analysis on the effect of drying rate showed that the rate of germination was also unaffected.

The standard deviations found with individual results were sometimes considerable, as was to be expected when using small numbers of grains (3 x 100) in each test. In addition, there were occasional large fluctuations in the results for which no explanation could be found. Re-testing of other parts of sub-samples showed adequate agreement with the initial results,

eliminating a statistical fluke as an explanation. The fluctuations could not be linked to any factors in the records of how the samples had been handled.

In the samples stored at 15°C the 3 day, 1 ml (agar) germination percentages increased steeply for about 21 weeks to around 60-70%, and then increased more slowly (Figs. 3.1 and 3.2). The 3 ml (agar) germination values increased very slowly indeed with storage time, so that after 48 weeks germination, values were only about 30% (Figs. 3.3 and 3.4). After even a years storage, at 15°C, it is clear that this barley was still too dormant (immature) to malt, and its germination energy figures were well below its germination capacity of around 97%.

In grain samples stored at 27°C both the 1 ml (agar) and 3 ml (agar) germination counts improved more rapidly than occurred in samples stored at 15°C (Figs. 3.5 and 3.6). After an initial rapid rise, to 60-70% in 6 weeks, the 1 ml (agar) count continued to increase more slowly, to 85-90% in 21-24 weeks, at which time the 3 ml (agar) count was about 60% (Fig. 3.6). Although in many samples the 3 ml (agar) count continued to increase, to around 80% in 48 weeks when the 1 ml (agar) count was around 90%, in one sample germinability suddenly fell to unacceptably low levels (Figs. 3.5 and 3.6). Dormancy having been broken, this is attributed to viability loss.

In samples stored at 38°C the initial increase in the rate of germination was most rapid of all, so that in 3 weeks storage the 1 ml (agar) germination values had increased to 60-85% (Figs. 3.7 and 3.8). However, at erratic intervals, and beginning after 3-27 weeks in store, the germinability and viability of various samples fell very rapidly indeed. Germination values (1 ml agar test) peaked or reached a plateau at various values (84%, 91%, 77%, 90%, 83%, 89%, 90%) before germination declined. All these values were below the viability of the grain, which was about 97%. Spot-checks were made on the viability of deteriorating batches of grains. While germinability and viability often declined together, in some instances germinability declined before viability indicating that a fall in vigour preceded death. It was apparent that two different, and probably independent, processes were operating: (a) a progressive, temperature dependent decline in dormancy (maturation), and (b) a progressive, temperature dependent loss of viability. In all probability, grains were losing vigour and beginning to die even while the net germinabilities of the samples was increasing rapidly.

It should be noted that at no stage during these trials were insect or major fungal infestations apparent.

## 3.2 Warm storage at different moisture contents

### 3.2.1 *Initial trials*

Clearly the factors related to loss of viability or the cause(s) of grain deterioration had to be identified and controlled or eliminated since, with extremely dormant barleys like Ack-Tr-88, dormancy will never be adequately reduced by extended periods of cool storage, while at the higher temperatures which are able to reduce dormancy there is the risk of catastrophic grain deterioration.

Two leads suggested how grain viability might be retained during warm storage:

(1) By chance a sample of Doublet barley, which had been extensively dried at 40°C, had been held at 40°C for more than 7 months in a closed container, yet its viability was 98% and it germinated vigorously. It was suspected that this grain sample had been extremely dry, perhaps with a moisture content of 7-8%.

(2) Roberts and his collaborators had demonstrated that the viability of grain samples, stored at constant temperatures, declined much more rapidly at higher moisture contents (Briggs, 1978; Ellis and Roberts, 1980a and b; 1981).

Following these leads it was thought likely that a moisture content of 12%, which is commonly chosen for grain in store to prevent microbial and mite infestations and deterioration of grain stored at U.K. ambient temperatures (15°C or less), was too high a value for 'safe' storage at 27°C and particularly at 38°C. To test this hypothesis samples of barley (Ack-Tr-88) were dried, using a constant drying regime (38°C; 10% RH), to a range of different moisture contents (9.4, 10.3, 11.0, 13.0 and 14.5%). These samples were stored at 38°C and at intervals sub-samples were taken for testing.

dormancy. In fact, close inspection of published data could not distinguish between the possible influences of various factors, including loss of vigour and viability, and, further, little account was taken of the influence of microbes remaining on the grain surface and proliferating at grain moisture contents exceeding around 12.5%. Hiltner (1901) found that treatment in an airflow at 40°C up to 10 days, followed by storage at room temperature for 2-3 weeks, broke dormancy in his barleys. However, many samples of dormant Triumph are not fully matured by this treatment. In addition, it appears that many, supposedly mature, barleys malt better after a warm treatment (Briggs, unpublished) so suitable warm treatments may improve barley vigour.

The work of Gordon (1968) attempts to quantify loss of dormancy by means of an Arrhenius relationship with temperature. In his work on rice seeds, Roberts (1961) first proposed a normally distributed dormancy period. The normal distribution has been more extensively used to describe viability through probit analysis (Ellis and Roberts, 1980a; Ellis and Roberts, 1981). Their work is particularly relevant, since the probit description of viability was tested with barley seeds (Ellis and Roberts, 1980b). The work conducted here builds on these earlier experiments to quantify germination changes in seeds.

It is our view that dormancy is regulated by two groups of factors - those exterior to the grain proper - the microbes and the film of surface water, as examples, and those within the grain. Understanding the influence of microbes would be greatly facilitated if it were possible to quantify the amount of metabolising/respiring microbial material on the grain. 'Plating' techniques cannot supply the data needed since (a) they are inevitably selective for the species they reveal, and (b) they will detect quiescent spores as well as vegetative, actively growing microbes, both single bacteria and large fungal mycelia.

We therefore, as a secondary objective, attempted to find a suitable method of quantification of the actively metabolising microbes present on the surfaces of grains.

Essentially nothing is known about the endogenous factors which regulate dormancy within barley grains. The report by Belderok (1968) that embryos from mature barley grain released glutathione and cysteine while embryos from immature grain did not and that there was a

(after 3 days) increase to a high value and then plateau, but also the rate of germination increases so that after 15 weeks storage the percentage germination of each sample is essentially maximum and is the same after 2, 3 and 4 days incubation. This is true of both the 1 ml and 3 ml (agar) germination test results (Figs. 3.11 and 3.12). Inspection of the other curves, for samples stored for 15-42 weeks, shows no sign of decreasing vigour or other grain deterioration.

When these results are compared with those obtained for Ack-Tr-88 dried to 12% moisture (at 38°C; 10% R.H.) and stored at 15°, 27° or 38°C a number of points emerge, or are emphasised (Figs. 3.13-3.18). In the samples stored at 15°C, while it is true that germination percentage (3 days) and rate of germination increased with storage time, even after 69 weeks storage the grain is not fit to malt (Figs. 3.13 and 3.14). In samples stored at 27°C the optimal germination percentage achieved after 27 and 30 weeks storage, approximately 95% in the 1 ml (agar) test is accompanied by markedly less good results with the 3 ml (agar) tests (75-80%) (Figs. 3.15 and 3.16). Even if the barley had been dried more, to eliminate the occurrence of deterioration, it would have had to be stored much longer to adequately reduce or eliminate 'water sensitivity' and have nearly coincident values for germination percentages in the 1 ml and 3 ml (agar) tests and in the grain viability tests. Comparison of these samples, stored at 38°C and a moisture content of 12% (Figs. 3.17 and 3.18), with those obtained from samples stored at 38°C and 9.4% moisture (Figs. 3.11 and 3.12), emphasise the unacceptable risks of grain deterioration at the higher moisture contents. These risks are clearly apparent after 18 weeks storage. Furthermore, at no stage is the percentage germination of this grain, after 2 days germination in the 1 ml or 3 ml (agar) tests as good as those achieved with grain stored at 38°C with a moisture content of 9.4%.

It is tentatively concluded that by drying dormant grain to about 9% moisture and storing it at 38°C its germinability will be improved to an extent which is unlikely to be matched, and perhaps cannot be matched, by grain stored for longer periods at 27°C or 15°C.
From the trend of the data, it was observed that the logarithm of the time taken for dormancy to decline by a given amount is inversely proportional to the storage temperature. Extrapolating from our own and published data (Gordon, 1968; Narziss *et al.* 1980a and b) it seems likely that when stored at 60°C barley should recover from dormancy in 1/10th -

1/12th time taken at 38°C. We have no information on how dry the grain would need to be, or indeed if any degree of drying would be sufficient to prevent heat damage to grain stored at this temperature. Using the computer model it is now possible to make better predictions (Section 4). Extrapolation with the model predicts a similar increase in the rate of break of dormancy at higher temperatures.

Lack of time prevented a systematic investigation of storage at higher temperatures for breaking dormancy, but some unsystematic observations were made. One batch of barley (Pen-Tr-90) was dried at 40°C, then stored in a closed container at 60°C for up to 9 days. After 9 days it germinated extremely rapidly in the 1 ml (agar) test and appeared to be nearly mature (Fig. 3.19). However, two other samples of barley stored at 70° improved in germinability much less well than expected and it seemed that the vigour of the grain had been reduced. Also a sample of another barley, stored at 60°C, subsequently showed signs of damage. Thus it seems that 60°C is probably a marginal temperature and 70°C is too hot for fresh barley that has been dried at 40°C and will probably have contained about 8% of moisture. It is not known if drying the grain to a lower moisture content would have made 60°C a safe temperature.

A sample of Kir-Tr-90 was dried to 9% moisture content in stages at 38°C then 48°C and subsequently stored at temperatures of 45, 50 and 60°C. The change in germinability during storage was tested using the 4 ml IoB test and is presented in Fig. 3.20. At 60°C the germination reached 80% in less than 1 day, plateaued and then declined to 40% after 3 days. It was concluded that this decline in germinability was due to a loss in vigour rather than a loss of viability, as germinability continued to increase as the germination test period was extended to 8 days. This maximum germination level was not reduced during the period 1.5 to 3 days. At 50°C the germination rose from 10% to 55% in the first day and reached 97% in 6 days. The behaviour at 45°C was similar to that at 50°C, although a little slower as would be expected.

Clearly it is extremely important that, to allow for the most rapid and safe maturation of grain lots, higher temperature/time/germinability studies must be carried out on a variety of grain samples of a range of varieties. It seems probable that some samples of less dormant barleys

would benefit in terms of germination % (3 days) and vigour (germination rate) from being carefully dried and then being given regulated, 'hot' treatments lasting only 1-2 days. The likely benefits to malting are very great indeed.

### 3.2.2 *Germination characteristics of other batches of barley*

Experiments were carried out on several other samples of grain, both those considered by the maltsters to show a limited tendency to dormancy such as Golden Promise (Fig. 3.21) and possibly Camargue (Fig. 3.22), and on samples of varieties considered more prone to deep dormancy, such as Doublet (Fig. 3.23) and other samples of Triumph (Figs. 3.24-3.32). All these samples were dried to 12% moisture, before storage.

A particular sample of Golden Promise lost all its dormancy in 1 week and all its water sensitivity in 3 weeks when stored at 38°C (Fig. 3.21b). At 27°C the rates of recovery were appreciably slower (Fig. 3.21a). With a sample of Camargue germinability stopped improving in both the 1 ml and 3 ml (agar) tests after 3 weeks at 27°C (Fig. 3.22a). At 38°C germinability in the 1 ml (agar) test reached its maximum in about 1-2 weeks, or in the 3 ml test in 3 weeks (Fig. 3.22b). At this high temperature the onset of deterioration was detectable after about 8 weeks and was obvious after 12 weeks. In contrast to the results found with Golden Promise and Camargue, a sample of Doublet remained extremely water sensitive (low 3 ml (agar) germination test results) after 10 weeks storage at 27°C, although the germination in the 1 ml (agar) test had been steady for several weeks at about 95% (Fig. 3.23a). In samples of Doublet stored at 38°C germination increased to slightly higher values of about 98%, in the 1 ml (agar) test, remained at this value for 3 weeks, and then declined (Fig. 3.23b). On the other hand the germination in the 3 ml (agar) test continued to increase until the fourth week of storage, and then declined sharply.

Thus the results confirm that the well-known varietal differences in dormancy are real, and that warm treatments are potentially useful for treating slightly dormant samples of grain.

Several samples of Triumph barley, other than Ack-Tr-88, were dried to 12% moisture and stored at 27°C and 38°C (Figs. 3.24-3.32). As with Ack-Tr-88, recovery of germination in

the 3 ml test ('water sensitivity') was inadequate at 27°C in all except one of the samples (Fig. 3.32a). In the samples stored at 38°C the germinabilities rose, peaked and in many instances declined. In general, the range of Triumph samples tested showed similar behaviour to Ack-Tr-88, the barley selected for the initial experimentation. It was observed that barley from commercial maltings (Figs. 3.31 and 3.32) achieved higher germination levels and appeared to behave more consistently than those from trial sites. The large standard deviations for 3 x 100 seeds was also noted. It was therefore decided, that for the work on quantification of rate of break of dormancy as a function of temperature (Section 4), barleys from commercial maltings should be used together with 9 x 100 seed counting.

# 4. PROBIT ANALYSIS OF DORMANCY

One of the main objectives of the project was to quantify the effects of grain temperature and moisture content on the rate of break of dormancy in malting barley. There is considerable evidence that the effects of storage temperature and moisture on seed viability can be quantified (Ellis and Roberts, 1980a and b; 1981; Roberts and Ellis, 1989; Ellis *et al.*, 1989; 1990; Dickie *et al.*, 1990). In a major investigation (Ellis and Roberts, 1980b) these effects were quantified for barley. It was demonstrated that seed lifespan is normally distributed and using the associated technique of probit analysis the parameters describing the effect of temperature and moisture were determined. In earlier work (Roberts, 1961; 1962; 1965) the dormancy period of rice seeds was shown to follow a normal distribution varying with temperature in a similar way to later data on viability. The logarithm of the mean dormancy period (time to 50% germination) was found to be a linear function of storage temperature. Later work on viability showed that given a normally distributed seed life the standard deviation of the distribution was a better parameter to use than the time to 50% germination as it allowed use of the whole curve in determination of the rate of seed deterioration.

A survey of papers on dormancy was conducted to find data to examine the hypothesis of a normally distributed dormancy period. The most distinguishing feature of a normally distributed dormancy period is the double curvature or sigmoid shape of the germination curve (Fig. 4.1b). A number of papers contained germinability recovery data for barley showing the sigmoid shape associated with a normal distribution (Gordon, 1968; Narziss *et al.*, 1980a and b; Palmer and Taylor, 1983;). Other work on the development of dormancy in the field (Strand, 1965; 1989; Burass and Skinnes, 1985) provided more evidence of a normally distributed dormancy period. The hypothesis of a normally distributed dormancy period was therefore tested using the data presented here.

## 4.1 Theory

Assuming a normally distributed dormancy period, the proportion of germinable seeds is given by the area under the normal curve. The normal curve is shown in Fig. 4.1(a) with the integrated form, Fig. 4.1(b), representing the germination curve. The equation of the normal

distribution curve is

$$\frac{dp}{dt} = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(t-\bar{t})^2}{2\sigma^2}} \qquad (4.1)$$

and the area under the curve is

$$p = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{t} e^{-\frac{(t-\bar{t})^2}{2\sigma^2}} dt \qquad (4.2)$$

where, for seed germination, p is the proportion of germinable seeds, t is storage time, and $\sigma$ is the standard deviation of the dormancy period distribution.

Let

$$X = \frac{t-\bar{t}}{\sigma} \qquad (4.3)$$

where X is called the normal equivalent deviate or probit value.

Substituting for X in Eqn (4.2) gives

$$p = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{X} e^{-\frac{X^2}{2}} dX \qquad (4.4)$$

The probability, p, is a function of X which transforms it into a linear function of time as shown in Fig. 4.1. The probit parameter, X, can be expressed in standard linear form

$$X = a + bt \qquad (4.5)$$

where $\bar{t} = -\frac{a}{b}$ *and* $\sigma = \frac{1}{b}$. In this way, if germination is transformed to X, the slope of the probit versus time curve is $1/\sigma$.

A sample of the data is shown in Fig. 4.2, where the points are the average values of 9 plates of 100 seeds, 4 ml Institute of Brewing test after 3 days (Section 4.2). The probit technique is essentially a weighted regression of the transformed germination percentages. The weights given the normal equivalent deviates are in proportion to the slope of the theoretical curve at

the equivalent germination value as in Eqn. 4.1. The weighting method used when there are replicate values at each time interval is to weight each replicate with a weighting based on the predicted germination value taken from an unweighted line regressed through all of the points (Finney, 1977). Details of the technique and the other statistical methods employed in determining $\sigma$ are presented in the Appendix.

Although the theoretical germination curve asymptotes to 100%, the mean percentage germination achieved in tests once dormancy has broken will be less than 100 due to sampling error and other factors. The theoretical probit line predicts a linearly increasing probit value with time, which represents the asymptoting germination curve. It is necessary, therefore, to truncate the data at a point at which the percentage germination begins to plateau in order to obtain a regression line representative of the major part of the curve as illustrated in Fig. 4.2. Another factor influencing the choice of cut-off point, in some cases, is deviation of germination values above 80% from the theoretical prediction by an amount not explained by sampling error. The exclusion of points from the fitting of lines to transformed germination curves has therefore been a subjective decision but in most cases the maximum percentage germination reached before the cut-off point is above 90%. Data deviating from the probit description will be considered later in Section 4.3, when the data and theoretical prediction are compared.

The inverse of the slope of each transformed germination curve, $\sigma$, is the standard deviation of the dormancy period distribution for that sample at a particular storage temperature and moisture. Work reported in Section 3 on variable moisture content storage demonstrated that, in the range 9% to 14% moisture (wet basis), the rate of break of dormancy is not affected by moisture content. This agrees with other work on dormancy in barley (Strand, 1965; Narziss *et al.* 1980a and b) and rice (Roberts, 1961). The final stage in the definition of the dormancy loss model is therefore the regression of the logarithm of the $\sigma$'s against storage temperature. Each $\sigma$ value is weighted in the log-linear regression by a function of the standard error of the slope of the original probit regression (Appendix).

For any set of data the time, $t = 0$ is defined relative to the theoretical curve by the initial germination. As an aid to comparing the predicted dormancy period distribution with the data

at each temperature a useful technique is to time-shift the data so that each experimental curve passes through the 50% germination point at time zero. Given that the normal equivalent deviate of 50% is 0, the amount by which each data set must be time-shifted, $t_s$, is given by

$$t_s = -a/b = -a\sigma \qquad (4.6)$$

The effect of time-shifting is shown in Fig. 4.2.

## 4.2 Experimental Method

The four barley samples used in the experiments to test the hypothesis of normally distributed dormancy were Triumph barleys obtained from maltings at Pencaitland and Kircaldy in Scotland during the 1990 and 1991 harvests. Three other Triumph barleys from experimental station trial sites were originally included in the experiment but the results for these samples were not included in the probit dormancy model for reasons explained below.

Each sample was dried in an apparatus, as described in Section 2.2, at 38°C, 10% relative humidity to 12% moisture content. Immediately after drying each sample was divided, sealed in glass containers and stored at 38°C, 27°C and 15°C. Two of the samples were also stored at 8°C. Each stored sample was subsampled at intervals of between one day and two weeks depending on the storage temperature. After sub-sampling each subsample was either tested immediately or chilled and stored at -18°C for later evaluation. The germination tests at Newcastle were carried out under conditions specified by the Institute of Brewing Analysis Committee (1986). Samples from tests were also sent to Birmingham for germination testing. The number of seeds used in each test was increased from 3 x 100 to 9 x 100. The duration of the test was extended in some instances to seven days to distinguish between viability and vigour. The storage period for each sample was determined by the initial quantity of seed stored. Maximum germinability was reached in all samples before stocks were used up.

## 4.3 Results

Each mean percentage germination is calculated after the nine test results have been filtered to remove extreme data points. The allowed limits of variation about each mean is based on

a global function determined from a dataset of all sets of replicates used in the analysis (Appendix I, Eqn (1.6)). Applying a 95% confidence limit based on this function to each set of replicates used in the model derivation resulted in the filtering out of 2% of the total dataset. These extreme results were, on the whole, below the test means and were probably the result of microbial infection during the test.

The transformed germination curves, time-shifted to 50% for each of the four storage temperatures, are shown in Figs. 4.3 and 4.4. The mean germinability at truncation for each of the data sets used in the subsequent regression analysis is given in Table 4.1. There is good agreement between the curves at each storage temperature. A significant departure from linearity above a normit value of 1.0 (85% germination) is observed in the samples stored at 38°C. This reduction in rate of dormancy loss is better observed on a percentage germination scale and is discussed later in this section. As Fig. 4.5 illustrates, a more significant deviation from linearity is observed in the trial site barleys, at a lower level of germination. The trial site barleys were dried and stored under the same conditions as the maltsters' barley. With these barleys the initial rate of dormancy loss is slower than for the maltsters' and there is also a decline in rate at higher levels of germination. This decline occurs earlier than for the maltsters' barleys and there is greater variation in germinability. This may be due to immaturity and moisture content of the barley at harvest, irregular growth phases in the field resulting in differences in germinability on the ear and between plants, and also harvesting operations. Whatever the causes of this behaviour, it was decided to base the dormancy model on the germination curves of the maltster's barleys given the agreement between samples grown in different seasons and at different locations.

The $r^2$ value for the regression of each sample probit germination curve is given in Table 4.1. Of those used in the model analysis, all except three are above 0.9 indicating good fits. These results support the hypothesis of a normally distributed dormancy period. A technique for comparing the curves with the sigmoid shape of the cumulative normal curve on a linear scale is to normalise the data by dividing the values of storage time (after time-shifting to 50%) by the $\sigma$ value from the probit regression for each curve. This allows all germination curves at all temperatures to be compared visually. The location of each data point is subject to error due to the error on $\sigma$ and the test error. The mean germination data for the samples

used in determination of the model are shown in normalised form in Fig. 4.6. There is reasonable agreement between the data and the theoretical curve. The double curvature or sigmoid shape of the data is very characteristic of the probit curve and therefore supports the use of this model.

The logarithm of $\sigma$ for each sample was plotted against storage temperature as shown in Fig. 4.7. The best fitted curve, determined as described in the Appendix, was linear. A quadratic fit did not reduce the error significantly. The fitted equation is

$$\log_{10}\sigma = 1.91 - 0.0352\,T \qquad (4.7)$$

where $T$ is the storage temperature in °C. The standard error of the intercept is 0.01, and the standard error of the slope is 0.0005. The dashed lines indicate the 95% confidence limits of the predicted curve. These limits define the limits of the predicted rate of break of dormancy for a particular temperature.

The predicted dormancy loss curve is compared with the time-shifted germination curves at each storage temperature in Figs. 4.8 and 4.9. All of the curves show good agreement with the predicted curve apart from the 38°C curves above 85% as noted above. When the germination tests were continued beyond the 3 day counts presented in Figs. 4.8 and 4.9 until maximum germination was achieved (in general, after 7 days) no reduction was observed in the maximum germinability (Fig. 4.10). The dotted line represents the 7-day germination curve estimated from the 3-day dormancy model by time shifting. No significant reduction in rate of recovery from dormancy or later loss in viability is observed for the 7 day count data. This indicates that at 38°C there is a loss in vigour or rate of germination rather than viability.

In comparison with the maltsters' barley, the germination curves of the trial site barleys shown in Fig. 4.11 do not follow the predicted curve as well. Two samples Inv-Tr-90 and Mot-Tr-90 depart from the predicted slope at between 60% and 70% germination. Also plotted is a sample of Triumph used in the earlier work (Ack-Tr-88) dried and stored in the same regime

as the other samples and tested using 900 seeds at each sub-sampling interval. The germination curves for this barley are very similar to those of other trial site barleys from later harvests. One sample (Abr-Tr-90) increased in germinability at a rate close to that predicted for the maltster's barley up to 80% germination at which point the rate of recovery decreased in relation to the predicted rate.

## 4.4 Comparison of the dormancy model with agar and 3-plate work

Duplicate subsamples of each of the barleys stored at 27°C and 38°C were taken at each storage interval and germinated using the 1 ml and 3 ml agar test (Doran and Briggs, 1992a) at Birmingham. The 3-day germination curves from the 1 ml tests, time-shifted and transformed to probit scale are shown for each storage temperature in Fig. 4.12. Apart from one of the maltsters' barleys, Kir-Tr-90, there is a difference in slope between the sample curves and the predicted line at both temperatures. The percentage germination curves for the trial site and maltsters' barley, at each storage temperature, are shown in Figs. 4.13 and 4.14 respectively. The departure of the trial site barleys from the model is most apparent at 27°C although there is consistency in rate of recovery from dormancy between samples. The curves for the maltsters' barley are closer to the predicted curve, particularly at 38°C. The difference between the observed and predicted is, again, greatest at 27°C.

The predictive value of the model was tested by using germination data for Triumph barleys of the 1990 harvest dried and stored at 27°C and 38°C as part of the controlled freezing experiment discussed in Section 2.4. This data is the result of 4 ml tests using 300 seeds per test and therefore can be compared directly to the method used by the malting industry. In these predictions the initial germinability is used as the start point of the predicted curve. The standard error of this initial value is taken as either that determined in this analysis (Appendix I, Eqn. (I.6) or the actual test error, whichever is the larger. The comparative plots are shown in Figs. 4.15 and 4.16. At 27°C, apart from two trial site barleys, the time to 95% germination is predicted reasonably well by the model. At 38°C, however, the model under-predicts the time to 95% germination in most cases. This is possibly due to the loss of vigour discussed previously.

The model is also compared with two other varieties, Doublet and Camargue, also from the 1990 harvest which were treated in the same manner as the barleys discussed above. The Doublet sample was from a trial site while the Camargue was from a commercial maltster. The comparative plots are shown in Fig. 4.17. Comparison of the predicted curve with the slower rate of recovery of Doublet is complicated by variable behaviour particularly at the lower temperature. At 38°C the predicted time to 95% germination is close to that observed given the large errors in the results. Camargue in comparison, recovered from dormancy at a faster rate than predicted for Triumph, particularly at the higher temperature. At 27°C the germination curve was at the upper limit of the model curve. Given the error bars for 3 x 100 seeds and the limited number of data points, further work is necessary to define more exactly whether the rate of recovery of Camargue or Doublet is significantly different from Triumph and whether it follows the same relationship with storage temperature.

## 4.5 Comparison of the dormancy model with other work

The model is compared with data from previous work on barley dormancy in Fig. 4.18. The line of Riis *et al.* (1989) is calculated from their model which is based on Triumph barley. No data or confidence limits are given with the model. The other data points are estimated from germination curves after transformation to probit form and visual fitting of probit curves.

The data of Narziss *et al.* (1980a and b) were the most extensive; the $\sigma$ values are subject to the least error and derive from a controlled study of cold and warm storage of the barley variety Igri. The data of Strand (1965) and Burass and Skinnes (1985) are derived from the results of experiments on Scandinavian barley varieties.

The data of Buras and Skinnes are calculated from the mean results for nine different lines. This work supports the idea that rate of dormancy development and loss is genetically determined and environmentally influenced in a similar manner before and after grain ripeness once the dough stage is completed. Since none of the data are based on Triumph barley a direct comparison with this work is not possible. The temperature effect on rate of dormancy loss does, however, appear to be similar for the data points presented. This similarity should not be treated as more than an observation since for predictive purposes this difference is quite large when the $\sigma$ is converted from the log to a linear scale.

## 4.6 A model of dormancy and viability

The work in this section has shown that the rate of recovery from dormancy can be described by the cumulative normal distribution curve. In their work on viability, Ellis and Roberts (1980b) presented a model for the viability loss of barley as a function of temperature and moisture content. This was also based on the cumulative normal distribution or probit technique. It is now possible to combine these two models to predict the germination history of a barley in storage.

Eqn (4.4) can be written

$$p = \phi(X) \qquad (4.8)$$

where $\phi$ is the cumulative normal distribution function. The percentage viability as a function of time can therefore be written

$$G_v = 100\ \phi(X_{vi} - t/\sigma_v) \qquad (4.9)$$

where $X_{vi}$ is the initial viability in probit form and $\sigma_v$ is the standard deviation of the normally distributed seed life given by Ellis and Roberts (1980b) as

$$\log_{10} \sigma_v = 9.983 - 5.896 \log_{10} M - 0.04\ T - 0.000428\ T^2 \qquad (4.10)$$

where M is the moisture content (% wet basis) and T is the temperature in °C. The percentage dormancy is similarly described by

$$G_d = 100\ \phi(X_{di} + t/\sigma_d) \qquad (4.11)$$

where $X_{di}$ is the initial germinability in probit form and $\sigma_d$ is the standard deviation of the normally distributed dormancy period as given in Eqn (4.7).

If the processes of loss of viability and break of dormancy are not linked, in the sense that a dormant or non-dormant seed is equally likely to lose viability, then we can write the apparent germinability as

$$G = G_d G_v / 100 \qquad (4.12)$$

Before using this equation, it should be noted that the dormancy model was derived from germination counts after 3 days, whereas the viability model is based on germination testing according to the International Seed Testing Association (1976) standard, which is concerned with the development of a healthy seedling. However, there is some evidence from Fig. 4.10 that the rate of break of dormancy during storage may be similar for a 3 day or a 7 day count. Nevertheless, the model gives a useful insight into the germination history of a barley. The calculations are straightforward and were placed on a microcomputer to give a graphical output of germinability, G with time in storage. The inputs to the model are storage temperature, storage moisture content, initial viability and initial germinability.

Figures 4.19 to 4.22 illustrate the use of the model. The sensitivity of the germination history to the input variables of initial viability, initial germinability, storage temperature and moisture content is examined. Figure 4.19 demonstrates the importance of barley quality, as defined by initial viability. Barleys with a 1% lower germination rate are considerably more vulnerable in this 99% region. This viability or germinative capacity refers to thermal ageing and not to other damage events. For example, mechanical damage in handling might reduce the count in a staining test but this would not imply an ageing of the undamaged seeds. A staining test is therefore likely to give an underestimate of seed quality from an ageing point of view.

The effect of moisture content is shown in Fig. 4.20, for a warm storage condition of 33°C. Malting barley stored at one percentage point above the normal 12% is considerably more at risk. In Fig. 4.21, the effect of increasing the storage temperature to 40°C can be observed. Barley at 12 and 13% moisture content is predicted to be more at risk, even assuming an initial viability of 99.9%. In both Figs. 4.20 and 4.21 the effectiveness of warm storage in breaking dormancy can be observed. It would appear from this work that an increase in germinability from 20% to 95% can be achieved in about 15 days at 33°C. At 40°C this period is reduced to around 8 days but with greater risk to viability damage.

Although rates of change are greatest at warm temperatures, Fig. 4.22 illustrates that over sufficiently long time periods there is deterioration even when the grain is stored cool (12°C). For long term storage, it is clear that again barleys with a high viability or germinative

capacity are far more durable.

The model gives useful insights into the interaction of storage conditions and barley quality and, within the limits of the assumptions on which it is based, serves as a guide to storage strategies.

## 5. MICROBES ON GRAIN

The evidence that microbes on the grain and in the surrounding film of water can reduce germinability, and so increase dormancy, is now overwhelming (Gaber and Roberts, 1969; Briggs, 1978; Briggs *et al.*, 1981; Kelly and Briggs, 1992a,b and c; Doran and Briggs, 1993b). It is well known that steeping in warm water can induce water sensitivity in grain (Lubert and Pool, 1964) and that warm, humid and wet weather can induce 'secondary dormancy' in grain standing in the field. We attribute these findings, at least in part, to increases in the population of microbes on the grains and in the surrounding water. Not infrequently fully viable grain becomes discoloured by heavy microbial populations on the husk.

We needed a method for quantifying metabolically active microbes (not dead individuals or colonies and not quiescent forms like spores). We failed to obtain convincing results when counting colonies of microbes from grain samples plated on a nutrient agar. In any case we needed a method to quantify the effectively metabolising or respiring mass of microbes and not a technique which might well 'score' a quiescent spore, a single bacterium and a massive fungal colony equally, and which would almost inevitably be selective, since particular nutrient media inevitably favour some species over others.

We attempted to quantify 'active microbial mass' in three ways: (a) by following the incorporation of radioactivity from [$^{14}$C]-glucose into insoluble material in the grains' surface layers. (b) By measuring the amount of formazan produced from the reduction of triphenyltetrazolium chloride in the surface layers of the grain. (c) By following the respiration rates of husk peeled from grain and of steeping liquor (Briggs and McGuinness, 1993). Measurements were made in thermostatted Hansatech cells using oxygen electrodes. All these approaches had strengths and weaknesses.

### 5.1 Experiments with [$^{14}$C]-glucose

We confirmed that [$^{14}$C]-glucose penetrates the husk and pericarp, but will not pass the testa and gain access to the interiors of undamaged grains. Thus radioactivity from [$^{14}$C]-glucose

'fixed' in the surface layers, has presumably been incorporated into microbial material, since the husk and pericarp are dead tissues.

Grains were soaked for a short period then were incubated with a solution of [$^{14}$C]-glucose and ammonium nitrate, which was used as a source of nitrogen. At the end of the incubation period the grains were thoroughly rinsed with a strong ethanolic solution of unlabelled glucose, to displace from the grains any 'unmetabolised' [$^{14}$C]-glucose and low-molecular weight microbial metabolites. Grains were then dried, ground and dissolved in 'Soluene', at 40°C. After bleaching the solution, with hydrogen peroxide in sunlight, the dissolved radioactivity was determined by scintillation counting. In some instances grain samples were decorticated with 50% sulphuric acid, to remove the husk, lodicules and pericarp, after incubation with [$^{14}$C]-glucose and washing, but before determining the remaining radioactivity. The results were unequivocal - samples that had been decorticated gave counts of 200 and 250 disintegrations *per* minute while comparable samples of grains that had not been decorticated gave counts of 6530 and 6780 d.p.m. When blanks were allowed for it was clear that all the 'fixed' radioactivity was in the surface layers of the grain that could be removed by treatment with sulphuric acid, the husk, lodicules and pericarp. Subjecting the grain to an antimicrobial treatment to reduce the population of microbes (rinsing with a solution of calcium hypochlorite followed by a solution of silver nitrate) reduced the subsequent level of fixation of [$^{14}$C] from radioactive glucose by more than 52%.

Thus our expectation (Briggs, 1978; 1987) that externally applied glucose will not penetrate the testa and gain access to the living tissues of the grains and that the microbes in the surface layers will metabolise it and 'fix' a proportion, have been confirmed. However, the techniques involved are expensive and slow and involve handling radioactive samples. We therefore sought an alternative method that would be rapid and inexpensive and might be suitable for use in routine laboratories.

## 5.2 Experiments with triphenyl tetrazolium chloride

The next approach was to try and quantify 'metabolising microbial mass' by its ability to reduce colourless triphenyl tetrazolium chloride (TTZ) to a dark red, water-insoluble formazan

(Briggs and McGuinness, 1993). Tetrazolium salts do not penetrate into the interiors of undamaged grains (where they are reduced in the embryo and aleurone layer as in the familiar viability test), so by incubating grain in a solution of TTZ buffered with phosphate and containing glucose and sodium succinate, that the microbes can use as reductants, it was hoped that the amount of red formazan, deposited in the surface layers, would be a measure of the amount of metabolically active microbes present. After rinsing and air drying the grain the formazan could be quantified by extracting it with dimethyl formamide (DMF) and estimating it spectrophotometrically, measured against appropriate blanks, by its absorbance at 485 nm.

Incubations were carried out at 25°C. Formazan colour increased progressively with time, but in some samples (not all) there was a 'kink' and then an acceleration in the rate of formazan deposition after about 2 h incubation. We tentatively concluded that microbial mass had started to increase significantly after 2 h and, as we wished to estimate the amount present at the start of the incubation, we routinely used 2 h incubation periods. The standard deviations of samples, tested in triplicate, were often larger than were easily tolerable. The tentative explanation for this is that individual grains vary widely in their degrees of microbial contamination, and this significantly influenced the results because of the necessarily small samples of grains used (usually 10 g). The 'stainability' of individual grains has not been investigated, but clearly this tetrazolium technique might be a suitable way of testing the variability of microbiological contamination on individual grains in a batch.

The test, as initially carried out, using a 0.2% solution of TTZ, gave low colour yields and was insensitive. In various trials, using several different grain samples, TTZ concentrations were varied in the range, 0.05 - 0.5%, 0.05 - 1%, and 0.2 - 2% (Fig. 5.1). These trials were successful, in contrast to later ones in which physically damaged grains were involved (see below). In each trial the maximum colour yield achieved in a 2 h incubation was with the highest concentration of TTZ. Colour yield did not increase in direct proportion to reagent concentration.

When the pH of the reagent mixture was varied in the range 3-8 colour yield varied relatively little, but was largest at pH 8.0. A pH of 6 was arbitrarily chosen for all standard tests as it was not too far removed from normal, initial steep pH values and it was feared that at

alkaline pH values non-specific chemical reduction of TTZ might be occurring. The influence of varying the supply of glucose and succinate in the reagent mixture was also investigated. Samples of barley were pre-steeped at 18°C for 64 h, with occasional mixing to allow an increase in the numbers of microbes. They were then incubated in solutions of 2% TTZ, buffered at pH 6, but in which the concentrations of glucose and sodium succinate were varied. The largest formazan colour (0.17) was obtained when glucose and succinate were present. Omission of glucose (0.1%) or succinate (5 mM) or both from the reagent gave rise, when incubated with samples of the same barley, to colour yields of 0.14, 0.14 and 0.12 respectively. However, increasing the concentrations of glucose and succinate did not increase the yield of formazan. The results indicated that colour yield was at least partly dependent on the metabolism of glucose and succinate by microbes which would, in addition, be gaining other nutrients from the surface layers of the grains.

When grains were steeped in water, at room temperature, for 18 h the colour yield (determined using 2 h incubations with TTZ reagent) increased from 0.037 to 0.103, as would be expected if the microbes were increasing in amount. Samples of another lot of grain were steeped in water or various very dilute solutions of hydrochloric acid (0.032 - 1 mM; final steep pH values, water, 6.4; acid solutions 6.3 - 3.8). The colours given in subsequent TTZ tests declined with increasing acid concentrations to 0.057. This finding, which has been confirmed, is consistent with the idea that slight acidity checks, but does not prevent, the multiplication and metabolism of microbes. Treatments with dilute mineral acids have been reported to improve the germinability of water-sensitive grains, presumably by antagonising the microbes (Doran and Briggs, 1992b).

In trials with another barley steeping at 18°C for 17 h caused no increase in colour formation (TTZ test) and the inclusion of hydrochloric acid in the steep did not reduce the colour to a value significantly less than that given by the unsteeped control. It is to be expected that the microbial population density, and probably the particular species present, will vary between batches of grain, and this might explain the difference in staining.

However, when steeping at 18°C was prolonged to 68 h in water or 10 mM hydrochloric acid, the pH values determined after steeping were 5.4 and 2.3 respectively and the colour yields

were 0.204 and 0.092. Unsteeped control grains gave colour yields of 0.066. Thus even 10 mM acid only checked, but did not prevent, an increase in the 'tetrazolium reducing microbes' over an extended steeping period. In shorter, 29 h steeps, acid concentrations of 1, 10 and 50 mM prevented the increase which occurred during steeping, but did not reduce it significantly below the value of the unsteeped control.

Aerating steeps and steeping at elevated temperatures both favour the proliferation of microbes (Kelly and Briggs, 1992a, b and c). Experiments were carried out at 18°C and 25°C with grains steeped in water or hydrochloric acid and shaken (aerated) or unshaken. The results of one experiment (Table 5.1) clearly indicate that the surface microbes on the grain increased considerably at 25°C but to a lesser extent at 18°C. The increase in the population of microbes was largely prevented by acidification of the steep water. Subjectively the turbidity of the steep water was related to the grain stainability, being greater for samples which stained more intensely. It seems likely that, in addition to improving aeration, shaking rubs grains together and dislodges microbes into the steep liquor. This would explain the discrepancy between shaken and unshaken samples incubated at 25°C (Table 5.1).

In an attempt to increase the sensitivity of the TTZ method a group of 4 samples of grain was steeped in buffered TTZ solutions (2%, 3%, 4% and 5%) for 2 h. The mean colours were 0.178, 0.227, 0.303 and 0.283 respectively. When samples were incubated in 2% and 4% solution of TTZ for 1, 2, 3, 4 and 22.5 h, the colours increase approximately linearly for the first 4 h, the intensities being much greater with the 4% solution of TTZ (Fig. 5.2). The lines did not extrapolate back to the origin of the graph. After 4 h incubation the colours were 0.313 and 0.475 for samples incubated with the 2% and 4% solutions of TTZ. However, it was noticeable that in the samples steeped in 4% TTC the DMF failed to dissolve all the formazan and bring all the colour into solution. It seemed that physical damage had allowed TTZ to penetrate the grains, and allow formazan deposition in the embryos and aleurone layers. Another observation which caused concern was that drying grain (at 40°C/3 days) and storing warm, at 60°C, for up to 9 days dramatically improved grain germination (Section 3) but the tetrazolium test colour declined relatively little compared to the undried control (0.130 against 0.155) although the warm storage treatment was expected to greatly reduce the microbial population on the grains. In another instance drying at 40°C and storing

at 70°C reduced the colour from 0.204 (undried) to 0.159. It is unclear if heat-killed microbes are still capable of reducing tetrazolium salts (as in the case with some heat damaged barley embryos) or if some other heat-resistant reducing system is present in the grains' surface layers.

These observations, combined with the substantial standard deviations of the results, suggested that although the TTZ method has its uses, it was too insensitive for our purposes and, indeed, when damaged grains were present, so that the TTZ could reach the embryo and aleurone layer, the results could be misleading.

### 5.3 Oxygen uptake experiments with husk preparations

As the third approach to assessing microbes on the grains, we elected to investigate the respiration rates of husks from dormant and non-dormant samples (Briggs and McGuinness, 1993). French workers have noted that husks stripped from dormant barley and oats take up oxygen (respire) more rapidly than those that have been taken from grains that have been 'after-ripened' by dry storage. The measurements were made on husks wetted with water in respirometers. With husk taken from grains of increasing maturity the initial respiration rates of the husk preparation decline. However, over a period of about 50 h the respiration rates of the samples rose, peaked and subsequently fell. The peak respiration rates were about the same for all the samples, but with increasing grain maturity the peaks occurred later during the incubation period (e.g. Corbineau *et al.*, 1986; Lenoir *et al.*, 1986). The French workers attributed their results to the activities of polyphenol oxidase activities in the husk. We could not reconcile their explanation with the fact that the husk is a dead tissue and so enzyme activity, derived from the husk itself, should not change during the experimental period. We therefore set out to test the hypothesis that the French observations were due to the activities of microbes and to see if measurements of husk respiration are a suitable way of quantifying the microbes that are present. A complication in interpreting the French results is that their germination tests were carried out on wet cotton wool and so it is not clear how, or if, they resemble the more usual germination tests (4 ml, paper; 1 ml agar) or 'water sensitivity' tests (8 ml paper; 3 ml agar) used in this country (Doran and Briggs, 1992a). However, it is likely that their results most nearly resemble those of our 'water sensitivity' tests. It is now

well known that suppressing microbes alleviates water sensitivity (Gaber and Roberts, 1969; Briggs, 1978; Kelly and Briggs, 1992a,b and c; Doran and Briggs, 1993b).

The 'model' we set out to test was that the husks of quiescent 'air-dry' grains contain quiescent, vegetative microbes, microbial spores, and substances which act as nutrients for the microbes. When the husks are wetted the vegetative forms of the microbes will begin to grow at once, spores will germinate after a lag period and the organisms derived from them, as well as the vegetative microbes, will multiply and respire as long as an adequate supply of nutrients is available. The microbes and the nutrients will become distributed between the husks and the surrounding water. When the nutrients become limiting the respiration rate of the microbial populations will check and will then decline. This decline will not necessarily indicate microbial death but may be caused by a lack of oxidizable nutrients. This model seemed to fit the French results since: (a) the more mature grains had husks which initially respired less, as would be expected if a decreasing proportion of the microbes survived storage and those which remained were present as spores, which would only germinate after a lag; (b) the peak respiration rate, which was essentially the same for all samples whatever their maturity, was followed by a decline which could be rationally explained if these were regulated by the nutrient supply. Our experiments were carried out in Hansatech cells, with the thermostat set at 25°C, and containing a Clark-type oxygen electrode that permitted the continuous measurement of the oxygen dissolved in the water (usually 2 ml) in which the strips of husks, peeled from damped grains pre-soaked for 1 h, were suspended. Usually 8-10 mg husk, stripped from 10 grains, were used in each experiment. The cell contents were continuously mixed with a magnetic stirrer. These conditions were significantly different to those occurring in the French experiments in which 100 mg of husk were wetted with 0.9 ml water and were held in air. These differences are sufficient to explain the quantitative differences between their results and ours. For technical reasons we could not exactly replicate the experimental conditions used by the French workers. Between measurements of respiration the 'cultures' were exposed to air. Respiration was measured by closing the cells and measuring the decline in oxygen content of the water with time, as recorded on a strip chart recorder.

The initial respiration rate of batches of husk from one batch of grain varied (e.g. 3-6 n mole

$0_2$/h./mg husk) as was expected since it had been deduced from the tetrazolium experiments that levels of microbial infestation vary between individual grains. During 50-60 h the respiration rates rose, peaked after about 24 h, and then declined. The greatest peak respiration rates measured were 27-28 n mole $0_2$/h./mg husk but usually the values were much less, around 12-16 n mole/h./mg husk. Changing the water, after about 23 h, when respiration was near its peak value, removed suspended microbes and soluble nutrients and immediately reduced the respiration rate, that of the husk strips suspended in fresh water was less by more than 50%, from 17 to 7 n mole $0_2$/h/mg in one instance. The steep water itself continued to respire, indicating that microbes were present in suspension and that these must have been supported by nutrients derived from the husk. The respiration of the husk in the fresh water continued to decline for about 48 h, suggesting that supplies of nutrients were being progressively used up. Adding glucose to the preparation *immediately* increased the respiration by a factor of x 2-3, showing that a lack of nutrients was indeed limiting the respiration rate, and that the respiration was almost certainly due to microbes.

Steeping husks in water containing a mixture of antibiotics chosen to suppress the growth of yeasts, fungi, gram-positive and gram-negative bacteria (penicillin G, streptomycin sulphate, amphotericin B and nystatin) reduced, but did not eliminate, husk respiration. The antibiotics did prevent any rise in respiration over a 48 h incubation period in this trial. Initial respiration rates were 4-5 n mole $0_2$/h/mg husk but during the incubation period they declined to 1-3 n mole/$0_2$/h/mg. In contrast controls, incubated in water only, respired at the same initial rate, but oxygen uptake peaked as usual after about 24 h, at about 27 n mole $0_2$/h/mg husk before declining to 8-11 mole $0_2$/h/mg at 52 h. Husk from grain that had been heat-treated, to break dormancy, respired less than husk from untreated control grain and in one trial the respiration rate remained less throughout a 49 h experiment, so that the initial rates (heat treated and control samples respectively) were initially 0.8 and 4 n mole $0_2$/h/mg, peaked (26-28 h) at 6-7 and 12-15 n mole $0_2$/h/mg. When, after 49 h, nutrient broth was added to the heat treated sample, the oxygen uptake jumped from 0-3 to 36 n mole $0_2$/h/mg. When, after 55 h, the husk was resuspended in fresh water it and/or the microbes had evidently taken up nutrients from the broth added to the medium as its respiration was then 14 n mole $0_2$/h/mg. It appeared that heating had sharply reduced the availability of nutrients for the microbes as well as reducing the initial microbial population. However, in another trial, while the initial

respiration rate of heat treated husk was less than that of the control, after about 22 h it exceeded that of the control so that after 28 h the values were 8 compared to 6 n mole $0_2$/h/mg. The addition of nutrient broth to the heat-treated and control samples, after 52 h, increased the respiration rates to 53 and 39 n mole $0_2$/h/mg respectively. Thus although heat treatment reduces the initial population of respiring microbes after a wet period of incubation the population can come to equal and even exceed that of the controls.

The respiration of another control after 49 h incubation was 8 n mole/h/mg and increased immediately to 52 n mole $0_2$/h/mg on addition of nutrient broth. Subsequently, over 4 h, the respiration increased further, to 65 n mole $0_2$/h/mg, indicating that initially nutrients limited respiration and that added nutrients immediately supported a higher rate of respiration followed by extra microbial growth.

After incubating a fresh sample of husk for 6 h rinsing and putting it into fresh water reduced the respiration relatively little, from 6 to 5 n mole $0_2$/h/mg. Subsequently, 25-28 h later, the respiration of the rinsed husk roughly equalled that of the husk in unchanged water, at about 9 n mole $0_2$/h/mg. Thus a wash, even of 6 h, is disappointingly ineffective at reducing the microbial population and nutrients. Thus, microbes are responsible for husk respiration and both the number of microbes on the husk *and* in the surrounding liquid and the availability of respiratory substrates control the respiration rate of a mixture of husk tissue in water.

Grains were steeped at 25°C either in still or shaken water. The grains in the shaken and aerated steep remained in the same water throughout, but the water in the unshaken steep was changed after 1, 5 and 8 h (Table 5.2). Shaking was by a mechanical device, and was much more efficient than that used in the TTZ experiments. More microbes were present in the husks from the shaken grains, as judged by the respiration rates, and many more were in the unchanged liquid of the shaken samples. However, the differences in respiration rates between husk samples (after rinsing) were not as large as anticipated, again indicating that washing grain is not as efficient as dislodging microbes and depleting nutrients as hoped. Clearly the interactions between so many factors makes the results of this, apparently simple, system hard to interpret in detail.

In a further trial some grains were steeped, by being shaken in water, others remained without disturbance under water, while still others were steeped in water that had initially been degassed (by being ultra-sonicated under vacuum) to remove dissolved oxygen. At intervals the respiration of samples of steep water or husks from the grains were determined, both before and after additions of nutrient broth, which was used to provide respiratory substrates. The results (Table 5.3) indicate that restricting access of oxygen to the steeping grain limited the multiplication of microbes both on the grain and in the steep liquor. Thus in malting it may be advantageous to avoid aeration in the early stages of steeping, when the respiration rate and oxygen requirements of the grain itself are known to be low. In subsequent trials it was shown that the addition of hydrochloric acid, to a final concentration of 10 mM, reduced or eliminated the respiration of both the husks and the microbes in the steep liquor. Microbial respiration always built up faster in husks and steep liquor from aerated samples. Steeping grain in hydrochloric acid (10 mM) always checked microbial respiration. Thus steep acidification combined with the avoidance of aeration is a feasible way of preventing the build up of microbes in the first steep. It has been shown that acidification with several mineral acids checks dormancy (e.g. Doran and Briggs, 1992b; 1993a).

The results shown in Table 5.4 (while showing two anomalous reductions in respiration after additions of nutrient broth) illustrate that merely changing steep liquor (Treatment B), when the grain is aerated, had a minor effect on the microbial population on the husk relative to continuous static steeping (Treatment A), although changing the steep liquor led to lower respiration rates in the liquor after addition of broth. However, air-resting *and* rinsing between water changes (Treatment D) reduced the respiration of the steep liquor (in the absence of added nutrients) relative to 'air rested and changed' steep liquor (i.e. without rinsing: Treatment C). Air resting between liquor changes appeared to reduce husk respiration to a level below that found when only the liquor was changed (Treatments C and D, relative to Treatment B). Clearly, the effects of changing the steep liquor, with the dilution of nutrients, removal of suspended microbes and re-aeration of the steep has complex effects.

In other trials, it was clear that subjecting grain to a period of warm storage, to reduce dormancy, reduced 'husk respiration' and the respiration of the microbes in the liquor when

the grains were steeped and that 'static steeping' in degassed water maintained the microbial population at a low level, while shaking and aerating encouraged an increase.

We conclude that microbes on the surface layers of grains and in the steep liquor are responsible for the observed oxygen uptakes. These microbes depend on nutrients leached from the husk and pericarp.

Measuring the respiration rates of husks and steep liquor is technically more complex and expensive than measuring formazan production from TTZ, but this approach can yield more information. It is proposed that when nutrient broth is added, to provide an excess of respiratory substrates, the oxygen uptake rate is a direct measure of the total amount of microbial mass that is able to respire. On the other hand, in the absence of added nutrients, the observed respiration rate is a complex reflection of both the 'respiring microbial mass' and the (usually limiting) supply of nutrients available to the microbes.

It is well established that microbes on grain and in the steep liquor can have major effects on grain germinability and maltability (e.g. Briggs, 1978; Briggs, 1987; Doran and Briggs, 1992a and b; 1993b; Kelly and Briggs, 1992a,b and c). Thoroughly washing grain and slightly acidifying the first steep liquor would be ways of reducing microbial activities. However, the most practical approach would be to carefully dry and store grain warm, then to carry out the first steep under anaerobic conditions, if possible using degassed water, to avoid the multiplication of microbes at this stage. This approach could be reinforced by carrying out the anaerobic steep in slightly acidified water. It seems probable that steeping anaerobically to a moisture content of about 35% then giving an air-rest would check the multiplication of microbes but not the subsequent growth of the grain which, indeed, in the absence of a substantial microbial population should be more vigorous and uniform (Briggs *et al.*, 1981).

# 6. SULPHUR-CONTAINING SUBSTANCES AND DORMANCY

The biochemical causes of dormancy are obscure. The present studies were initiated in the belief that two sets of observations pointed firmly to the involvement of thiol-containing compounds in barley dormancy. Firstly, Belderok (1968) reported that unripe barley embryos were unable to release glutathione and cysteine but that when the embryos were taken from ripe grains, release of these thiol containing substances did occur. Furthermore, he reported a good correlation between the sum of the amounts of the thiols present in the embryos after a 24 h soak and the percentage of grains germinated after three days. Secondly, Pollock and Kirsop (1956) reported that soaking dormant barleys in solutions of thiols (including hydrogen sulphide and isopropyl mercaptan) overcame profound dormancy but not water-sensitivity. We have confirmed some of these observations (Doran and Briggs, 1993a; see below).

The objective, therefore, was to investigate the role of thiols and their oxidised products, disulphides, in dormancy. The first requirement was to identify the thiols and disulphides present in the grain and develop methods for assessing them quantitatively. Then, armed with the necessary experimental techniques, we intended to investigate the thiol and disulphide-containing materials in the embryos of dormant and non-dormant grains under a variety of conditions.

## 6.1 The analysis of thiols and disulphides in parts of barley grains

The original intention was to employ a combination of published analytical methods. However, it became apparent that, as originally formulated, these were incapable of delivering reliable results when applied to extracts of barley embryos. Considerable developmental work was required to obtain reliable data, but this will only be referred to briefly to explain particular procedures used in the final methods.

Initially, thin layer chromatography and thin layer electrophoresis, in single- and two-dimensional separations, were used in attempts to identify the highly fluorescent monobromobimane derivatives of the thiols present in extracts of barley embryos. The clear resolution of these derivatives, reported by the originators of the method (Fahey *et al.* 1980)

were not achieved. However, it was apparent that the main thiols present were likely to be cysteine and glutathione. No way was found of improving the resolution of these methods and so their use was discontinued.

Soluble thiols were extracted with a solution of sulphosalicylic acid containing EDTA. This reagent minimises the auto-oxidation of thiols and thiol-disulphide exchange and precipitates proteins. The thiols (total) were estimated in these extracts by a method based on the reports of Ellman (1959) and Jocelyn (1987). Under alkaline conditions thiols react with 5,5'-dithiobis (2-nitrobenzoic acid), otherwise DTNB or Ellman's reagent, undergoing thiol-disulphide exchange, giving a derivative in which the 5-thio 2-nitrobenzoic acid is linked via a disulphide link to the original compound and 5-thio-2-nitrobenzoic acid is quantitatively liberated (Fig. 6.1). Under alkaline conditions the latter molecule ionises to give the thionitrobenzoate ion, which is bright yellow and can be quantified by its absorbance at 412 nm. Our results confirm those of Silverstein (1975) that the molar extinction coefficient for the thionitrobenzoate ion is 13,600. From this the amount of thiol originally present can be calculated. We separated the DTNB derivatives of the thiols by reverse-phase h.p.l.c. and quantified them, with reference to internal standards, by their absorbance at 330 nm. Using purified, authentic derivatives this wavelength was shown to be the absorbance maximum for these substances.

Thiols were separated from other substances in crude extracts of grain by allowing them to bind to beads of cross-linked agarose substituted with side chains to which *p*-hydroxymercuribenzoate residues were attached. The methods used for preparing the *p*-HMB agarose beads were based on those of Cuatrecasas (1970) and Axén *et al.* (1975). The Sepharose 6B gel beads were first reacted with epichlorohydrin to 'activate' the gel by providing side chains carrying epoxy-groups (Fig. 6.2). In turn this 'epoxy-activated' agarose was allowed to react with an excess of ethylene diamine, which gave rise to a side chain terminating in an amino group (Fig. 6.2). *p*-Hydroxymercuribenzoic acid was coupled to the amino group, via a peptide link formed in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Fig. 6.3). With a binding capacity of about 8 μmol reduced glutathione/ml packed *p*-HMB agarose the gel had about twice the thiol-binding capacity of preparations made from commercial 'amino-ethyl agarose'. While short columns of *p*-HMB agarose bound thiols

from standard solutions they failed to achieve quantitative binding when the thiols were present in crude grain extracts. Eventually it was demonstrated that the *p*-HMB-binding groups were being blocked with non-thiol substances in the grain extracts. These appeared to be polyphenols since, with time, the columns turned brown. By treating the crude extracts with the solid adsorbent PVPP (polyvinylpolypyrrolidone), to bind polyphenols, grain extracts were obtained from which the thiols were removed quantitatively by the *p*-HMB agarose columns (Fig. 6.4). The bound thiols were subsequently released from the column with DTT (dithiothreitol) (Fig. 6.5) and after derivatisation with DTNB, were analysed by h.p.l.c.

The original intention was to reduce the disulphides (oxidised glutathione, GSSG; cystine, CysSSCys), which emerged from the *p*-HMB agarose column, by passing the mixture through a second column containing bound dihydrolipoyl agarose. The resulting thiols were to be trapped in a second *p*-HMB agarose column. However, the performances of the 'reducing' columns were erratic and their use was discontinued. Attempts were made to reduce the disulphides in the eluate from the *p*-HMB agarose directly, using DTT or $NaBH_4$. These attempts failed. Eventually it was shown that this failure was due to raffinose and sucrose, or their breakdown products, in the eluate. Thus before the disulphides could be reduced they had to be separated from the neutral substances which were also present. This was achieved by ion-exchange chromatography. Several systems were tested and discarded. Eventually pre-packed small columns containing a silica-based, strongly acidic medium having sulphonic acid substituents proved successful. Cystine, oxidised glutathione and cystamine (the internal standard) were quantitatively retained on two SCX columns, used in series, and were all eluted, to about 80% extent, with a strong phosphate buffer containing EDTA and methanol (Fig. 6.6). The disulphides in the eluate were no longer mixed with sugars and could be quantitatively reduced with DTT without difficulty (Cleland, 1964), and after derivatisation with DTNB they were analysed by h.p.l.c.

Analysis of the DTNB derivatives of the grain thiols and internal standards was carried out on a reverse-phase LiChrosorb RP-18 h.p.l.c. column held at 30°C, using a gradient system with solvents of ammonium formate and methanol. Absorbance was determined at 330 nm and peak areas were calculated with an electronic integrator. From this data the amounts of the DTNB derivatives were calculated. Various trials were made to achieve optimal

separation of the DTNB-thiol derivatives in the shortest possible time. The system finally adopted is illustrated in Fig. 6.7. It was found that commercial samples of DTNB contained an impurity which absorbed at 330 nm and co-eluted with the derivative of glutathione, preventing its quantitative estimation. This impurity was eliminated by recrystallising the DTNB from a complex solvent. A range of solvent pH values and gradients were tried to achieve the separation of the DTNB derivatives of the grain thiols and the internal standards from each other, from the thionitrobenzoate ion and from unreacted DTNB. The thiol derivatives involved were cysteine, γ-glutamylcysteine, cysteinylglycine, glutathione, N-acetyl cysteine (internal standard) and cysteamine (internal standard) (Fig. 6.7). In grain extracts the levels of the dipeptides γ-glutamylcysteine and cysteinylglycine were so low that they could not be quantified reliably, and so they were ignored. Thiols, released from precipitated protein in the extracted pellet by DTT, were at such low levels that they, also, were ignored.

## 6.2 Thiols and disulphides in barley embryos

Initial experiments were carried out on a dormant, undried sample of Doublet barley and on a sub-sample in which dormancy had been broken by drying and warm storage. The thiol levels were determined in the embryos of dormant and mature samples before wetting and at intervals during steeping and germination, while the grain was being micromalted. In these samples the levels of reduced glutathione and cysteine were essentially the same in the dormant and in the mature quiescent grains (Fig. 6.8). The most notable occurrence was the sharp fall in the levels of glutathione during the initial stages after steeping and the subsequent rise in this, and in the level of cysteine in the mature grain as the grains began to germinate.

The use of the Doublet barley was suspended while the analytical method for disulphides was perfected. During this 6-month period the grain was stored at -18°C. Re-analysis showed that during the period of storage the levels of glutathione, GSH, in the dormant embryos, from undried grain, had declined from 1150 nmol/100 embryos to 790 nmol/100 embryos. The level of oxidised glutathione, GSSG, was high, at 490 nmol (reduced equivalent)/100 embryos. In the embryos from the mature (and dried) grain the level of GSH was relatively unchanged, at 1040 nmol/100 embryos while the level of GSSG was 245 nmol reduced equivalents/100 embryos. Thus it appeared that storing the damp (21% moisture) dormant grain at -18°C had

permitted the oxidation of GSH to GSSG. Thus the use of this grain was discontinued and dormant and mature samples of Triumph barley (both 12% moisture) were employed in subsequent studies.

The germinative energies (1 ml agar test) of the Triumph barley samples were 98% for the mature sample and 25% for the dormant sample. In each case the germinative capacity was 99%. There were no significant differences between the GSH levels in the embryos of the two samples of quiescent grain (Fig. 6.9).

In contrast to the result with Doublet barley, the GSH level fell by about 35% in the first steeping period in both samples of Triumph when these were micromalted (Fig. 6.9). Subsequent experiments showed that this drop in thiols was independent of whether or not the steep liquor was fully aerated or was de-aerated. The changing levels of GSH and cysteine were essentially the same in both the dormant and non-dormant samples until the mature grain had germinated to an appreciable extent, when the levels of GSH and cysteine in the embryos increased. The levels of oxidised glutathione (GSSG) and cystine were also determined (Fig. 6.9). Again there were no appreciable differences between the results from the mature and the dormant grains. Perhaps the most striking observation is that the initial decline in GSH was accompanied by a decline in GSSG, indicating that the loss of GSH was not due to oxidation, and may have been due to export from the embryos. In the dry embryos the ratio of cysteine:cystine was ca 1:4 while the ratio of GSH:GSSG was ca 3:1. Thus these thiol/disulphide pairs were not in redox equilibrium with each other.

Because the germination of the mature grain was only 60 $\pm$ 4% after 3 days 'germination' the micromalting trial was repeated with both the dormant and the mature grain, but with the removal of surface moisture at the end of the steeping period. The improved procedure induced better germination both in the dormant grain and in the mature, which germinated completely in 3 days. In a subsequent experiment with the mature barley, the thiol and disulphide levels were determined as before (Fig. 6.10) and also in the degermed grains to determining if the initial 'loss' of "total" glutathione (GSH + GSSG) from the embryo was matched by a gain in the rest of the grain. The decline in total glutathione (GSH + GSSG) in the embryos which occurred during steeping was about 310 nmol reduced equivalents/100

embryos while the rise in the degermed grain was about 490 nmol reduced equivalents/100. Thus it seems that a significant transfer of glutathione from the embryo to the degermed grain had occurred and possibly the total had been augmented by synthesis, although this is uncertain because of the errors inherent in the determinations. The comparatively small changes in cysteine and cystine do not suggest a transfer of these substances to the endosperm. In this trial germination was even and comparatively rapid. GSH rose in the embryo and declined in the degermed grain as germination occurred. Graphs showing the changes in total thiol and disulphide levels are shown in Fig. 6.11.

## 6.3 The location of thiols in barley grains

The location of the GSH found in degermed grain was unknown. Isolated aleurone layers, prepared from degermed grains using enzyme digestion, contained about 740 nmol/100 aleurones. It appears probable that initially all the GSH found in degermed grains is located in the aleurone layer and not the starchy endosperm.

Grains that had been steeped were cut, and the exposed surfaces were treated with monobromobimane (MBBr). The positions of thiols were detected by the intense fluorescence of their MBBr derivatives when viewed under ultraviolet light. The cut embryo, and particularly the scutellum, fluoresced very brightly indeed and so did the cells of the aleurone layer although, with this tissue, the intense fluorescence of the ferulic acid in the cell walls complicated the picture.

The ability of embryos to release thiols was confirmed by incubating isolated embryos placed scutellum-down on monobromobimane solution (20 µl/embryo). The fluorescence of the solution after 24 h incubation showed that thiols had been released from the embryos. The question arose whether the enzyme glutathione reductase, which reduces GSSG to GSH at the expense of oxidising NADPH, was involved in the observed changes. Despite the changes in the GSH:GSSG ratio which occurred during steeping and germination glutathione reductase activity was nearly constant until 24 h after the end of steeping, after which the enzyme activity slowly increased with germination time. Thus the observed changes in the GSH:GSSG ratio could not be attributed to changing levels of this enzyme.

## 6.4 The effects of steeping barley in solutions of dormancy-breaking compounds

It is well established that steeping dormant barley in solutions of hydrogen peroxide or various thiols tend to overcome dormancy (Pollock and Kirsop, 1956; Briggs, 1978; Doran and Briggs, 1993a). Preliminary tests indicated that steeping 'mature' grains in water or various concentrations of propyl mercaptan then spreading the steeped grain on agar led to the grain chitting only to an extent of about 60% in 3 days, as in the 8 ml (paper) water sensitivity test. Under these conditions germination was not improved by the thiol, confirming Pollock and Kirsop's (1956) conclusion that thiols have no influence on water sensitivity. Subsequent trials were carried out using the conditions specified by Pollock and Kirsop (1956), in which the steeped grains were drained and then incubated on two layers of filter-paper wetted with 2 ml water. Under these conditions the mature Triumph barley germinated completely in 3 days, while the dormant barley germinated only 17%. Three thiols, a disulphide and a 'potential thiol'-containing substance (thiourea) were tested at a range of concentrations for their abilities to overcome dormancy. Germination increased with concentration until a 'threshold' concentration was reached. At higher concentrations germination was inhibited. The threshold concentrations were:- propylmercaptan, 0.4%; British anti-Lewisite (BAL; 2,3-dimercaptopropanol),0.1%; 2-mercaptoethanol, 0.1%; for thiourea, 2% and for bis-(2-hydroxyethyl) disulphide, 0.2%. It was found that steeping in a solution of BAL (0.1%) significantly delayed the germination of mature Triumph barley.

Hydrogen peroxide (0.75%) is routinely used as a steeping agent to induce the germination of dormant barley (Institute of Brewing Analysis Committee, 1986). Samples of mature and dormant barley were steeped in hydrogen peroxide and at intervals embryos were separated and the thiol and disulphide levels were determined (Fig. 6.12). The levels of oxidised glutathione and cystine were essentially the same in the dormant and mature grains. However, the cysteine levels were somewhat higher and the reduced glutathione levels were somewhat lower in the mature grains. As hydrogen peroxide generated *in vivo* in plants is believed to be 'detoxified' by reaction with reduced glutathione it was interesting that the levels of GSH and GSSG approximated to those usually found in the grains steeped in water. Thus, there is no indication that hydrogen peroxide entered the grains and oxidised the GSH.

The effects of steeping in BAL (0.1%) were tested on the thiol and disulphide levels in samples of dormant and mature grain. Preliminary experiments showed that the DTNB derivative of BAL separated from the other derivatives on h.p.l.c. and so did not interfere with the estimation of endogenous thiols. Various steeping regimes were tested. That finally adopted was a 32 h steep in BAL (0.1%) followed by a 16 h steep in water. At intervals the levels of thiols and disulphides in the embryos were determined (Fig. 6.13). Relative to water-steeped controls the germination of the mature barley was delayed but that of the dormant grain was greatly enhanced. The changes in the levels of GSH were very similar to each other in the two grain samples and to those seen in other samples (Figs. 6.9 and 6.10). However, there appeared to be a most dramatic increase in cysteine during steeping in BAL, but there are doubts about this (see below). There were no significant differences between the cysteine levels in the dormant and the mature grains. Steeping in BAL reduced the levels of the endogenous disulphides (GSSG and cystine) to vanishingly low levels and they did not reappear until the second day of germination. It appears that these were fully reduced by the BAL (Fig. 6.13).

The dramatic increase in 'apparent' cysteine in embryos, consequent on steeping in BAL, could have been due to proteolysis. If this idea was correct the apparent increase in the level of cysteine should have been accompanied by an increase in the amounts of other free amino acids. To test this idea amino-acids were extracted from the embryos of barley that had been steeped in water or BAL. The amino acids were analysed by ion exchange chromatography, by Alta Bioscience. There were no massive differences between the amino-acids from the different samples nor were the levels of cysteine elevated by BAL. To explain this anomaly it must be supposed that a metabolite for BAL, when derivatised with DTNB, co-elutes with the cysteine-DTNB derivative on h.p.l.c. To test this hypothesis material was collected from the h.p.l.c. and sent for examination by mass-spectrometry. However, no clear spectra have been obtained, and so confirmation of this hypothesis must await further studies.

Based on preliminary trials samples of grain were steeped in water (2 x 24 h) or in BAL (0.1%, 24 h, water, 24 h), and then were micromalted. The samples, grown for 3 or 5 days, were analysed by Paul's Malt Ltd. (Table 6.1). Comparison between the two sets of results obtained with mature grain emphasises that BAL slowed germination, leading to lower levels

of extract, diastatic power, and (after 5 days) soluble nitrogen (TSN), which is reflected in the soluble nitrogen ratio (SNR). The wort viscosity figures also indicate that BAL has slowed the degradation of viscous materials, probably chiefly ß-glucans. With dormant grains the 3-day analyses show that BAL has slowed modification, relative to the water-steeped samples, but after 5 days modification is greater in the BAL-treated grain. It is notable that even after 5 days germination the BAL-treated malt was not as well modified as that from the BAL treated mature grain, and was substantially inferior to the malt prepared from the mature grains steeped in water.

## 6.5 The uptake and release of dormancy-breaking, sulphur-containing substances by steeped barley

While indirect evidence indicated that thiols act within the grain to break dormancy there was no direct evidence that they enter grains. By steeping decorticated, surface sterilised grains of barley in solutions of BAL it was shown that washed grains subsequently released BAL into solutions provided that the initial concentration had been sufficiently high. Furthermore, it was shown that at low concentrations all the BAL was taken up by grains and none was released when the grains were subsequently steeped in water. Thus it is likely that BAL was being quantitatively converted into a non-diffusable metabolite in the grain.

We had assumed that thiols were responsible for breaking dormancy, and so we had been surprised to find that the disulphide bis-2(-hydroxyethyl)-disulphide overcame dormancy. We tested whether this substance was reduced to mercaptoethanol in the grain, since this thiol might be the effective dormancy-breaking agent. Under many conditions grains were found to reduce the disulphide to a thiol that was released into the steeping medium.

It was demonstrated that isolated embryos and aleurone layers both had the ability to reduce bis-(2-hydroxyethyl)disulphide to a thiol.

## 6.6 Discussion

The difficult and protracted developmental work provided us with analytical methods for thiols

and disulphides. These methods appear to be both sensitive, accurate and reliable.

The application of these methods to determining the endogenous thiols of dormant and mature barley grains has not provided any evidence that the levels of these substances are connected with the regulation of dormancy. The only stage during micromalting when divergence occurs between the levels of the thiols and disulphides of dormant and mature samples of grain is when the non-dormant grains begin to germinate. Presumably it was this change that was detected by Belderok (1968) although the analytical techniques he used were comparatively primitive and were incompletely described and seem not to have given results comparable to ours. Despite this discouraging conclusion it remains a fact, that we have confirmed, that soaking grains in solutions of thiols, or substances that are able to give rise to thiols, while it initially delays the germination of mature grains, eliminates dormancy. The evidence available suggests that the endogenous grain thiols are compartmentalised, since the glutathione and cysteine are not in redox equilibrium. However, the exogenous dormancy-breaking thiols probably permeate all parts of the grain and all the cellular compartments. The most effective concentrations of these substances are high (e.g. 0.1% for ß-mercaptoethanol), perhaps because the 'dormancy breaking' function that they perform involves their taking part in a relatively 'unfavourable' reaction requiring high levels of thiols to 'push' it to completion.

The results of the single malting trial, in which dormant and mature grains were steeped in water or BAL, showed that, at least under the conditions tested, the BAL did not improve the malting performances of the dormant grain to such an extent that it equalled that of the mature grain. Furthermore, as expected from germination tests, the BAL slowed the modification of the mature barley. More trials are required to decide whether, under other conditions, thiol treatments would permit dormant grain to be malted successfully.

# 7. CONCLUSIONS

## 7.1 Thermal processing and probit analysis

The main observations on the effects of thermal processing are presented below. It should be noted that the work presented was conducted almost entirely on the variety, Triumph.

(i) Drying temperature and drying rate did not affect germinability or subsequent rate of break of dormancy during storage at temperatures of 15, 27 and 38°C.

(ii) At a temperature of 38°C, moisture contents in the range 9.4 - 14.5% did not affect rate of break of dormancy.

(iii) Storage temperature is the dominant parameter in controlling the rate of break of dormancy.

(iv) There is evidence that storage at higher temperatures and lower moisture contents, produces a barley that germinates more rapidly and has less water sensitivity than normally observed.

(v) Cooling and freezing post-harvest before warm storage did not affect germinability or rate of break of dormancy during storage, as compared with unfrozen samples.

(vi) Cooling and freezing of sub-samples after warm storage did not affect germinability, as compared with unfrozen sub-samples.

(vii) The hypothesis of normally distributed dormancy period was supported by the sigmoid shape of the germination versus storage time curves.

(viii) A model of dormancy was developed giving the slope ($1/\sigma$) of the probit plots as a function of temperature.

(ix) The slope, which is a measure of rate of emergence from dormancy, was not affected by the site or season of harvest.

(x) Barleys sampled from commercial maltings showed more consistent behaviour than barleys from trial sites.

(xi) A model has been developed, combining the probit description of dormancy with previous work on the probit description of viability, to predict the germination history of a barley in storage.

These observations have a number of implications. Lower moisture levels than 12% would enable the use of higher storage temperatures and would not only break dormancy more quickly but may produce a more maltable barley. This, however, needs to be costed against the additional drying costs.

The minimal effect of drying regime and sudden cooling are observations that are surprising to maltsters, who are wary of sudden moisture removal or temperature drop. These observations contribute to the overall picture and if accepted may reduce the constraints on the handling of malting barley.

The combined model of dormancy and viability gives a useful insight into germination change during storage. The model indicates how critical the quality of the barley, as defined by viability (germinative capacity), is to the maximum germinability level that can be achieved through warm storage. Use of the model should be of considerable benefit in the development of storage strategies for malting barley.

## 7.2 Microbes on grain

Our results confirm that microbes on the surfaces of grains are significantly deleterious to germination, probably because they compete with the tissues of the grain for oxygen.

[$^{14}$C]- Glucose was metabolised exclusively by the microbes on the surfaces of ungerminated

grain. None penetrated the testa to reach the living grain tissues.

The quantitative spectrophotometric measurement of the insoluble red formazan, produced by the microbes in the surface layers of the grain reducing triphenyl-tetrazoliumchloride, provided a rough measurement of 'metabolising microbial mass'. The method finally developed still lacked sensitivity but, by using it, it was possible to demonstrate that, for example, the microbial population increased with steeping time, and increased more rapidly at 25°C than at 18°C. Acidification of steep water and degassing steep water, to deoxygenate it, both checked microbial growth.

The oxygen uptake of husk stripped from grains was shown to be caused by the associated microbes. These were supported by nutrients gained from the husk. The oxygen uptake rate at any time might be limited by the supply of nutrients and/or the size of the microbial population. Some microbes remained attached to the husk while others multiplied in the surrounding water.

The results suggest that, in malting, it is desirable to use warm-stored barley on which the microbial population has been reduced. Then, to minimise the multiplication of microbes during steeping, the first steep should be anaerobic and the liquor could, with advantage, be slightly acidified with a mineral acid. When the steep is drained, at the start of the first air-rest, it would be an advantage to rinse the grain downwards, as the water is withdrawn, to replace the film of water around the grains, which contains microbes and materials leached from the husk, with fresh water.

### 7.3 Sulphur-containing substances and dormancy

Reliable methods have been developed for quantitating thiols and disulphides extracted from barley grains or their parts. Total thiols were determined colorimetrically with a DTNB reagent, using appropriate blanks. Individual thiols were determined by collecting them from solution, to which an internal standard had been added, on a column containing *p*-hydroxymercuribenzoate substituted gel, eluating and then, after derivatization, quantitating by h.p.l.c. The major thiols were glutathione and cysteine but traces of γ-glutamyl-cysteine

and cysteinyl-glycine were also present. The disulphides were assayed in the eluate from the column by trapping on an ion-exchange matrix, to separate the sugars, elution, reduction to thiols and, after derivitization, quantitation by h.p.l.c. The major disulphides were oxidised glutathione and cystine.

In malting grain glutathione and, to a lesser extent, cysteine levels in embryos fell markedly in the early stages.

The glutathione was released into the endosperm. The levels changed in the same ways, and to the same extent in dormant and mature samples until germination began when the levels increased more in the mature and faster growing grain and less in the dormant. The disulphide levels changed little until germination began.

The thiols were concentrated in the living tissues, the embryo and the aleurone layer, in quiescent grains.

Dormancy can be overcome by soaking grains in a solution of hydrogen peroxide or some thiols. When dormant and mature grain samples were soaked in hydrogen peroxide germination was induced, but was slower in the dormant grains; differences in the levels of the thiols and disulphides were minimal. A range of thiols were tested for their abilities to overcome dormancy. Dormant and mature barleys were steeped in a solution of BAL (2,3-dimercaptopropanol) and were micromalted. Difficulties were encountered in assaying cysteine due, it is believed, to the derivative of a thiol-containing metabolite of BAL co-chromatographing with the derivative of cysteine on h.p.l.c. No differences were observed between the thiols and disulphides of embryos of the dormant and mature grains, which germinated at the same rate. However, steeping in BAL reduced the initial levels of the disulphides to very low values. Thus the role(s) of thiols in regulating dormancy remains an enigma.

Samples of dormant and mature barley, steeped in water or BAL, were micromalted with germination periods of three and five days. The samples were analysed by Pauls Malt Ltd. As expected, the dormant grain malted much less well than the mature, when they had both been steeped in water.

Steeping in BAL reduced the rate of malting in mature grain but accelerated it in dormant grain. Both the malts from BAL-steeped barley were inferior to those prepared from water-steeped, mature grain.

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# APPENDIX

## Method of probit analysis for break of dormancy

Testing a seed lot for level of germination can be considered as a process of sampling from a population exhibiting a binary characteristic, i.e. the ability to germinate or not. If all other factors influencing the result of a test are ignored, the expected error will be solely that due to sampling. This error is defined as

$$s_s = \sqrt{\frac{p(1-p)}{n}} \qquad \text{(I.1)}$$

where n is the total number of seeds tested, and p is the proportion of seeds germinated. The test mean percentage germination

$$\bar{g} = 100p \qquad \text{(I.2)}$$

is the same as the mean of k plates of x seeds where n = kx so that if the test error is due only to sampling then

$$\sigma_m = \frac{s_t}{\sqrt{k}} = \sqrt{\frac{p(1-p)}{kx}} \qquad \text{(I.3)}$$

and it follows that

$$s_t = \sqrt{\frac{p(1-p)}{x}} \qquad \text{(I.4)}$$

where $\sigma_m$ is the standard error of the estimated population mean and $s_t$ is the standard error of the test mean. When $s_t$ is greater than the sampling error then the residual variance may be calculated as

$$s_r^2 = s_t^2 - s_s^2 \qquad \text{(I.5)}$$

A negative residual variance is treated as zero residual error.

A suitable method of quantifying the residual error, as defined in Eqn. (I.5), was found to be as a multiple of the sampling error (Eqn. (I.1); this relationship is suggested in Eqn. (I.4). Two datasets were analysed, 206 sets of nine plate replicates and 85 sets of three plate

replicates, all from 4 ml paper tests. Approximately 60% of the standard deviation of the means in both sets was larger than the expected sampling error. The best estimate of the test error function for both sets of data was

$$s_t = 1.5\sqrt{\frac{\bar{g}(100-\bar{g})}{100}} \qquad \text{(I.6)}$$

When applied to a set of test replicates using a 5% rejection level, a 100 seed test result was rejected if

$$|g - \bar{g}| > 2\,s_t \qquad \text{(I.7)}$$

This criterion was applied to the data point furthest from the mean first and if rejected the mean was recalculated before reapplying the criterion to the next furthest and so on.

The method of probit analysis used is similar to that proposed by Finney (1977) for probit analysis of tests comprising a single replicate and log-linear analysis of tests using multiple replicates in bioassay (Finney, 1978). After extreme values were filtered out, the proportion of germinated seeds in each replicate was transformed to a probit scale and an unweighted linear regression of probit germination against storage time was carried out. The weight, W given to all of the replicates in a test is a function of the probit value, X, of the provisional regression line at the corresponding value of the abscissa (storage time) for the test

$$W = \frac{100\,e^{-X}}{\sqrt{2\pi p(1-p)}} \qquad \text{(I.8)}$$

where p is the proportion of germinable seeds as estimated by the provisional line.
A weighted linear regression (Baker, 1986) of the probit value of all replicate germination percentages with storage time was then carried out using a weight, W, for each set of replicates. The function which defines the germination curve of a sample on a probit scale is

$$X = a + bt \qquad \text{(I.9)}$$

where X is probit germination, t is storage time in days, a is the intercept and b is the slope of the probit regression line. The standard deviation of the distribution of dormancy loss period among seeds of the sample, $\sigma$, is $1/b$. Successive regressions were carried out with weights calculated from Eqn. (I.8) using values of Y taken from the previous regression line

until the residual sum of squares reached a minimum. The statistical software package used for the analysis was GLIM (Baker, 1986). The normal link rather than the probit link function, which did not allow for iterative weighting, was employed (Baker, 1986).

The goodness-of-fit of a probit line to the data was determined using two parameters: (i) correlation coefficient, r and (ii) an F-test of the proportion of the deviance due to the variation of all of the points about the line against the variation of the replicate values about the means (Bliss, 1967). In the latter case a statistically significant F-value is an indication of either an inappropriate choice of model, recognised by a trend in the residuals, or of heterogeneity caused by factors inherent to the test and test subjects (Finney, 1978).

The parameter used as a measure of the amount of variation about the probit regression line above that due to sampling variation was the heterogeneity factor (Finney, 1977). This is equivalent to Pearson's $X^2$, divided by the degrees of freedom, also termed the scale parameter (Aitkin *et al.* 1989). Finney (1977) recommends multiplying all variances by the heterogeneity factor when it is greater than unity, provided systematic lack of fit is not the cause of the high $X^2$ value. Systematic lack of fit was examined by plotting the residuals, weighted by the same values as used in the regression, against the fitted probit germination values. Where no trend in the residuals was observed (as was the case for all probit regressions in this analysis) the variance of the slope of each probit line, $Var(1/\sigma)$, was increased by the heterogeneity factor.

A weighted regression of the $\sigma$ values from all samples against storage temperature was carried out to determine the constants $K_b$ and $C_d$ in the equation

$$\log_e \sigma_d = K_b - C_d T \qquad \text{(I.10)}$$

Each $\sigma$ value (on a log scale) was weighted by its variance after transformation to the log scale as follows:

If $y = f(x)$

then $Var(y) = Var(x)\,(f'(x))^2$ (Meyer, 1970),

where $(f^1(x))^2$ is the first differential operator,

$$Var(\sigma) = \sigma^4 \, Var(1/\sigma)$$

and it follows that $Var(\log_e \sigma) = \sigma^2 \, Var(1/\sigma)$

giving a factor, $w = 1/(\sigma^2 \, Var(1/\sigma))$ (I.11)

as the weight. Confidence limits for $\sigma_d$ predicted from the temperature function are calculated from

$$Var(\log_e \sigma_d) = Var(K_b) + (T - \bar{T})^2 Var(C_d) ,$$

using the same transformation function as above (Meyer, 1970), such that

$$Var(\sigma_d) = Var(\log_e \sigma_d)\sigma^2_d$$

giving $Var(1/\sigma_d) = Var(\log_e \sigma_d)/\sigma^2_d$ (I.12)

as the variance of the slope of a probit curve predicted from the dormancy model, where $\bar{T}$ is the mean storage temperature from the regression.

Table 2.1 Samples from the 1988 harvest.

| Sample Code | Variety | Origin | Harvest Date | Total weight in store (kg) | Initial Moisture Content (%) |
|---|---|---|---|---|---|
| Ack-Tr-88 | Triumph | Acklington Cockle Park Exp. Stn. | 25.8.88 | 200.0 | 25.8 |
| Ack-Tr-88s | Triumph | Acklington Cockle Park Exp. Stn. | 7.9.88 | 31.7 | 24.4 |
| Inv-Tr-88 | Triumph | Aldroughty, Inverness. N.S.C.A. | 22.8.88 | 26.0 | 28.3 |
| Inv-Gp-88 | Golden Promise | Aldroughty, Inverness. N.S.C.A. | 22.8.88 | 25.7 | 28.8 |
| Ack-Db-88 | Doublet | Acklington, Cockle Park Exp. Stn. | 24.8.88 | 38.3 | 25.9 |
| Abr-Tr-88 | Triumph | Sunnybrae, Aberdeen. N.S.C.A. | 30.8.88 | 23.7 | 20.5 |
| Abr-Gp-88 | Golden Promise | Sunnybrae, Aberdeen. N.S.C.A. | 30.8.88 | 21.8 | 17.5 |
| Edn-Tr-88 | Triumph | Lr. Fulford, Edinburgh. S.C.A.E. | 12.9.88 | 24.3 | 20.8 |
| Edn-Db-88 | Doublet | Lr. Fulford, Edinburgh. S.C.A.E. | 12.9.88 | 24.0 | 22.5 |
| Mot-Tr-88 | Triumph | High Mowthorpe, Yorks. A.D.A.S. | 26.8.88 | 25.0 | 22.1 |

N.S.C.A. : North of Scotland College of Agriculture*
S.C.A.E. : Scottish Centre of Agricultural Engineering
A.D.A.S. : Agricultural Development and Advisory Service

* now Scottish Agricultural College of Aberdeen.

Table 2.2 The germination characteristics of the freshly harvested (1988), undried barley samples when placed into cold store.

| Sample Code | Variety | GERMINATIONS (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Agar (Mean) 3 days | | $H_2O_2$ 3 days | Kiln, 40°C/4 days Stored 40°C/3 days $H_2O_2$ + $GA_3$ (50 mg/1) 3 days | Decorticated 4 ml $GA_3$ (50 mg/1) 3 days | Kiln 40°C/3 days Store 40°C/25 days Room temp. 7 days $H_2O_2$ 3 days |
| | | 1 ml | 3 ml | | | | |
| Ack-Tr-88 | Triumph | 5 | 2 | 32 | 91.5 | 95 | 97.5 |
| Ack-Tr-88s | Triumph | 10.3 | 4.3 | 57.5 | 90 | - | 94 |
| Inv-Tr-88 | Triumph | 12 | 2.3 | 69 | 76.5 | - | 90.5 |
| Inv-Gp-88 | Golden Promise | 11.7 | 5.7 | 65 | 78 | - | 88.5 |
| Ack-Db-88 | Doublet | 10.7 | 3 | 67.5 | 97.5 | - | 100 |
| Abr-Tr-88 | Triumph | 12.7 | 5.3 | 49 | 93 | - | 97.5 |
| Abr-Gp-88 | Golden Promise | 18.3 | 8 | 72 | 97.5 | - | 98.5 |
| Edn-Tr-88 | Triumph | 16 | 4.7 | 73 | 92 | - | 96 |
| Edn-Db-88 | Doublet | 27.3 | 7 | 65 | 87 | - | 90 |
| Mot-Tr-88 | Triumph | 2.3 | 1.3 | 43 | 96 | - | 98 |

1. All germination tests 18°C
2. 1 ml and 3 ml agar germination tests (3 x 100 corns)
3. $H_2O_2$ standard I.O.B. test 200 grains.
4. $H_2O_2$ + $GA_3$ standard test, but 50 mg/1 gibberellic acid included.
5. 4 ml $GA_3$ test. As Nutfield 4 ml test, but with gibberellic acid included.
6. 40°C kilning in continuous rapid airflow.
7. 40°C storage in plastic bags, in incubator.
8. All grains > 2.18 mm.

Table 2.3 Samples from the 1989 harvest.

| Sample Code | Variety | Origin | Harvest Date | Total weight in Store (kg) | Initial Moisture Content (%) |
|---|---|---|---|---|---|
| Edn-Db-89 | Doublet | Lr. Fulford, Edinburgh. S.C.A.E. | 15.8.89 | 25.0 | 16.4 |
| Edn-Tr-89 | Triumph | Lr. Fulford, Edinburgh. S.C.A.E. | 15.8.89 | 25.0 | 16.5 |
| Inv-Tr-89 | Triumph | Aldroughty Inverness. N.S.C.A. | 17.8.89 | 25.0 | 16.4 |
| Inv-Gp-89 | Golden Promise | Aldroughty Inverness. N.S.C.A. | 17.8.89 | 25.0 | 15.7 |
| Mot-Tr-89 | Triumph | High Mowthorpe Yorks A.D.A.S. | 17.8.89 | 23.0 | 16.5 |
| Ack-Tr-89 | Triumph | Acklington Cockle Park Exp. Stn. | 23.8.89 | 175.0 | 16.3 |
| Abr-Tr-89 | Golden Promise | Sunnybrae Aberdeen N.S.C.A. | 14.9.89 | 12.5 | 16.8 |
| Abs-Tr-89 | Triumph | Sunnybrae, Aberdeen N.S.C.A. | 14.9.89 | 15.7 | 17.2 |

N.S.C.A. : North of Scotland College of Agriculture*
S.C.A.E. : Scottish Centre of Agricultural Engineering
A.D.A.S. : Agricultural Developmental and Advisory Service

* now Scottish Agricultural College of Aberdeen.

Table 2.4 Samples from the 1990 harvest

| Sample Code | Variety | Origin | Initial Moisture Content (%) | Initial Germination (%)* |
|---|---|---|---|---|
| Ack-Tr-90 | Triumph | Acklington | 24.7 | 6.0 |
| Ack-Db-90 | Doublet | | 26.0 | 9.7 |
| Inv-Tr-90 | Triumph | Inverness | 21.6 | 25.3 |
| Inv-Gp-90 | G. Promise | | 19.2 | 61.7 |
| Abr-Tr-90 | Triumph | Aberdeen | 20.1 | 27.3 |
| Abr-Gp-90 | G. Promise | | 14.5 | 77.0 |
| Edn-Tr-90 | Triumph | Edinburgh | 16.5 | 56.3 |
| Edn-Db-90 | Doublet | | 15.8 | 70.0 |
| Mot-Tr-90 | Triumph | High Mowthorpe | 16.5 | 12.3 |
| Kir-Tr-90 | Triumph | Kirkaldy | 18.1 | 8.5 |
| Kir-Bl-90 | Blenheim | (maltings) | 17.4 | 53.3 |
| Kir-Ca-90 | Camargue | | 19.7 | 20.7 |
| Kir-Gp-90 | G. Promise | | 18.6 | 47.3 |
| Pen-Tr-90 | Triumph | Pencaitland | 12.7 | 12.3 |
| Pen-Bl-90 | Blenheim | (maltings) | 15.9 | 46.7 |
| Pen-Ca-90 | Camargue | | 15.8 | 99.3 |

*Institute of Brewing, 4 ml test.

Table 2.5 Samples from the 1991 harvest.

| Sample Code | Variety | Origin | Initial germination* (%) | Initial Moisture Content (%) |
|---|---|---|---|---|
| Inv-Tr-91 | Triumph | Inverness$^{+}$ | 32 | 17.1 |
| Inv-Ca-91 | Camargue | Inverness$^{+}$ | 53 | 17.1 |
| Kir-Tr-91a | Triumph | Kirkaldy | 12 | 18.6 |
| Kir-Tr-91b | Triumph | Kirkaldy | 64 | 18.2 |
| Kir-Bl-91 | Blenheim | Kirkaldy | 41 | 17.3 |
| Kir-Ca-91 | Camargue | Kirkaldy | 6 | 19.4 |
| Pen-Tr-91a | Triumph | Pencaitland | 8 | 16.9 |
| Pen-Tr-91b | Triumph | Pencaitland | 30 | 16.2 |
| Pen-Ma-91 | Magie | Pencaitland | 77 | 17.5 |
| Pen-Db-91 | Doublet | Pencaitland | 22 | 18.1 |
| Pen-Bl-91 | Blenheim | Pencaitland | 58 | 16.5 |
| Pen-Ca-91 | Camargue | Pencaitland | 32 | 16.1 |
| Pen-Ty-91 | Tyne | Pencaitland | 55 | 16.9 |
| Pen-Pr-91 | Prisma | Pencaitland | 63 | 19.1 |
| Pen-Sh-91 | Sherpa | Pencaitland | 36 | 14.2 |
| Abr-Ca-91 | Camargue | Aberdeen$^{+}$ | 89 | 18.6 |
| Abr-Tr-91 | Triumph | Aberdeen$^{+}$ | 28 | 20.4 |

*Institute of Brewing, 4 ml test

$^{+}$ Trial site barleys, all others from maltings

Table 2.6 Combinations of drying and storage conditions in the first experiments with Ack-Tr-88.

| Drying Temp. (°C) | Drying Air R.H. (%) | Drying Time (h) | Storage Temp. (°C) |
|---|---|---|---|
| 48 | 10 | 4.35 | 15 |
| 48 | 10 | 4.25 | 27 |
| 48 | 10 | 4.25 | 38 |
| 48 | 15 | 4.35 | 15 |
| 48 | 20 | 5.25 | 15 |
| 38 | 10 | 6.80 | 15 |
| 38 | 10 | 6.75 | 27 |
| 38 | 10 | 6.85 | 38 |
| 38 | 15 | 8.00 | 27 |
| 38 | 20 | 8.75 | 15 |
| 27 | 20 | 12.25 | 15 |
| 27 | 20 | 12.75 | 27 |
| 27 | 22 | 13.50 | 38 |
| 27 | 30 | 24.30 | 15 |
| 27 | 30 | 24.25 | 27 |
| 27 | 30 | 24.20 | 38 |

Table 4.1 Regression of probit plots showing the germination value above which points were discarded.

| Sample | Storage Temperature (°C) | Number of points in regression | Mean germinability at truncation (%) | $r^2$ |
|---|---|---|---|---|
| Pen-Tr-90 | 38 | 6 | 98.8 | 0.9490 |
| Kir-Tr-90 | 38 | 4 | 85.4 | 0.9523 |
| Pen-Tr-91a | 38 | 6 | 90.4 | 0.9498 |
| Pen-Tr-91b | 38 | 7 | 96.8 | 0.9195 |
| Inv-Tr-90 | 38 | 5 | 94.9 | 0.8178 |
| Mot-Tr-90 | 38 | 4 | 86.0 | 0.8890 |
| Abr-Tr-90 | 38 | 5 | 94.6 | 0.8430 |
| Ack-Tr-88 | 38 | 6 | 89.6 | 0.9334 |
| Pen-Tr-90 | 27 | 10 | 94.7 | 0.9464 |
| Kir-Tr-90 | 27 | 9 | 96.0 | 0.9406 |
| Pen-Tr-91a | 27 | 7 | 82.9 | 0.9498 |
| Pen-Tr-91b | 27 | 8 | 95.2 | 0.9294 |
| Inv-Tr-90 | 27 | 9 | 89.7 | 0.8867 |
| Abr-Tr-90 | 27 | 7 | 81.3 | 0.9366 |
| Ack-Tr-88 | 27 | 12 | 88.8 | 0.9493 |
| Pen-Tr-90 | 15 | 12 | 97.0 | 0.9567 |
| Kir-Tr-90 | 15 | 8 | 91.9 | 0.8630 |
| Pen-Tr-91a | 15 | 8 | 86.2 | 0.9268 |
| Pen-Tr-91b | 15 | 8 | 95.1 | 0.9104 |
| Pen-Tr-91a | 8 | 12 | 95.3 | 0.9384 |
| Pen-Tr-91b | 8 | 11 | 98.1 | 0.8287 |

Table 5.1 Formazan Colour (485 nm)

| | 18°C | | 25°C | |
|---|---|---|---|---|
| Steeping | Final Steep pH | TTZ colour | Final Steep pH | TTZ colour |
| Water (still) | 5.9 | 0.196 | 5.5 | 0.515 |
| Water (shaken) | 5.6 | 0.214 | 5.6 | 0.359 |
| HCl (10mM;still) | 2.2 | 0.080 | 2.1 | 0.085 |
| HCl (10mM;shaken) | 2.3 | 0.074 | 2.3 | 0.070 |
| Unsteeped control | - | 0.067 | - | 0.062 |

Grains were steeped for 69 h in water or in hydrochloric acid (10 mM) at the temperatures shown. Final steep pH values are given. The grains were rinsed with distilled water and the TTZ test (2.0%, pH 6.0) was carried out. Shaken samples were agitated by hand twice a day. Results are the means of triplicates.

Table 5.2 The respiration of husk samples and the incubation liquid.

| Treatments | Rate of oxygen uptake (n mole $O_2$/h/mg husk or 2 ml steep water) | | | | |
|---|---|---|---|---|---|
| | Control | | | Shaken | |
| Sample time = | 1 h | 5 h | 8 h | 1 h | 8 h |
| Husk blotted | 4.2 | 4.9 | 4.1 | 5.4 | 9.8 |
| + broth | 8.6 | 10.5 | 4.2 | 6.9 | 12.9 |
| Liquid | 0 | 0 | 2.6 | 3.2 | 25.9 |
| + broth | 4.7 | 3.1 | 3.9 | 4.8 | 30.6 |
| Rinsed husk | 4.6 | 8.2 | 6.0 | 5.6 | 10.1 |
| + broth | 7.5 | 9.7 | 16.0 | 10.3 | 16.3 |

In the first, control group of samples grains were soaked in water, without agitation, at 25°C. At intervals, after 1, 5 and 8 h, grains were removed and husk was peeled from them. The water was changed after each sampling. In the second group of samples grains were agitated in water at 25°C. Grain and liquid samples were taken after 1 h and 8 h. The respiration rates of the husks were determined, after blotting, both before and after the addition of nutrient broth and again after rinsing and then again after further additions of broth.

Table 5.3 The respiration of various husk and liquid samples.

| Treatments | Rates of oxygen uptake (n mole $O_2$/h/mg husk or 2 ml steep water) | | |
|---|---|---|---|
| Sample time = | 2.5 h | 4.5 h | 6.0 h |
| A. Shaken husk | 8.5 | 11.3 | 11.3 |
| " + broth | 11.2 | 13.7 | 16.4 |
| Liquid | 6.4 | 15.4 | 33.7 |
| " + broth | 8.6 | 16.2 | 49.0 |
| B. Static husk | 5.2 | 6.2 | 5.3 |
| " + broth | 5.4 | 7.4 | 5.9 |
| Liquid | 3.2 | 0 | 17.8 |
| " + broth | 3.2 | 7.7 | 24.3 |
| C. Static; degassed husk | 5.8 | 3.8 | 5.1 |
| " + broth | 5.3 | 6.2 | 7.0 |
| Liquid | 0 | 0 | 0 |
| " + broth | 0 | 1.5 | 14.0 |

Grains were steeped at 25°C.

A - Shaken in a large conical flask.

B - Remaining static in a small conical flask closed with a bung, but with a substantial air headspace.

C - Remaining static, immersed in degassed water held in a boiling tube and closed with a bung. The headspace was small. At intervals samples were taken and the respiration rates were determined on husks and steep liquor both before and after additions of broth.

Table 5.4 The respiration rates of samples of barley husk and steep liquor.

| Treatments | Rate of oxygen uptake (n mole $O_2$/h/mg husk or 2 ml steep water) | | |
|---|---|---|---|
| Time from wetting | End 1st Steep (18 h) | End 2nd Steep (42 h) | End 3rd Steep (66 h) |
| A. Husk | 4.1 | 4.0 | 10.8 |
| " + broth | - | 10.6 | 20.8 |
| Liquid | 0 | 9.5 | 10.7 |
| " + broth | - | 4.5* | 5.4* |
| B. Husk | 3.6 | 8.9 | 6.8 |
| " + broth | - | 13.1 | 9.5 |
| Liquid | 0 | 13.4 | 17.7 |
| " + broth | - | 40.3 | 33.5 |
| C. Husk | 1.9 | 3.3 | 3.9 |
| " + broth | - | 10.9 | 8.1 |
| Liquid | 0 | 16.7 | 16.1 |
| " + broth | - | 22.0 | 32.7 |
| D. Husk | 3.0 | 2.7 | 3.8 |
| " + broth | - | 10.3 | 5.7 |
| Liquid | 0 | 0.4 | 1.2 |
| " + broth | - | 19.3 | 10.2 |

A - steeped continuously in the same water; B - had the steep liquor changed after each sampling; C - had the steep liquor changed after a 1½ h air rest, after each sampling; and D - were treated as C but were also rinsed before fresh steep water was supplied. Treatments were all at 15°C, but respiration rates were measured at 25°C.

* Anomalous results

Table 6.1 Analyses of micromalts prepared from dormant and mature Triumph barley steeped in water or in a solution of BAL.

| Barley sample Steep solution Germination day | | Extract (L°/Kg, d.b.) | DP (°IoB) | TN (% of dm) | TSN (% of dm) | SNR | Wort viscosity (cSt) | Total malting losses (% dry weight basis) |
|---|---|---|---|---|---|---|---|---|
| Dormant | Replicate 1 | 181 | 30 | 1.55 | 0.28 | 17.1 | 2.04 | 1.65 |
| Water | Replicate 2 | 165 | 27 | 1.66 | 0.28 | 16.9 | 1.98 | 1.59 |
| Day 3 | Replicate 3 | 177 | 26 | 1.58 | 0.30 | 19.0 | 2.24 | 1.77 |
| | Mean ± S.D. | 174±7 | 28±2 | 1.60±.05 | 0.29±.01 | 17.7±1.0 | 2.09±.11 | 1.67±.07 |
| Dormant | Replicate 1 | 223 | 41 | 1.58 | 0.34 | 21.5 | 1.82 | 3.54 |
| Water | Replicate 2 | 231 | 41 | 1.46 | 0.38 | 26.0 | 1.93 | 3.77 |
| Day 5 | Replicate 3 | 221 | 42 | 1.61 | 0.34 | 21.1 | 1.90 | 2.93 |
| | Mean ± S.D. | 225±5 | 41±1 | 1.55±.06 | 0.35±.02 | 22.9±2.2 | 1.88±.05 | 3.41±.35 |
| Mature | Replicate 1 | 203 | 34 | 1.48 | 0.30 | 20.3 | 2.11 | 2.32 |
| Water | Replicate 2 | 205 | 29 | 1.50 | 0.32 | 21.3 | 2.04 | 2.29 |
| Day 3 | Replicate 3 | 196 | 29 | 1.47 | 0.30 | 20.4 | 2.15 | 2.32 |
| | Mean ± S.D. | 202±4 | 31±3 | 1.48±.01 | 0.31±.01 | 20.7±.5 | 2.10±.05 | 2.31±.02 |
| Mature | Replicate 1 | 274 | 79 | 1.52 | 0.73 | 48.0 | 1.45 | 6.65 |
| Water | Replicate 2 | 280 | 71 | 1.57 | 0.70 | 44.6 | 1.46 | 6.06 |
| Day 5 | Replicate 3 | 287 | 74 | 1.47 | 0.70 | 47.6 | 1.50 | 6.44 |
| | Mean ± S.D. | 280±6 | 75±4 | 1.52±.04 | 0.71±.02 | 46.7±1.5 | 1.47±.02 | 6.38±.24 |
| Dormant | Replicate 1 | 159 | 37 | 1.70 | 0.34 | 20.0 | 2.10 | 1.46 |
| BAL | Replicate 2 | 159 | 37 | 1.72 | 0.34 | 19.8 | 2.35 | 1.64 |
| Day 3 | Replicate 3 | 160 | 43 | 1.76 | 0.34 | 19.3 | 2.24 | 1.38 |
| | Mean ± S.D. | 159±1 | 39±3 | 1.73±.03 | 0.34±0 | 19.7±.3 | 2.23±.10 | 1.49±.11 |
| Dormant | Replicate 1 | 236 | 53 | 1.71 | 0.48 | 28.1 | 1.78 | 4.04 |
| BAL | Replicate 2 | 239 | 61 | 1.62 | 0.47 | 29.0 | 1.71 | 3.97 |
| Day 5 | Replicate 3 | 214 | 47 | 1.73 | 0.38 | 22.0 | 1.98 | 3.99 |
| | Mean ± S.D. | 230±12 | 54±6 | 1.69±.05 | 0.44±.05 | 26.4±3.1 | 1.82±.11 | 4.00±.03 |
| Mature | Replicate 1 | 176 | 31 | 1.60 | 0.32 | 20.0 | 2.31 | 1.53 |
| BAL | Replicate 2 | 172 | 29 | 1.63 | 0.32 | 19.6 | 2.24 | 1.67 |
| Day 3 | Replicate 3 | 159 | 28 | 1.73 | 0.32 | 19.4 | 2.38 | 1.56 |
| | Mean ± S.D. | 169±8 | 29±2 | 1.63±.05 | 0.32±0 | 19.7±.3 | 2.31±.06 | 1.59±.06 |
| Mature | Replicate 1 | 252 | 52 | 1.55 | 0.50 | 32.3 | 1.75 | 4.31 |
| BAL | Replicate 2 | 268 | 58 | 1.63 | 0.54 | 33.1 | 1.66 | 4.65 |
| Day 5 | Replicate 3 | 263 | 56 | 1.61 | 0.52 | 32.3 | 1.63 | 4.53 |
| | Mean ± S.D. | 261±7 | 55±3 | 1.60±.03 | 0.52±0.2 | 32.6±.4 | 1.68±.05 | 4.25±.27 |

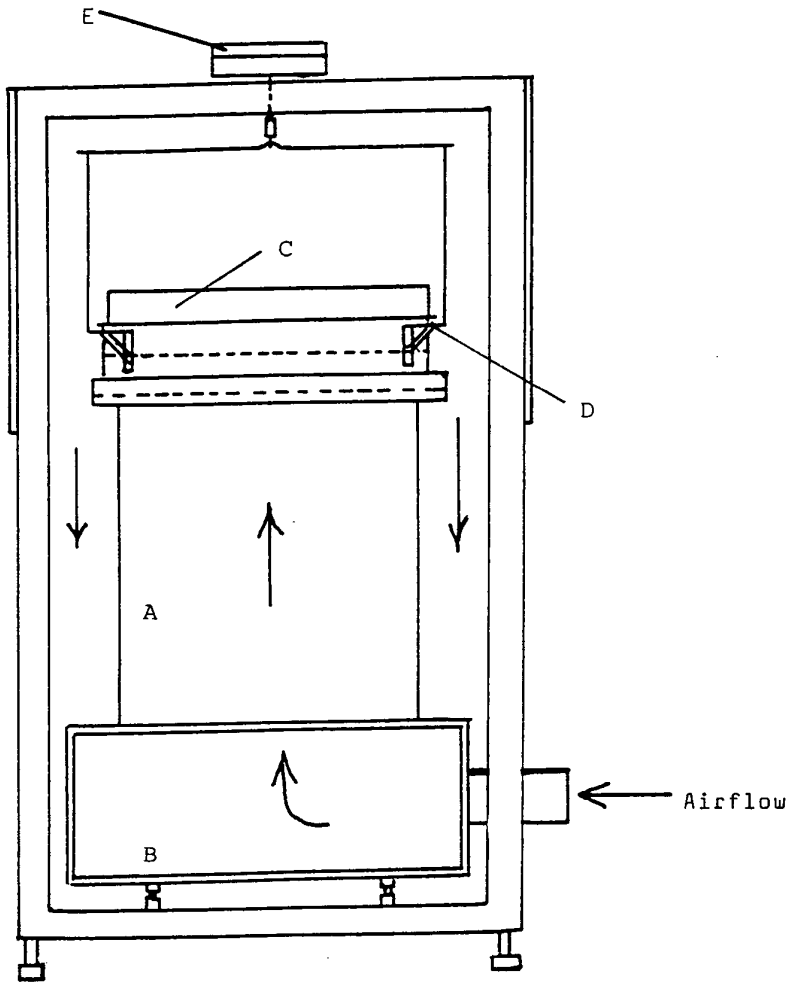



Fig. 2.1 Apparatus for drying samples of malting barley under controlled conditions; (A) plenum, (B) insulated chamber, (C) drying tray, (D) support collar, (E) digital balance

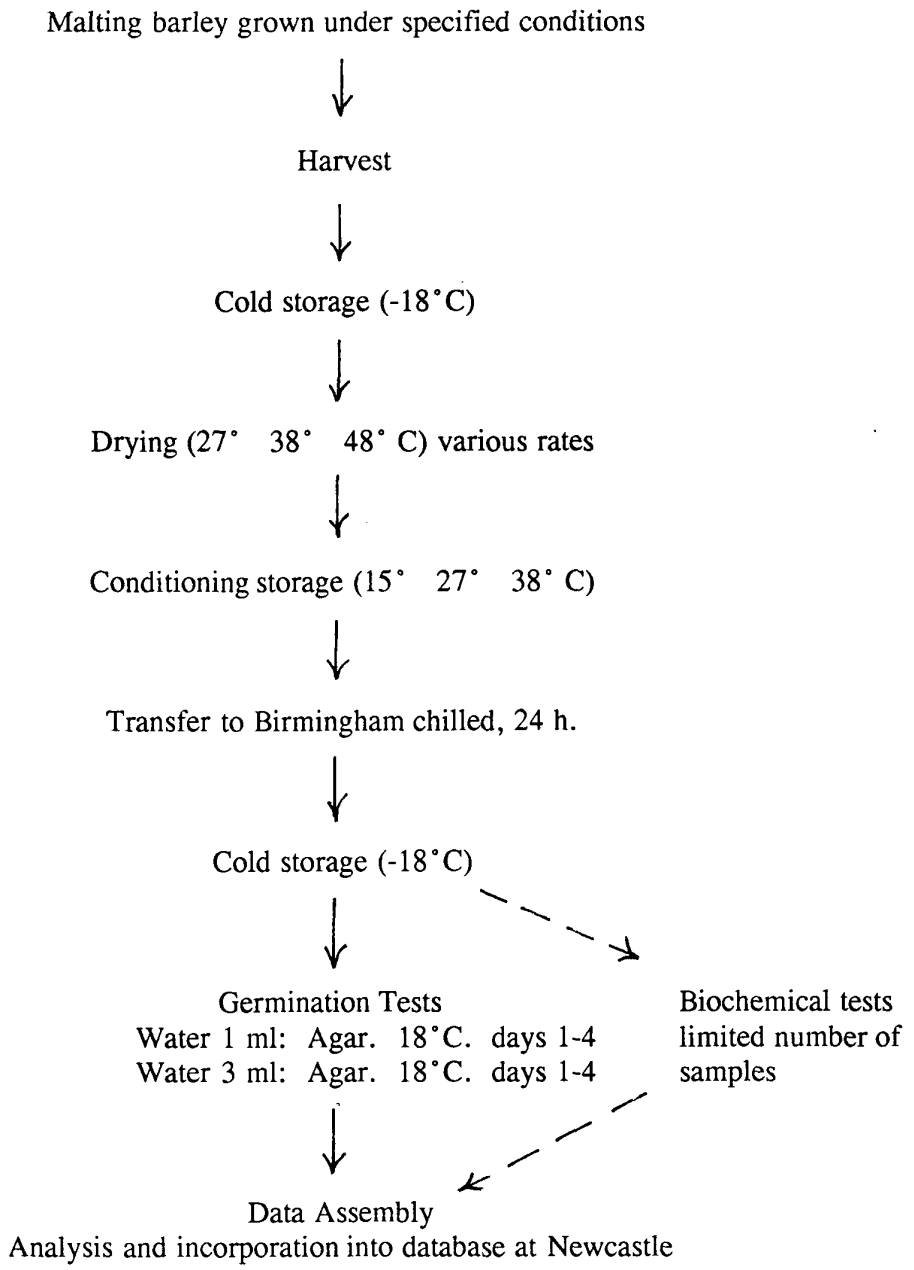



Fig. 2.2 Project flow chart

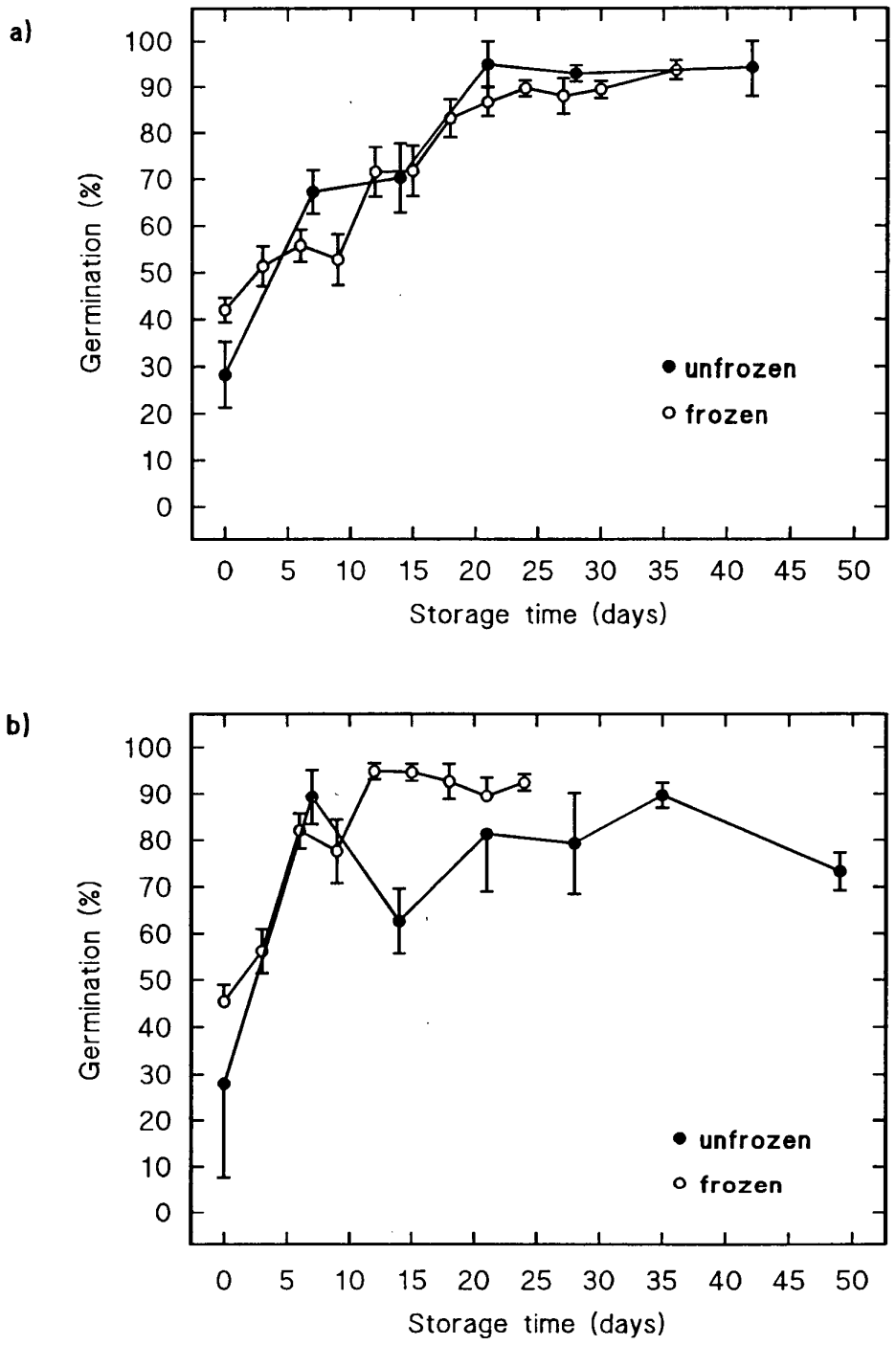



Fig. 2.3 Postharvest storage freezing trial with Inv-Tr-90 subsequently stored at (a) 27°C and (b) 38°C (4ml l.o.B. test; unfrozen, 3x100 seeds; frozen, 9x100 seeds).

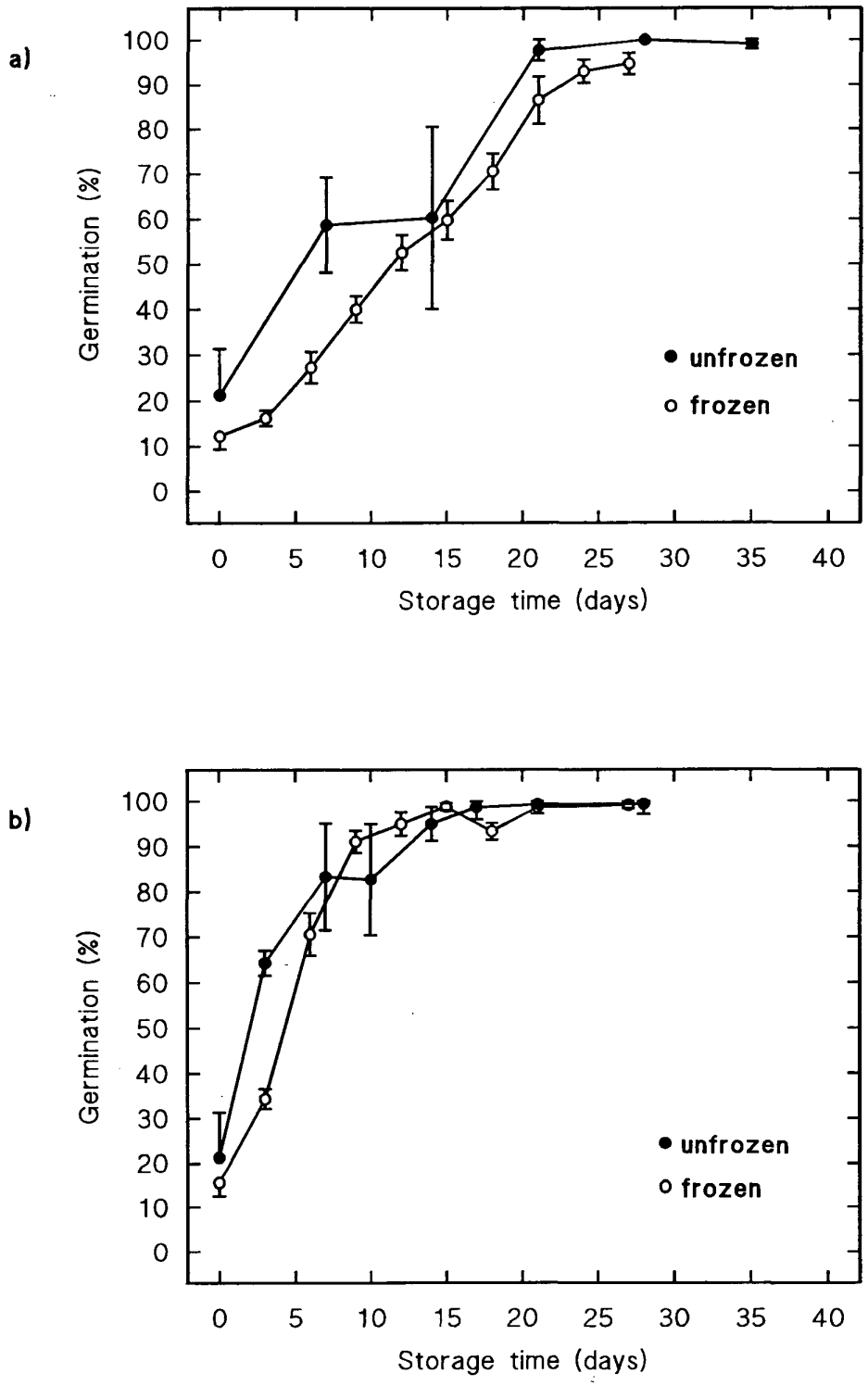



Fig. 2.4 Postharvest storage freezing trial with Pen–Tr–90 subsequently stored at (a) 27°C and (b) 38°C (4ml l.o.B. test; unfrozen, 3x100 seeds; frozen, 9x100 seeds).

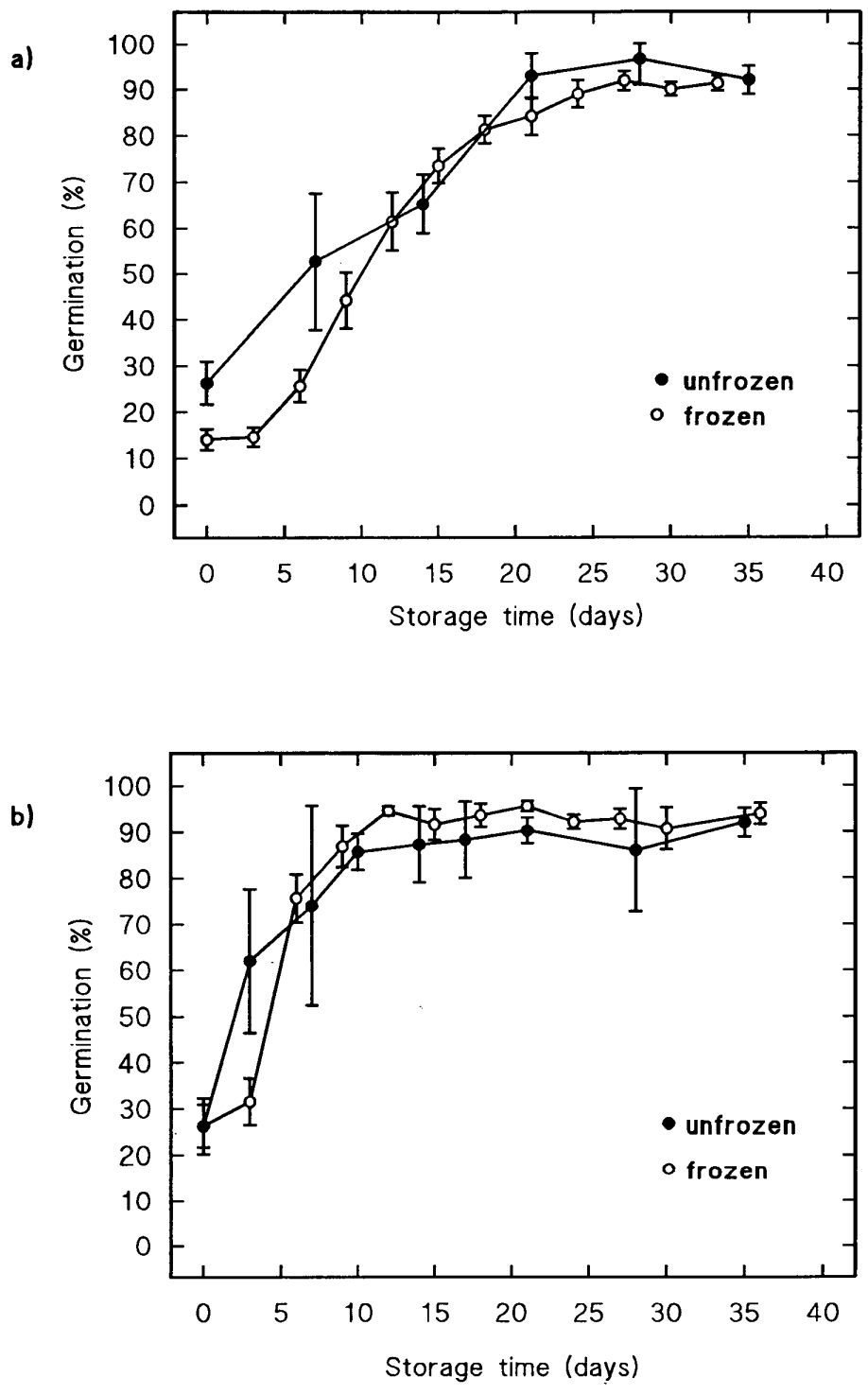



Fig. 2.5 Postharvest storage freezing trial with Abr-Tr-90 subsequently stored at (a) 27°C and (b) 38°C (4ml I.o.B. test; unfrozen, 3x100 seeds; frozen, 9x100 seeds).

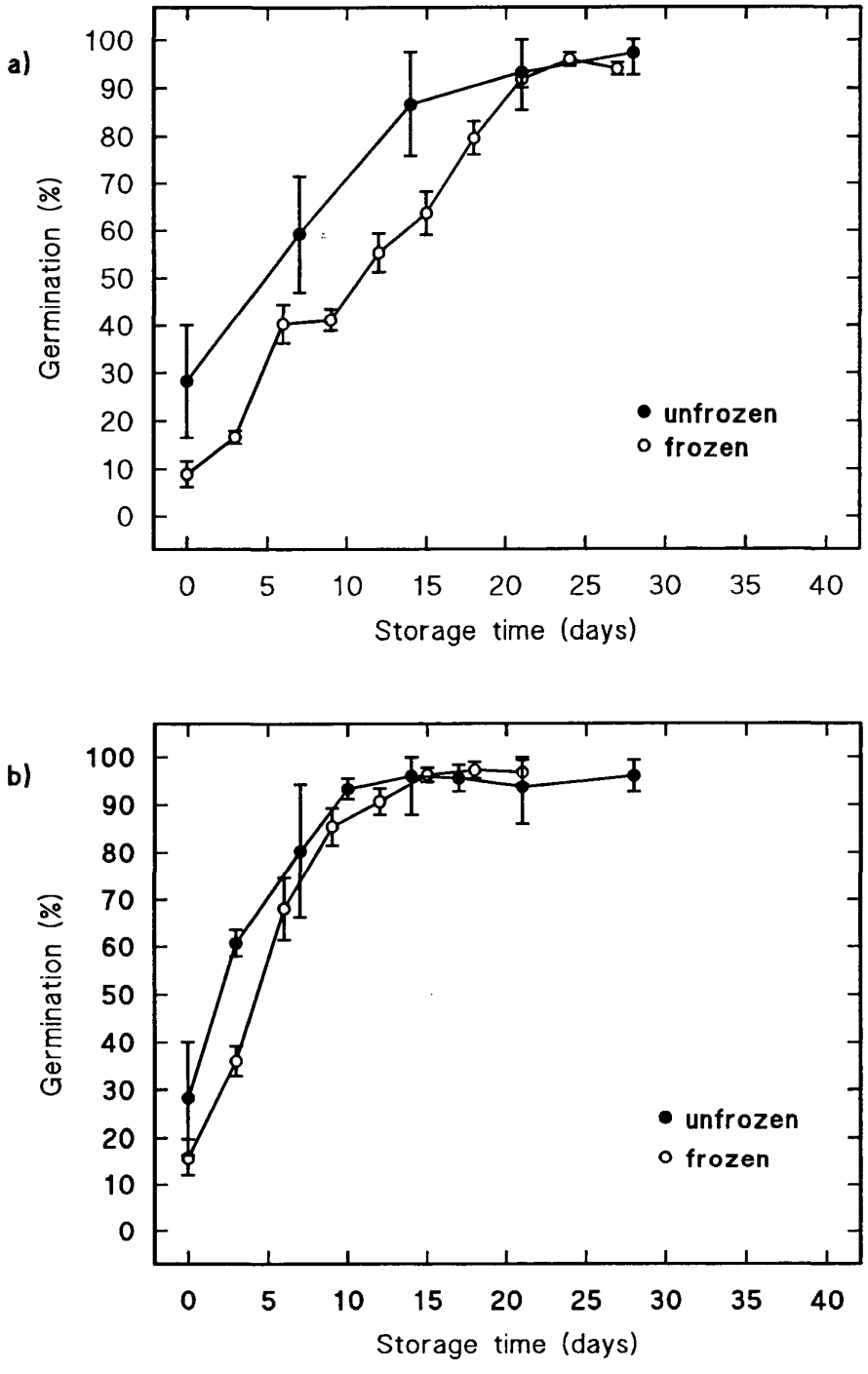



Fig. 2.6 Postharvest storage freezing trial with Kir–Tr–90 subsequently stored at (a) 27°C and (b) 38°C (4ml l.o.B. test; unfrozen, 3x100 seeds; frozen, 9x100 seeds).

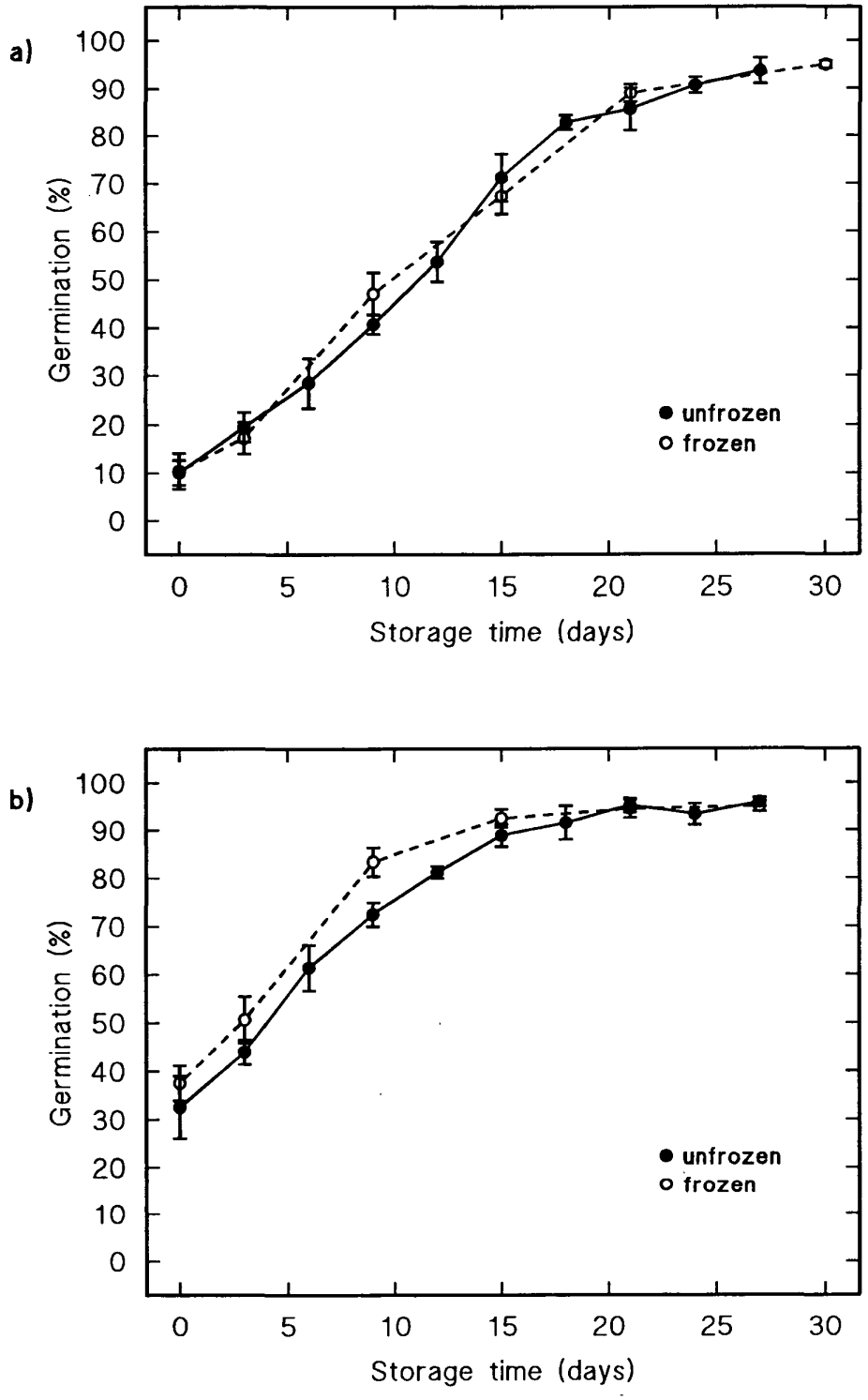



Fig. 2.7 Freezing trial on subsamples of (a) Pen-Tr-91a and (b) Pen-Tr-91b stored at 27°C (4ml l.o.B. test, 9x100 seeds).

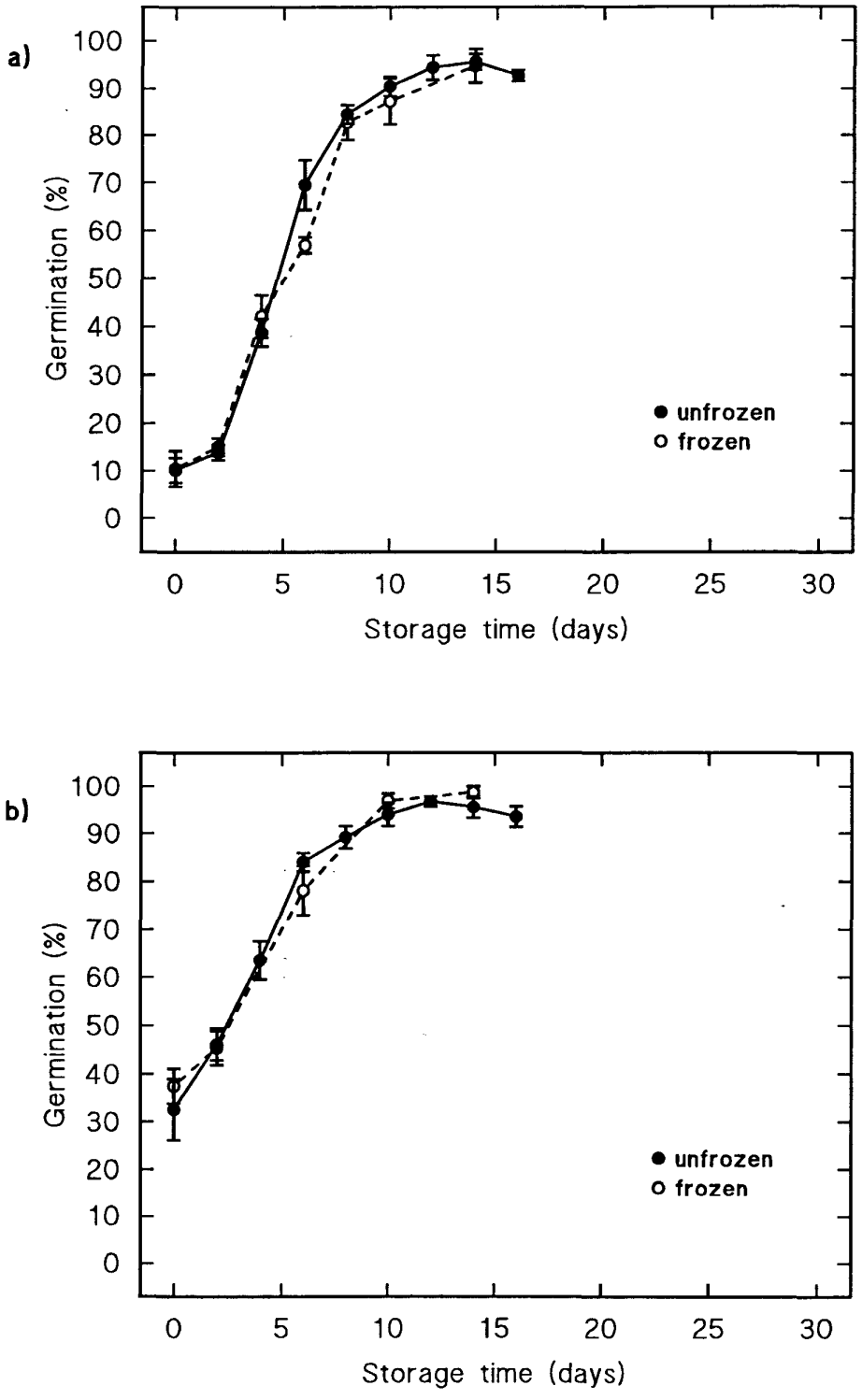



Fig. 2.8 Freezing trial on subsamples of (a) Pen–Tr–91a and (b) Pen–Tr–91b stored at 38°C (4ml I.o.B. test, 9x100 seeds).

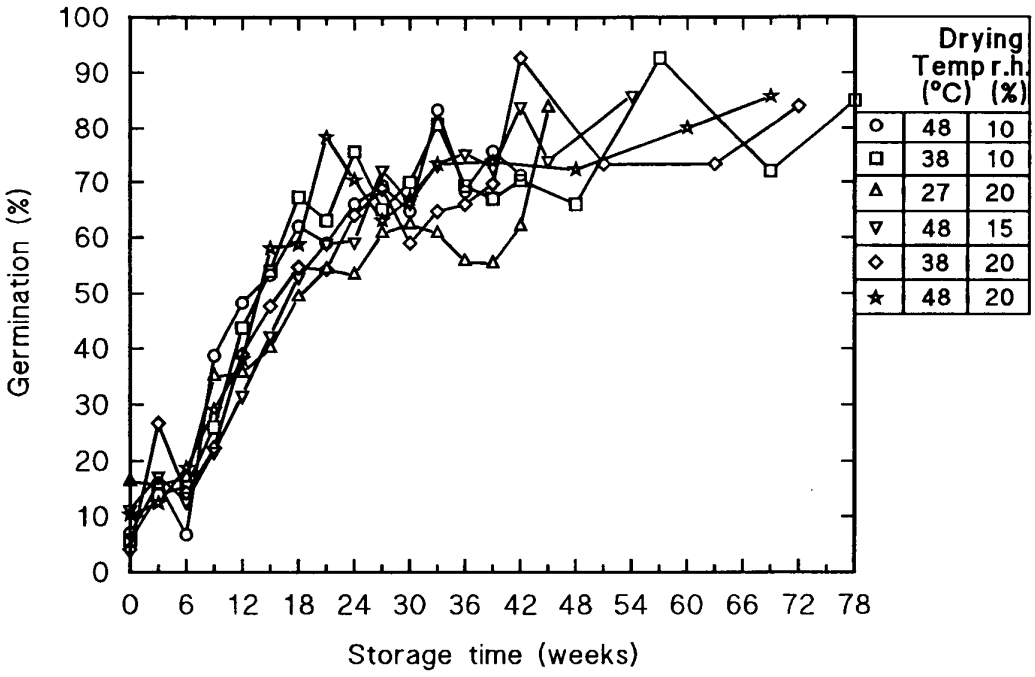



Fig. 3.1 Germination of Ack–Tr–88 during storage at 15°C, 12 % moisture content (1 ml agar test, 3x100 seeds)

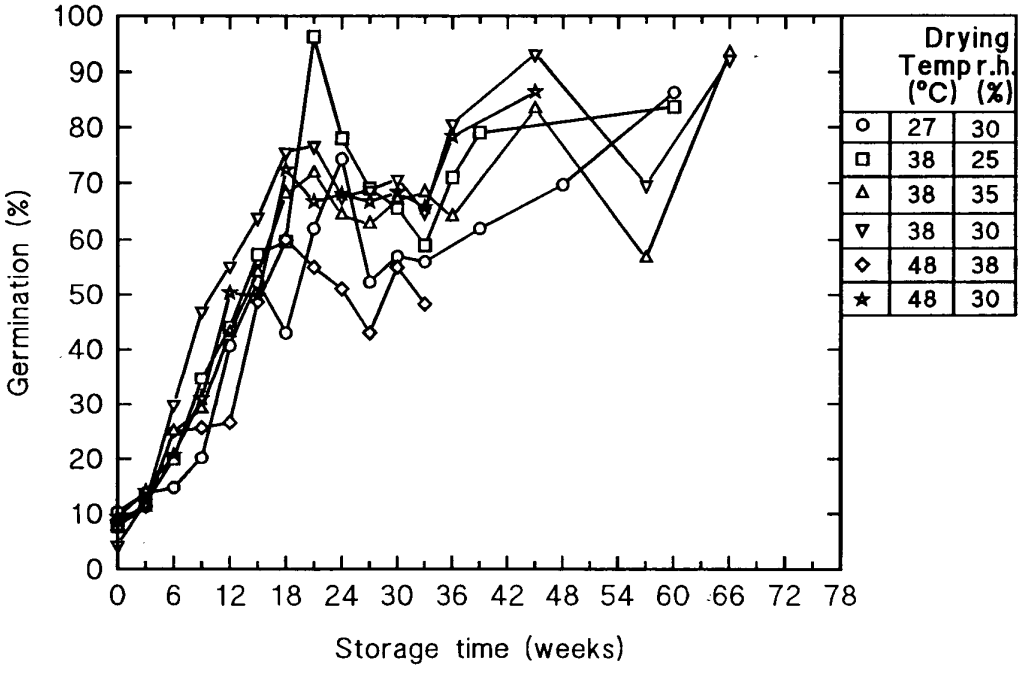



Fig 3.2 Germination of Ack–Tr–88 during storage at 15°C, 12% moisture content (1 ml agar test, 3x100 seeds)

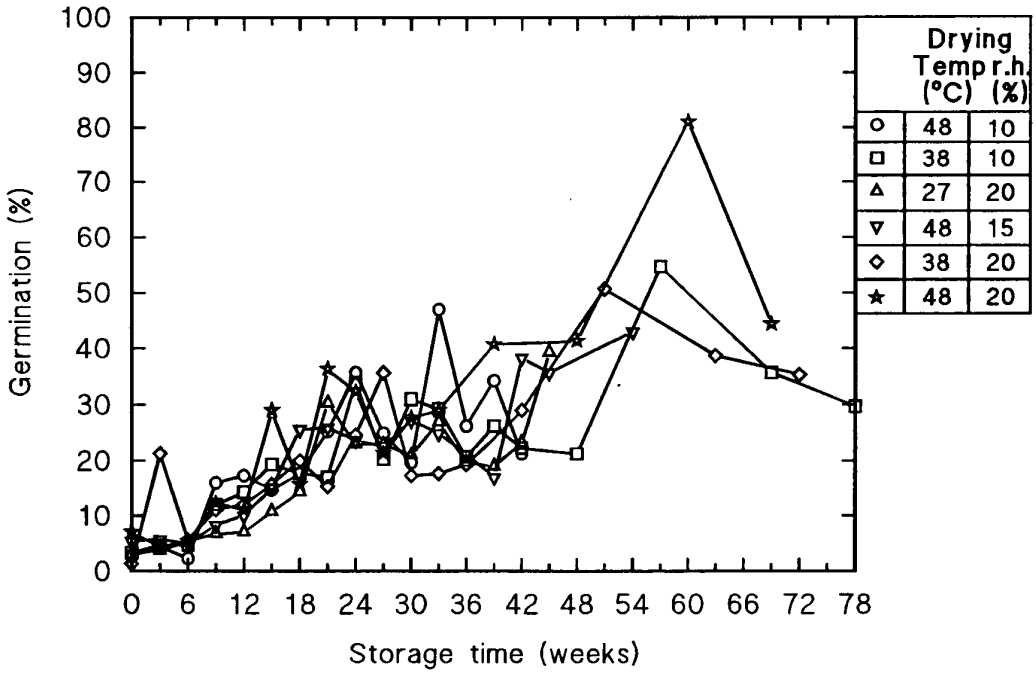



Fig. 3.3 Germination of Ack–Tr–88 during storage at 15°C, 12 % moisture content (3 ml agar test, 3x100 seeds)

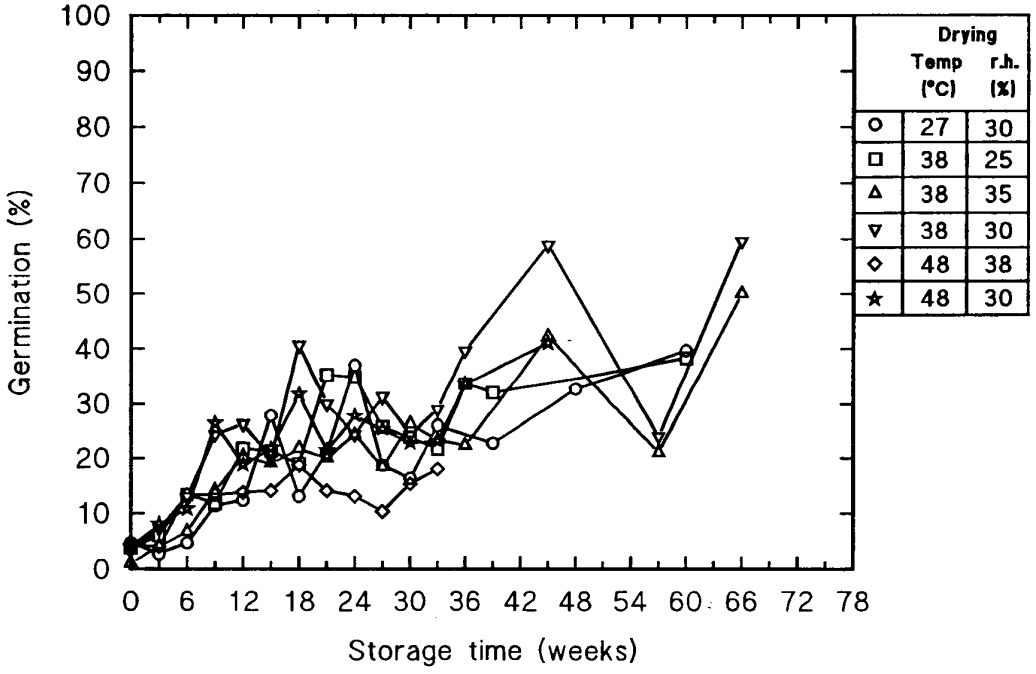



Fig. 3.4 Germination of Ack–Tr–88 during storage at 15°C, 12 % moisture content (3 ml agar test, 3x100 seeds)

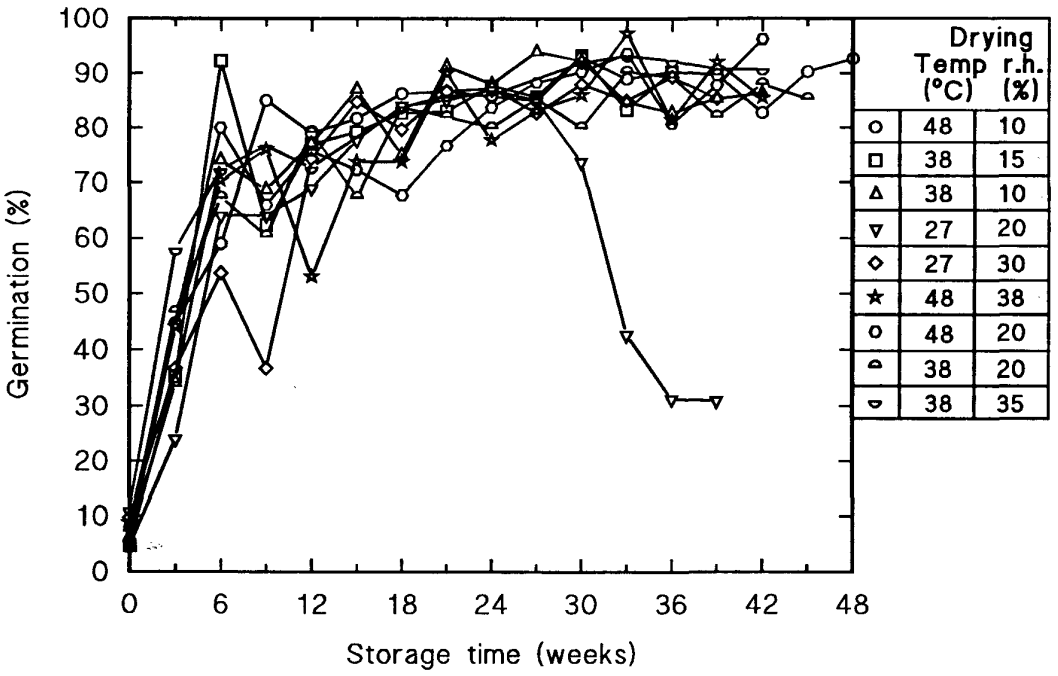



Fig. 3.5 Germination of Ack–Tr–88 during storage at 27°C, 12 % moisture content (1 ml agar test, 3x100 seeds)

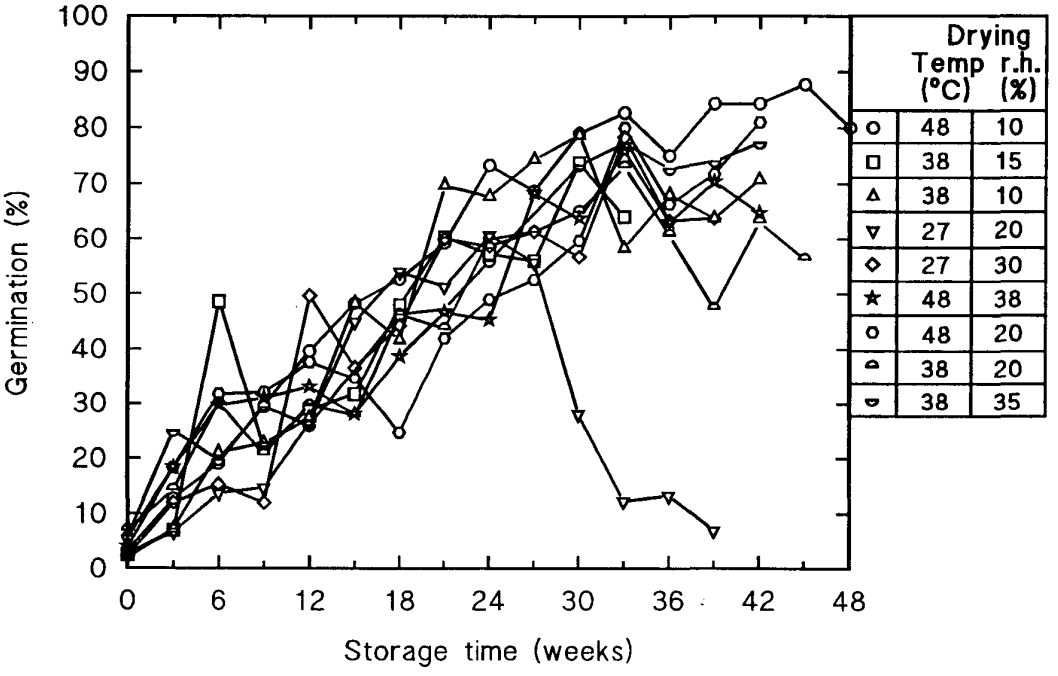



Fig. 3.6 Germination of Ack–Tr–88 during storage at 27°C, 12 % moisture content (3 ml agar test, 3x100 seeds)

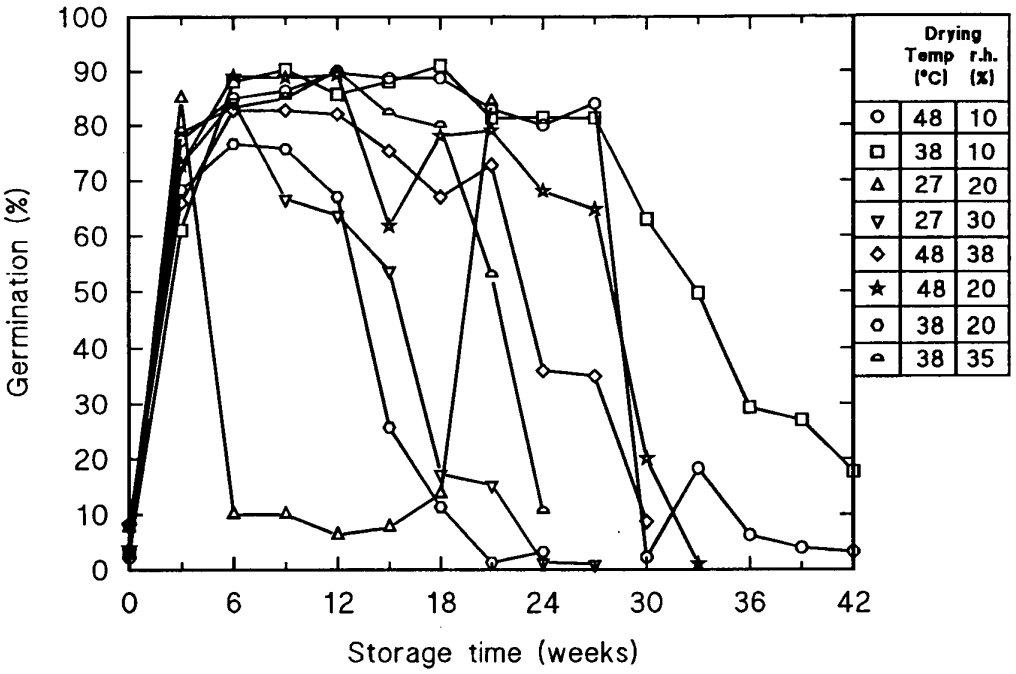



Fig. 3.7 Germination of Ack–Tr–88 during storage at 38°C, 12 % moisture content (1 ml agar test, 3x100 seeds)

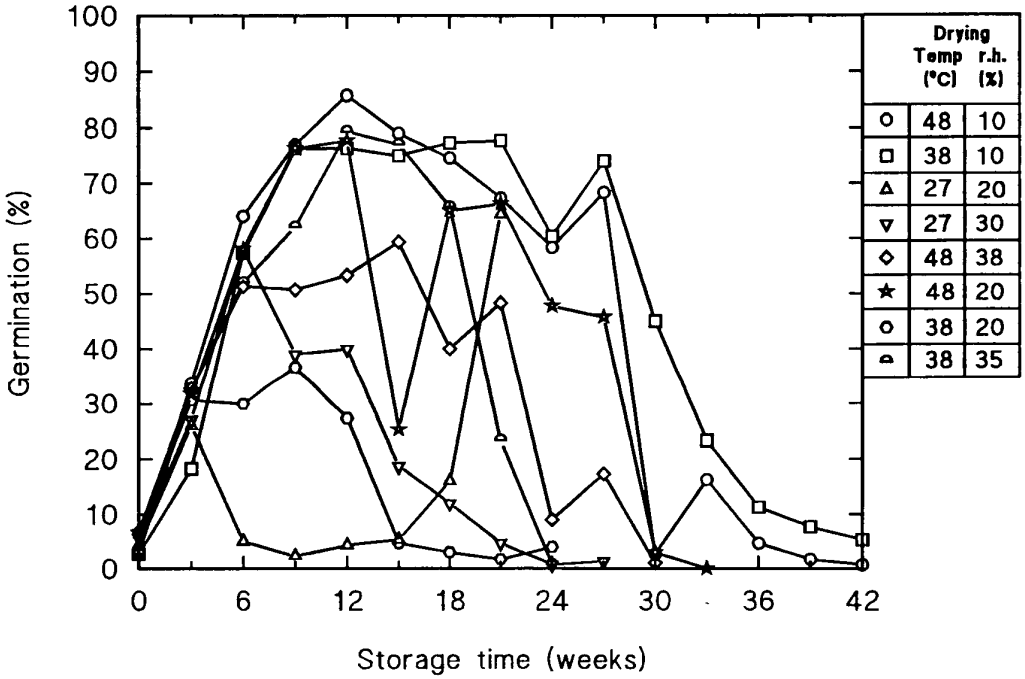



Fig. 3.8 Germination of Ack–Tr–88 during storage at 38°C, 12 % moisture content (3 ml agar test, 3x100 seeds)

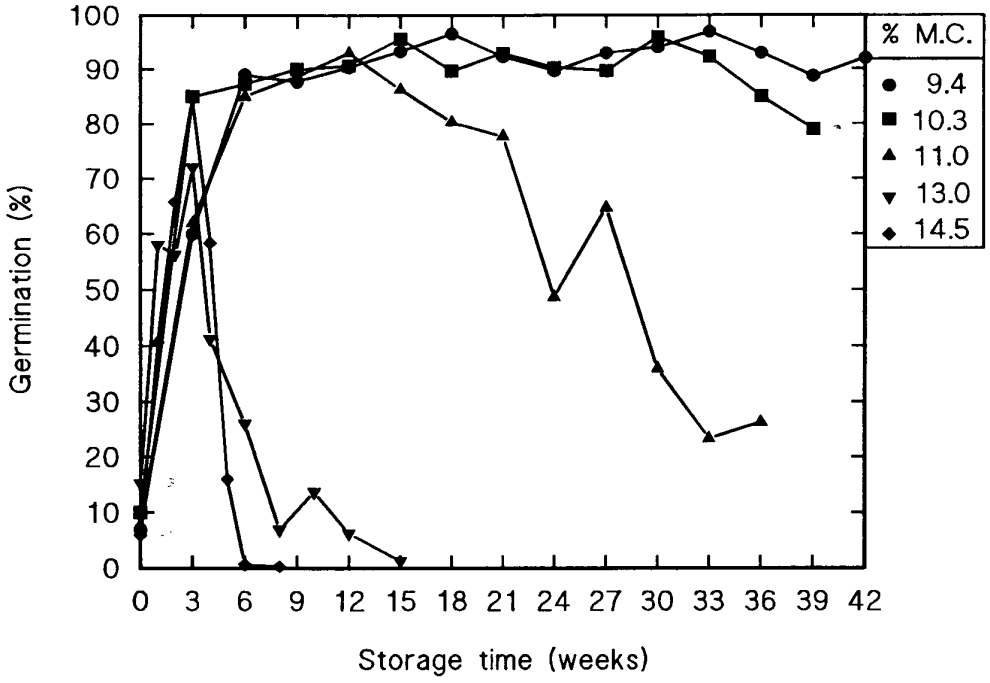



Fig. 3.9 Germination of Ack–Tr–88 dried with air at 38°C, 10 % r.h., during storage at 38°C (1ml agar test, 3x100 seeds)

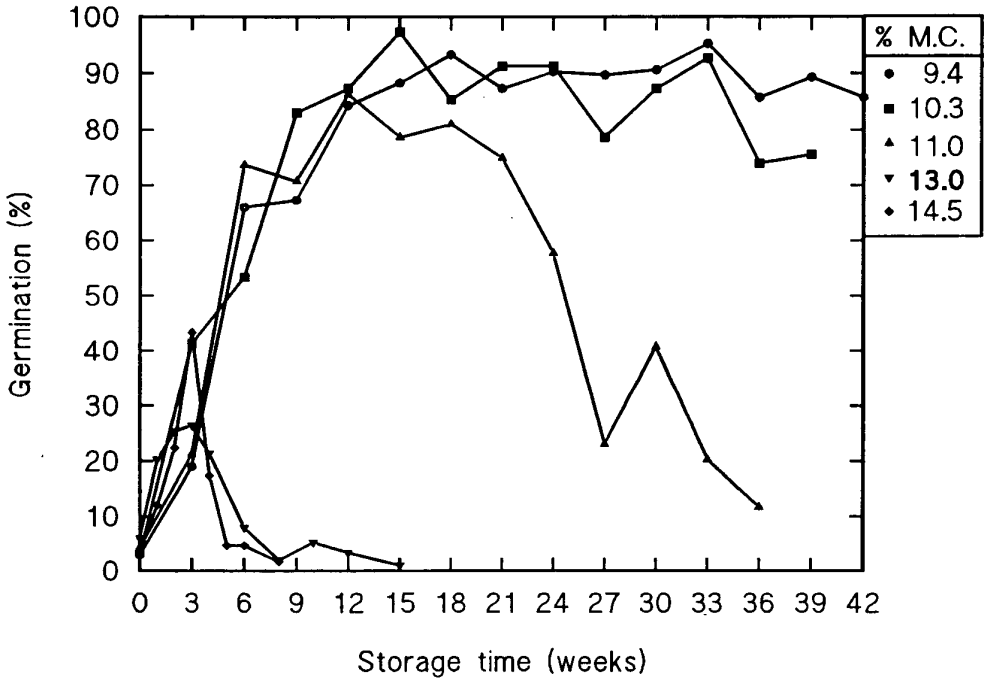



Fig. 3.10 Germination of Ack–Tr–88 dried with air at 38°C, 10 % r.h., during storage at 38°C (3 ml agar test, 3x100 seeds)

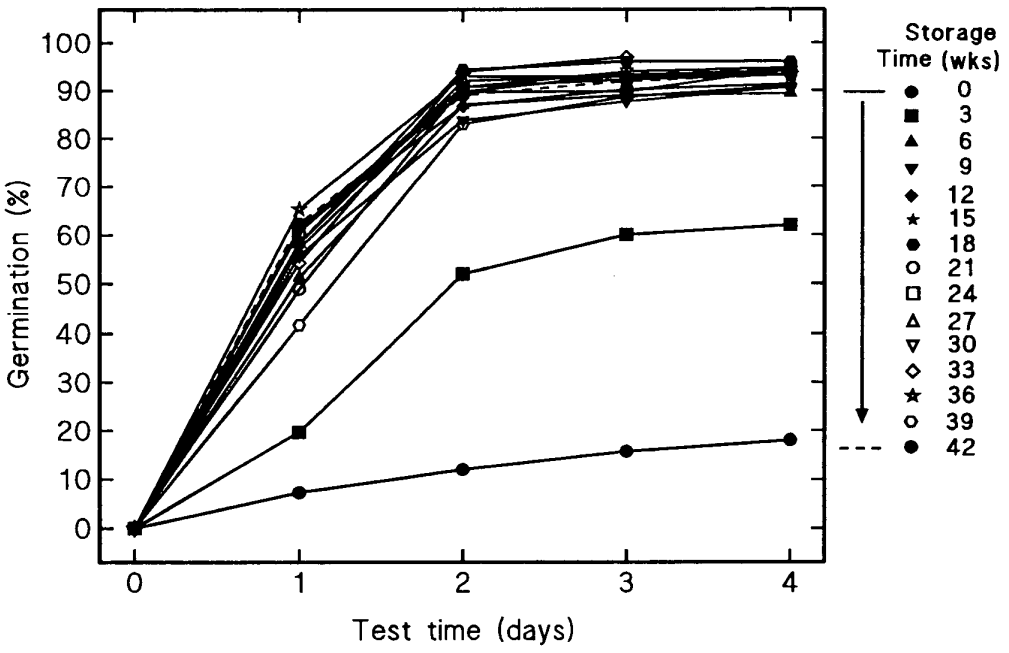



Fig. 3.11 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 1ml agar tests (3x100 seeds), during storage at 38°C, 9.4 % moisture content

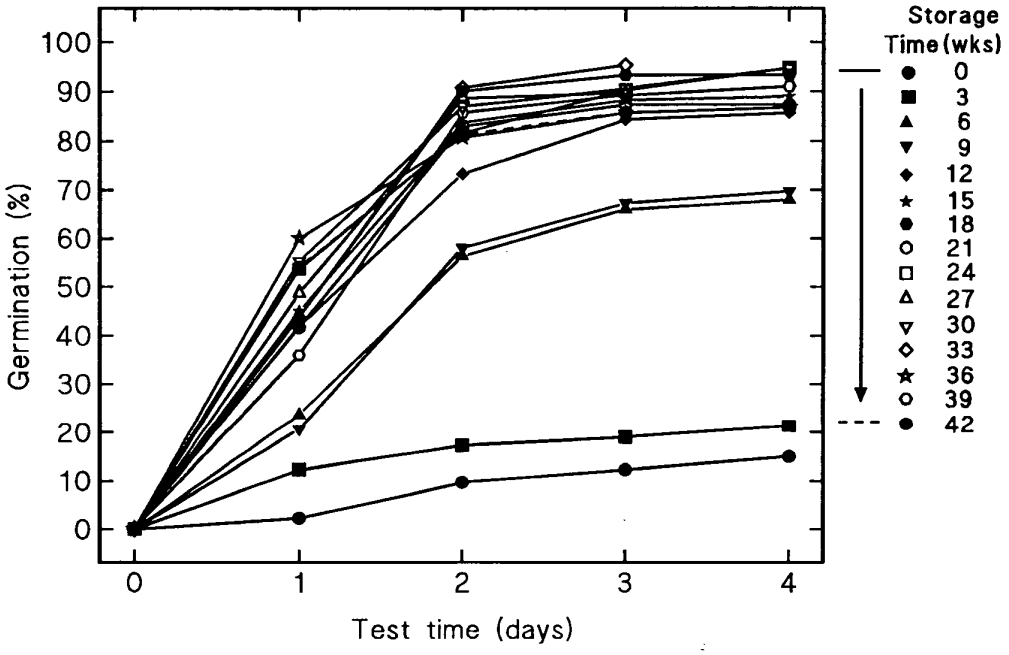



Fig. 3.12 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 3 ml agar tests (3x100 seeds), during storage at 38°C, 9.4 % moisture content.

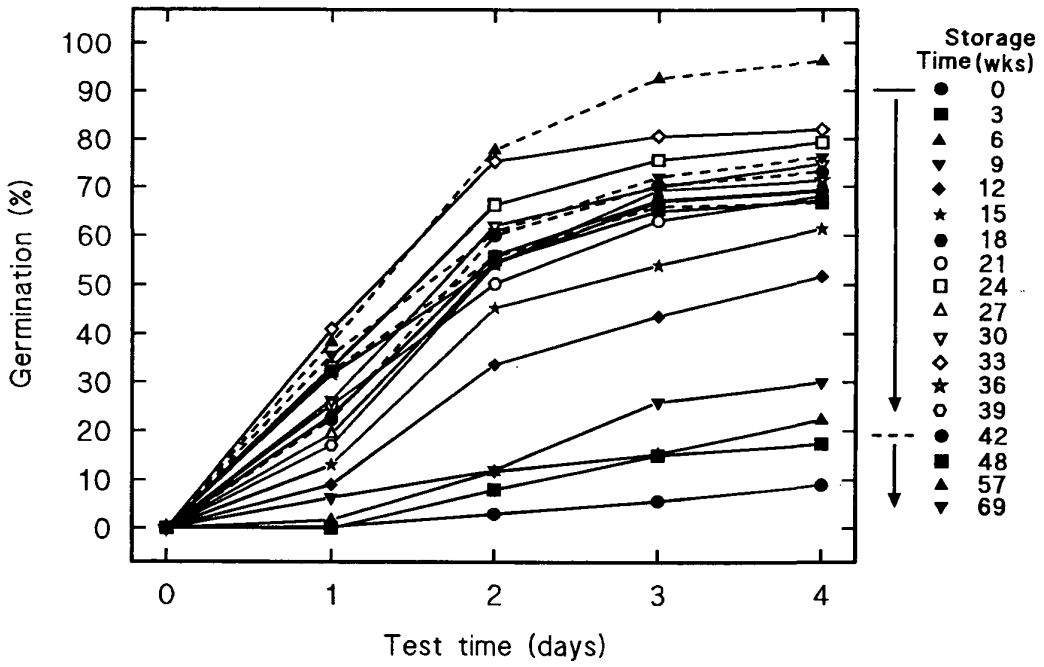



Fig. 3.13 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 1ml agar tests (3x100 seeds), during storage at 15°C, 12 % moisture content.

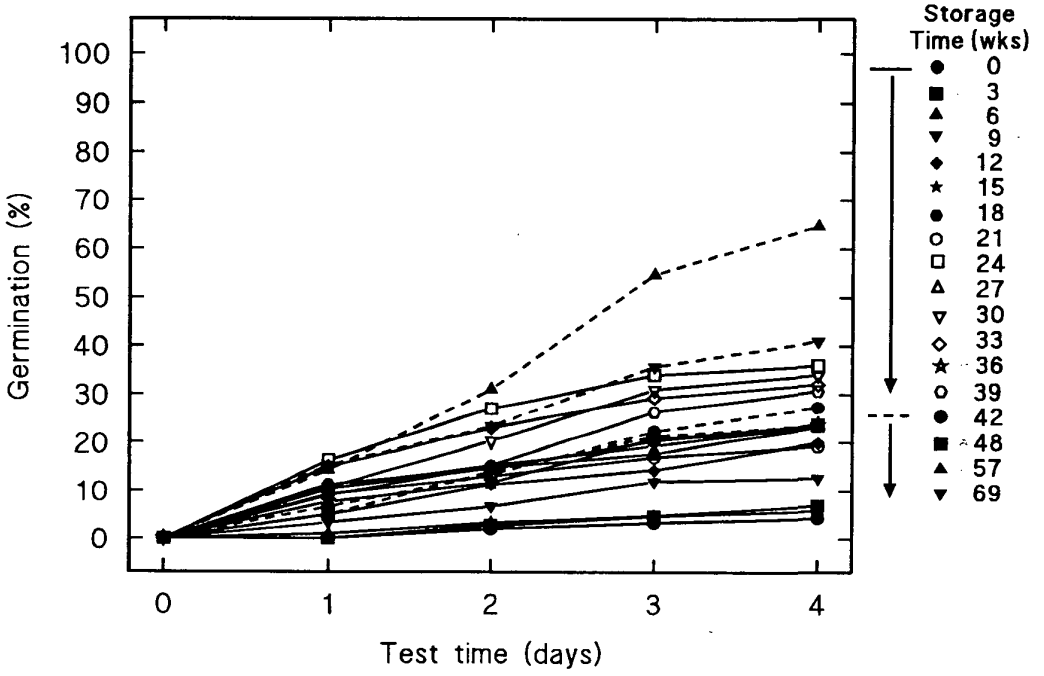



Fig. 3.14 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 3 ml agar tests (3x100 seeds), during storage at 15°C, 12 % moisture content.

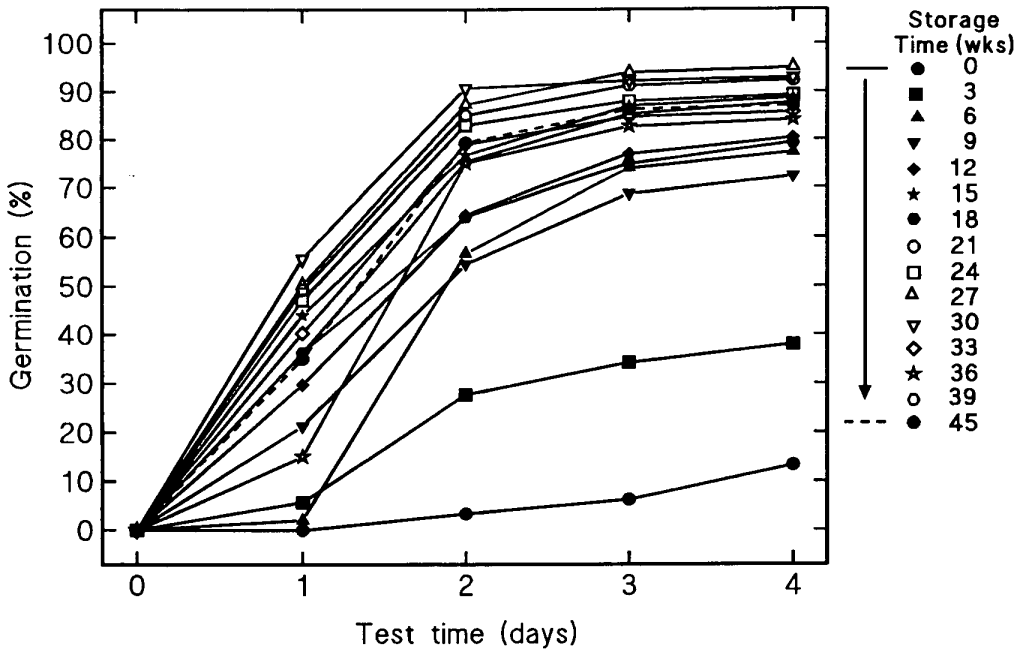



Fig. 3.15 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 1 ml agar tests (3x100 seeds), during storage at 27°C, 12 % moisture content.

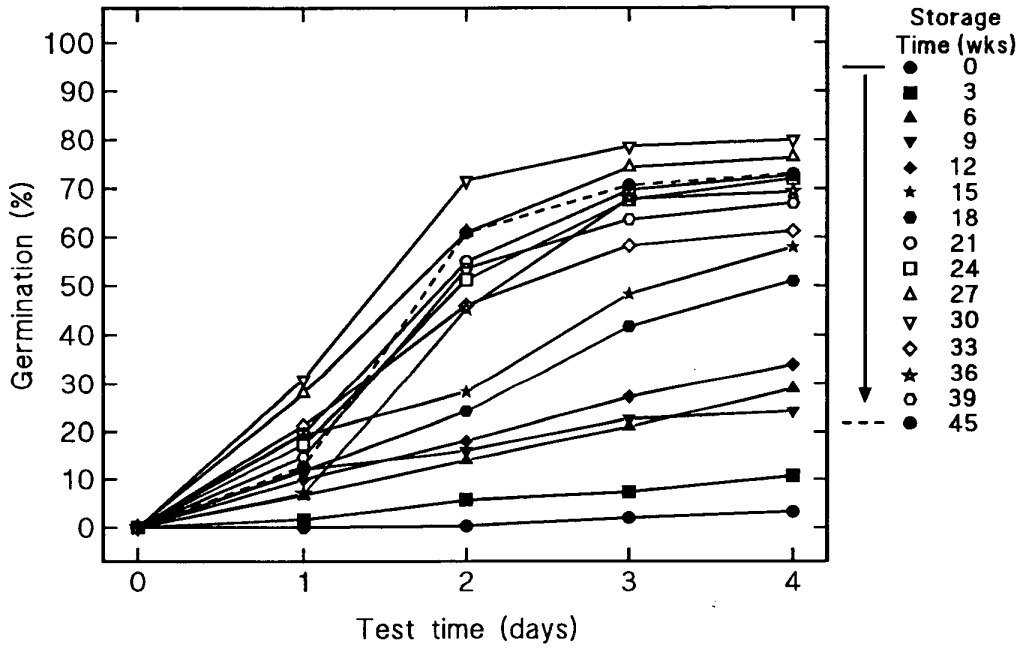



Fig. 3.16 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 3 ml agar tests (3x100 seeds), during storage at 27°C, 12 % moisture content.

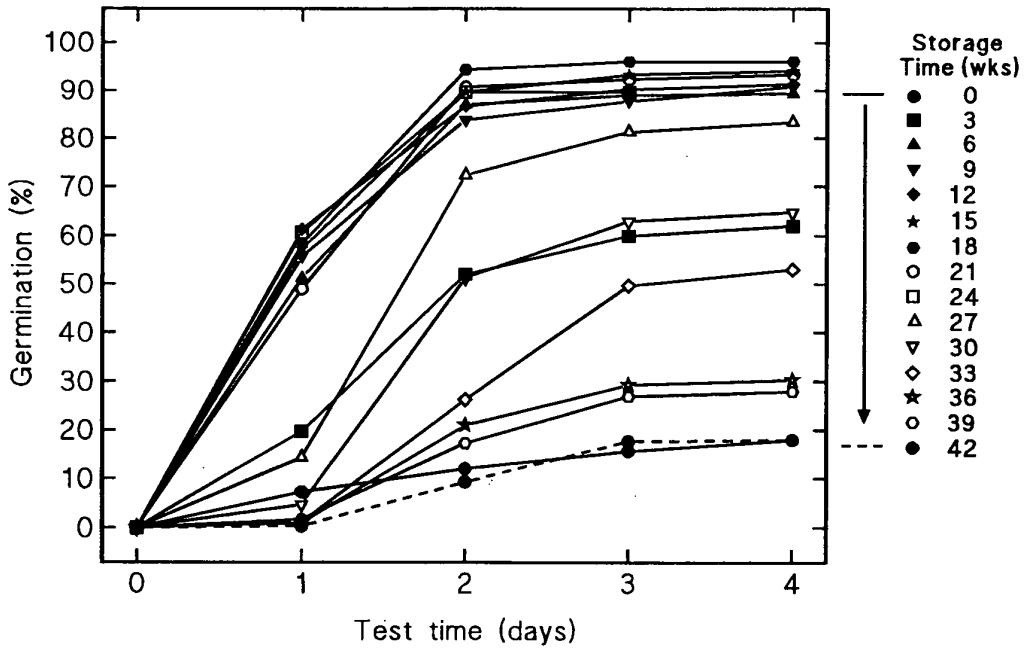



Fig. 3.17 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 1 ml agar tests (3x100 seeds), during storage at 38°C, 12 % moisture content.

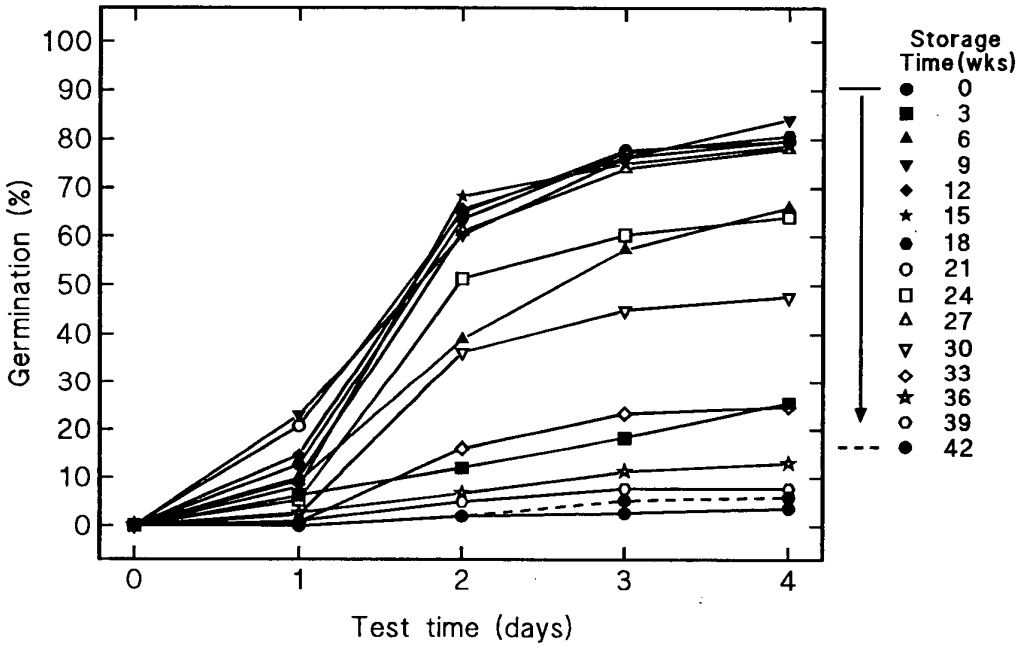



Fig. 3.18 Cumulative germination of Ack–Tr–88 dried at 38°C, 10 % r.h., in 3 ml agar tests (3x100 seeds), during storage at 38°C, 12 % moisture content.

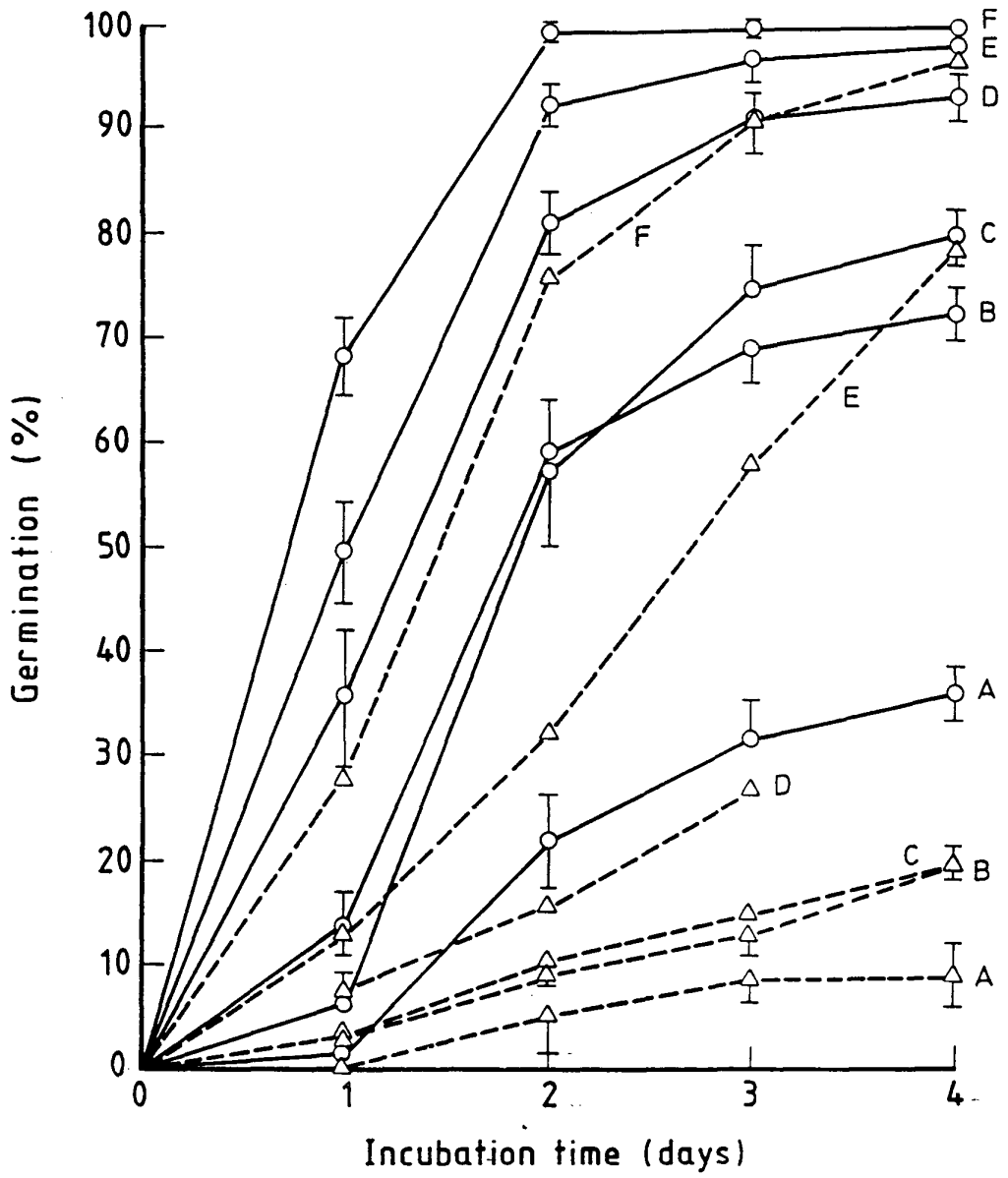



FIG 3.19

The germination of sub-samples of a batch of Triumph barley (Pen-Tr - 90) , in the 1 ml (agar) test 0——0 and in the 3 ml (agar) test Δ- - -Δ. A, undried samples; B, samples dried at 40°C for 3 days. Samples dried at 40°C for 3 days, then stored at 60°C for 7 h, C; for 2 days at 60°C, D; for 4 days, E, and for 9 days, F, at 60°C. The grains were tested immediately at the end of the warm period. The bars show the standard deviations of the results (300 seeds)

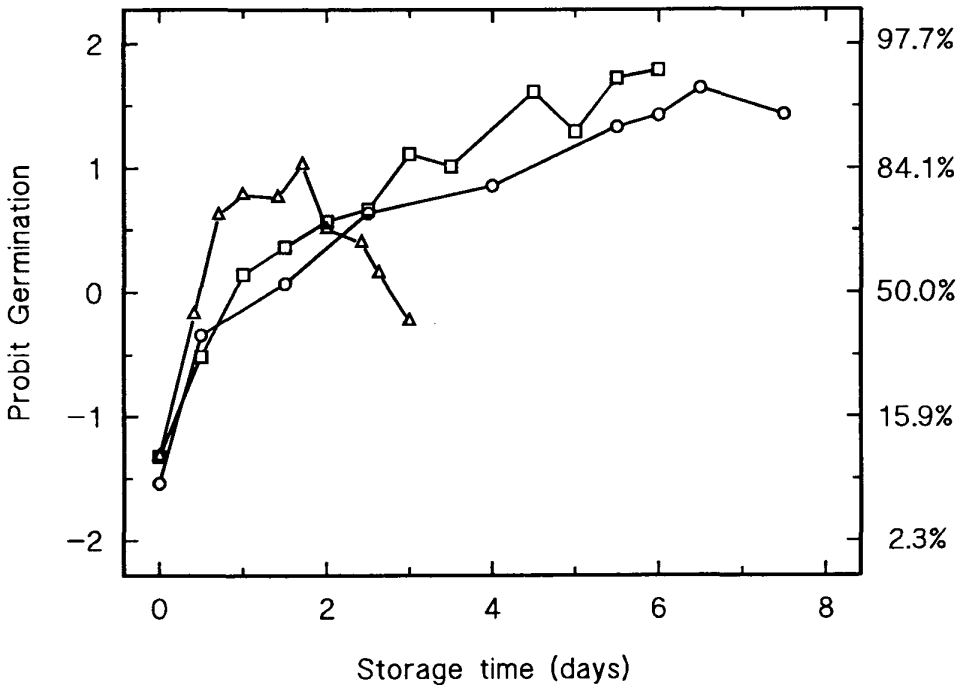



Fig. 3.20 Probit germination of Kir−Tr−90 during storage at (o) 45°C, (□) 50°C and (△) 60°C, 9.0 % moisture content (4 ml l.o.B. test, 9x100 seeds)

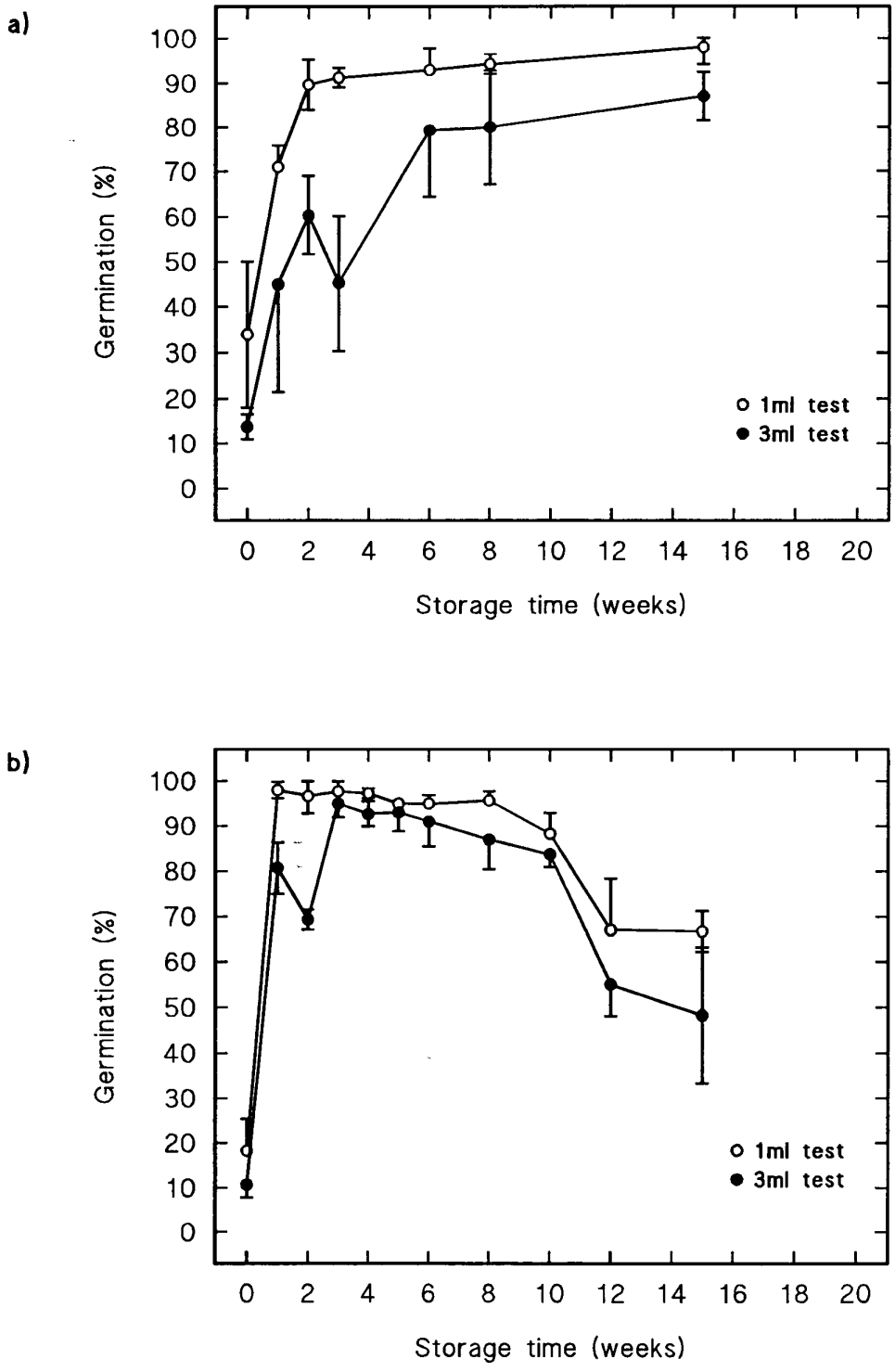



Fig. 3.21 Germination of Abr–Gp–88 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

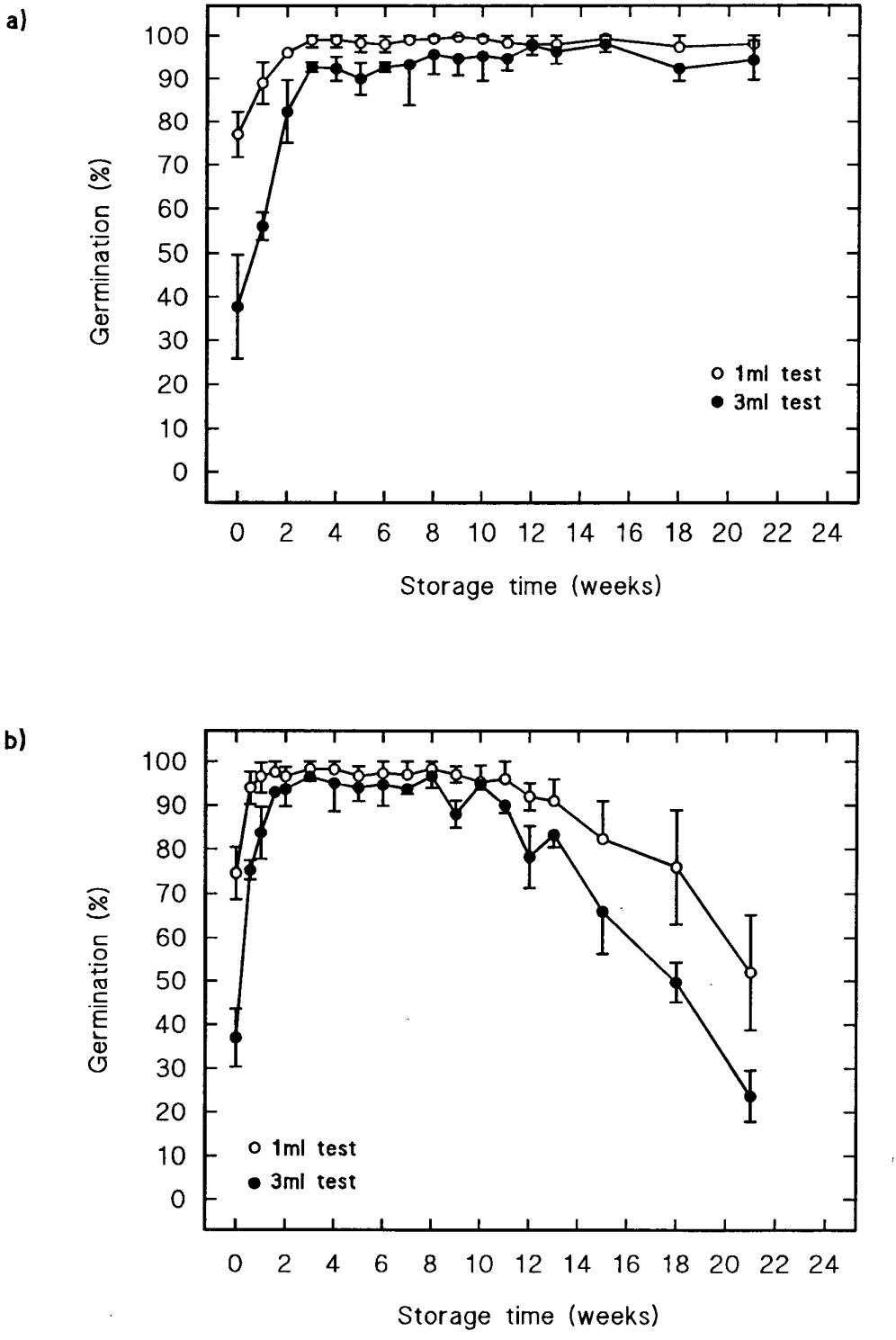



Fig. 3.22 Germination of Kir–Ca–90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

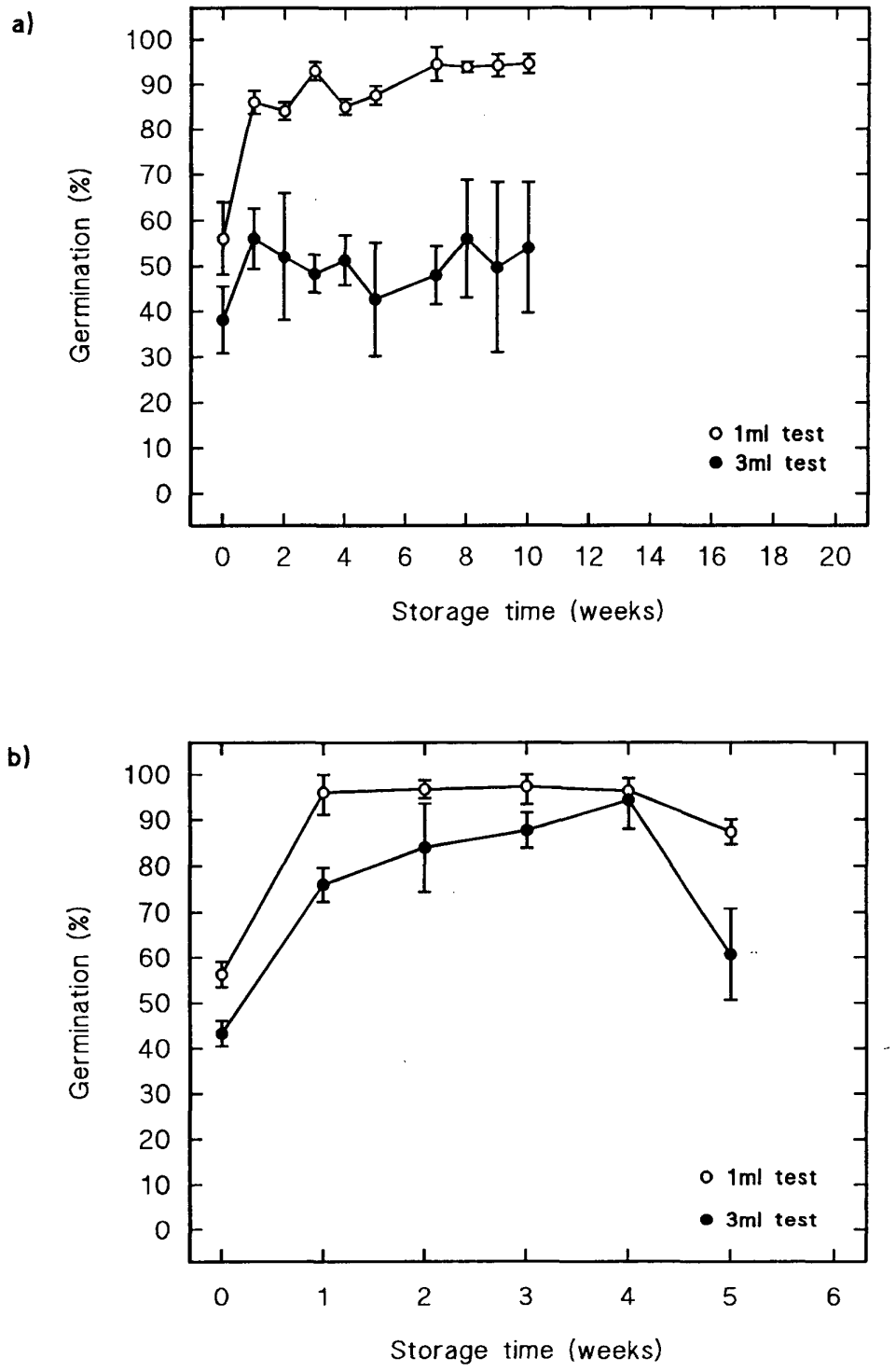



Fig. 3.23 Germination of Ack-Db-90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

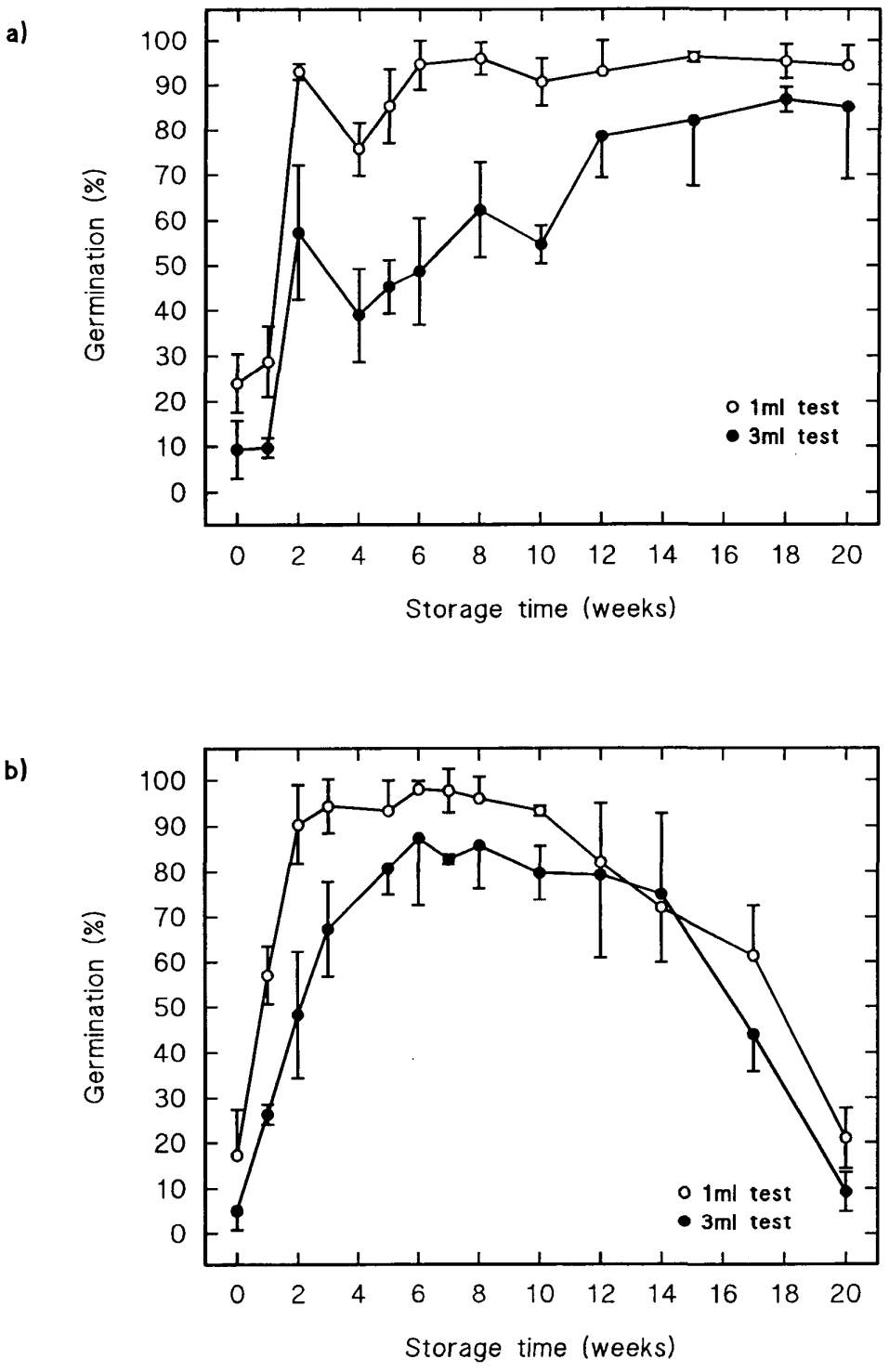



Fig. 3.24 Germination of Abr-Tr-88 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

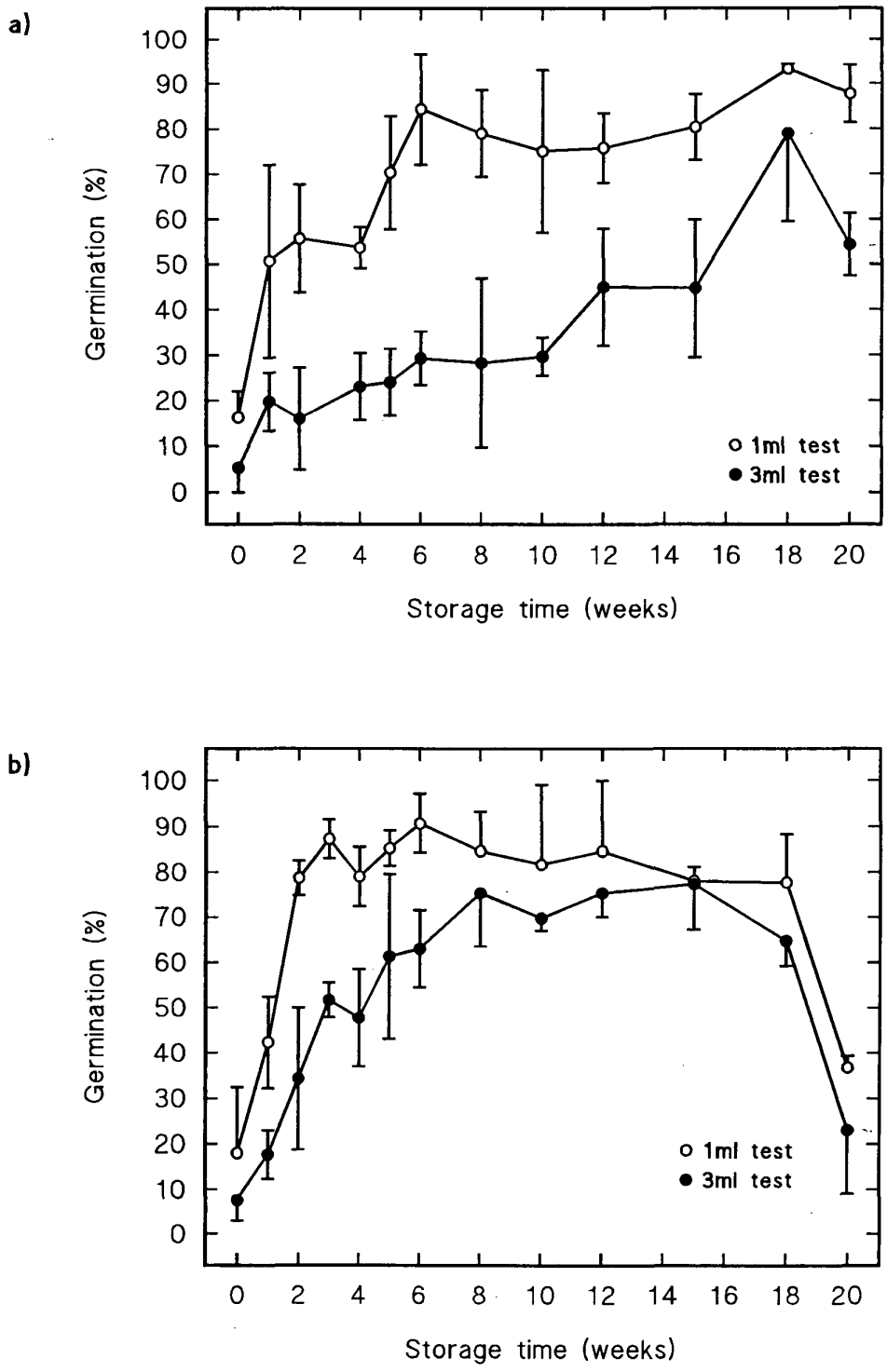



Fig. 3.25 Germination of Edn-Tr-88 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

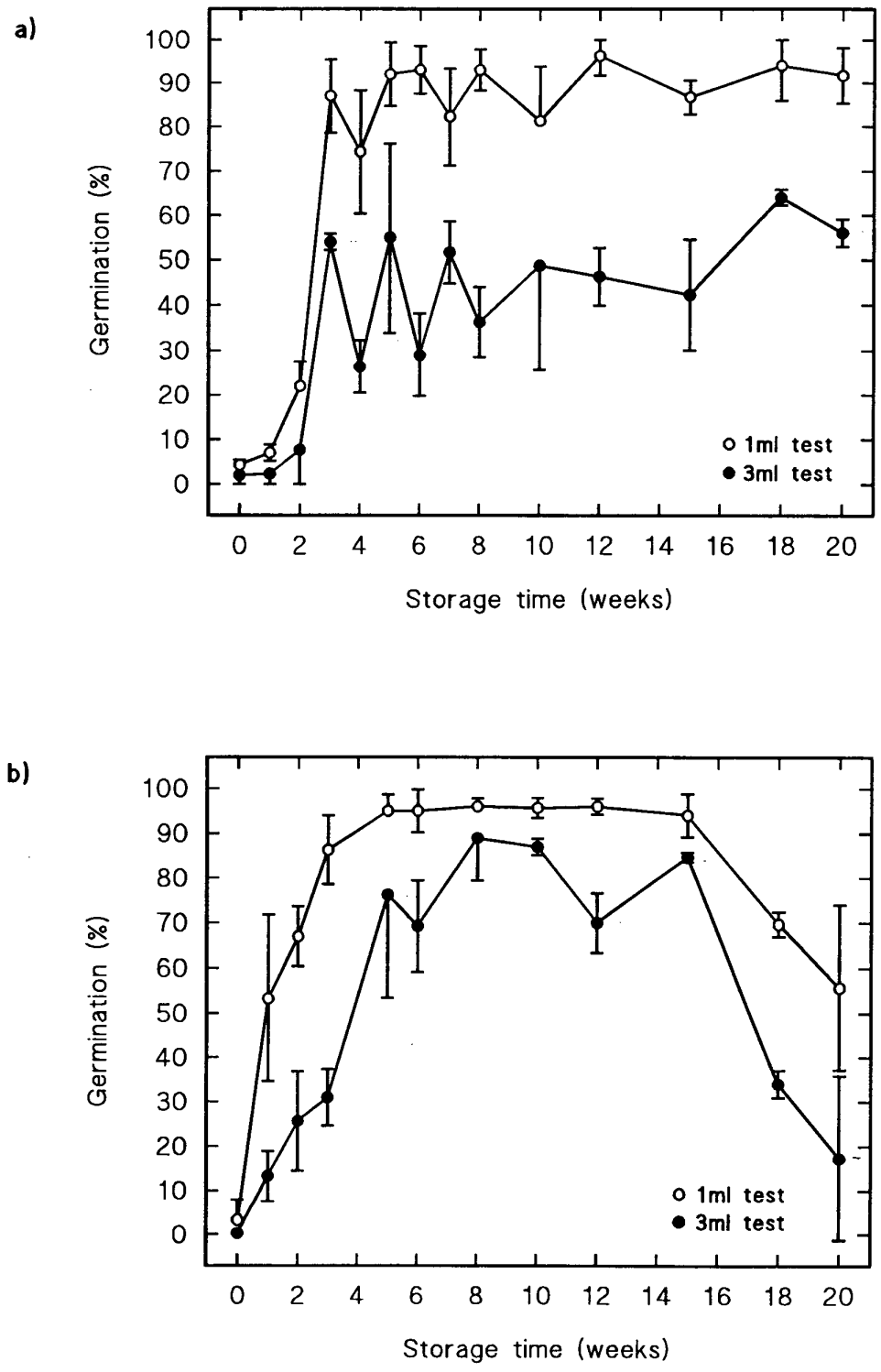



Fig. 3.26 Germination of Mot-Tr-88 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

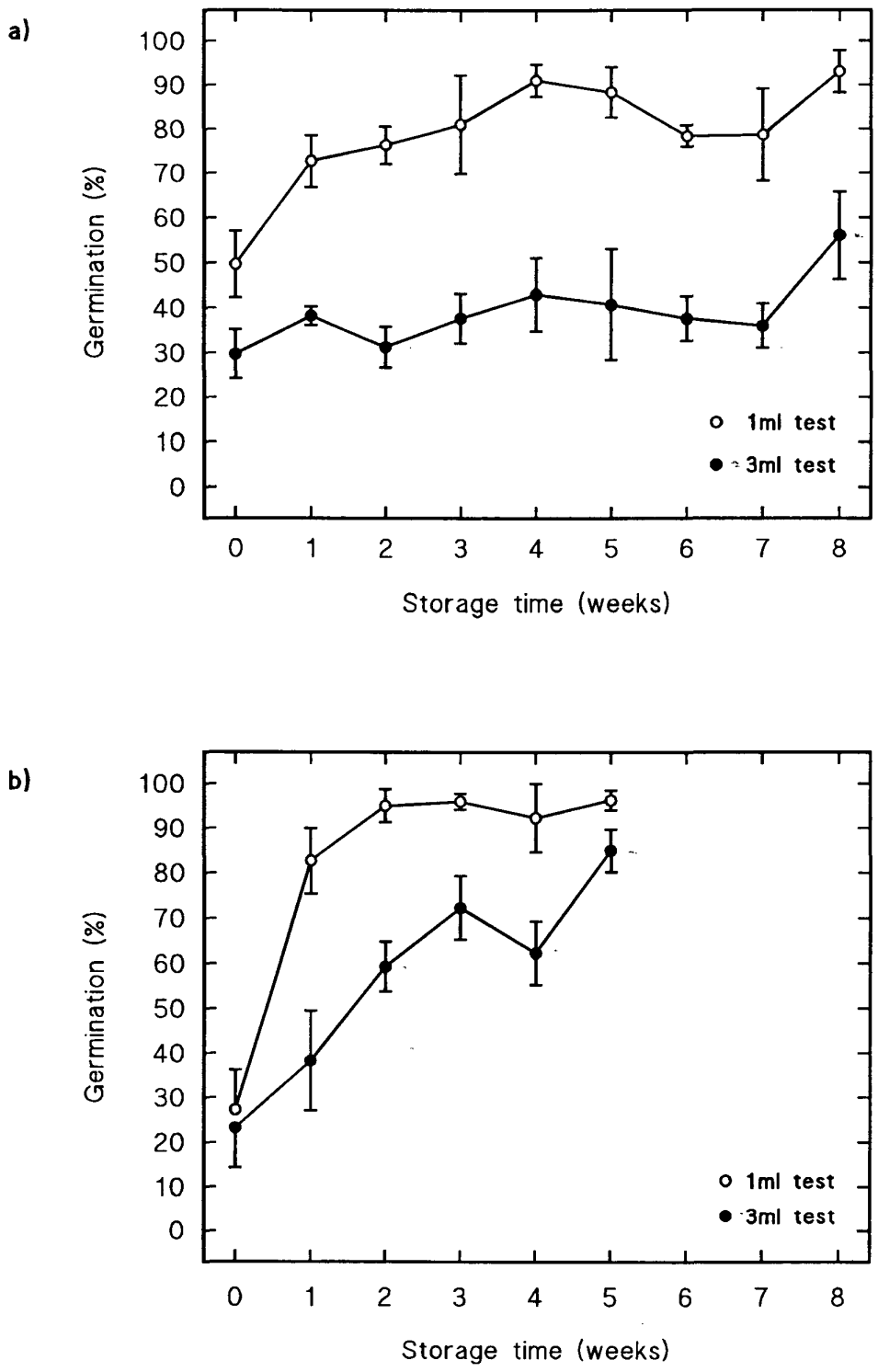



Fig. 3.27 Germination of Ack-Tr-90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

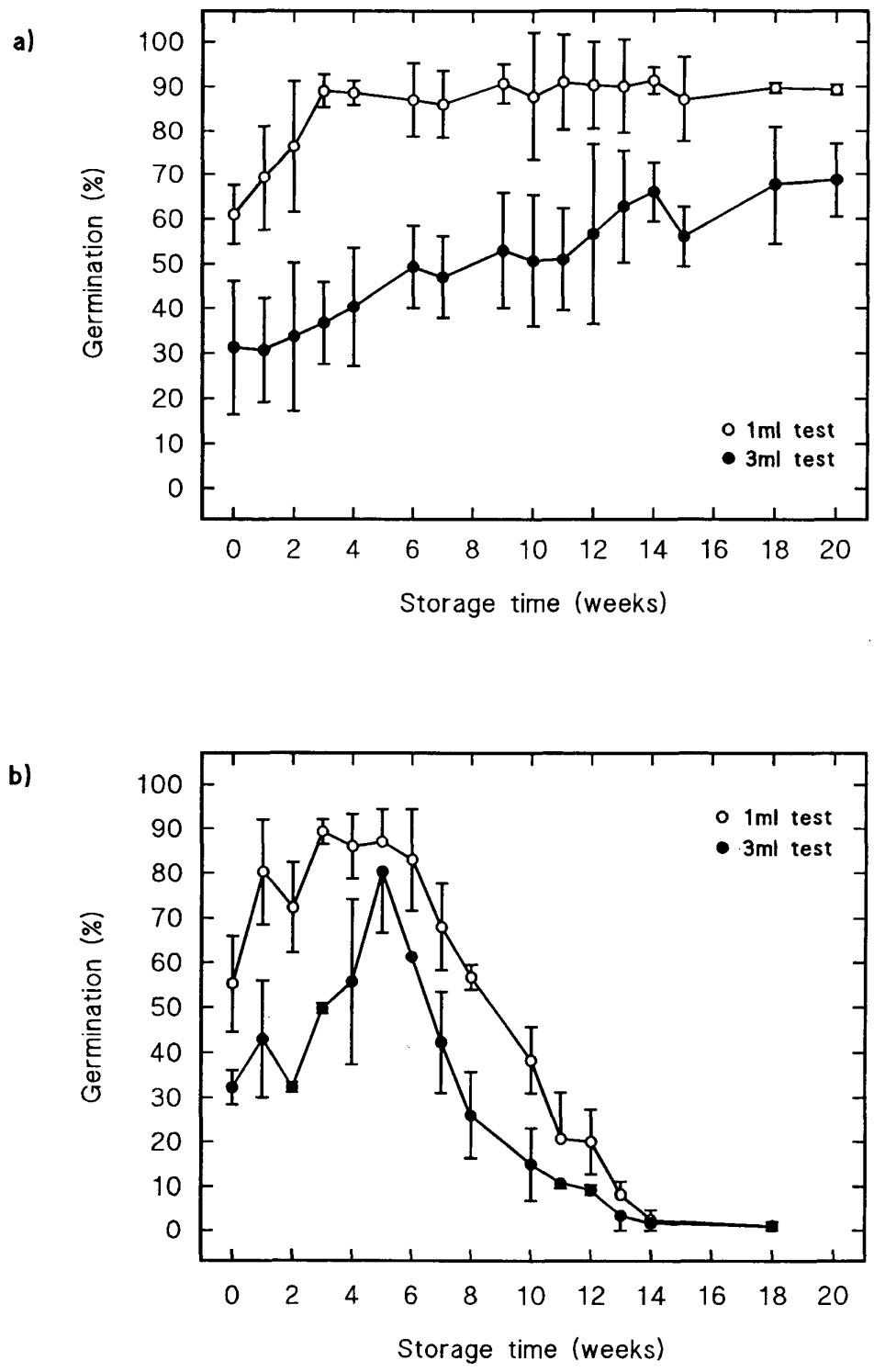



Fig. 3.28 Germination of Inv-Tr-90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

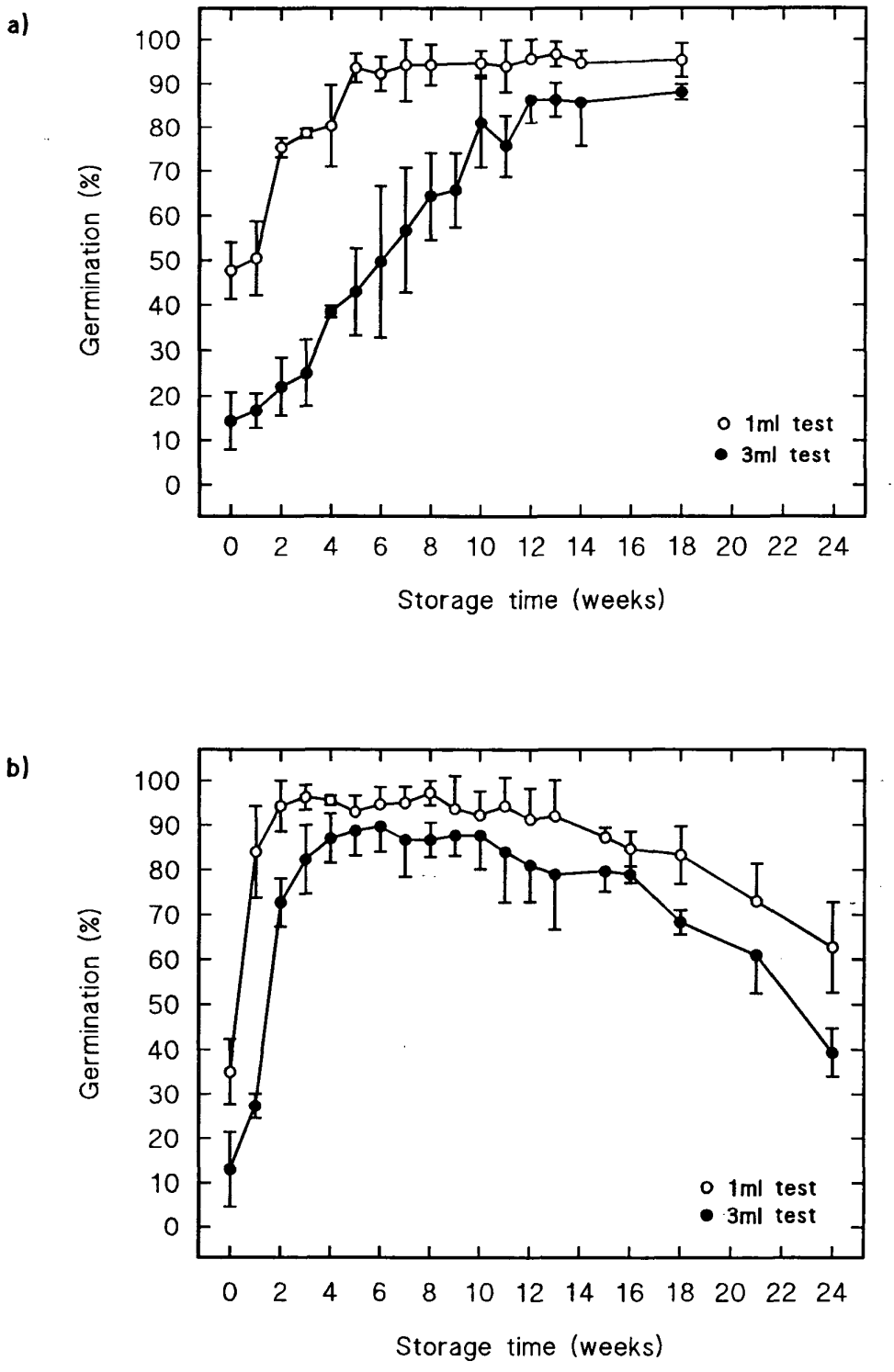



Fig. 3.29 Germination of Mot-Tr-90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

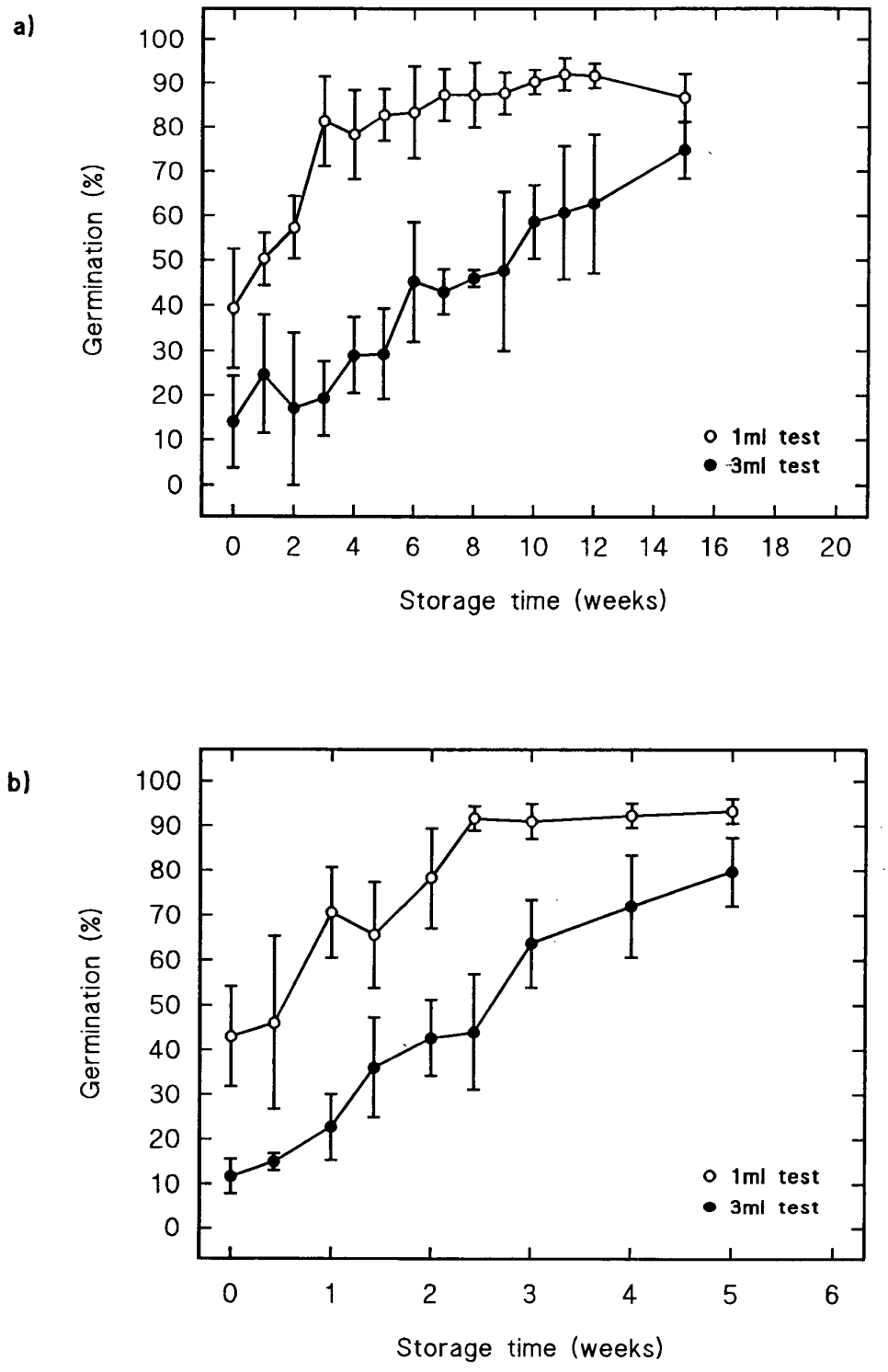



Fig. 3.30 Germination of Abr–Tr–90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

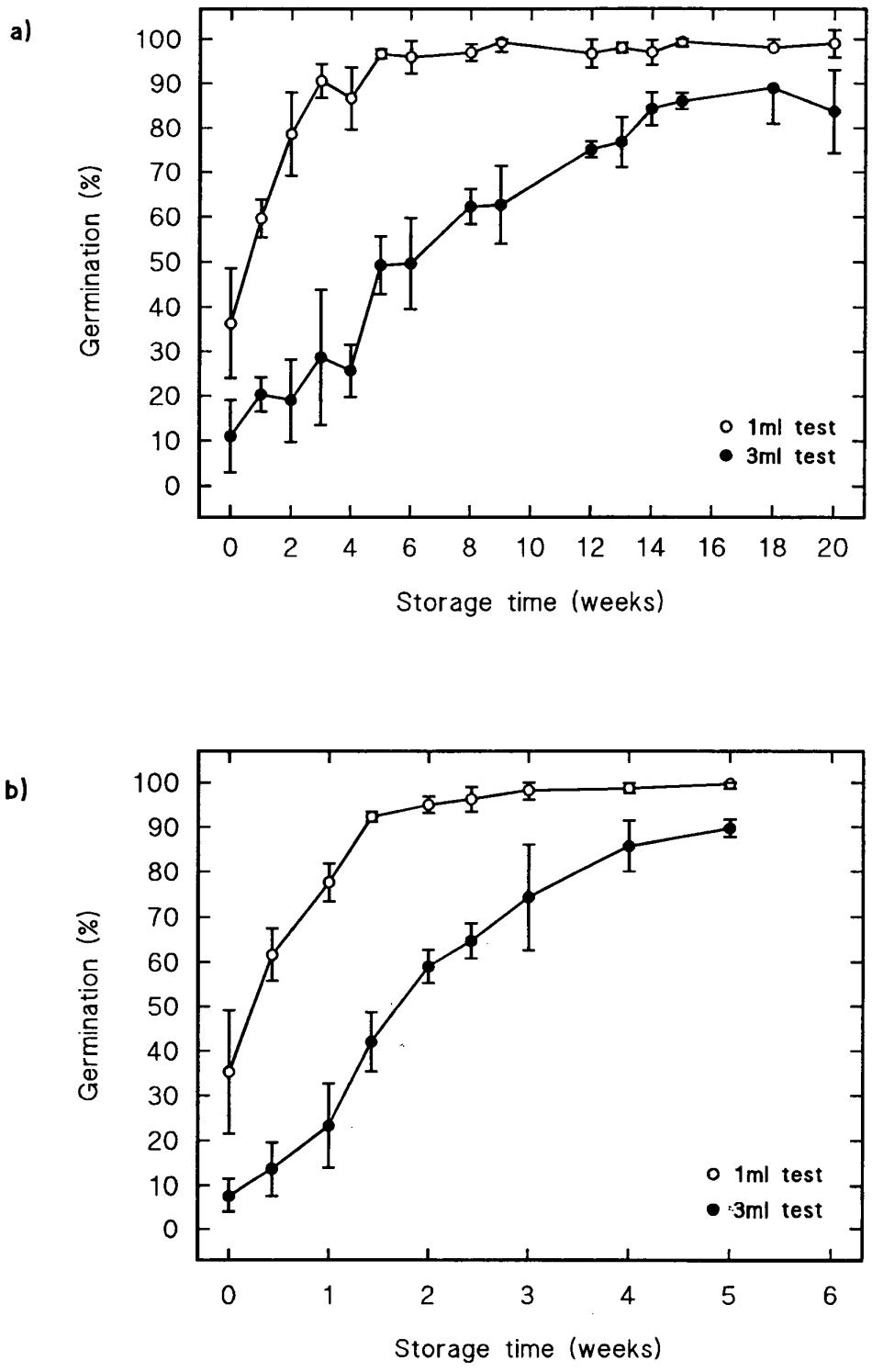



Fig. 3.31 Germination of Pen–Tr–90 during storage at (a) 27°C and (b) 38°C, 12 % moisture content (3x100 seeds).

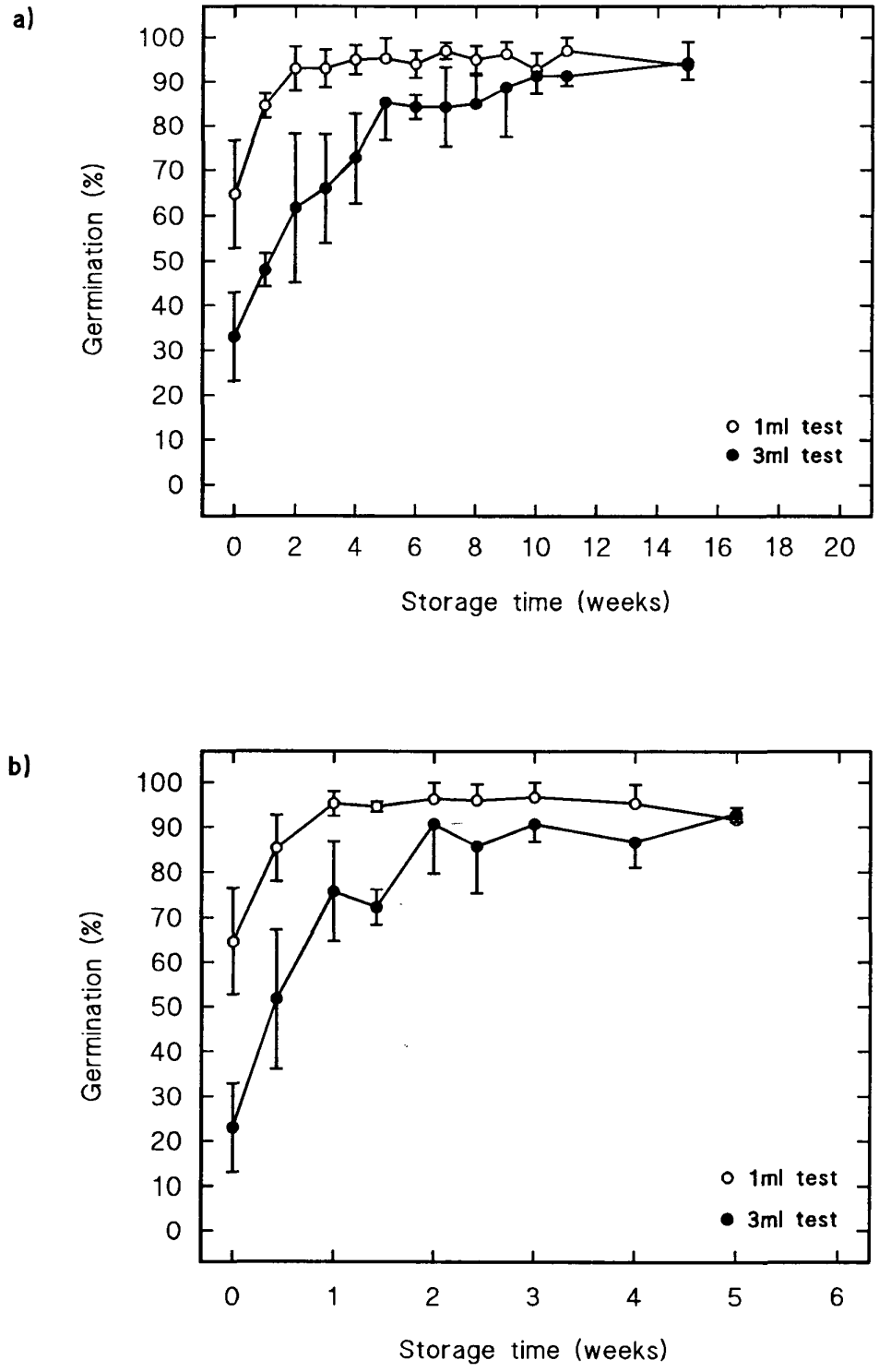



Fig. 3.32 Germination of Kir-Tr-90 during storage at (a) 27°C and (b) 38C, 12 % germination (3x100 seeds).

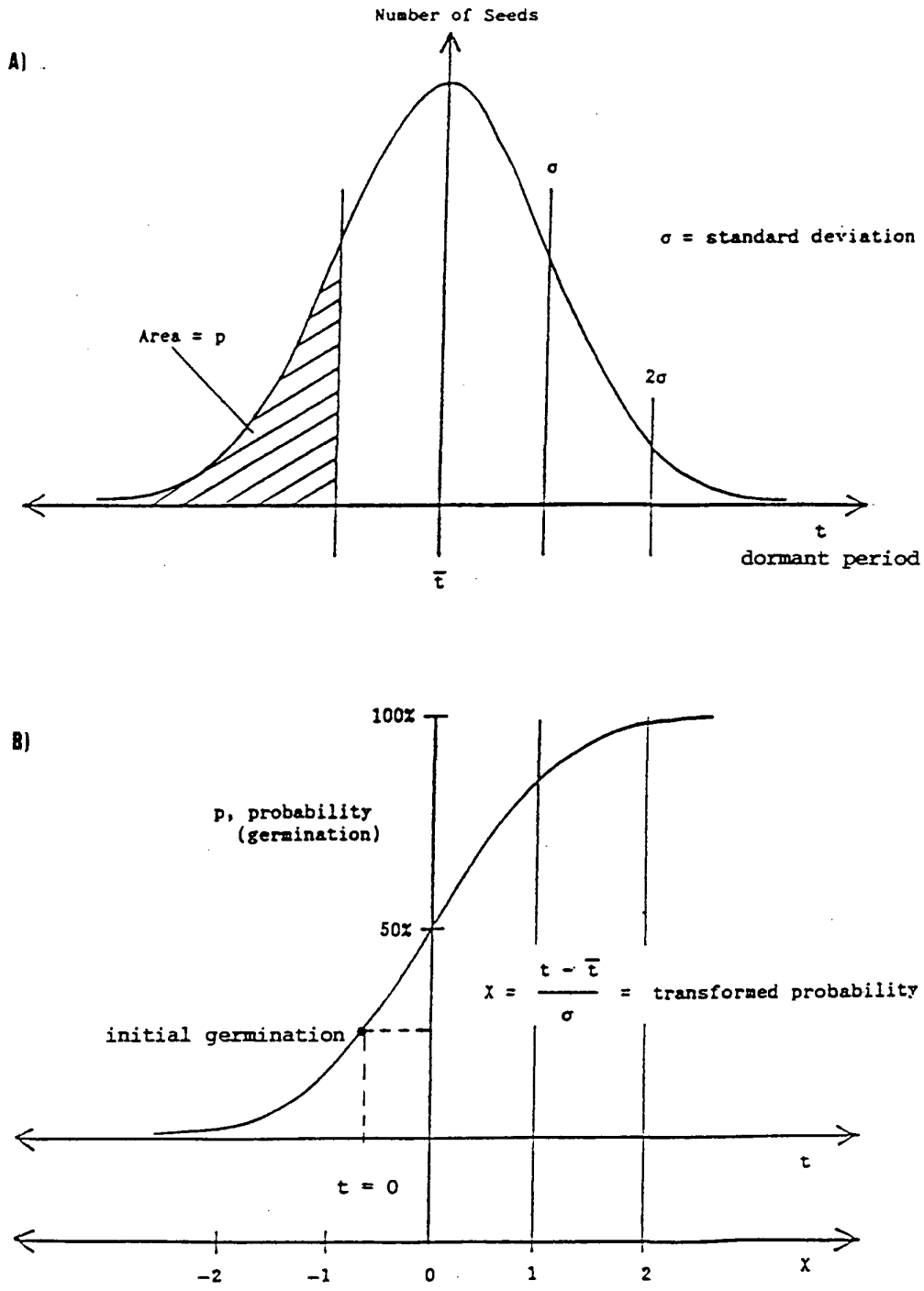



Fig. 4.1 Probit analysis, the normal distribution and zero time defined by initial germinability.

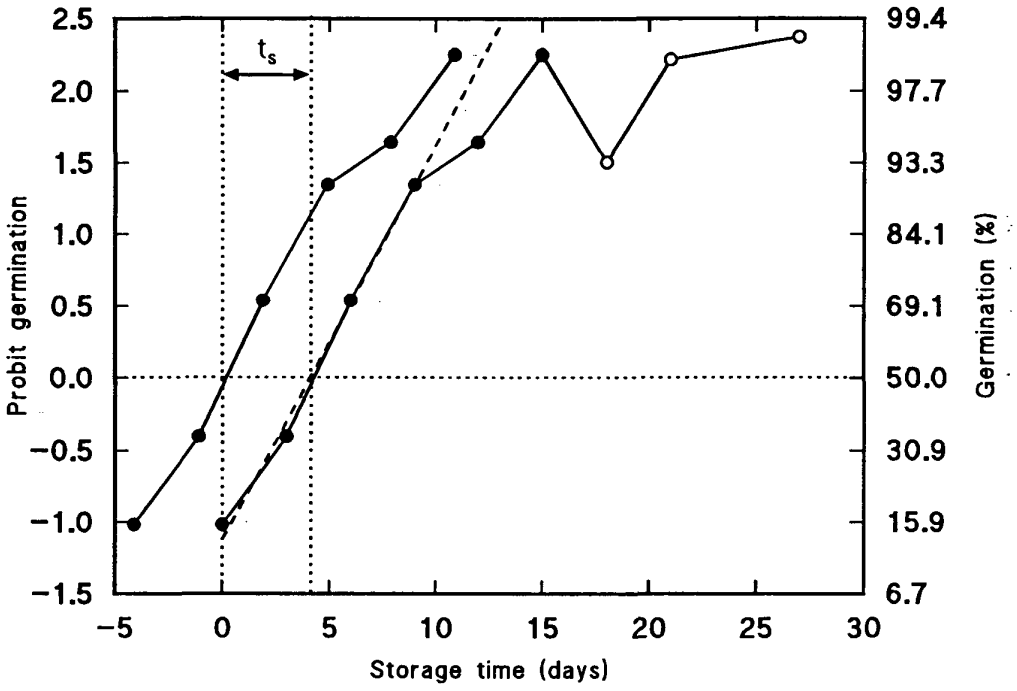



Fig. 4.2 Germination curve time–shifted by $t_s$ days to 50% germination at zero time. (●), data points used in the regression; (○), data points excluded from the regression; dashed line is the regression line.

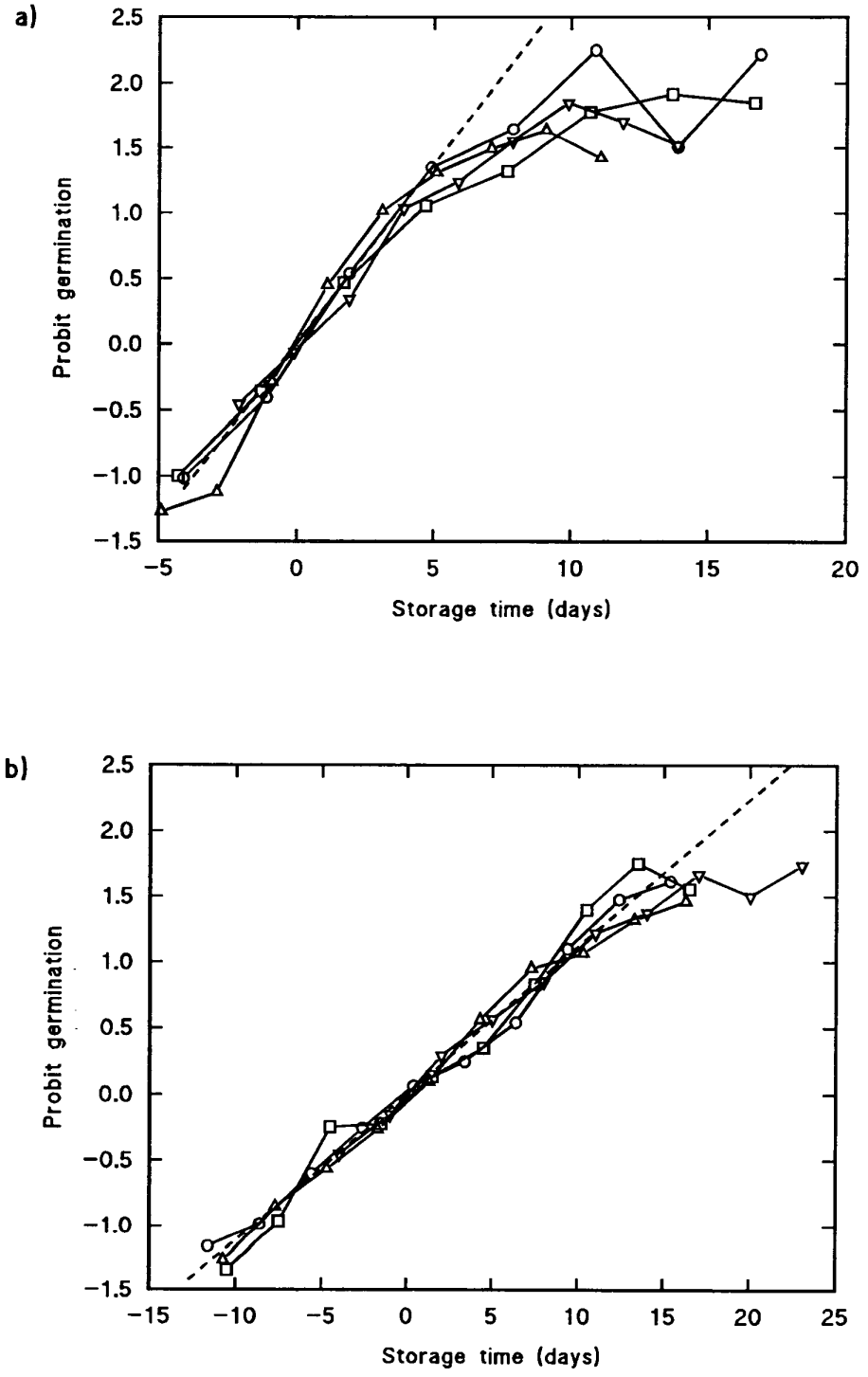



Fig. 4.3 Probit germination of (o) Pen–Tr–90, (□) Kir–Tr–90, (△) Pen–Tr–91a and (▽) Pen–Tr–91b during storage at (a) 38°C and (b) 27°C, 12% moisture content (4ml l.o.B. test, 9x100 seeds). Dashed line is calculated from the dormancy model.

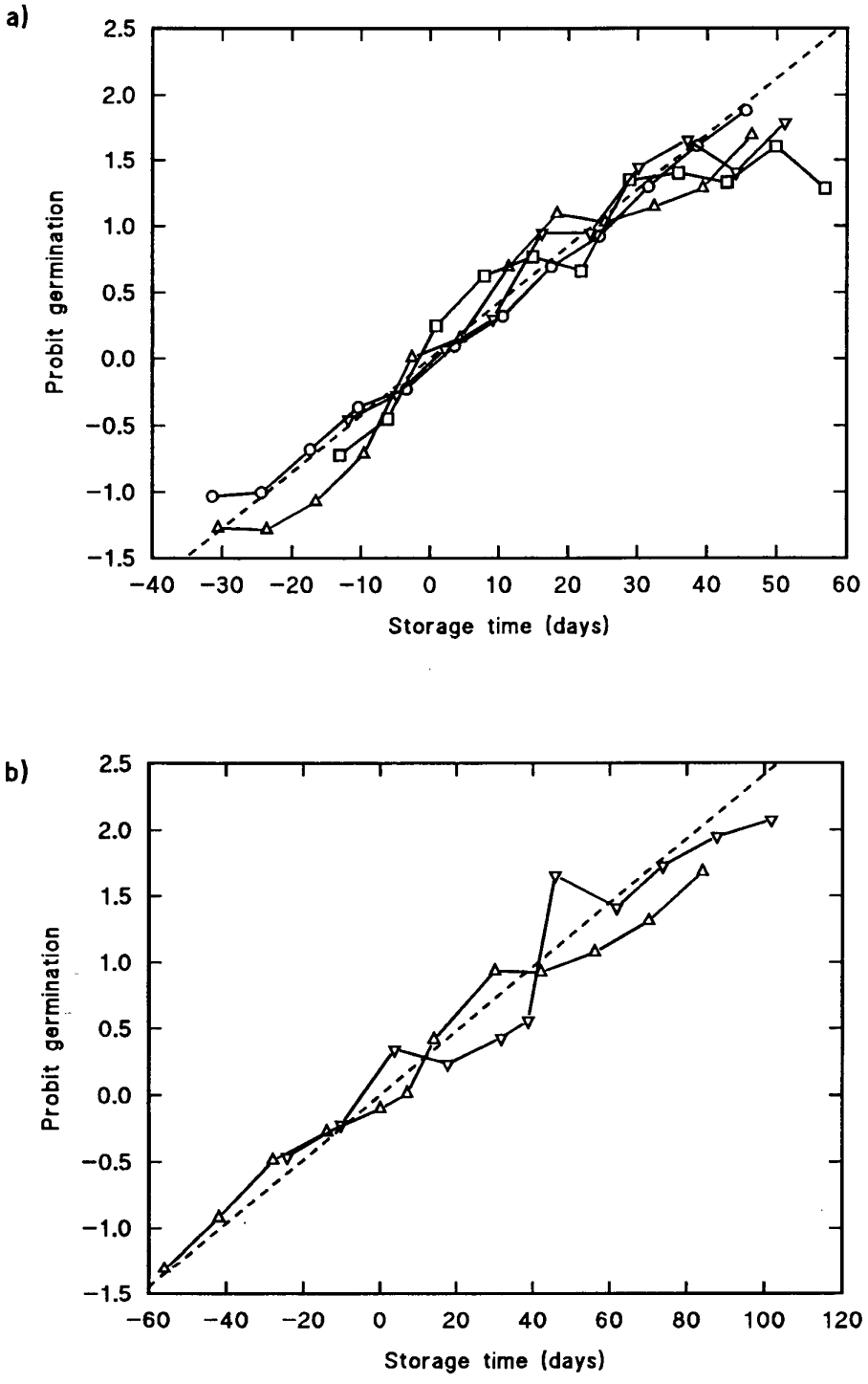



Fig. 4.4 Probit germination of (○) Pen−Tr−90, (□) Kir−Tr−90, (△) Pen−Tr−91a and (▽) Pen−Tr−91b during storage at (a) 15°C and (b) 8°C, 12% moisture content (4ml l.o.B. test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

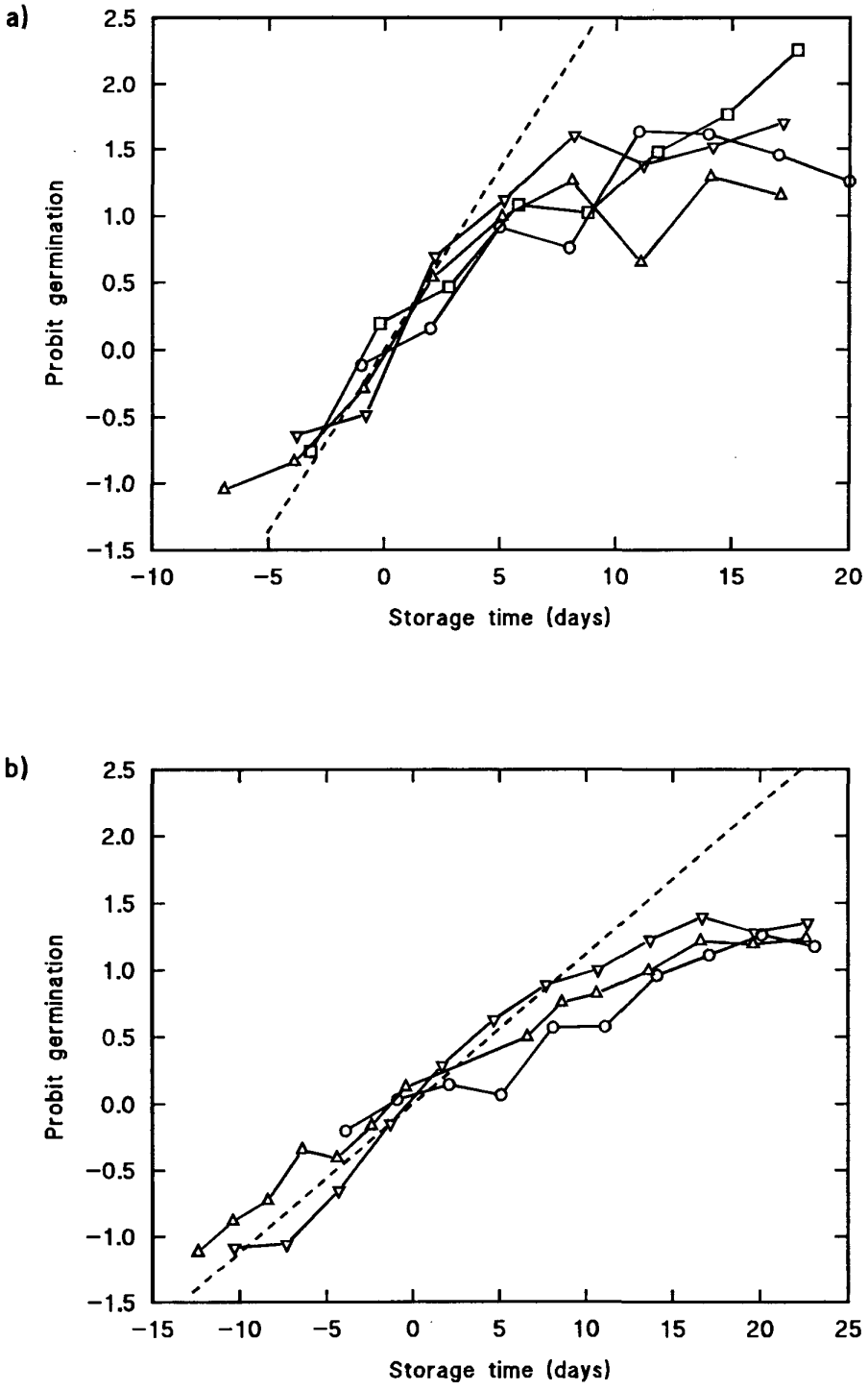



Fig. 4.5 Probit germination of (○) Inv−Tr−90, (□) Mot−Tr−90, (△) Ack−Tr−88 and (▽) Abr−Tr−90 during storage at (a) 38°C and (b) 27°C, 12 % moisture content (4ml l.o.B. test, 9x100 seeds) Dashed line is the curve calculated from the dormancy model.

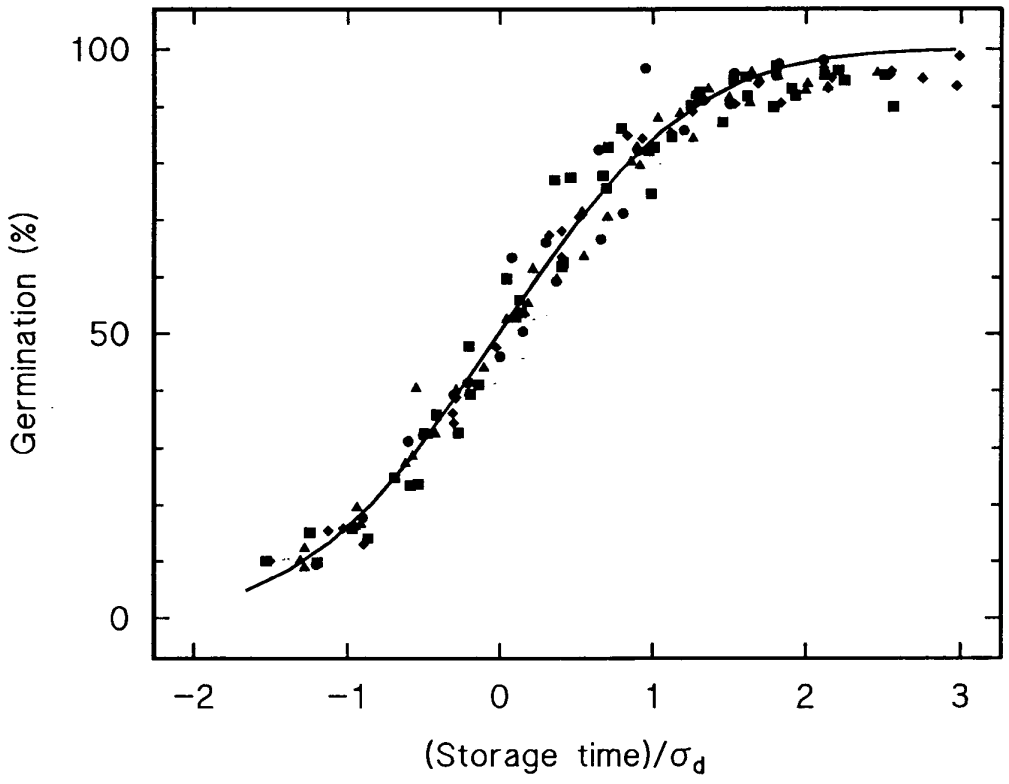



Fig. 4.6 Normalised germination data (4ml I.o.B. test, 9x100 seeds) of maltster s' barley during storage at (●) 8°C, (■) 15°C, (▲) 27°C and (◆) 38°C, 12 % moisture content. Solid line is the theoretical cumulative normal distribution curve.

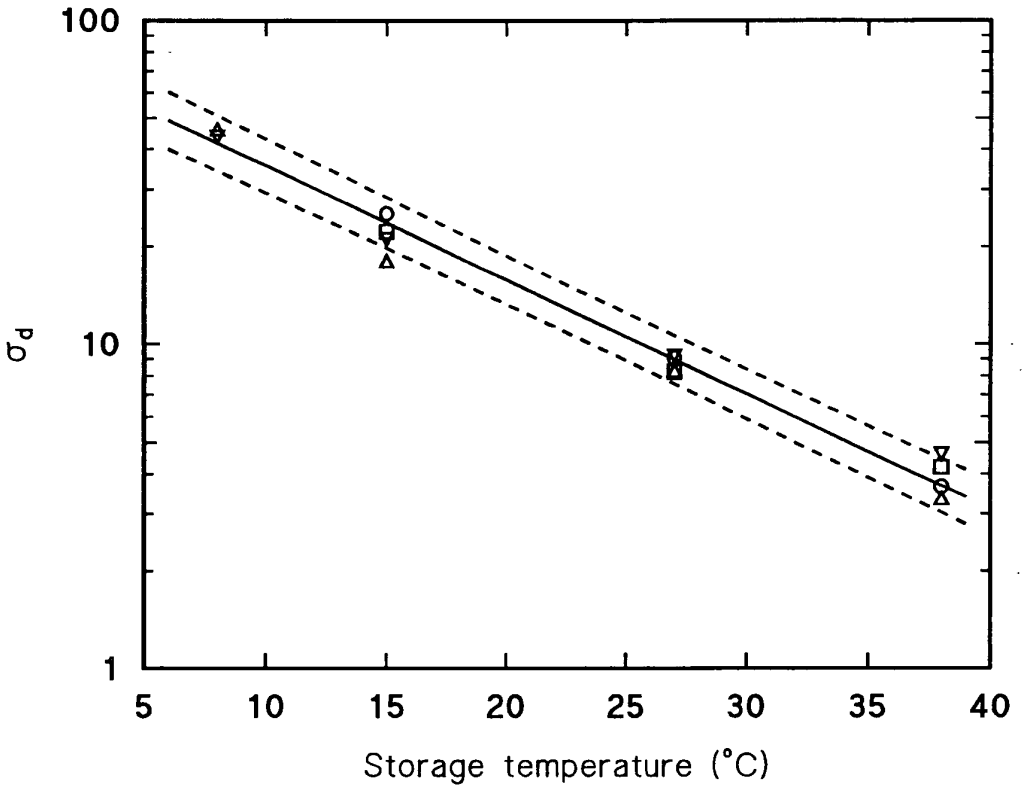



Fig. 4.7 Effect of storage temperature on the standard deviation of the dormancy period distribution, $\sigma_d$, of the maltster's barley. Solid line is the fitted curve; dashed lines are the 95% confidence limits.

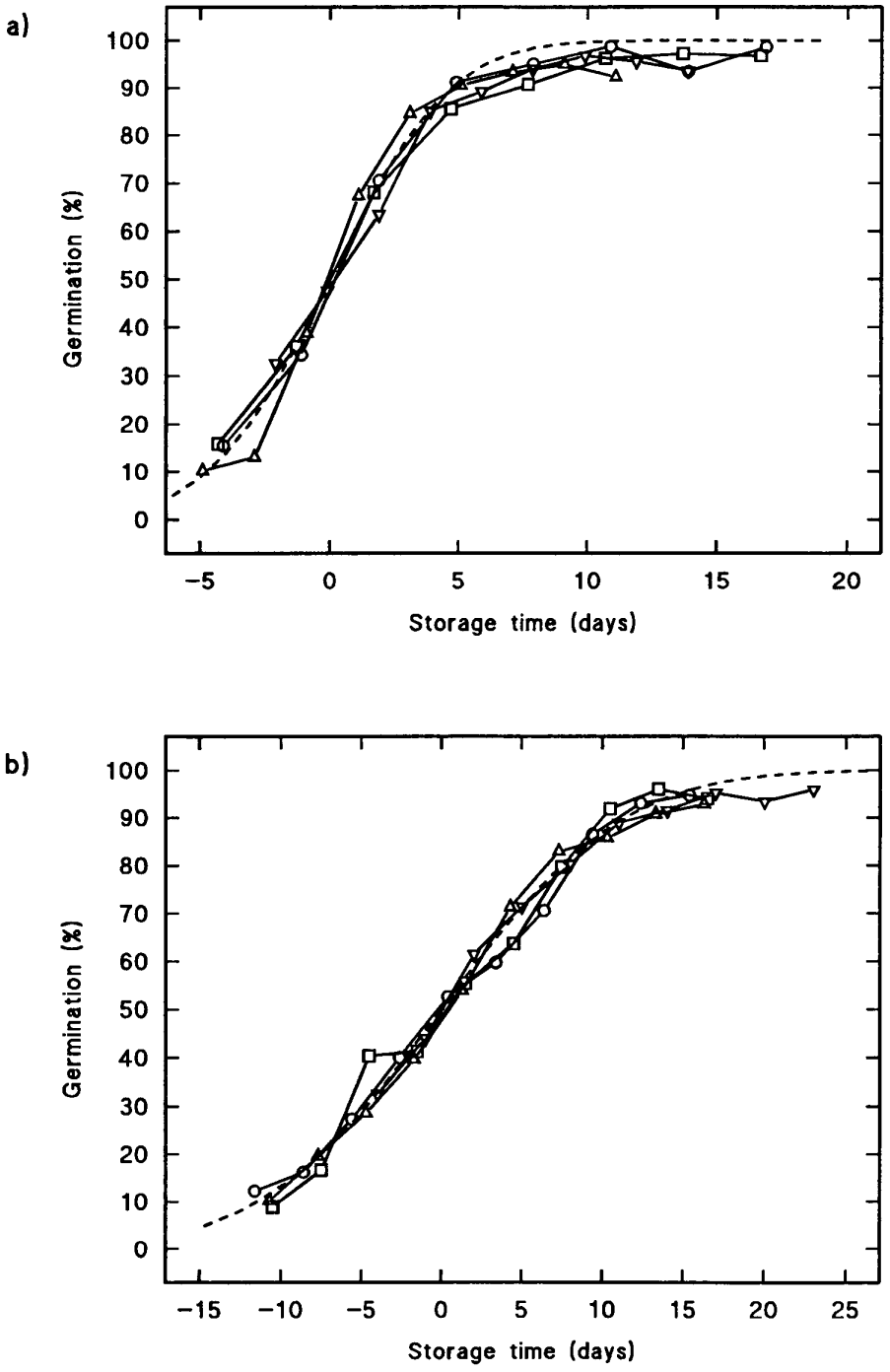



Fig. 4.8 Germination of (○) Pen–Tr–90, (□) Kir–Tr–90, (△) Pen–Tr–91a and (▽) Pen–Tr–91b during storage at (a) 38°C and (b) 27°C, 12 % moisture content (4ml l.o.B. test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

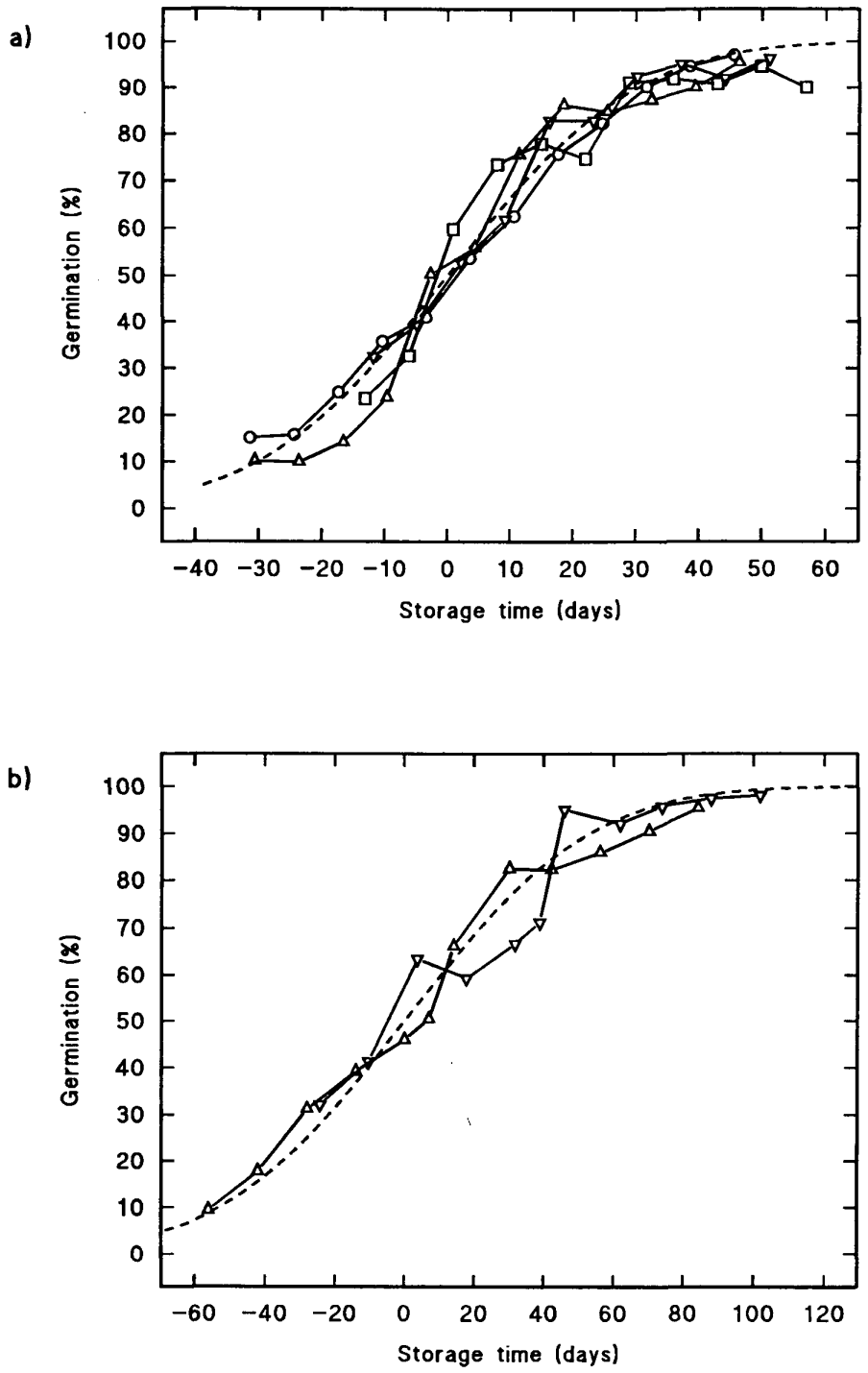



Fig. 4.9 Germination of (○) Pen–Tr–90, (□) Kir–Tr–90, (△) Pen–Tr–91a and (▽) Pen–Tr–91b during storage at (a) 15°C and (b) 8°C, 12 % moisture content (4ml l.o.B. test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

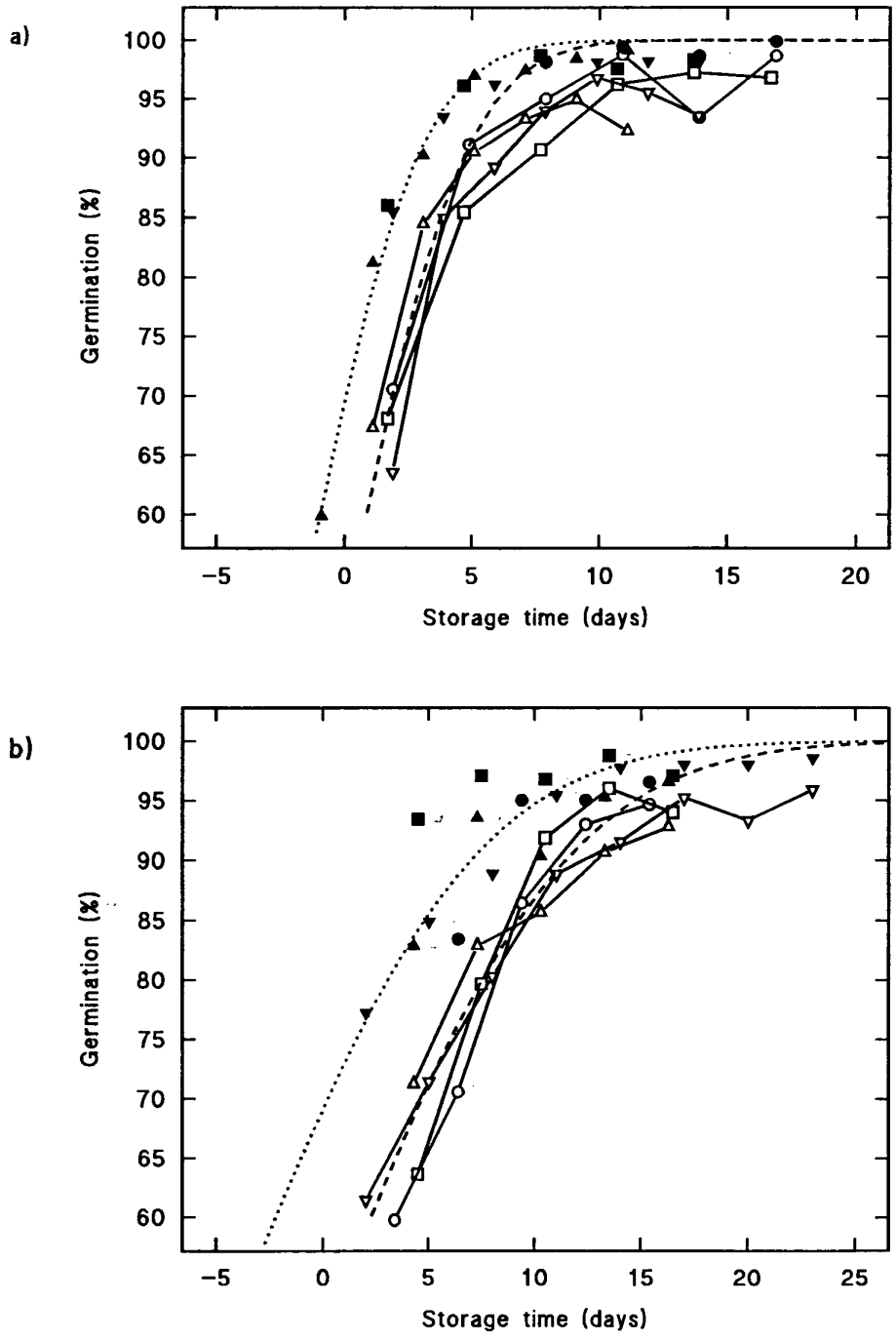



Fig. 4.10 Comparison of 3-day with 7-day germination of (o) Pen-Tr-90, (□) Kir-Tr-90, (△) Pen-Tr-91a and (▽) Pen-Tr-91b during storage storage at (a) 38°C and (b) 27°C, 12 % moisture content (4ml l.o.B. test, 9x100 seeds). Solid symbols are 7-day data; dashed line is the calculated 3-day germinability curve; dotted line is the estimated 7-day germinability curve from the dormancy model.

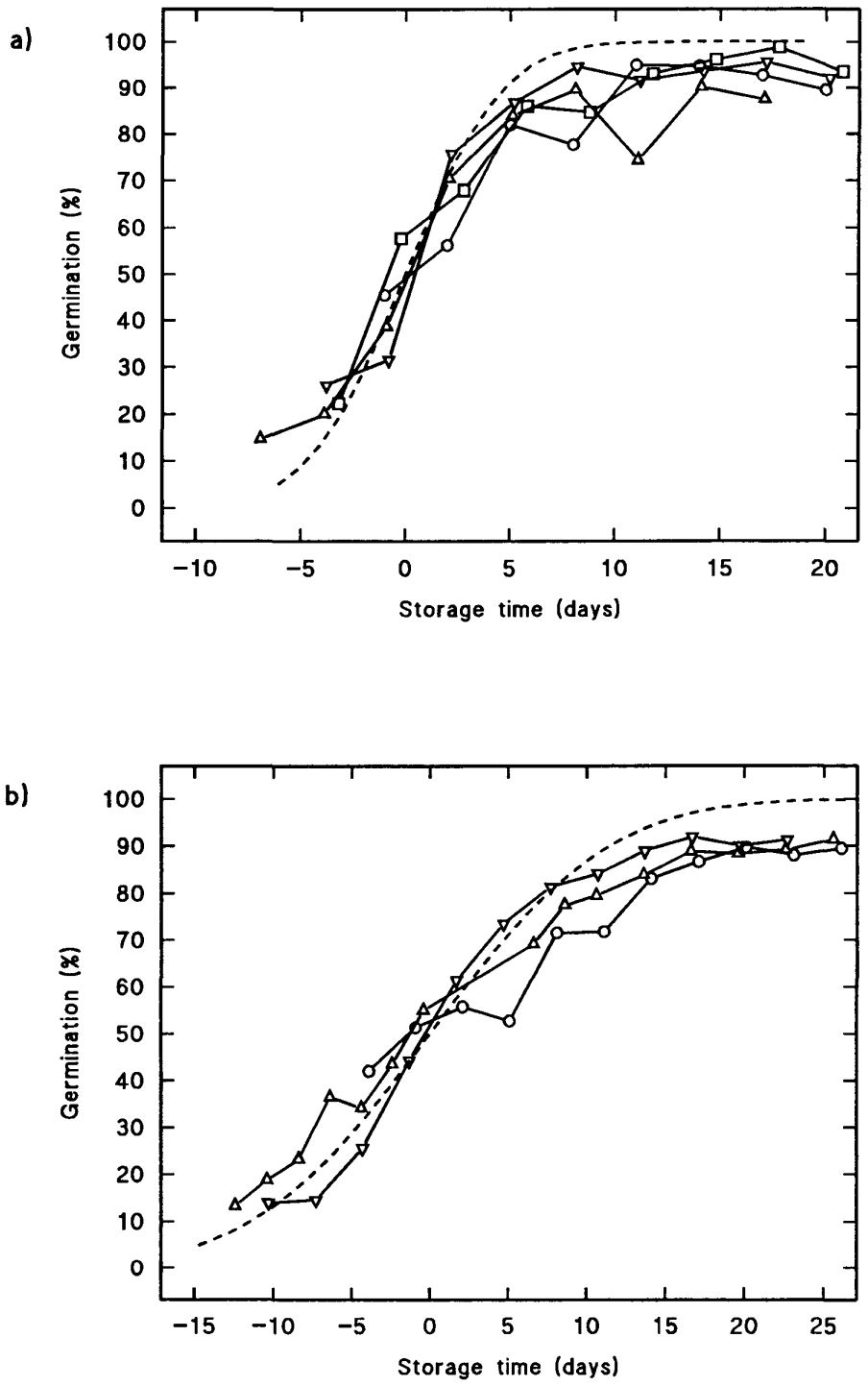



Fig. 4.11 Germination of (○) Inv–Tr–90, (□) Mot–Tr–90, (△) Ack–Tr–88 and (▽) Abr–Tr–90 during storage at (a) 38°C and (b) 27°C, 12 % moisture content (4ml l.o.B. test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

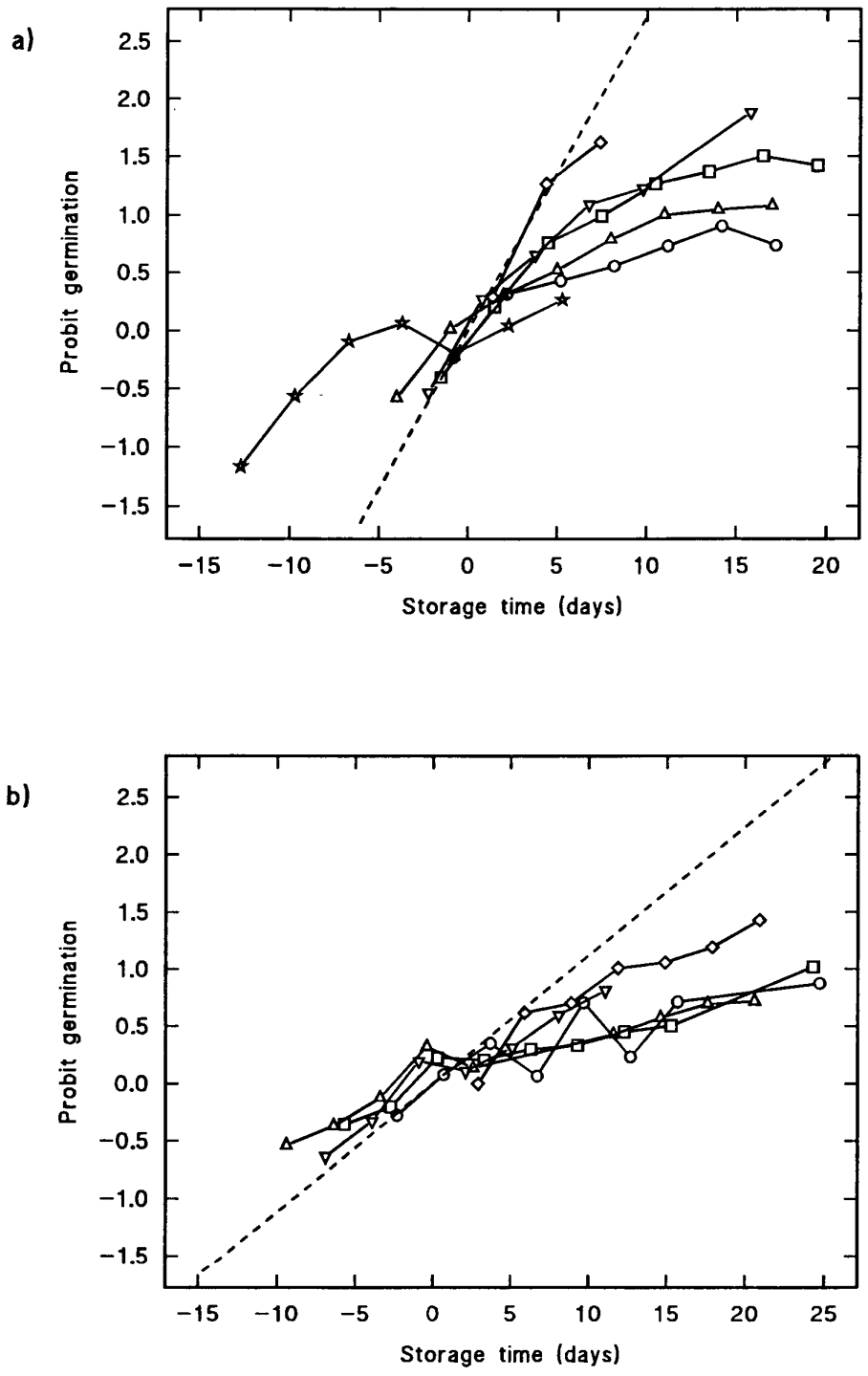



Fig. 4.12 Probit germination of (○) Inv−Tr−90, (□) Mot−Tr−90, (△) Abr−Tr−90, (☆) Ack−Tr−88, (▽) Pen−Tr−90 and (◇) Kir−Tr−90 during storage at (a) 38°C and (b) 27°C, 12 % moisture content (1ml agar test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

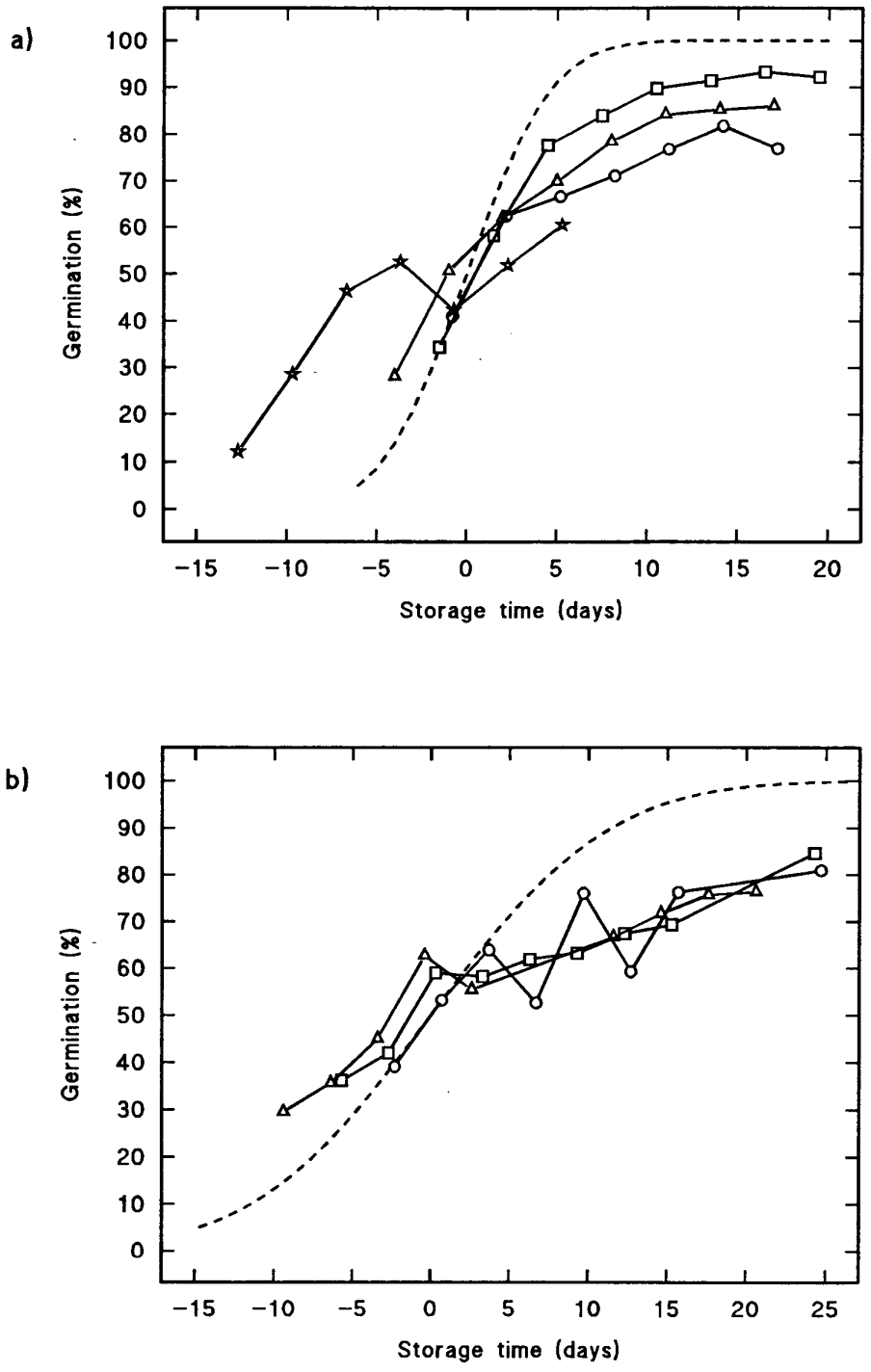



Fig. 4.13 Germination of (○) Inv–Tr–90, (□) Mot–Tr–90, (△) Abr–Tr–90 and (☆) Ack–Tr–88 during storage at (a) 38°C and (b) 27°C, 12 % moisture content (1ml agar test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

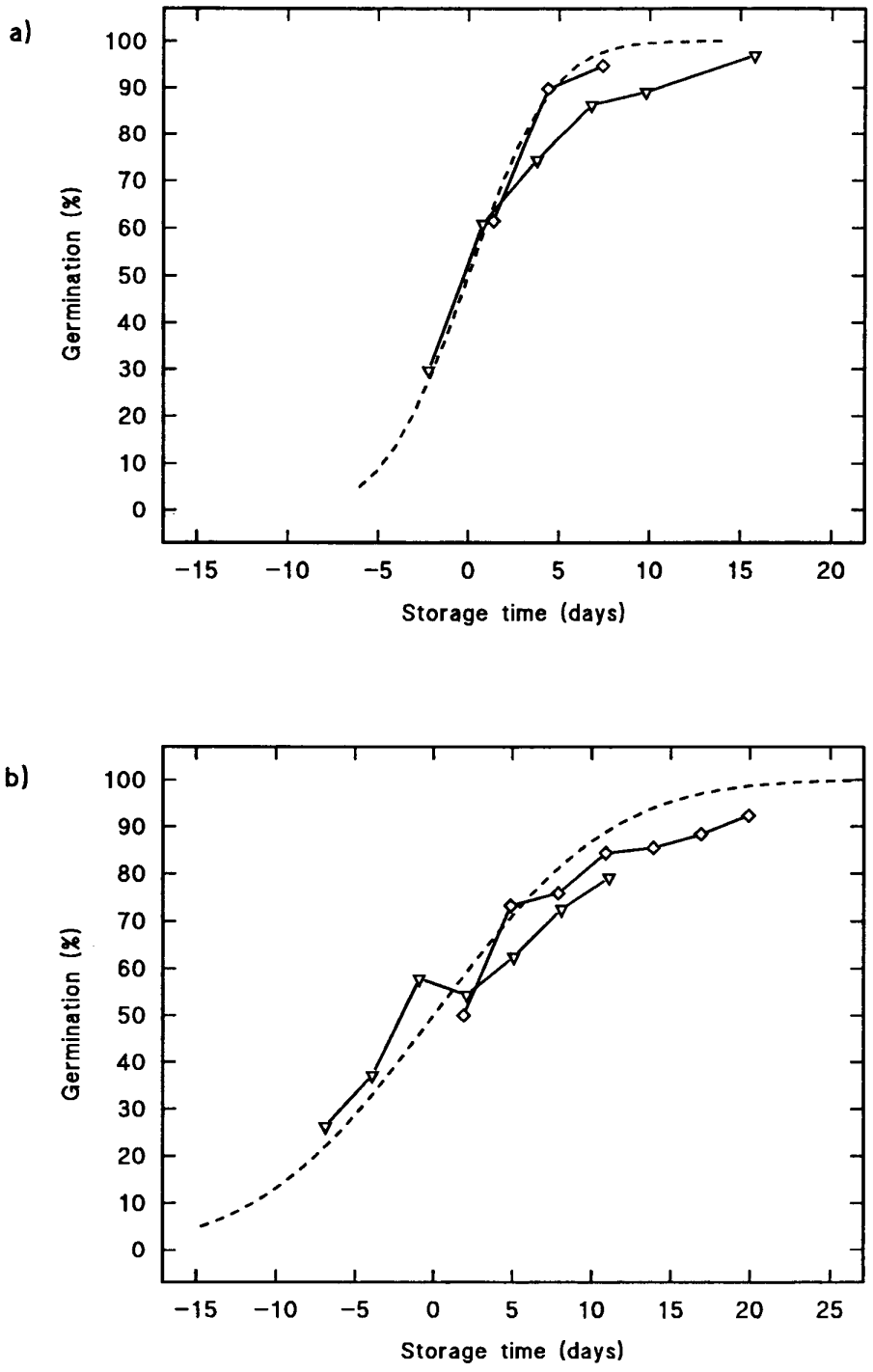



Fig. 4.14 Germination of (▽) Pen−Tr−90 and (◇) Kir−Tr−90 during storage at (a) 38°C and (b) 27°C, 12% moisture content (1ml agar test, 9x100 seeds). Dashed line is the curve calculated from the dormancy model.

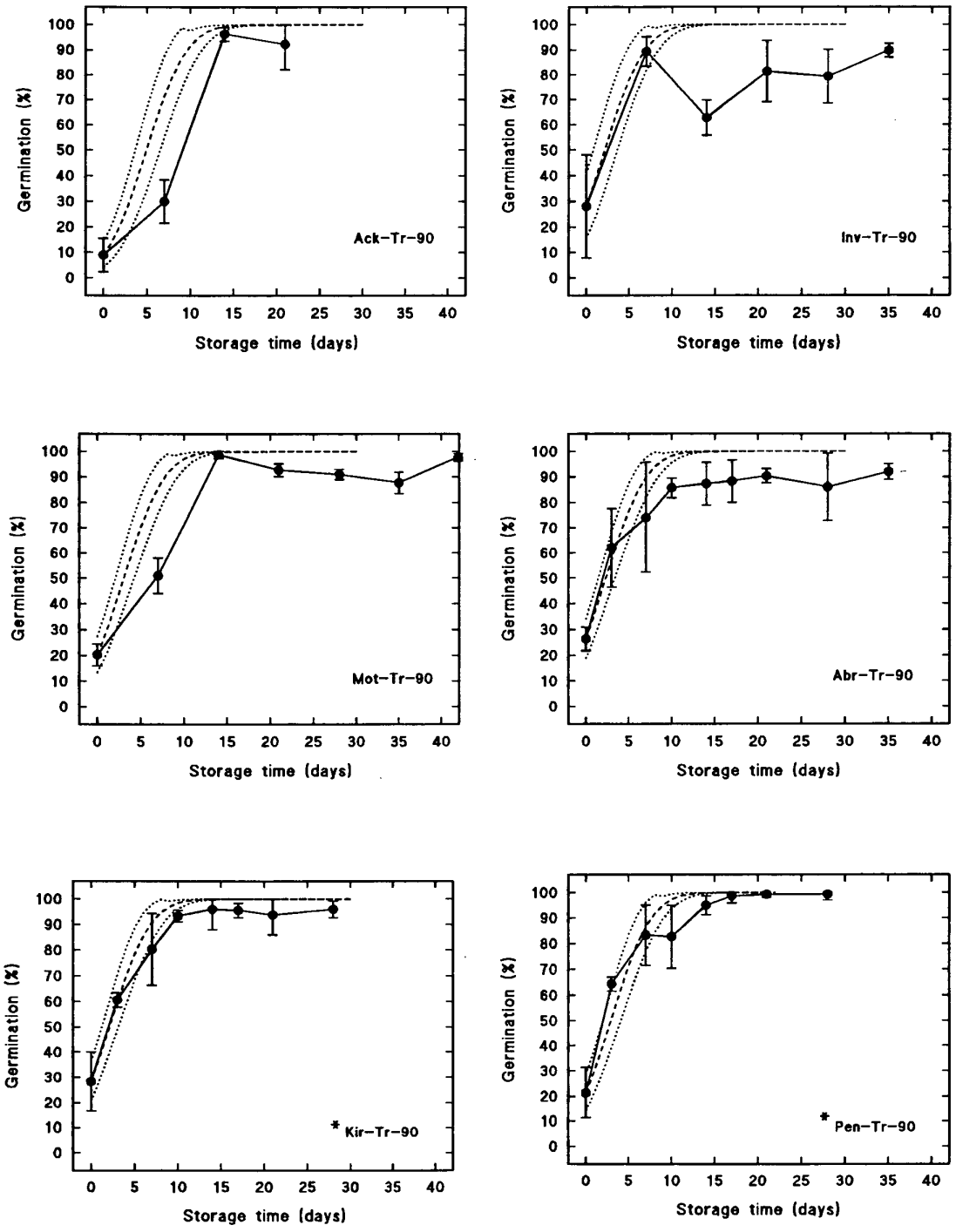



Fig. 4.15 Comparison of germination during storage at 38°C, 12 % moisture content (4ml l.oB. test, 3x100 seeds) of trial site and maltster's (*) barley with the curve predicted from the dormancy model. Dashed line is the predicted curve; dotted lines are the 95% confidence limits of the predicted curve.

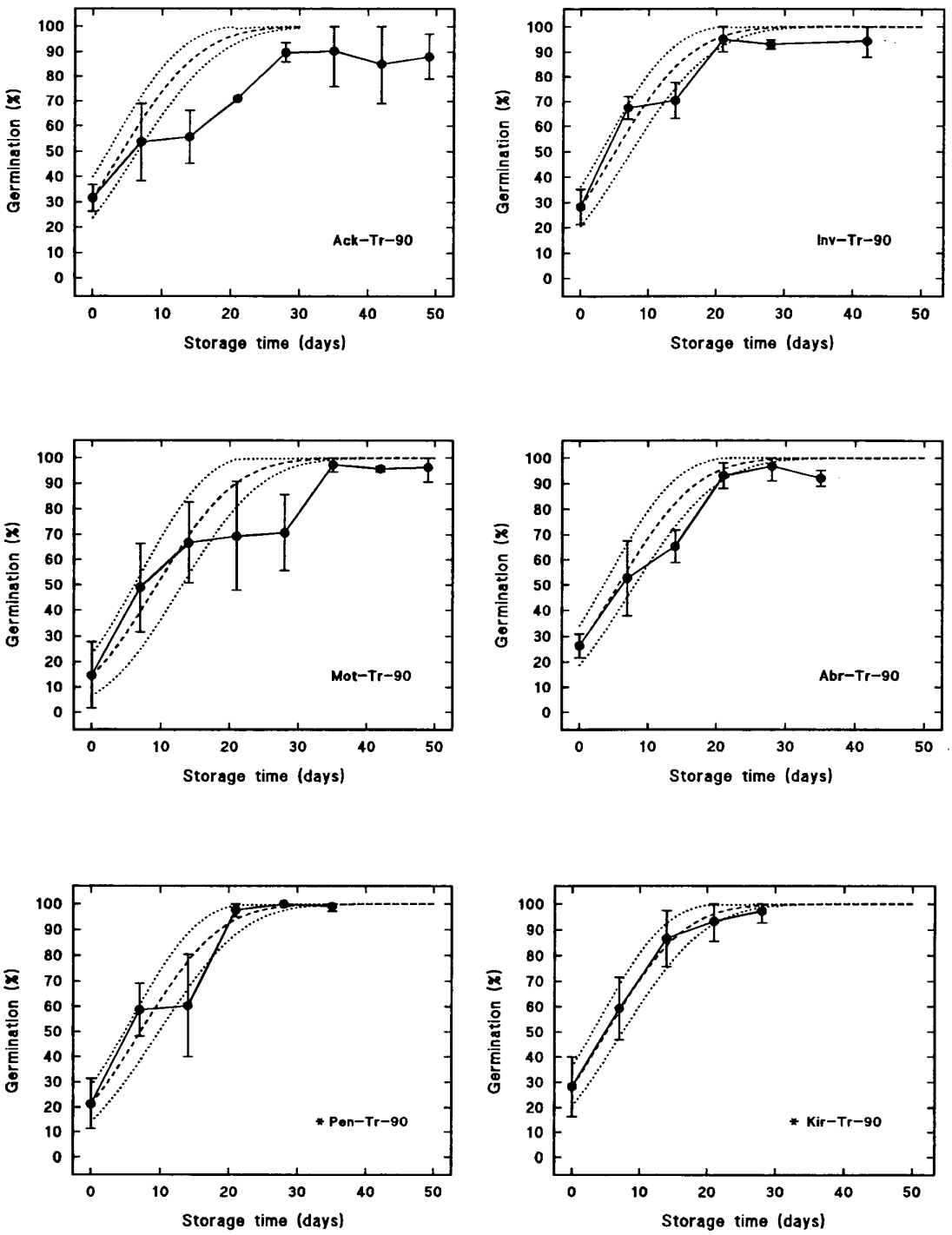



Fig. 4.16 Comparison of germination during storage at 27°C, 12 % moisture content (4ml I.oB. test, 3x100 seeds) of trial site and maltster s' (*) barley with the curve predicted from the dormancy model. Dashed line is the predicted curve; dotted lines are the 95% confidence limits of the predicted curve.

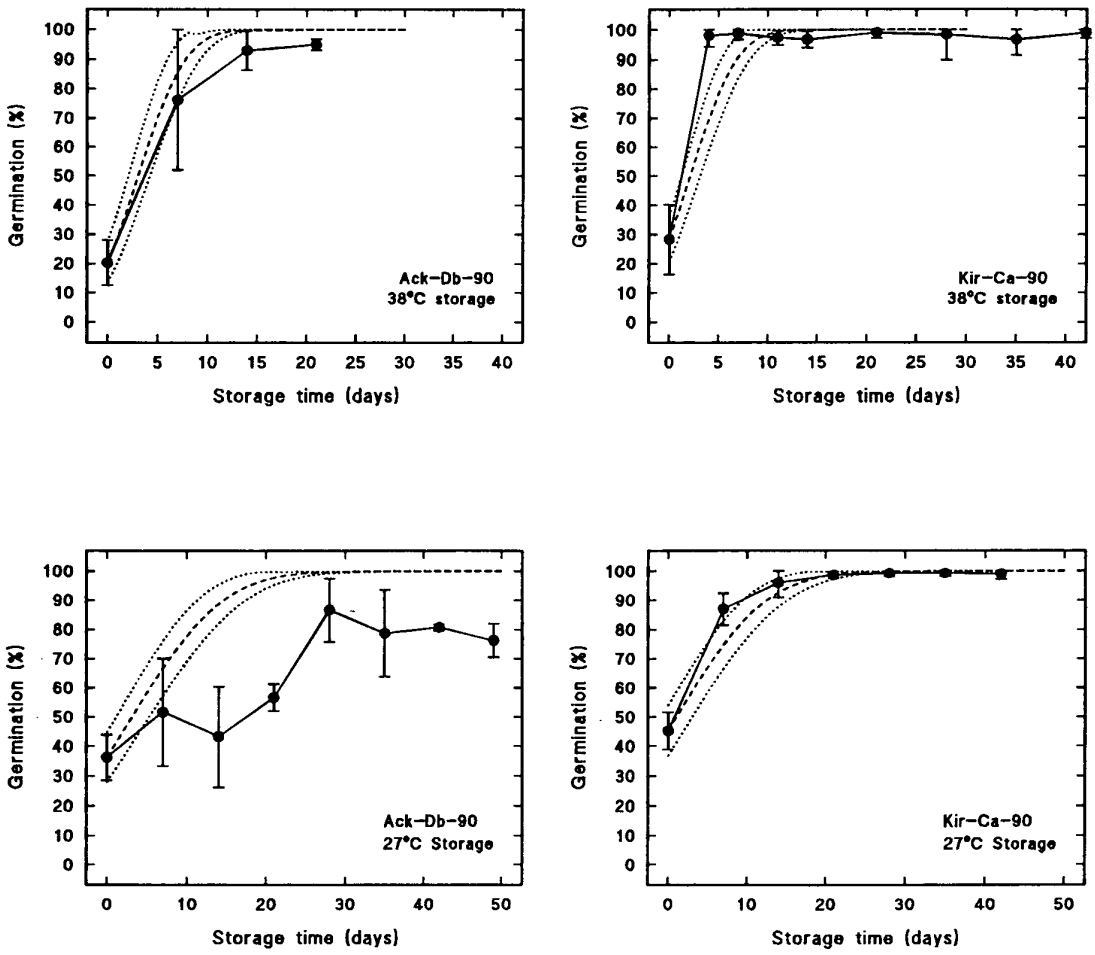



Fig. 4.17 Comparison of germination during storage at 27°C and 38°C, 12% moisture content (4ml I.oB. test, 3x100 seeds) of trial site Doublet and maltster s' Camargue barley with the curve for each predicted from the dormancy model. Dashed line is the predicted curve; dotted lines are the 95% confidence limits.

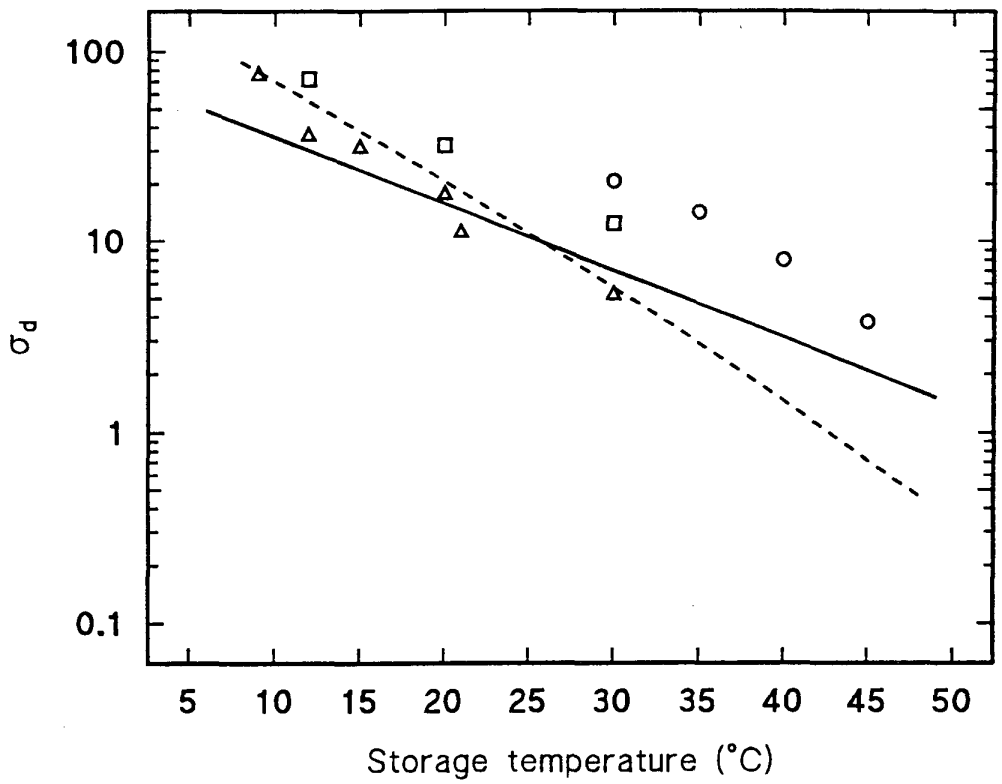



Fig. 4.18 Effect of storage temperature on the standard deviation of the dormancy period distribution, $\sigma_d$. Solid line, HGCA project model; dashed line, model of Riis et al. (1989); (○) Narziss et al. (1980); (□) Strand (1965); (△) Burass and Skinnes (1985).

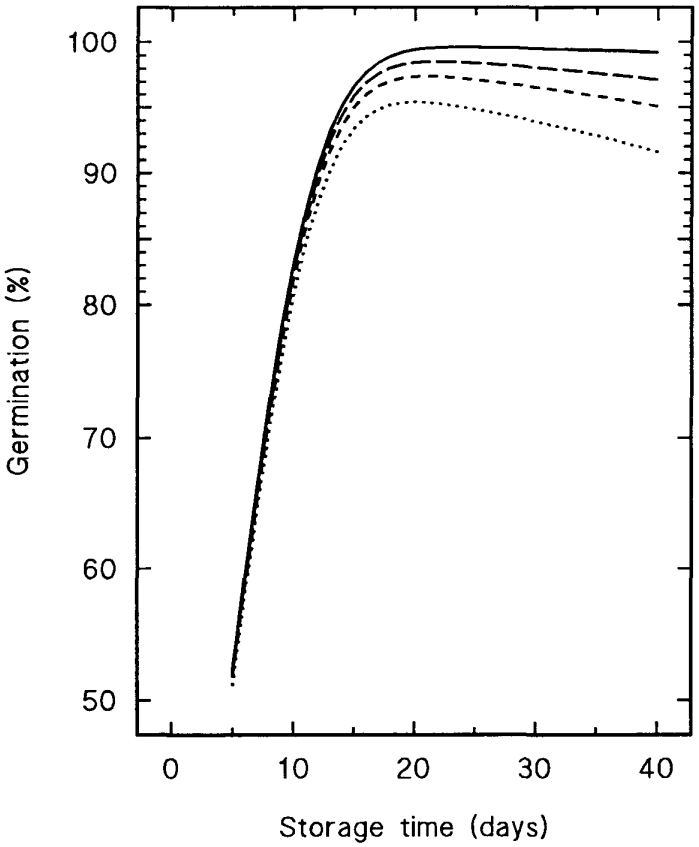



Fig. 4.19 Effect of a storage temperature of 33°C on the germinability of barley with an initial germinative energy of 20%, 12% moisture content and initial germinative capacity of 99.9% (——) 99.5% (— —), 99.0% (- - -) and 98.0% (·····), predicted using the dormancy/viability model.

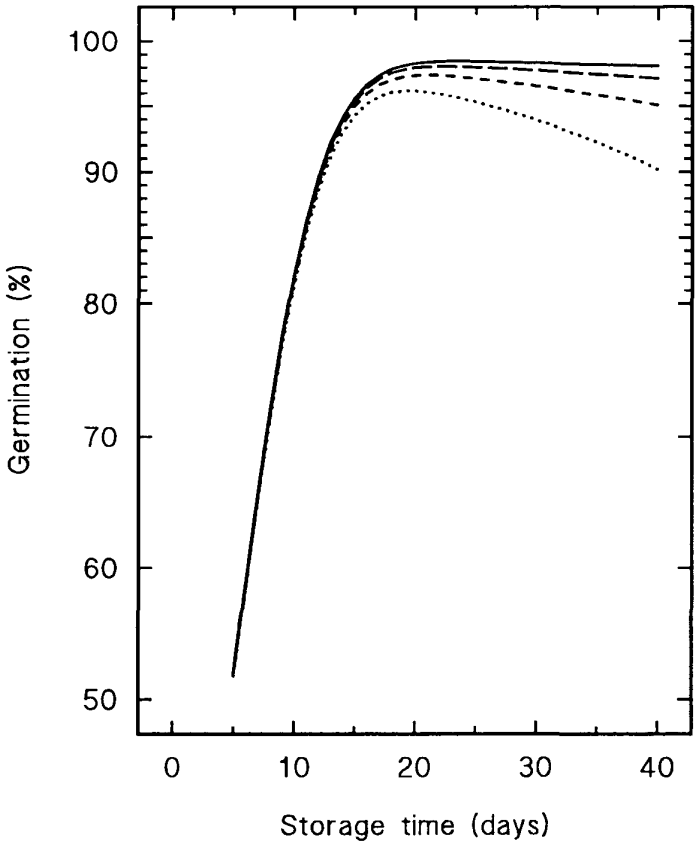



Fig. 4.20 Effect of a storage temperature of 33°C on the germinability of barley with an initial germinative energy of 20%, initial germinative capacity of 99.0% and a moisture content of 10% (——), 11% (— —), 12% (- - -) and 13% (·····), predicted using the dormancy/viability model.

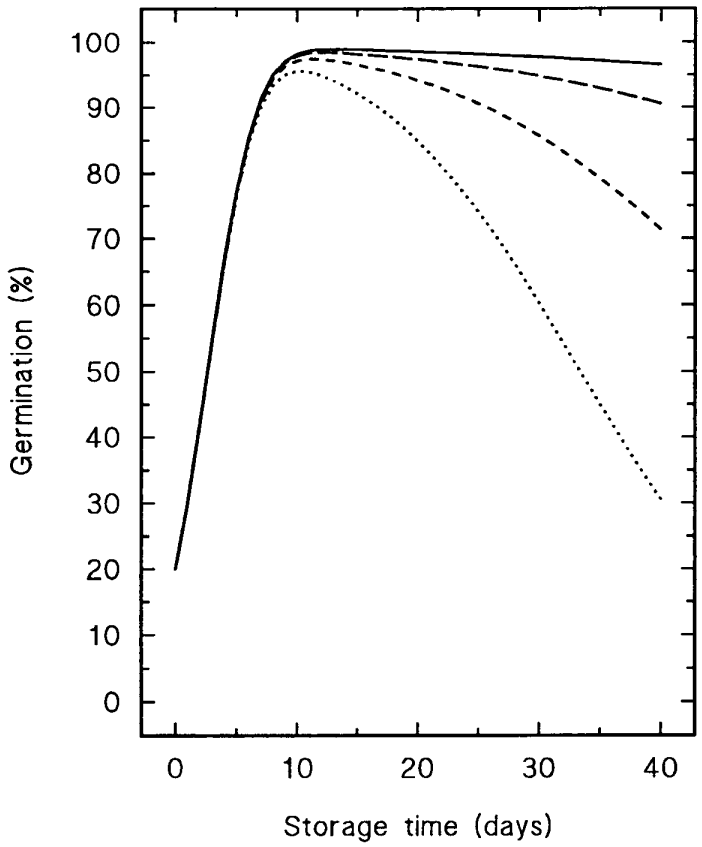



Fig. 4.21 Effect of a storage temperature of 40°C on the germinability of barley with an initial germinative energy of 20%, initial germinative capacity of 99.9% and a moisture content of 10% (——), 11% (— —), 12% (- - -) and 13% (·····), predicted using the dormancy/viability model.

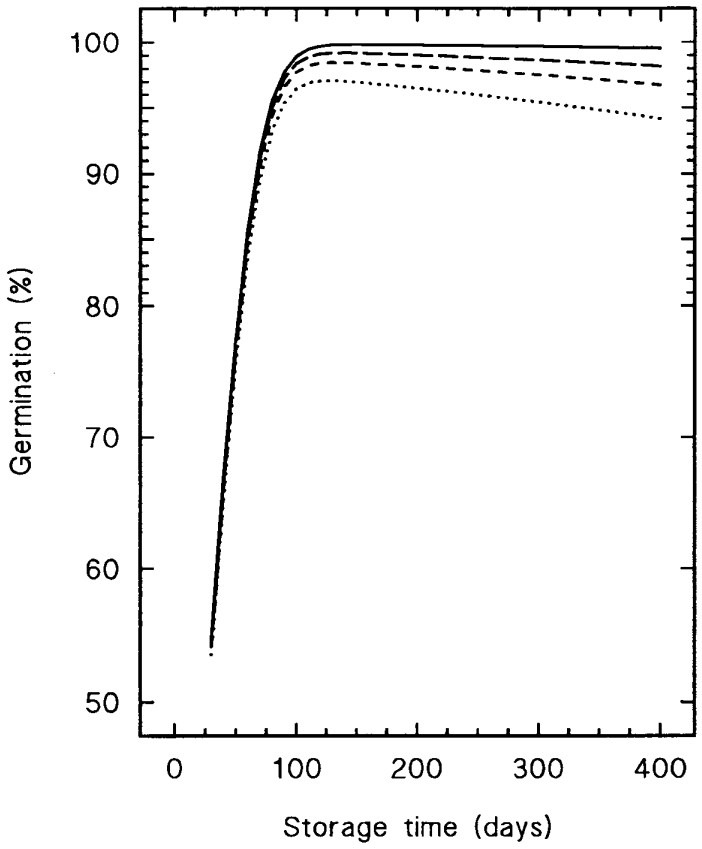



Fig 4.22 Effect of a storage temperature of 12°C on the germinability of barley with an initial germinative energy of 20%, moisture content of 12% and an initial germinative capacity of 99.9% (——), 99.5% (— —), 99.0% (- - -) and 98.0% (·····), predicted using the dormancy/viability model.

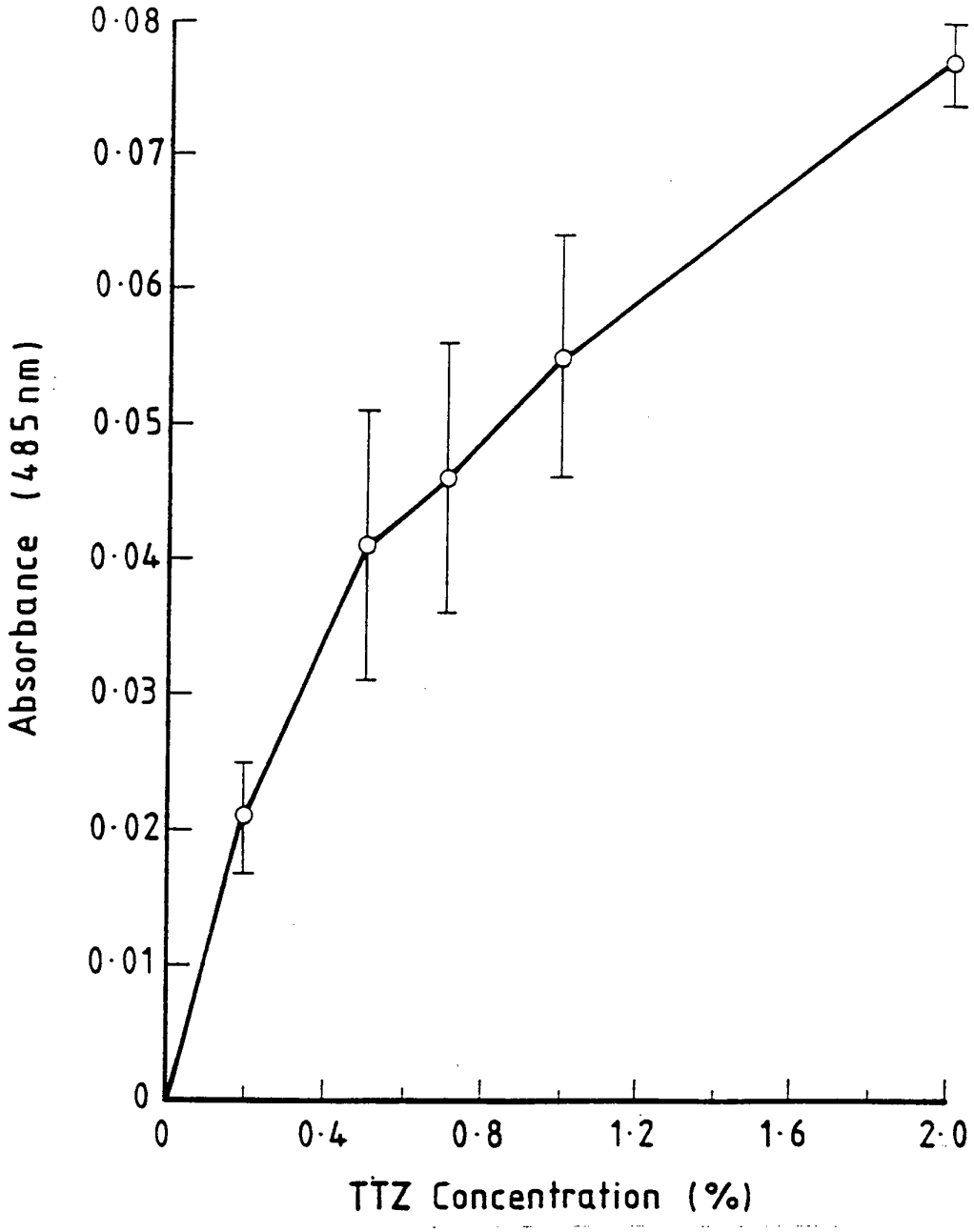



Figure 5.1

Formazan production on grains during a 2 h incubation period in reagents buffered with phosphate and containing glucose, sodium succinate, and various concentrations of triphenyltetrazolium chloride (TTZ). The bars show the standard deviations.

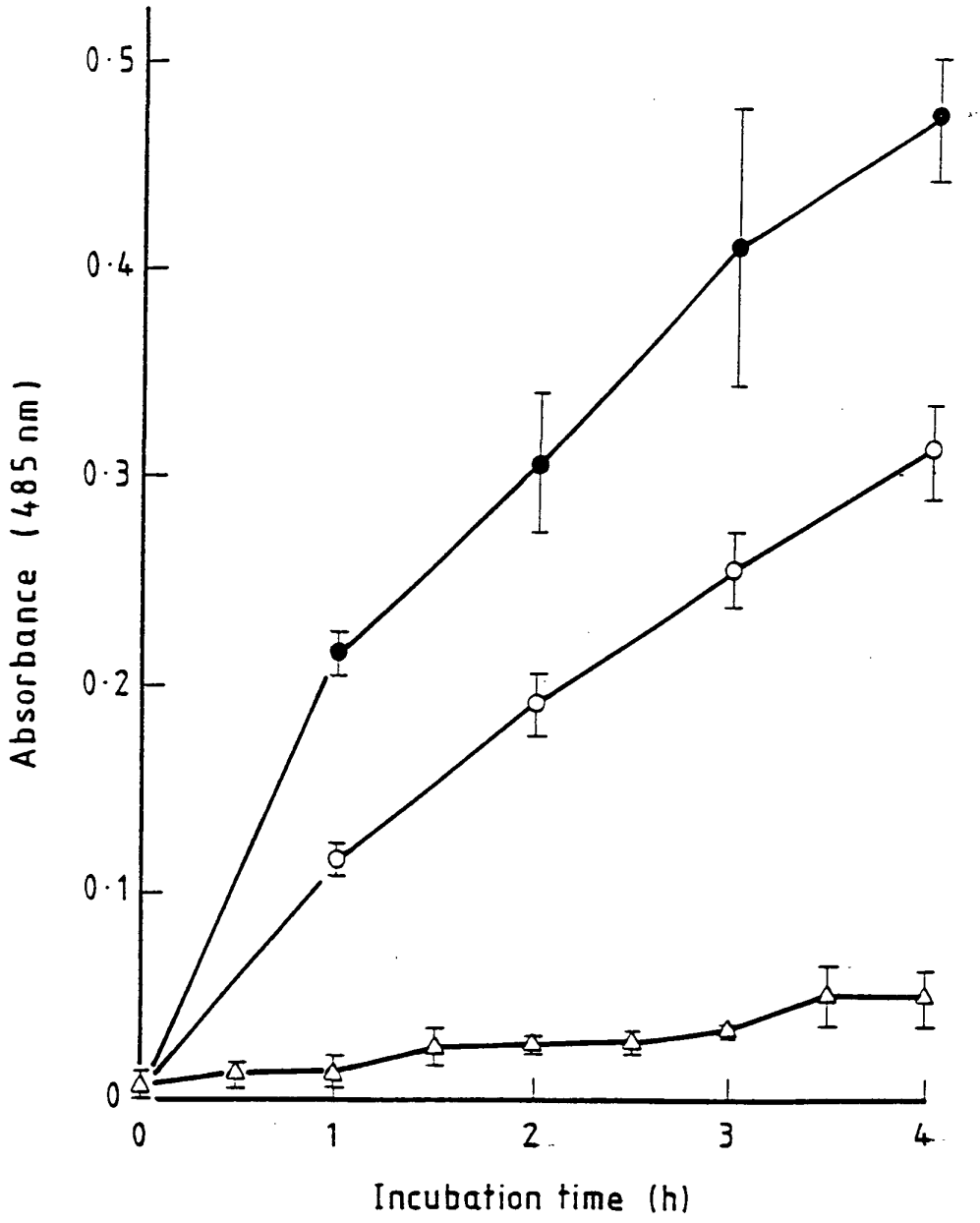



Fig. 5.2.

Formazan colours developed by grain samples incubated for different periods in reagents containing different concentrations of triphenyltetazolium chloride (TTZ) △, 0.2% TTZ; ○, 2% TTZ; ●, 4% TTZ. Two different samples of grain were used, one with the lowest concentration of TTZ, the other or the two higher concentrations. After 22.5h. incubations in 2% and 4% TTZ the formazan colours were 0.97±0.08 and 0.83±0.07 respectively. The bars indicate the standard deviations.

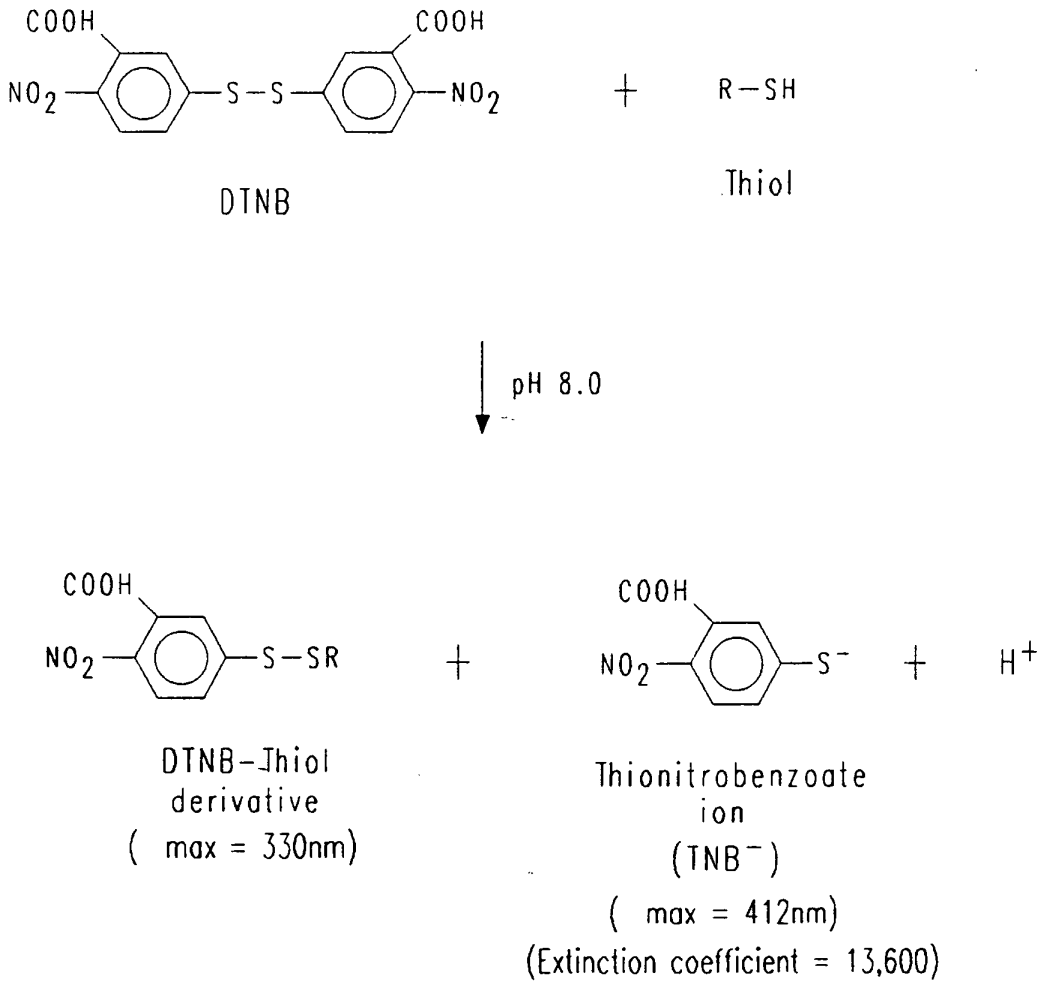
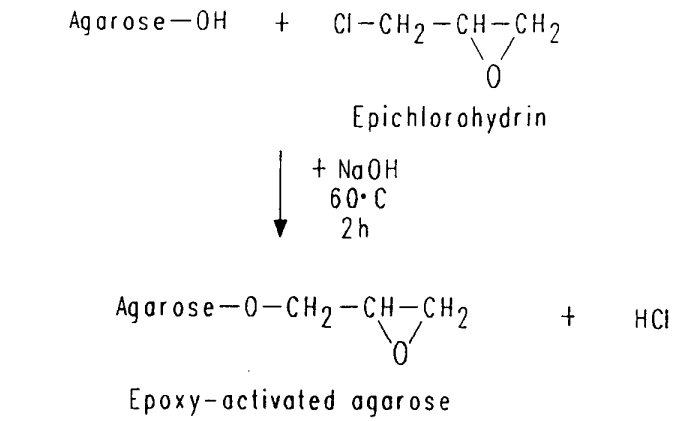
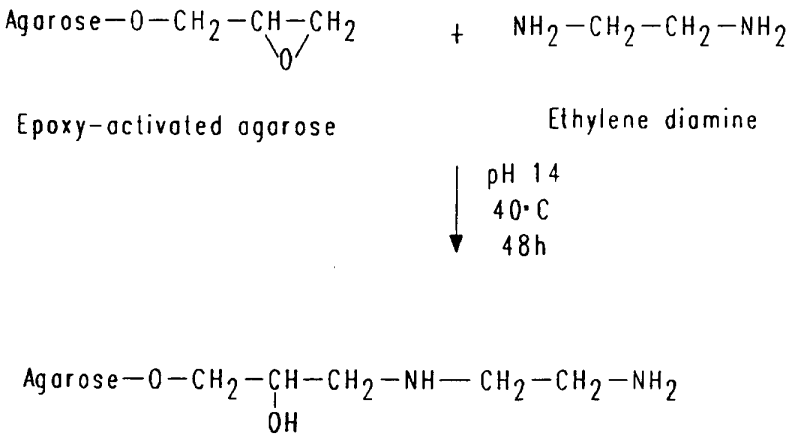



Fig. 6.1 The derivatisation and quantitation of thiols with DTNB, 5,5'-dithiobis (2-nitrobenzoic acid).

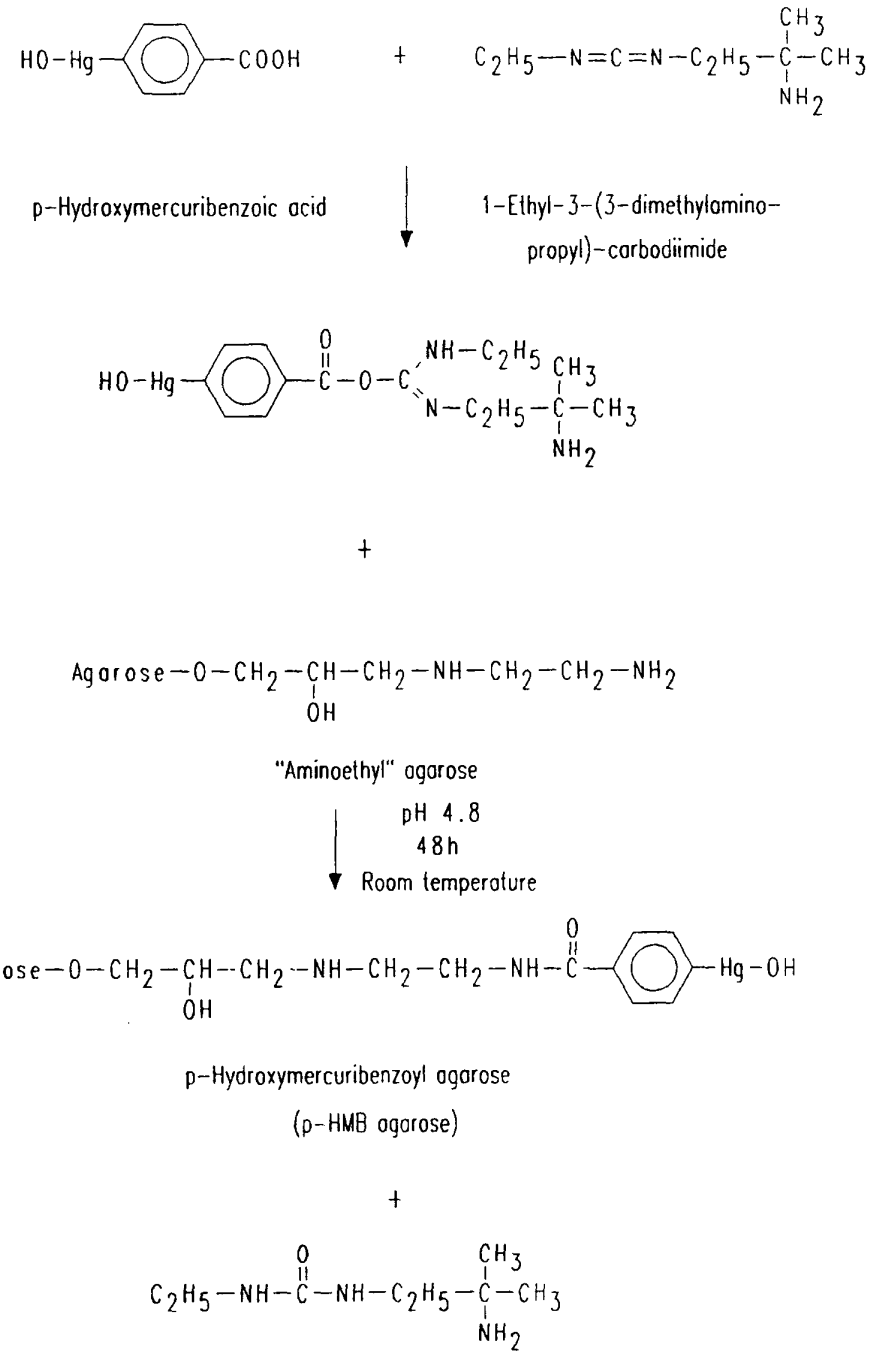



Preparation of epoxy-activated agarose




Preparation of "aminoethyl" agarose

Fig. 6.2 The preparation of epoxy-activated Sepharose and its conversion into aminoethyl-Sepharose.




Preparation of p-hydroxymercuribenzoyl agarose

Fig. 6.3 The conversion of aminoethyl-Sepharose into p-hydroxymercuribenzoyl-Sepharose.

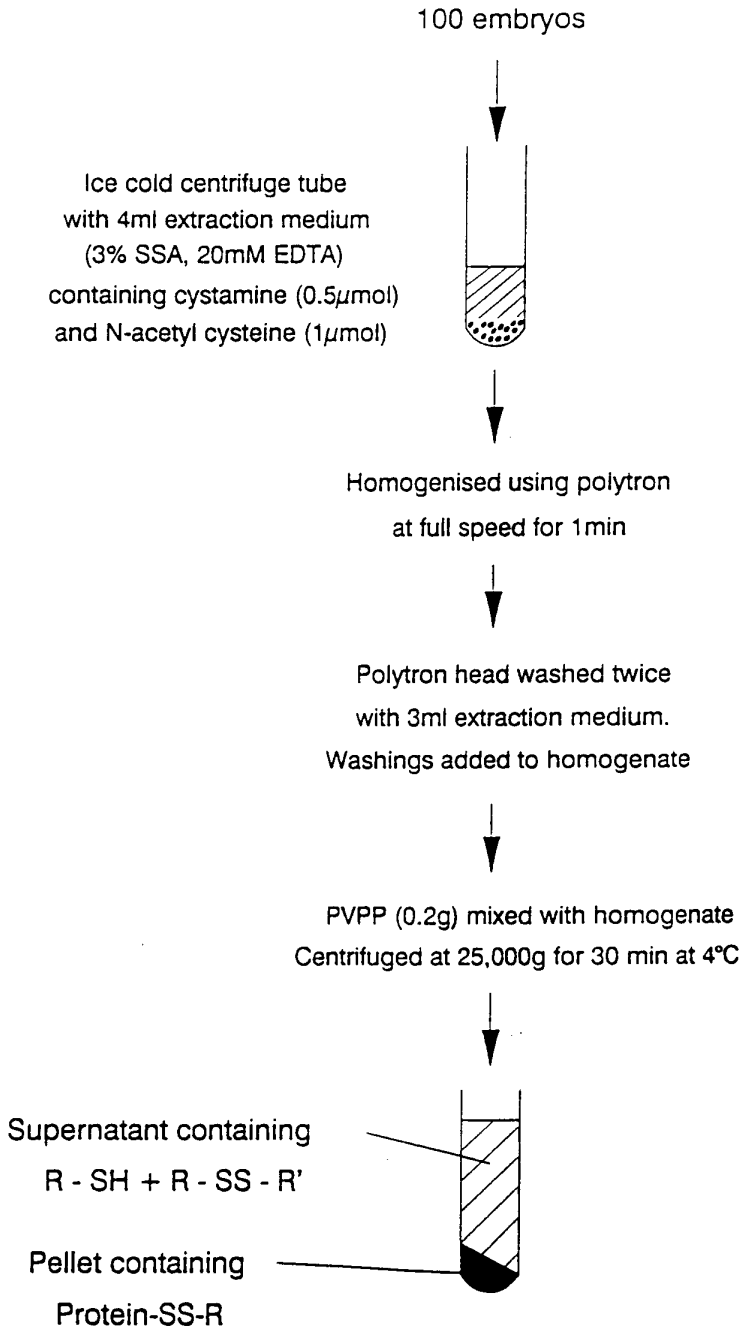



Fig. 6.4 An outline of the procedure for the extraction of soluble thiols and disulphides from barley embryos and their separation from protein-bound thiols.

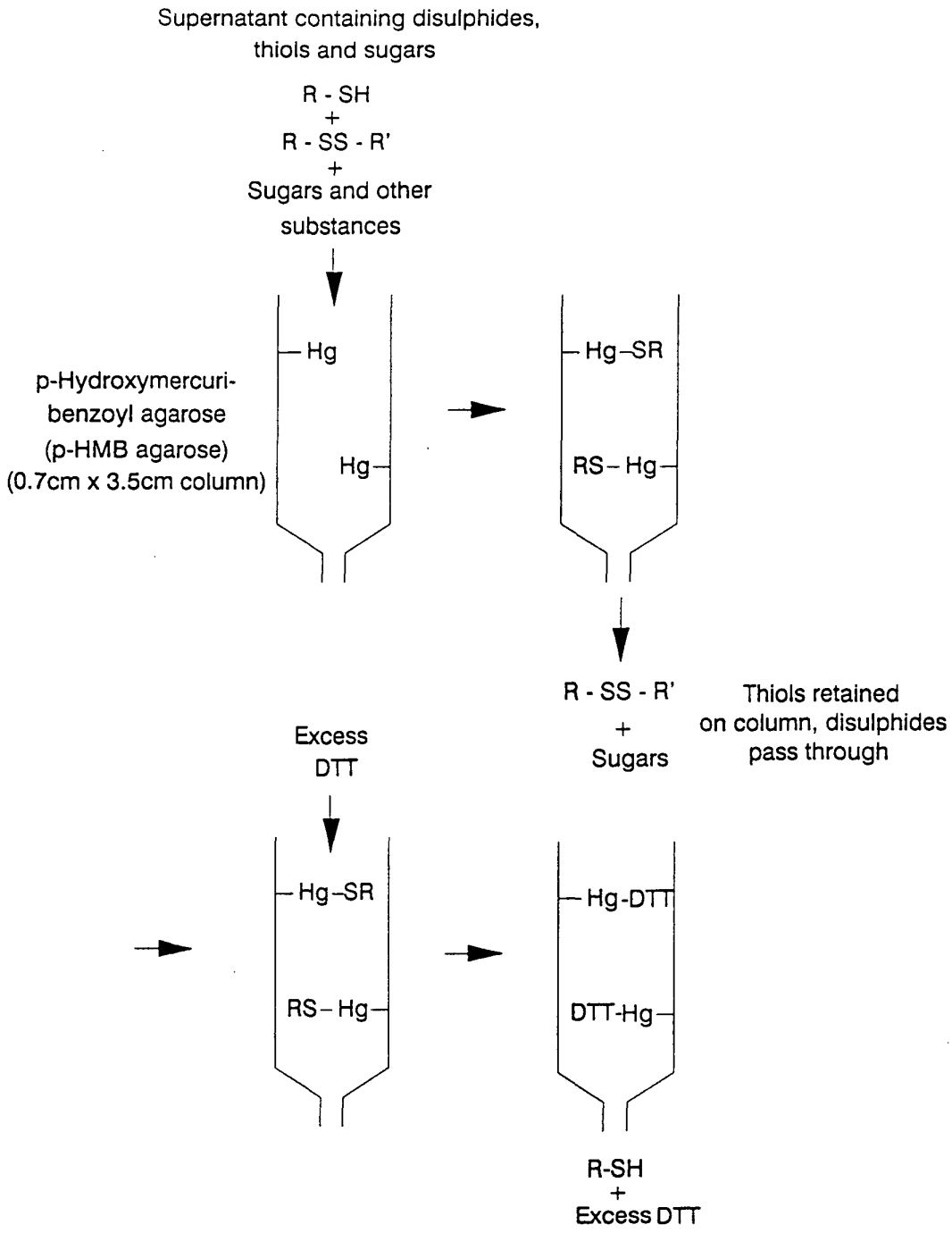



Fig. 6.5 The separation of thiols from disulphides and other soluble, non-thiol containing substances in embryo extracts using columns of p-hydroxymercuribenzoyl-agarose beads.

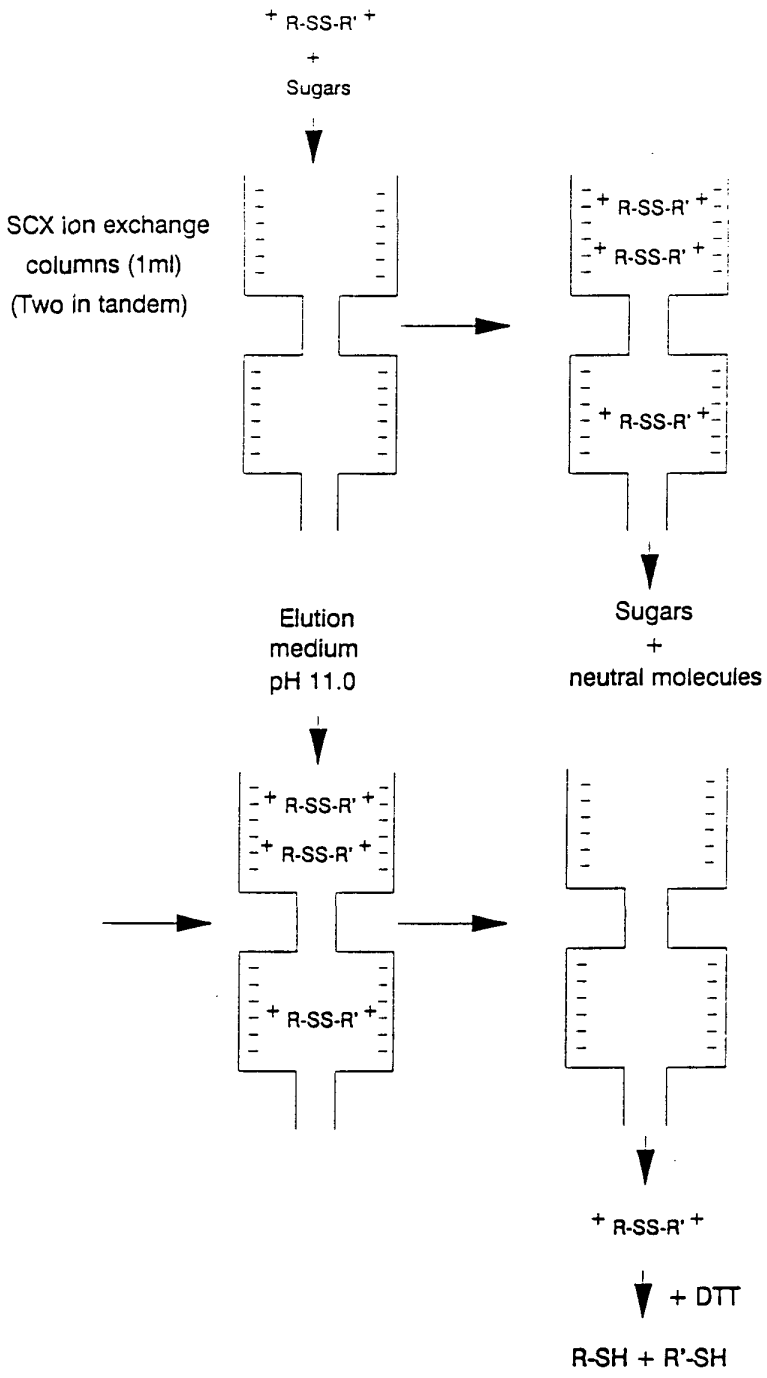



Fig. 6.6 The separation of the disulphides and other molecules with positive charges from negatively charged and neutral molecules, including sugars, using ion exchange chromatography.

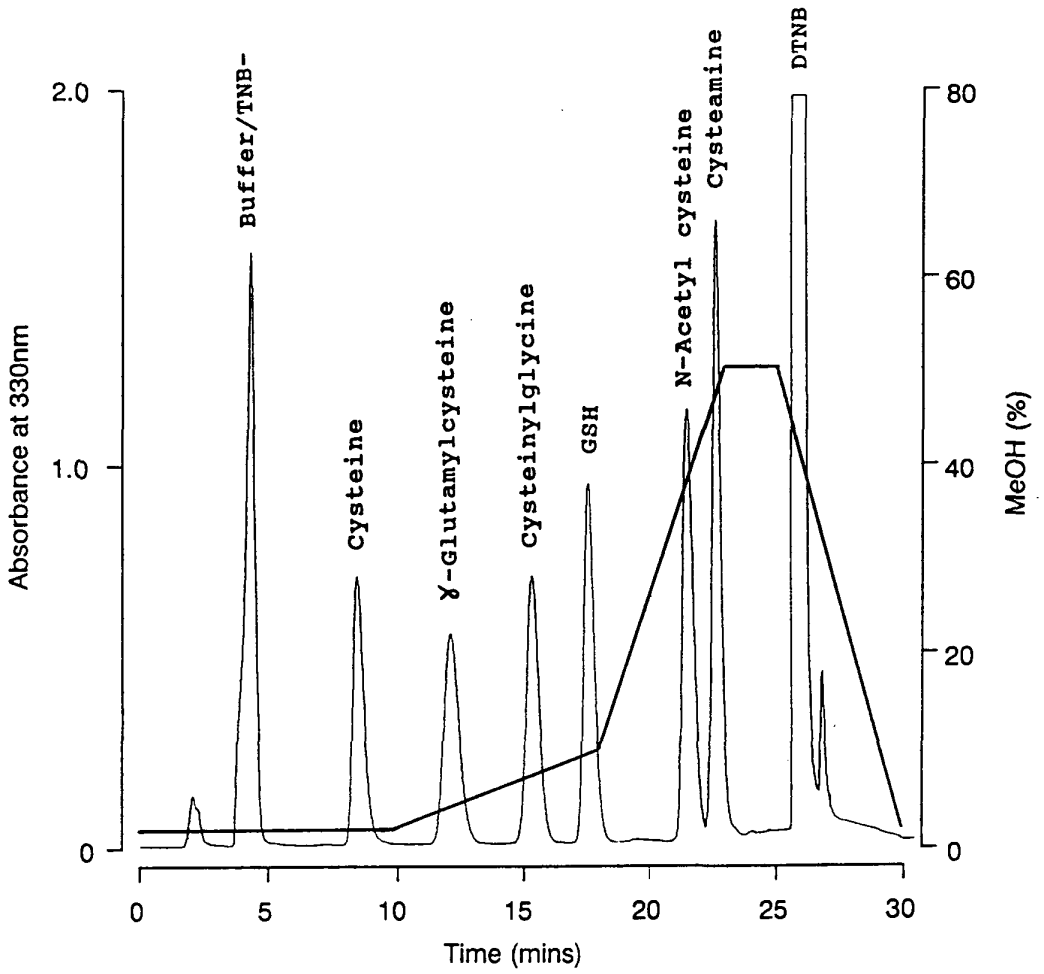



Fig. 6.7 The separation of the DTNB derivatives of barley thiols and two internal standards by reverse-phase h.p.l.c. using a gradient of methanol and a second solvent of ammonium formate.

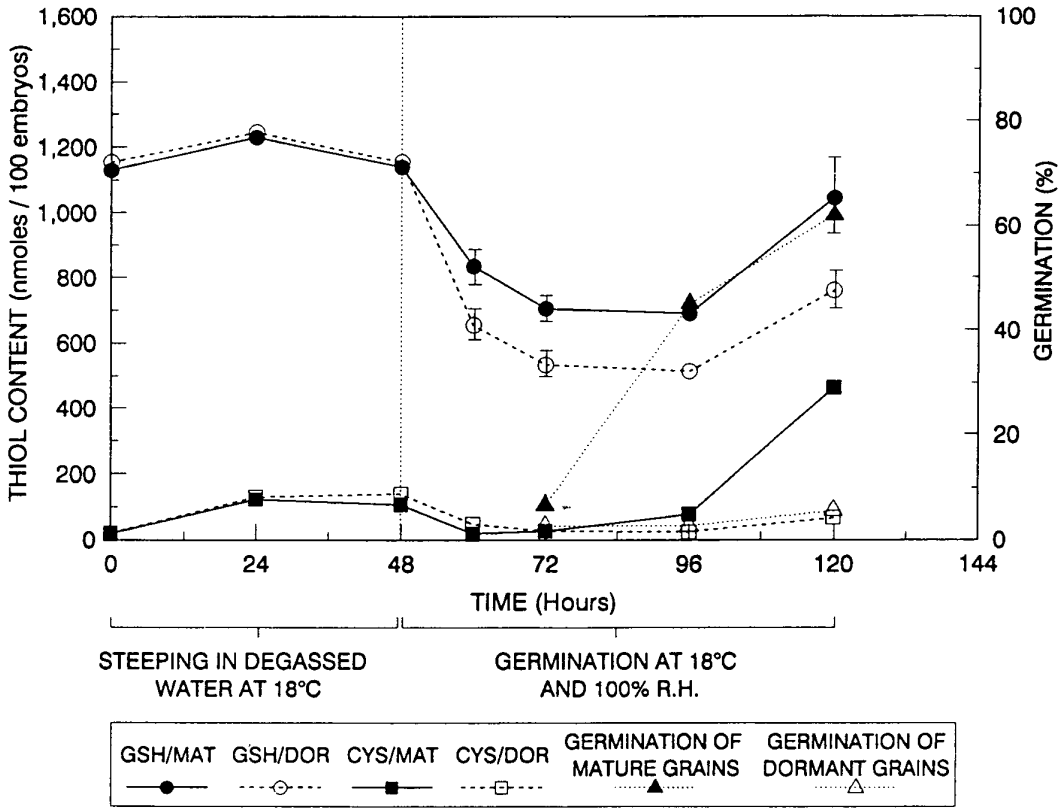



Fig. 6.8 Changes in the thiols in the embryos of dormant and mature Doublet barley and the percentage germination during micromalting.

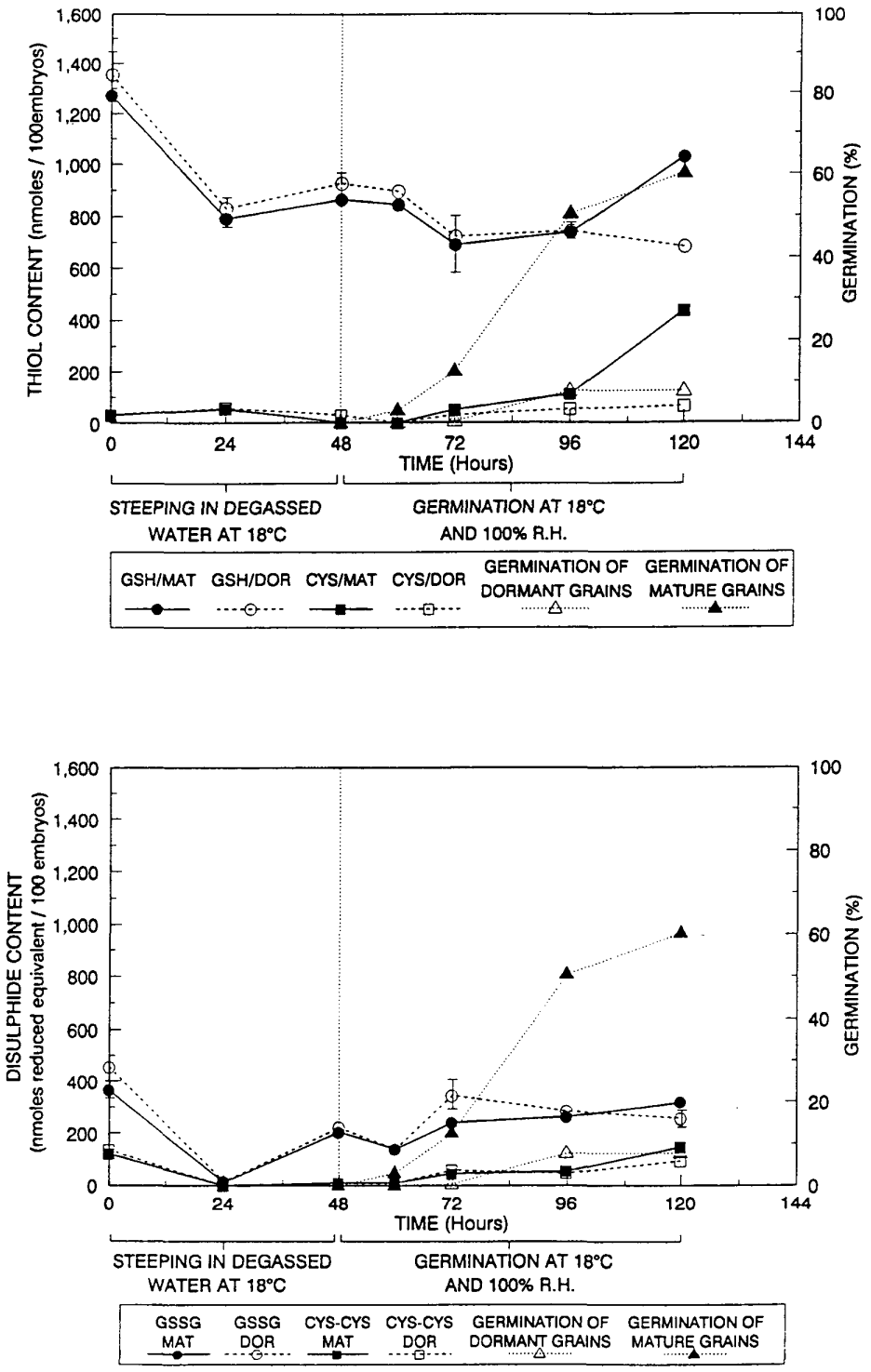



Fig. 6.9 *Changes in the levels of thiols and disulphides in the embryos of dormant and mature Triumph barley and the percentage germination during micromalting.*

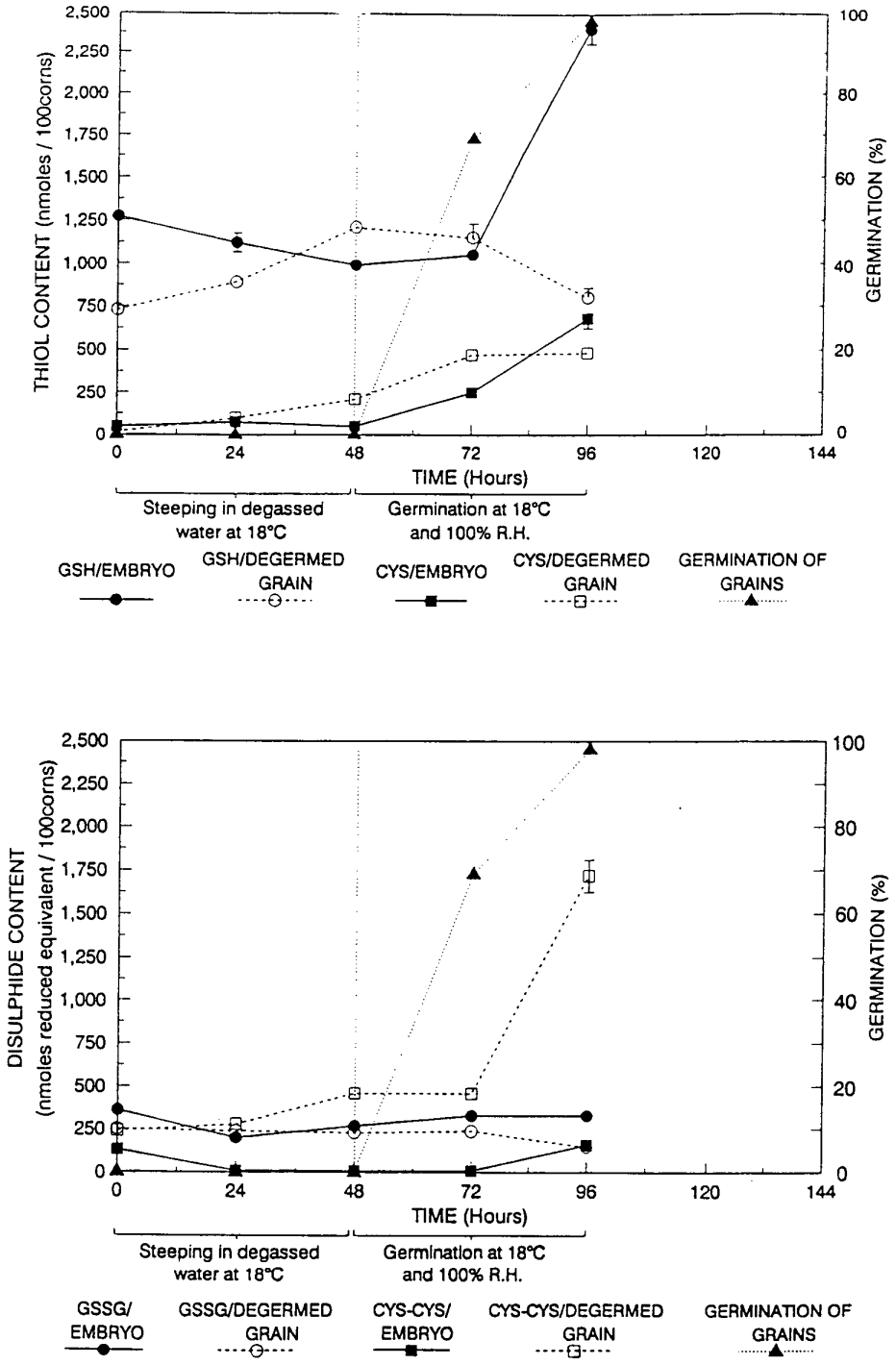



Fig. 6.10 Changes in the levels of thiols and disulphides in the embryos and degermed grains of mature Triumph barley and the percentage germination during malting using an improved schedule involving the removal of surface moisture from the grain at the end of steeping.

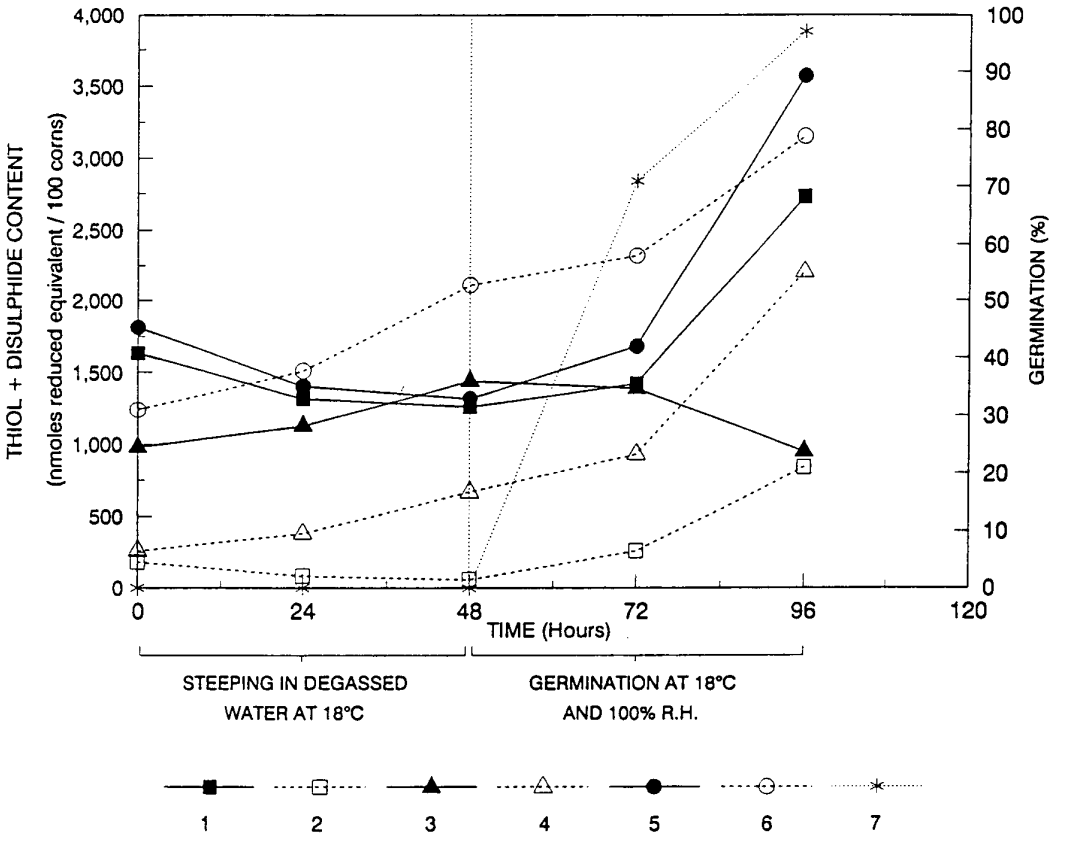
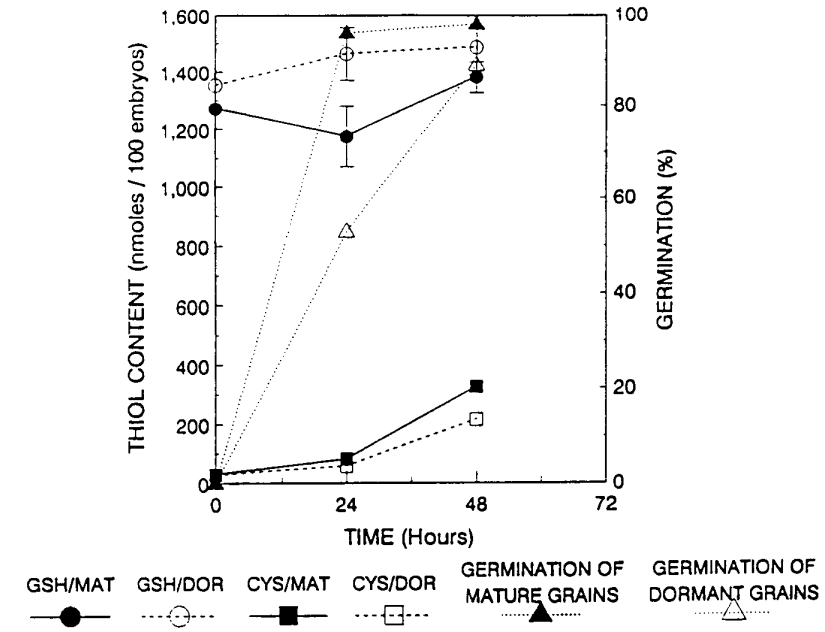



Fig. 6.11 The changes in thiols, disulphides and 'total thiols' in the embryos and degermed grains of mature Triumph barley and the percentage germination during micromalting. Data derived from that presented in Fig. 6.10.



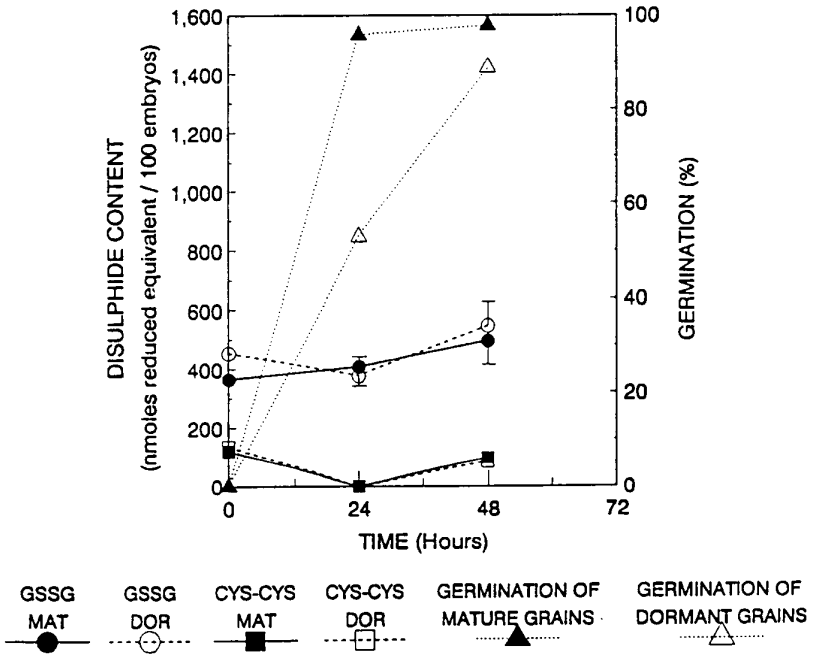




Fig. 6.12 The percentage germination and the level of thiols and disulphides in the embryos of dormant and mature grains of Triumph barley steeped in dilute hydrogen peroxide (0.75%).

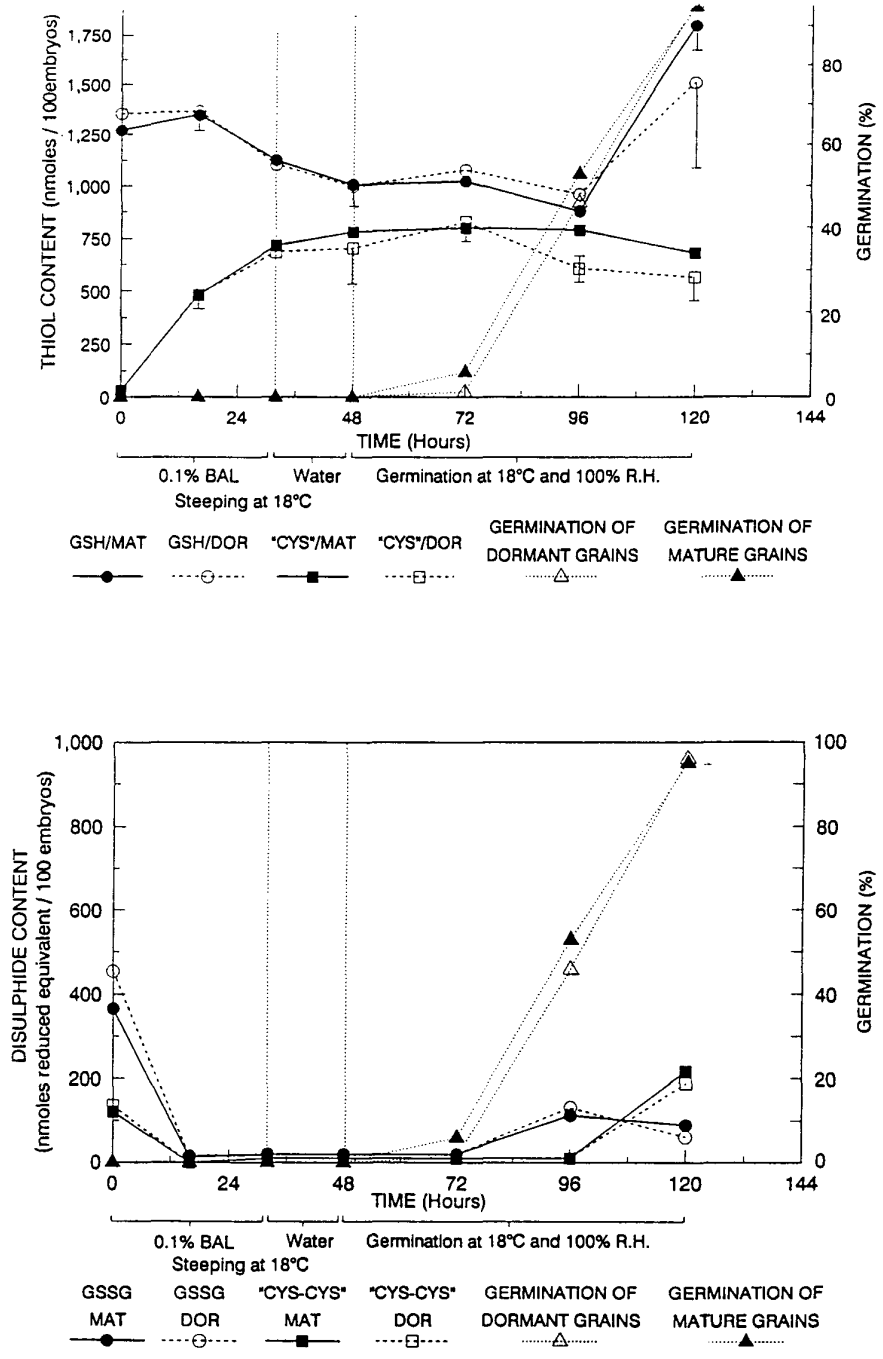



Fig. 6.13 Changes in the percentage germination and the levels of thiols and disulphides in the embryos of dormant and mature Triumph barley during micromalting, with a steep in dilute BAL (0.1%).