HGCA

PROJECT REPORT No. 61

OVERCOMING DORMANCY IN MALTING BARLEY

JULY 1992

PRICE £7.00

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by

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Final report of a three year project at the School of Biochemistry, University of Birmingham, Edgebaston, Birmingham B15 2TT. The work commenced in October 1987 and was funded by a grant of £19,829 from the Home-Grown Cereals Authority (Project No. 0006/2/87).

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ABSTRACT

The objectives of the project were to find means by which dormancy could be reduced or eliminated so that insights might be gained into the fundamental causes of dormancy and possibly ways to eliminate it under commercial conditions would appear. The treatments, usually applications of chemicals, were to be applied (a) to 'green grain', before it was dried; (b) to dried grain', during storage, and (c) to grain in the 'pre-steep period'. Treated samples of grain were stored, in parallel with untreated controls, at ambient temperatures. At intervals sub-samples were taken and tested for germinability.

The first experiments were to develop more reliable germination tests than those already available. These trials were successful, our 1 ml and 3 ml (agar) tests being superior to the 4 ml and 8 ml (paper) tests of the Institute of Brewing (Doran and Briggs, (1992), Journal of the Institute of Brewing, 98 (3), 193-201).

About a hundred different treatments were applied to dormant samples of grain in the 'green grain' and 'dry storage' periods. Only two treatments were beneficial. Applications of gibberellic acid stimulated germination as would be expected, since this substance is non-volatile. Spraying grain with sulphuric acid (0.5%) before drying also led to more rapid germination. Experiments indicated that it probably functioned by damaging microbes in the grains' surface layers. Some of the negative results obtained were also of interest. For example availability

of oxygen during storage was not a necessary condition for a decline in dormancy.

Steeping samples of grain in various solutions for 6 h. then air-resting for 18 h. before testing showed that under these conditions dilute solutions of sulphuric acid, hydrochloric acid, phosphoric acid and various sulphur-containing compounds alleviated dormancy.

It was confirmed that antibiotics improved grain germinability. It was shown that microbes in the surface layers of grains consumed considerable amounts of oxygen and that the embryos of dormant grains need a more ready supply of oxygen to germinate than those from mature grains. Consequently we support the view that microbes in the surface layers of grain check germination, at least in part, by competing with the embryo for the oxygen.

Gibberellin contents were determined by bioassay and abscisic acid contents by capillary GLC (ECD), in mature and dormant sub-samples of three different lots of grain. We conclude that endogenous levels of these hormones are unlikely to be connected with grain dormancy.

Introduction

Post harvest dormancy is costly to British Maltsters and Farmers. Dormancy creates the necessity for Maltsters to hold 'carry over" stocks from season to season to allow malting to proceed until the new seasons crop has lost its dormancy, that is it has undergone post-harvest maturation or ripening. Dormancy

also creates problems when barley is to be exported, as overseas customers are frequently unwilling to purchase grain which is dormant.

The problem is not just that of germination or non-germination. Dormant grain may germinate but more slowly and irregularly than mature grain held under the same conditions. Usually dormancy is overcome by modifying malting conditions and by storing grain, preferably warm, for more or less extended periods. Almost nothing is known of the causes of dormancy other than its intensity varies between varieties, Triumph and Doublet, as examples, being prone to dormancy, so there is a genetic component. It is greatly influenced by the growing season, there are endogenous (within grain) and exogenous factors involved (including the populations of grain microbes), and the expression of dormancy is strongly dependent on the conditions of growth, (such as germination temperature and the availability of water) (Briggs, 1978; Briggs et al., 1981; Pollock, 1962). A consequence of the latter is that the conditions used in germination tests must be rigidly controlled. We were not satisfied with the tests available to us, and so a first priority was to develop a more rigourously standardised set of germination test conditions.

Using the new germination tests the next two approaches, which are closely interconnected, were: (i) to test the effects of a wide range of chemicals, and a few other treatments, on the rate of recovery from dormancy of samples of barley during

storage, and (ii) to carry out experiments designed to clarify the causes of dormancy.

A considerable number of chemical substances have been tested for their abilities to overcome dormancy. However, these have usually been tested by adding them to the water wetting the supporting medium in germination tests. It is not immediately clear how results of such an approach can be extrapolated to the malting process, or of what value such results might be to farmers. A further complication is that where particular chemical treatments have been successful in reducing or eliminating dormancy with some grain lots they have been ineffective with others. This is understandable if dormancy is a 'multi-factorial' problem and if different chemicals overcome different factors that are responsible for grains' failures to germinate on different occasions. However it is at first sight surprising that dormancy may be reduced by treatments with acids or bases; with oxidising agents or reducing agents; with thiols and with substances which vigorously interact with thiols. We hypothesize that many agents do not penetrate into grains, but act by damaging the microbes on the grains surfaces. Other agents may penetrate and act on the embryo within the grain. The embryos of "water sensitive" grains have a higher oxygen requirement for germination than embryos of mature grains (Crabb and Kirsop, 1969). Microbes compete for oxygen and adversely influence malting, embryo germination and the response of aleurone tissue to gibberellic acid (Kelly and Briggs, 1992a,b,c). Microbes occur associated with the husk and in the

surrounding film of liquid (Briggs and Woods, 1992). Agents and treatments which are reported to alleviate or overcome dormancy include grain drying and warm storage; dilute mineral acids or acid salts such as phosphoric acid, sulphuric acid and acid sodium fluoride, lime water, sodium carbonate and bicarbonate, sodium and potassium hydroxides, soluble nitrates, nitrites and bromates, sulphites, sodium azide, tungstates, manganese sulphate, hypochlorites, hydrogen peroxide, elevated levels of oxygen, many thiols or potential thiols, such as propyl mercaptan and thiourea, iodoacetamide, N-ethylmaleimide, mercuric chloride, various salts of heavy metals, mixtures of antibiotics, sulphur dioxide, carbon monoxide, formaldehyde, brief exposures to organic solvents and applications of gibberellic acid. It should be pointed out that often excessive doses or prolonged exposures to many of these agents actually damage grains or kill them.

We adopted a novel approach in attempting to overcome dormancy. Mindful of the practicalities of handling grains in a maltings, treatments were made (i) at the green grain stage, (ii) in the dry storage period, and (iii) in the pre-steep period.

When grain arrives at a maltings it usually has to be held in ventilated stores until it can be dried sufficiently to permit long term storage without aeration. It is feasible, therefore, to expose grain to gases or to spray it with solutions of substances at this stage, and to dry it subsequently. On the other hand dried grain in store might be subjected to various physical treatments or be exposed to gases or applications of materials that do not increase its moisture content. If

successful either of these approaches would be of value to maltsters, farmers or seed merchants. The third approach would probably only be of value to maltsters. At the 'pre-steep' stage grain might be exposed to gases or be given various treatments or be wetted, provided that it was adequately ventilated until it was committed to the steeping process. Such "wet" treatments, together with the others mentioned, might also be of value to breeders or farmers anxious to improve the vigour of seed immediately before sowing.

If microbes are present in such quantities on the grain surface layers that they compete with the embryo and aleurone for oxygen to a significant extent then this should be detectable by suitable measurements of respiration. It was decided that the possibility should be tested.

There is a long-standing hypothesis that dormancy in seeds is due to a balance between endogenous levels of a growth promoting hormone and a growth inhibiting hormone. It has also been presumed that the hormones involved are gibberellins and abscisic acid respectively. In a number of seeds the amount of ABA present in the embryos seems to correlate with the degree of dormancy and in some cases, during maturation and the decline of dormancy, the levels of ABA decline (Milborrow, 1974; Sondheimer et al., 1968; 1969; Galston et al., 1974). In wheat and barley levels of ABA are highest at the milky and waxy stages of grain formation, then the levels decline as the grains ripen (Slominski et al., 1974; Goldbach and Michael, 1975; King, 1976; McWha, 1976).

We intended to test this hypothesis by comparing the levels of gibberellins (determined by bioassay) and of ABA (GLC - ECD; capillary columns) in dormant grains and in sub-samples of the same grain lots that had been after-ripened by drying and warm storage.

MATERIALS AND METHODS

Samples of barley

Samples of barley were kindly supplied by Paul's Malt Ltd and Wolverhampton and Dudley Breweries Ltd.

Each sample was thoroughly mixed, using a sample divider, and sub-samples (approx 1.5 kg) were double wrapped in waterproof plastic bags and stored at -18°C. Grain quality did not alter appreciably in 2 years when stored in this way. When taken for use each bag was allowed to warm up to room temperature (approx. 18°C). Bags were opened after 1 h. and grain was subsequently attemperated at 18°C or 25°C, as appropriate, for 4 h.

Storage

Barleys were treated, as indicated in the text, then stored in airtight glass or plastic bottles or, when gases were used, in mild steel cylinders with inlet and outlet tubes, closed by taps or a half-inflated rubber balloon that allowed for changes in atmospheric pressure. Containers of grains stored at ambient temperatures were enclosed in a waterproof shed outside, but protected from the sun and rain by an overhanging part of a

building. Temperatures were noted with a recording thermometer. Naturally the temperatures varied throughout the year, consequently the rates of recovery from dormancy varied in different trials. Consequently untreated, control batches of grain were stored in parallel with treated batches in each trial. Applications of agents to green grain samples were made by spraying on solutions or suspensions (6 ml/50g grain) then, 6 h. later, drying the grain to a moisture content of approx. 12%, before putting the samples into store.

Grains were exposed to gases in mild steel containers or sealed glass bottles, all 'buffered' with a partly inflated balloon. The gases used were ethylene, oxygen, ozone, air, nitrogen, carbon dioxide and ammonia. Generally each gas was passed through the container for 2 h., with grain mixing, before the vessels were sealed. However ammonia and ethylene were injected into the storage vessels. While most samples were stored at ambient temperatures a few were stored at 40°C.

Grain was treated in the 'pre-steep' period by immersing 100 grains, in boiling tubes, in 10 ml of the test solution for 6 h. at 15°C. Samples were drained, air rested for 18 h. at 15°C and then placed in Petri dishes and tested in the 1 ml, 3 ml and 5 ml (25°C) agar tests. Some grain samples were subjected to low-pressure treatments. Grain to be treated was spread on a perforated metal plate in a vacuum desiccator. This was evacuated and maintained at a low pressure using a motorised, oil-sealed vacuum pump. After treatments the samples were stored in air, in sealed glass bottles, at ambient temperatures.

Germination tests

Germination tests were routinely carried out on agar using the methods we developed (Doran and Briggs, 1992) in comparison with the 4 ml (paper) and 8 ml (paper) tests carried out according to the Institute of Brewing (1986), but with more rigid control of temperature (18°C±0.8°C), and relative humidity (normally more than 85%) and the 10 ml (paper), 25°C high-stress test (Kelly, 1990; Kelly and Briggs, 1992a).

Barley samples, prepared using a sample divider, were graded on a set of shaking EBC-type screens. Grains of 2.18 mm or less were separated and discarded. Samples of 100 grains were counted into boiling tubes and were attemperated, at 18°C and 25°C, for 4 h., together with Petri dishes containing agar or paper wetted with distilled water as appropriate. At the start of each test the corns were tipped into the dishes (100/Petri dish) and were spread out evenly at random, but not touching. No attempt was made to ensure that all the corns were ventral or furrow-side down. The tests, as finally adopted, were carried out in plastic Petri dishes (9 cm) containing a layer of purified agar gel (Oxoid No L28, 0.75%, 10 ml). The tests, equivalent to the IoB 4 ml and 8 ml tests, were carried out with 1 ml or 3 ml distilled water initially added to the surface of the gel. In the test equivalent to the 10 ml (paper), 25°C high-stress test (Kelly, 1990), 5 ml of water was added to the agar and germination was carried out at 25°C. In all cases incubations were carried out in fully humidified streams of air, through inner chambers held within the incubators. Humidity was always over 85% (electronic

hygrometer; Protimeter) and temperatures were within 0.8°C of the selected values. The numbers of chitted (germinated) grains were determined after 1,2,3 and 4 days incubation.

Results, of 3 replicates of 100 corns, were noted as mean ± standard deviation. Where appropriate pairs of results, obtained after 72 h. incubation, were compared using the standard one-way analysis of variance (Doran and Briggs, 1992).

In some specialised trials grains that had been decorticated (husk and pericarp removed) were incubated on agar in flat-bottomed crystallizing dishes, closed with Petri dish lids, with 1, or 20 or 25 or 30 ml water/dish.

In other trials the microbes on the surface of the grains were controlled by steeping, for 90 min. at 15°C, in a solution/suspension of a mixture of antibiotics before blotting and submitting to a named germination test. Controls were steeped similarly, but in water. The antibiotics used were: amphotericin B, 0.01%; nystatin, 0.04%; benzyl penicillin, 0.08% polymyxin B, 0. 0.4% and streptomycin sulphate, 0.08%.

Respiration studies

For various purposes the oxygen uptake rates of finely milled samples of grain (entire or decorticated; with or without an antimicrobial treatment) were determined in water saturated with air, at 15°C. Special plastic 'Perspex' chambers were constructed, each being sealable and having a water-jacket. The contents of each were agitated with a magnetic stirrer. Oxygen partial pressures were determined using Clark-type, membrane-

covered platinum electrodes, using a potassium chloride electrolyte. Each electrode was calibrated by immersion in water saturated with air and with water deoxygenated by saturation with sodium sulphite. Corrections were made for changes in the atmospheric pressure, which was variable and usually less than that of a "standard atmosphere". As respiration proceeded the partial pressure of oxygen declined, and this was recorded on a chart recorder. Oxygen uptake rates were calculated from the gradients of the lines obtained.

The extraction and bioassay of gibberellins

The methods used were based on those used by Raynes (1983) and Smith and Briggs (1980). However, instead of measuring α-amylase production the release of reducing sugars from degermed, decorticated grain was used as an indication of a response to gibberellins.

The barley to be investigated was ground to a fine grist. The grist was extracted twice with cold aqueous methanol (80%). The pooled extracts were concentrated *in vacuo* (max. 30°C) and the aqueous residue was stirred with PVPP (polyvinyl poly pyrrolidone, 50mg/ml). The PVPP was separated by centrifugation. The supernatant, combined with water washes of the PVPP, was adjusted to pH 2.5 and was partitioned against ethyl acetate (5x50 ml). No biologically active gibberellins were detectable in the aqueous residue. Samples of ethyl acetate solution were loaded onto Whatman No. 1 chromatography paper (19 mm wide). Known amounts of gibberellic acid were similarly loaded onto

paper strips. The chromatograms were developed with the solvent iso-propanol/0.880 ammonia/water (10:1:1). In this system gibberellins A_1 and A_3 run in the region Rf 0.4-0.6 or 0.6 - 0.8, while the 'inhibitor ß-complex' runs near the front. Chromatograms were cut into sections. Sections were equivalent to 0.2 Rf units in length. Gibberellins present in the strips corresponding to Rf segments 0.4-0.6, 0.6-0.8 and 0.8-1.- were bioassayed . Degermed, decorticated half-grains were incubated, for 48 h. at 25°C, in a water-saturated atmosphere on the chromatogram sections, with a buffer (0.5 ml; sodium succinate, 20 mM; calcium sulphate, 1mM; disodium EDTA, 0.1 mM; pH6.0). At the end of the incubation period the assay plates were frozen, at -18°C. Subsequently the quantities of reducing sugars released were determined colorimetrically using the PAHBAH (p-hydroxybenzoic acid hydrazide) reagent of Lever (1972). The results, calculated with reference to a calibration curve, are expressed as gibberellic acid (GA_3) equivalents. Experiments with radioactive, tritiated gibberellin GA_1 (assayed by scintillation counting) indicated that better than 90% was recovered in the Rf zone 0.6-0.8 on the chromatogram from the entire procedure.

Extraction and assay of abscisic acid, ABA

The methods developed were based on those of Vermeer et al., (1987). Corns, finely ground in a Moulinex mill were extracted, while being treated with a Polytron Plus P10A ultrasonicator, with aqueous methanol (80%) containing the antioxidant butylated

hydroxytoluene (100mg/litre). The extracts were successively passed through a Millipore AP-25 prefilter, then a C-18 Maxiclean cartridge solvated with methanol and water. Each filtrate and methanolic washings off the column were combined and evaporated and the residue was partitioned between potassium dihydrogen phosphate (0.5M, pH 3.0) and ethyl acetate. The acids in the ethyl acetate layer were converted to methyl esters, using diazomethane. The dried residue was dissolved in hexane-ethyl acetate (9:1) and aliquots were analysed by gas-liquid capillary chromatography (GLC).

The recovery, in the hexane-ethyl acetate, of radioactive [^{3}H]-ABA that has been added to grains before extraction, as determined by liquid scintillation counting, was 75%(±1.7%;n=6). GLC analysis was carried out on a Pye Unicam 304 instrument equipped with an electron capture detector (ECD; highly sensitive for ABA) and a Phillips computing integrator. The column (30 m x 0.25 mm i.d.) was fused silica WCOT coated with SE30, film thickness 0.25 μm (Alltech Associates). Carrier gas was filtered, oxygen-free nitrogen. Injection of samples onto the column was via a stream splitter.

The response factors of the ECD towards ABA and the internal standard DIHBA (3,5-diiodo-hydroxybenzoic acid) were determined. The considerable problems encountered, of irregular detector response and base line instability, suggest that further work needs to be done on this system, possibly including a further sample-purification step using HPLC, as advocated by others. However, time did not permit us to extend this work.

RESULTS

The development of improved germination tests

As a preliminary to the main study it was necessary to develop a closely regulated method for determining the germinability of barley. The intention was to develop methods that would give results closely similiar to those given by the 4 ml and 8 ml tests of the Institute of Brewing (Analytical Committee, 1986) and the 10 ml (25°C) high-stress test (Kelly, 1990; Kelly and Briggs, 1992) but which would be more robust, being less prone to differences caused by variations in atmospheric relative humidity, corn size, etc. It was only practical to carry out determinations in triplicate, i.e. with 100 corns in each of 3 dishes. This carries the inevitable statistical penalty which follows from dealing with relatively small numbers of grains (Briggs, 1978).

The approach chosen was to use agar gel as a perfectly flat germination substratum with an inherently high water content, in Petri dishes, with water added to the surface. The dishes were to be incubated in a pump-driven, water-saturated atmosphere at a closely controlled temperature (18°C) and not in an atmosphere with uncontrolled relative humidity, and a temperature range (18-21°C) as used in the Institute of Brewing methods. Comparative studies were always made, in parallel, with 4 ml and 8 ml tests carried out according to the IOB, except that the relative humidities were always high (unless otherwise specified) and the temperatures were set at 18° and 25°C, as appropriate . These studies were successful and are being published (Doran and

Briggs, 1992). Fuller details of the development of the method are given in the publication.

The germinability of dormant grains is greatly influenced by temperature. We were aware that the temperature control of many commercial incubators was poor. To prevent icing on the cooling surfaces we found it necessary to install high-humidity, metal chambers, fitted with perforated shelves, inside our incubators to contain the moisture. Air from the incubator was driven, by an aquarium pump, through an air-stone' and a Buchner flask of distilled water (also housed in the incubator) and into the inner, high humidity chamber. Excess humid air was vented into the laboratory. Relative humidity within the inner chamber routinely exceeded 85% (Protimeter electronic hygrometer), but temperatures were only maintained to within ± 0.8°C, a variability substantially greater than our target of ± 0.1°C. The first trials were to determine what volumes of water, added to the surfaces of the agar plates, would allow germination percentages, at 18°C and 25°C, equal to those using the 4 ml, 8ml and 10 ml (25°C) tests on paper. In the cases of both mature and dormant barleys, at 18°C, the germination percentages fell as the water additions increased from 1 to 4 ml/dish. At 25°C germinations were low, but tended to declined as water additions increased from 4 to 8 ml/dish. The agar tests that gave the most similar results to the 4 ml and 8 ml (18°C) tests and 10 ml (25°C) test carried out on paper were those with water additions of 1 ml and 3 ml (18°C) and 5 ml, (at 25°C), to the agar. This tentative conclusion was tested with more extensive trials in

which two operators, working with ten samples of barley differing in maturity, tested each sample in triplicate (3 x 100 corns) using all the agar and paper tests in a water-saturated atmosphere. In most instances the results of the two operators agreed closely, so for statistical purposes they were pooled. The most variable results were obtained using the 4 ml (paper) test as improved for these trials by using a high relative humidity and a well controlled temperature (18°C).

Comparisons between the tests made on paper and the equivalent tests made on agar showed that the results were generally similar. However, detailed comparisons between the results obtained after 72 h incubation showed that occasionally large discrepancies occurred, with higher germination percentages being found with the 4 ml (paper) tests relative to the 1 ml (agar) test. The results of the other two pairs of tests (8 ml, paper vs. 3ml, agar and 10 ml, paper, 25°C vs. 5 ml, agar, 25°C) were very close.

To test the importance of humidification of the incubation chambers comparative trials were carried out, at 18°C, between the 1 ml (agar) and 4 ml (paper) tests and the 3 ml (agar) and 8 ml (paper) tests, both in highly humid atmospheres and in unhumidified atmospheres (R.H.<35%). In nearly all cases the results of the comparisons made in high-humidity atmospheres were very similar. In the trials made at low relative humidities agreement was much less good, as would be expected as the papers tended to dry, but the disagreements occurred in different "senses" with the six different barleys. However, the standard

deviations of the results were not usually appreciably greater than those encountered in the 'high humidity' trials. Thus reproducibility between trials is best attained if incubations are carried out in fully humidified atmospheres.

To determine the reproducibility of tests carried out at different times three lots of barley were tested in saturated and unsaturated atmospheres at 18°C, in the 1 ml (agar), 4 ml (paper), 3 ml (agar) and 8 ml (paper) tests, carried out three times, at two-week intervals. The means and standard deviations of the results were determined. In general reproducibility was best in tests carried out in humid atmospheres and on agar. The results given by the 1 ml (agar) and 4 ml (paper) tests were not identical. It should be noted that some barley samples gave highly variable results.

Thus the tests carried out on agar are more 'robust' and so are slightly preferable to the tests on paper, even when these are improved over the 'Recommended Methods' by being carried out in a water-saturated atmosphere and at a closely regulated temperature.

Dormancy recovery in grain samples treated with agents 6 h. before drying and putting into store

Various grain samples received a wide variety of different treatments during the 'green grain' (predrying) stage. Grains were then placed in store, together with untreated controls, and germinability was determined at intervals. Although a few of the compounds tested had adverse effects (e.g. thiolactic acid) most

had no effect on the rate of recovery from dormancy. Many of the substances tested reportedly, at least partially, relieve dormancy if applied immediately before, or during germination tests. Grains (16% moisture) received sprayed applications of the solutions or dispersions (6 ml/50g) 6 h before drying to 12% moisture. The substances tested without beneficial effects and the doses used, were as follows:

tert - Butyl alcohol (0.01, 1.0%); tert - butyl peroxide (0.01,0.1,1%); tert-butyl hydroperoxide (0.01, 0.1, 1.0%); cumene hydroperoxide (0.01,0.1,1.0%); hydrogen peroxide (0.5, 0.75%); sodium metabisulphite (0.5, 1.0, 2.0%); sodium nitrate (1,10,20 mM); sodium nitrite (1.10,20 mM); hydroxylamine (1,10,20 mM); sodium hydroxide (0.5%); putrescine (5,10,20 mM); spermine (5,10,20 mM); spermidine (5,10,20 mM) 2-aminoethane thiol (0.1,1,10 mM); cystamine dihydrochloride (0.1,1,10 mM); 2,2'-thiodiethanol (0.1,1,10 mM); 3,3'-thiodipropanol (0.1,1,10 mM); dithiothreitol (DTT;0.1,1,10mM); trans-1,2-dithiane-4,5-diol (oxidised DTT; 0.001, 0.01, 0.1 mM); N-acetyl-L-cysteine (1,10,100 mM); L-cysteine (1,10,100 mM); 2-mercaptoethanol (0.1,1,10 mM); mercaptoethanoic acid (0.1,1,10 mM); 2-mercapto-propanoic acid (0.1,1,10 mM); 3-mercaptopropanoic acid (0.1,1,10 mM); 3-mercapto-1,2-propanediol (0.1,1,10mM); 2,3-dimercaptopropanol (BAL; British anti-lewisite; 0.001,0.01,0.1 mM); 2,3-dimercaptopropanol tributyrate (0.001,0.01,0.1 mM); VFA, mixture of propanoic acid, butanoic acid and 2-methylpropanoic acid (ratios 2:1:1;0.2% and 0.4% v/w); methanol (1,3, 5%); ethanol (1,3,5%).

Two "green grain" treatments did accelerate the rate of recovery from dormancy. Applications of gibberellic acid (10μM; 100μM) reduced the time taken for germination to exceed 75% to 9 weeks (controls over 14 weeks) in the 1 ml (agar) test. As gibberellic acid is non-volatile this is equivalent to an addition in the germination test, where it is well known for its ability to stimulate germination. The other beneficial treatment was spraying the green grain with sulphuric acid (0.5%) which accelerated the rate of recovery such that germination exceeded 75% in the 1 ml (agar) test in 4-9 weeks, and in the 3 ml (agar) test in 9-14 weeks. In both instances the control values exceed 14 weeks. These results are shown graphically in Fig. 1. Germination increased more rapidly in both the 1 ml and 3 ml (agar) tests, in the samples treated with sulphuric acid.

Sulphuric acid is non-volatile, and is well known for its ability to decorticate barley. We know of no other acid that is able to do this. There were no signs of husk separation or disintegration in the treated grains. This lead was followed using some pre-steep treatments. It seems probable that sulphuric acid exerts its beneficial effects by killing microbes on the surface of the grain (see below).

<u>The effects of treatments during dry storage on the rate of decline of dormancy</u>

Grains were stored at ambient temperatures in air, atmospheres enriched with ozone, in oxygen (100%), nitrogen (100%), carbon dioxide (100%) or in air supplemented with ethene

(ethylene; 200 µl/litre, 400 µl/litre). Ozone damaged the grain, particularly that near the gas inlet. The other treatments had no effects whatsoever, relative to the air controls, on the rates of recovery from dormancy. This result was not immediately accepted since it was believed that, for dormancy to decline, oxygen had to be present during the storage period. Some of trials were therefore repeated on grain samples stored at ambient temperatures and at 40°C. In addition, in view of the reports of Cairns and de Villiers (1986a,b) that exposure to atmospheres rich in ammonia gas were astonishingly successful in breaking the dormancy of wild oats and some wild Hordeum species, and the report of Forward (1958) that exposure to low pressures greatly improved the germinability of dormant oats, treatments with ammonia and high vacuums were tested. The results are shown Table 1 (Briggs, 1990).

At ambient temperatures none of the treatments had any significant effects on the rates of decline in dormancy. Some of these results have now been confirmed independently (Baxter and Dawe, 1990). However, in the samples stored at 40°C, which recovered relatively rapidly from dormancy, carbon dioxide appeared to be absorbed by the grain (the balloon used to balance the gas pressure against the air pressure collapsed and was drawn into the storage vessel; this happened repeatedly in successive trials) and the germination of the grain in the 3 ml (agar) test was substantially depressed (Table 1).

These findings are important in that: (i) They demonstrate that the recovery from dormancy during storage cannot be due to

an oxygen-dependant reaction. (ii) Storage of grains in inert atmospheres, to check insect infestations and mould growth, will not check recovery from dormancy but (iii) storage at elevated temperatures to accelerate the decline in dormancy will be acceptable in atmospheres of nitrogen, but possibly not in atmospheres of carbon dioxide.

Grain treatments during the presteep period

A number of trials were made to test whether the intensity of dormancy could be altered by 'triggering' the phytochrome system. Dormant grains, resting on a wet substratum, were illuminated with red and far red lights. No effects on the germinability of the grains were detected.

With the beneficial effects of sulphuric acid sprays applied in the 'green' grain stage in mind, tests were undertaken to determine the value of steeps in acids of various strengths. The results (Table 2) show that steeping in hydrochloric, sulphuric and phosphoric acids (each 0.1%) enhanced grain germination. Since it is known that these acids will not penetrate undamaged, ungerminated grains (Briggs, 1978, 1987), and since only sulphuric acid (50%) causes the disintegration of the husk and pericarp, it seems that these acids exert their beneficial effects on the outer layers of the grains, most probably by inhibiting the surface microbes. Later studies confirmed this hypothesis (see below).

Pollock and Kirsop (1956) tested the abilities of a wide range of substances to overcome dormancy during germination

tests. Among the most successful were a range of thiol-containing substances including some that we had tried unsuccessfully in the 'green grain' treatments. We therefore tested a limited range of thiols, or substances which can isomerise to give thiols (thiourea) or are likely to be hydrolysed to give them (BAL-tributyrate), applied in pre-steeps. Of the many possible compounds available we chose those that we judged likely to be able to penetrate into grains (Table 3). In addition we tried other substances that, because of their polarity, we thought might not get into grains or which, although they contained sulphur, do not contain actual or potential thiol groups (Table 4).

The results astonished us. At particular doses some of the thiols, e.g. 2,3-dimercaptopropanol (BAL) came close to eliminating dormancy, as determined in the 1 ml (agar) test, and enormously increased germination in the 3 ml and 5 ml (25°C) agar tests. In addition it was clear that, at some doses, some compounds damaged the grain and killed it (Table 3).

In contrast, the more polar thiol-containing compounds (DTT; L-cysteine; N-acetyl L-cysteine) and the other S-containing substances that were tested were without effect or were harmful (Table 4).

It is tentatively concluded that to be active in breaking dormancy S-containing substances need to contain an actual or a potential thiol (sulphydryl) group and to be able to penetrate into the grain. If this is so then they must act on the living tissues within the grain and not on the microbes in the surface

layers. By comparison with the results of the 'green grain' treatments we also conclude that the thiols must be applied to the hydrated grains, when their tissues are actively metabolising.

The influence of antibiotics on grain germination

In initial trials, in which a cocktail of antibiotics (chosen to check fungi, yeasts and gram-positive and gram-negative bacteria,) was added to the germination dishes, incorporated in the agar and surface water, no beneficial effects were seen. However, inspecting the dishes it was clear that antibiotics in suspension had settled on the agar, and not on grains.

In an alternative approach grains were steeped for 90 min., at 15°C, in a solution/suspension of antibiotics. The controls were steeped in water. Results of replicate trials, run in successive weeks, were good. The germination of a mature barley (1986 crop) in the 1 ml test was not stimulated by antibiotics. On the other hand germination in the 3 ml test was stimulated (Fig 2). In contrast antibiotics applied to samples of dormant grains stimulated germination in both the 1 ml and 3ml (agar) tests, the stimulation being relatively greater in the 3 ml test (Fig 3,4). The results of the 3 ml tests (with antibiotics) came close to those of the 1 ml tests with and without antibiotics. However the germination in the 1 ml test was well below that of the viability of the grain. This finding adds weight to our conclusion that microbes and (independently) the film of surface

water, together with other 'endogenous' factors acting within the grain, are responsible for dormancy (Kelly and Briggs, 1992). In the 5 ml (25°C; agar) test antibiotics seemed to marginally reduce germination in one trial (Fig 3), but stimulated it significantly with a different barley (Fig 4).

Germination of decorticated grains, under water

In principle microbes may inhibit grain germination by competing for oxygen and/or by generating phytotoxic materials which poison the embryo. We thought that with grains resting on 10 ml. agar gel any toxic substances generated by the microbes would probably be 'diluted' by diffusing into the gel, and so the beneficial effects of controlling microbes were likely to be due, at least mainly, to preventing their competing with the grain tissues for oxygen. A model system was used to test the hypothesis that oxygen availability was important.

Samples of three lots of grains, one mature and two dormant, were decorticated with 50% sulphuric acid so that the husks, pericarps and most of the microbes were removed. The grains were surface sterilised with calcium hypochlorite and were put to germinate on agar in crystallising dishes to which 1, 20, 25 or 30 ml of sterile, distilled water had been added. The larger volumes of water presented a significant barrier to oxygen diffusion from the air down to the grains.

With 1 ml. water the mature grain reached maximum germination in 1 day, with 20 ml it took 2 days, and with larger amounts of

water it took longer still (Fig. 5). However, with a dormant grain sample germination was much less good, and was depressed to a much greater extent when the grains were immersed in larger volumes of water (Fig 6). This was also true of another, different sample of decorticated, dormant barley (Fig. 7). Feeding oxygen gas into the germination cabinets largely eliminated the inhibition of germination caused by 30 ml water (relataive to 1 ml), showing that the inhibition caused by the large amount of water in experiments conducted in air was due to the restriction of the availability of oxygen to the grain. There was an indication that calcium hypochlorite treatment damaged the grains slightly, as in one instance where surface sterilisation was omitted germination was greater than in the comparable, surface sterilised sample (Fig 6).

The decortication of samples of grain that had been pre-treated with sulphuric acid (0.5%) eliminated the advantage of enhanced rates of germination, when compared to controls that had also been decorticated. Thus it is likely that the acid treatment (0.5% sulphuric acid) exerts its benefits by acting on the surface layers of the grains and, most probably microbes associated with the husk.

In contrast, treating decorticated grains with sodium hydroxide (0.5%) damaged them, reducing their germinability, as was expected (Briggs, 1978).

Thus the germination of dormant, decorticated grain is depressed much more by restricting access of oxygen to the grain than is the germination of mature, decorticated grain. This

agrees with the finding, made with isolated embryos incubated under various partial pressures of oxygen, that embryos from water-sensitive barleys have a higher oxygen requirement for germination than embryos from mature grains (Crabb & Kirsop, 1969).

Respiration of whole and decorticated, milled grains

Experiments in the previous sections demonstrated that: (a) controlling microbes improved the germination of dormant grains, and (b) decorticated mature grains germinate much better when the oxygen supply is limited by a layer of water than do decorticated, dormant grains. It remained, therefore, in order to test a remaining part of the hypothesis that microbes on the surface of the grain compete for oxygen, to demonstrate that these microbes have a significant oxygen uptake rate. It is well known that removing the surface film of moisture from grains or decorticated grains increases their oxygen uptake rates, because the moisture film, the husk and the pericarp are barriers to gas exchange, specifically to oxygen diffusion from the atmosphere to the living and respiring tissues of the grain. It is impossible to decide, by measuring the oxygen uptake rates of whole corns, to what extent the microbes contribute to oxygen uptake by whole-grains. To overcome the ambiguities caused by the diffusion barriers, samples of entire grains, of surface sterilised grains, of decorticated grains and of surface sterilised decorticated grains were milled and the respiration rates of the milled preparations were determined. Two sub-samples of three lots of

grain were investigated. In each case 1 subsample was dormant, the other had been matured by a drying and warm storage treatment at 40°C. Surface sterilisation was with calcium hypochlorite and silver nitrate (Raynes, 1983). Samples of the flour (10g) were suspended in air-saturated water at 15°C, and the oxygen uptake rates were found using Clark-type electrodes. Oxygen uptake rates were expressed per corn, per hour (Table 5).

The antimicrobial treatment is unlikely to have truly sterilised the treated grains, but it will have substantially reduced the microbial populations. It did not reduce grain germinability, but there is the risk that some agent may have been carried over and so reduced the oxygen uptake of the samples of ground grains. In every case decortication, which will have removed most of the microbes from the surface of the grains, substantially reduced the respiration rates of samples (Table 5). In each pair of samples the reduction between the entire and decorticated grains was greater for the dormant relative to the mature samples. In every case warm storage/grain maturation caused a drop in respiration, as would be predicted from the fact that warm storage reduces the population of microbes. Similarly the 'sterilisation' treatment applied to intact grains reduced respiration significantly in dormant samples, but less, or not to a significant extent in mature samples.

Decortication almost eliminated the differences in respiration rates between mature and dormant pairs of grain samples. The 'sterilisation' treatment tended to reduce the respiration of two samples of decorticated grains, but seemed to

increase it in one case, Triumph 87. Thus, although there are difficulties in interpreting these results in detail, it is clear that normal grains, especially those that are dormant, have a very substantial, strongly respiring population of microbes associated with their surface layers.

Gibberellins and abscisic acid in dormant and mature grain samples

To test the hypothesis that dormancy is regulated by a balance between endogenous growth promoters and inhibitors, specifically gibberellins and abscisic acid, these substances were determined in dormant and mature (warm stored) sub-samples of the same grain lots.

The gibberellins were extracted, partitioned into ethyl acetate, separated from inhibiting substances by paper chromatography and bio-assayed using sugar release from degermed grains as the test method. Recoveries of [^{14}C]-gibberellin A_1 were excellent. A dose response curve, using gibberellic acid (GA_3) as a standard was used to calibrate the method (Fig 8).

Comparisons between dormant and mature sub-samples of the same lots of barley, either quiescent or after 1 or 6 h. steeps, demonstrated that there were no significant differences in gibberellin - like substances (GA_3 equivalents, by bioassay) between the pairs of grain samples (Table 6,7). Thus differences in initial gibberellin - like substances or changes during the initial wetting stages cannot explain differences between dormant and mature samples of grain.

Abscisic acid, ABA, was extracted from ground samples of grains. The crude extract was passed through a C-18 cartridge, adjusted to pH 3.0, and partitioned into ethyl acetate. After methylation, using diazomethane, the methyl- ABA was assayed by temperature programmed GLC using a capillary column and an electron capture detector with a peak height integrator. Recovery of [^{3}H]-ABA, added to grain, in the solution used for GLC was 75%. 3,5-Diiodo-hydroxybenzoic acid (DIHBA) was used as an internal standard. Even with standards and the best temperature programme found the irregularity of the base-line and instability of the ECD gave problems (Fig 9). Consequently there is a doubt about the reliability of the analyses. However, the results indicate that there was marginally more ABA in the dormant grains than in the mature, and indeed in one case the dormant grain seemed to contain significantly more ABA than the mature (Table 8). It will be noted that even when 'matured' Triumph 1989 contained more ABA than Triumph, 1988 and that both contained more than the 'dormant' sample of Doublet.

Thus the evidence indicates that the relative levels of gibberellins and ABA are not the causes of dormancy in barley.

CONCLUSIONS

The most important conclusions from this work are:

1. That with the exceptions of applications of sulphuric acid and gibberellic acid none of the treatments applied to the 'green grain' and during dry storage accelerated the maturation of barley.

2. Maturation during storage is not an oxygen-dependent process.
3. Atmospheres of carbon dioxide but not nitrogen are deleterious to grain stored at 40°C.
4. Some thiols applied to grain as a pre-steep, followed by an air rest, break dormancy very efficiently. These results are highly dose dependent. The effective agents are probably those which penetrate to interiors of the grains.
5. Microbes adversely effect grain germination. It is likely that they compete with the grain for oxygen. Decorticated dormant grains have a higher requirement for oxygen for germination than decorticated, mature grains.
6. No convincingly significant differences were detected between the gibberellin and abscisic acid contents of mature and dormant sub-samples of different lots of grain.

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Table 1

Treatments applied to grain samples and the storage times taken to reach 75% germination after 72 h. incubation in two germination tests.* The barley was a sample of Triumph (1987 harvest; 12% moisture).

Treatment	concentrations used	Time (wk.) to 75% germination after 72 h	
		1 ml test	3 ml test
40°C			
Oxygen	100%	2	2-6
Nitrogen	100%	2	2-6
Carbon dioxide	100%	2	(44% at 6 wk)
Air	100%	2	2-6
Ambient temperature			
Oxygen	50%, 100%	18-22	26-30
Nitrogen	100%	18-22	26-30
Carbon dioxide	100%	18-22	26-30
Air	100%	18-22	26-30
Ambient temperature			
Ammonia	15%, 25%	20-24	28-32
Air	-	20-24	28-32
Ambient temperature			
Vacuum	24 h., 48 h.	20-24	28-32
Control	-	20-24	28-32

*Unless listed separately no differences exist between different concentrations or treatments.

Table 2

The germination of samples of Triumph barley (1988 harvest) steeped in the solutions shown for 6 h. at 15°C, drained and air-rested for 18 h. at 15°C, and then put to germinate in the various agar tests. The germination values shown were for 72 h. incubation times. Results: means of triplicates (standard deviation).

		Germination (%)		
Substance	Concentration (%)	1 ml, 18°C	3ml, 18°C	5ml, 25°C
Control (water)	-	73.7 (1.5)	47.0 (2.0)	33.7 (1.5)
Sulphuric acid	1.0	37.5 (4.9)	39.5 (3.5)	2.3 (1.2)
	0.5	47.3 (6.8)	44.7 (3.0)	18.3 (1.5)
	0.1	88.0 (2.0)	78.7 (3.1)	62.7 (5.5)
	0.01	87.3 (2.1)	60.0 (5.6)	59.3 (0.6)
Hydrochloric acid	1.0	40.0 (3.5)	39.0 (3.0)	3.5 (2.1)
	0.5	78.0 (4.0)	53.3 (3.5)	12.0 (4.2)
	0.1	94.0 (1.0)	87.7 (2.1)	67.3 (9.5)
	0.01	84.3 (3.1)	60.7 (2.1)	53.0 (2.0)
Phosphoric acid	1.0	52.7 (7.2)	46.3 (2.5)	12.0 (2.8)
	0.5	63.0 (4.6)	55.0 (7.2)	43.3 (2.5)
	0.1	89.3 (1.5)	87.7 (1.5)	76.0 (3.5)
	0.01	87.7 (1.2)	45.3 (3.5)	46.0 (5.3)

Table 3

The germination (after 72 h) of samples of Triumph barley (1988 harvest) after steeping in the solutions shown (6h., 15°C), air resting (18 h., 15°C) and then incubating in the 1 ml, 3 ml and 5 ml (25°C) agar tests. Results: mean of triplicate (standard deviation).

		Germination, (%)					
Substance	Concentration	1ml, 18°C		3ml, 18°C		5ml, 25°C	
Control (water)	-	46.0	(3.0)	15.5	(1.5)	16.0	(4.4)
2,3 Dimercapto-propanol (BAL)	10mM	0	(0)	0	(0)	0	(0)
	1mM	93.7	(2.1)	88.3	(1.5)	66.7	(2.1)
BAL-tributyrate	1mM	21.7	(8.4)	0	(0)	0.6	(0.6)
	0.1mM	86.3	(5.0)	72.3	(5.5)	51.7	(2.6)
Thiolactic acid	10mM	0	(0)	0	(0)	0	(0)
	1mM	81.7	(4.0)	56.7	(1.2)	37.0	(2.6)
3-Mercaptopropionic acid	10mM	0	(0)	0	(0)	0	(0)
	1mM	69.0	(7.0)	50.3	(5.1)	24.3	(3.2)
Mercaptoacetic acid	10mM	0	(0)	0	(0)	0	(0)
	1mM	92.3	(2.5)	68.3	(11.8)	40.3	(6.7)
3-Mercapto-1,2,-propane diol.	10mM	0	(0)	0	(0)	0	(0)
	1mM	72.0	(7.5)	9.3	(9.2)	-	-
Thiourea	0.3%	96.0	(2.0)	94.3	(4.2)	81.7	(5.9)

Table 4

The germination of samples of Triumph barley (1988 harvest), steeped in the solutions shown for 6 h. at 15°C, drained and air-rested for 18 h. at 15°C and then set to germinate in the various tests. The germination values shown were for 72 h. incubations. The results: mean of triplicates (standard deviation).

Substance	Concentration (mM)	Germination, (%) 1ml, 18°C	3ml,18°C
Control (water)	-	46.0 (3.0)	15.5 (1.5)
Dithiothreitol (DTT)	10	34.0 (2.5)	4.3 (0.6)
	1	51.3 (3.5)	2.3 (1.2)
	0.1	19.0 (8.2)	3.3 (1.2)
Oxidised DTT	10	31.0 (1.7)	4.3 (1.5)
	1	45.7 (3.8)	4.0 (1.7)
	0.1	17.0 (3.6)	6.0 (1.0)
L-Cysteine	10	43.0 (7.0)	26.3 (9.7)
	1	38.7 (4.2)	6.0 (4.4)
	0.1	16.3 (3.8)	4.0 (0)
N-Acetyl L-Cysteine	10	37.0 (3.6)	15.7 (4.7)
	1	50.3 (14.6)	3.7 (2.1)
	0.1	15.0 (2.6)	7.3 (2.1)
2,2'-Thio-diethanol	10	21.0 (5.3)	7.3 (1.5)
	1	25.3 (10.1)	4.7 (1.5)
	0.1	17.7 (2.1)	5.0 (1.0)
3,3'-Thio-dipropanol	10	18.7 (5.0)	5.3 (2.1)
	1	45.3 (18.8)	4.0 (2.6)
	0.1	14.3 (4.5)	4.3 (1.2)
Cysteamine	10	17.7 (4.7)	4.3 (0.6)
	1	40.0 (1.7)	1.7 (1.5)
	0.1	12.7 (5.5)	6.7 (1.5)

Table 5

Oxygen uptake by six milled samples of barley, being three pairs of samples each pair having a dormant and a warm stored/matured sample of the same lot of barley. Values: mean (standard deviation) to 2 significant figures.

	Respiration rate (μmole oxygen/grain/h)			
	Intact -	Intact sterilised	Decorticated -	Decorticated sterilised
Doublet (88)				
Dormant	0.20 (0.02)	0.14 (0.01)	0.11 (0.01)	0.10 (0.02)
Mature	0.16 (0.04)	0.12 (0.02)	0.11 (0.01)	0.06 (0.02)
Triumph (88)				
Dormant	0.20 (0.04)	0.17 (0.03)	0.11 (0.01)	0.10 (0.01)
Mature	0.10 (0.01)	0.11 (0.01)	0.06 (0.01)	0.06 (0.01)
Triumph (87)				
Dormant	0.20 (0.01)	0.16 (0.01)	0.14 (0.00)	0.18 (0.02)
Mature	0.13 (0.01)	0.14 (0.01)	0.14 (0.01)	0.18 (0.03)

Table 6

The amount of gibberellin-like material extracted from corns of quiescent samples of barley. Germination percentages and amounts of GA-like material are given as the mean ± standard deviation (n=6). Samples are from the 1986, 1987 and 1988 harvests. Tri, Triumph; Do, Doublet. The sack from which the sample was taken is designated as a, b or c. No significant differences exist between dormant and mature sub-samples of the same sack. Differences between varieties are also not significantly differenct from each other (Student's t-test).

Sample	Germination after 72 h (%)		Gibberellin like material (GA_3-equivalents; ng $corn^{-1}$)
	1 ml (agar)	3 ml (agar)	
Tri86a	96	82	0.10 ± 0.02
Tri86a	68	46	0.08 ± 0.03
Tri87a	99	86	0.09 ± 0.03
Tri87a	55	41	0.10 ± 0.05
Tri87b	99	93	0.10 ± 0.02
Tri87b	61	37	0.07 ± 0.04
Tri87c	96	94	0.09 ± 0.06
Tri87c	59	40	0.11 ± 0.03
Tri88a	94	96	0.08 ± 0.01
Tri88a	51	36	0.06 ± 0.04
Tri88b	98	87	0.04 ± 0.03
Tri88b	52	38	0.05 ± 0.03
Do88a	98	89	0.06 ± 0.02
Do88a	46	22	0.06 ± 0.03

Table 7

The amounts of gibberellin-like material extracted from steeped corns of two samples of barley. Germination percentages and amounts of gibberellin like material (mean ± standard deviation (n=6)) are given. Samples are from the 1987 and 1988 harvests. Tri, Triumph; Do, Doublet. No significant differences exists between dormant and mature sub-samples of the same sack that were steeped for the same length of time. N.S. indicates that no significant difference exist between samples steeped for 1 h. and the same sample steeped for 6 h. *indicates significance at the 5% level and ** indicates significance at the 1 % level.

Sample	Germination after 72 hr (%)		Steeping Time (h.)	GA-like material (GA_3-equivalents; ng $corn^{-1}$)
	1 ml	3 ml		
Tri87a	55	41	1	0.17 ± 0.05(N.S.)
			6	0.14 ± 0.05
Tri87a	99	86	1	0.09 ± 0.10(N.S.)
			6	0.19 ± 0.10
Do88a	46	22	1	0.04 ± 0.03**
			6	0.14 ± 0.06
Do88a	98	89	1	0.04 ± 0.04(N.S.)
			6	0.15 ± 0.10

Table 8

Abscisic acid present in various samples of grain. Results, mean (standard deviation).

	ABA (pg/g dry weight)	
Barley	Dormant	Mature
Triumph, 1989	2850 (96)	1465 (383)
Triumph, 1988	1052 (288)	752 (55)
Doublet, 1988	361 (63)	235 (41)

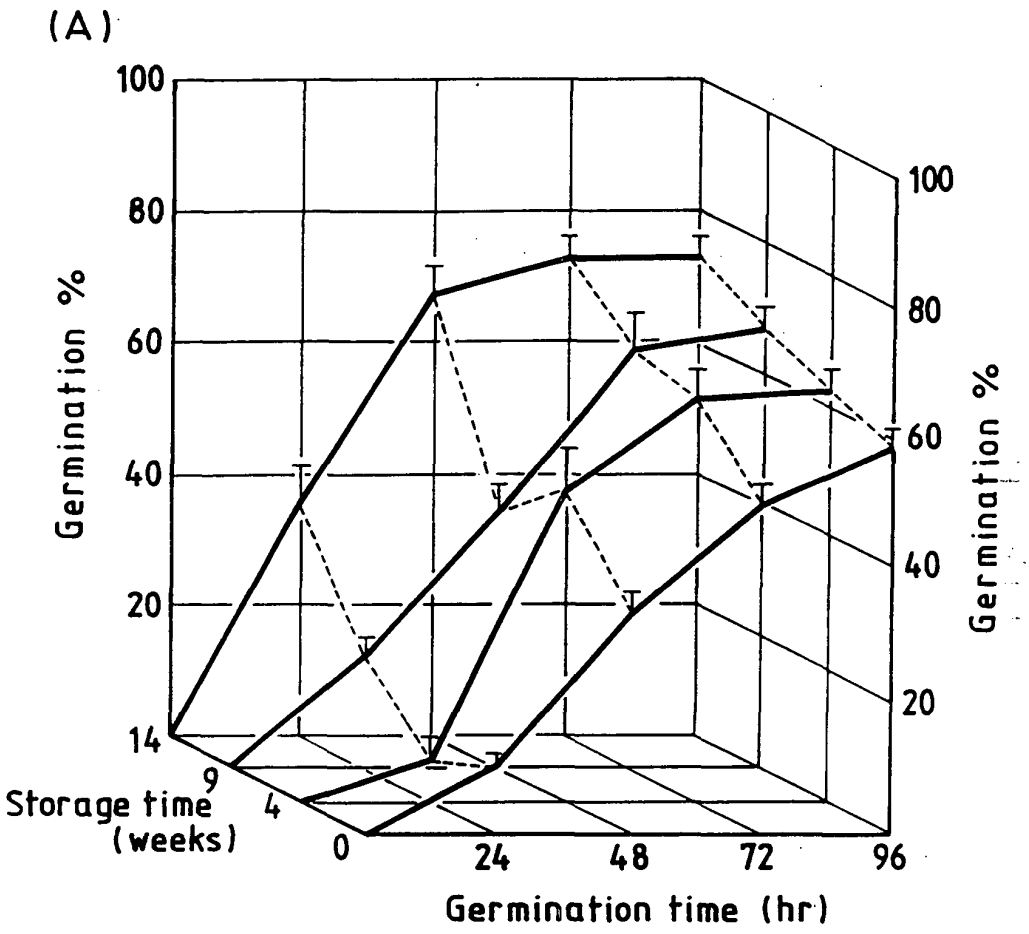
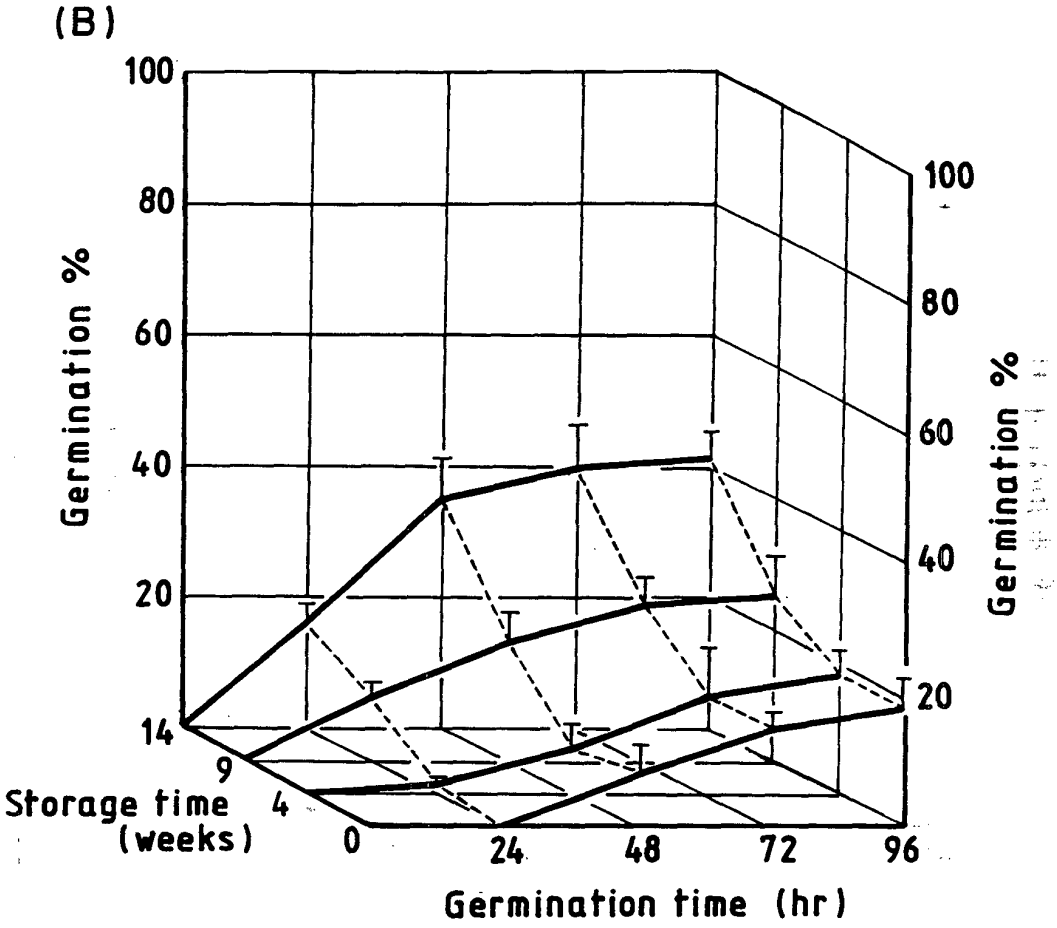
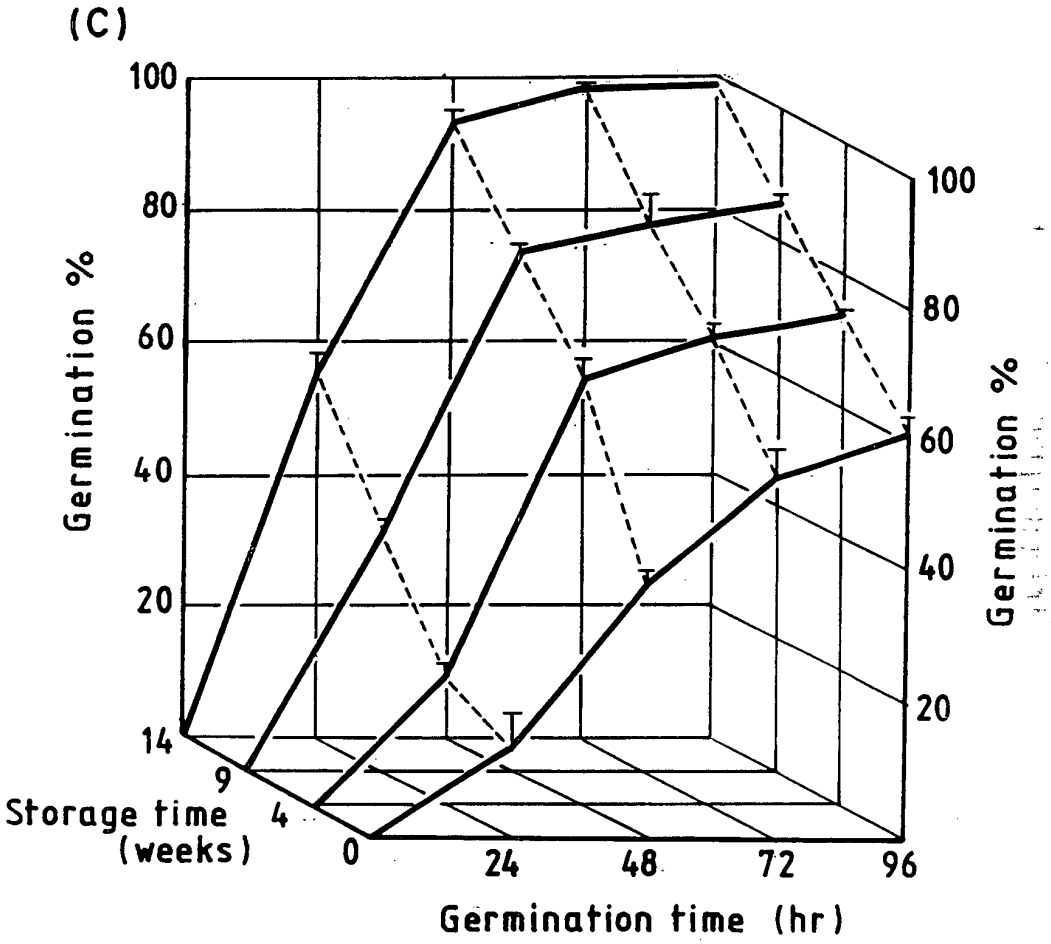
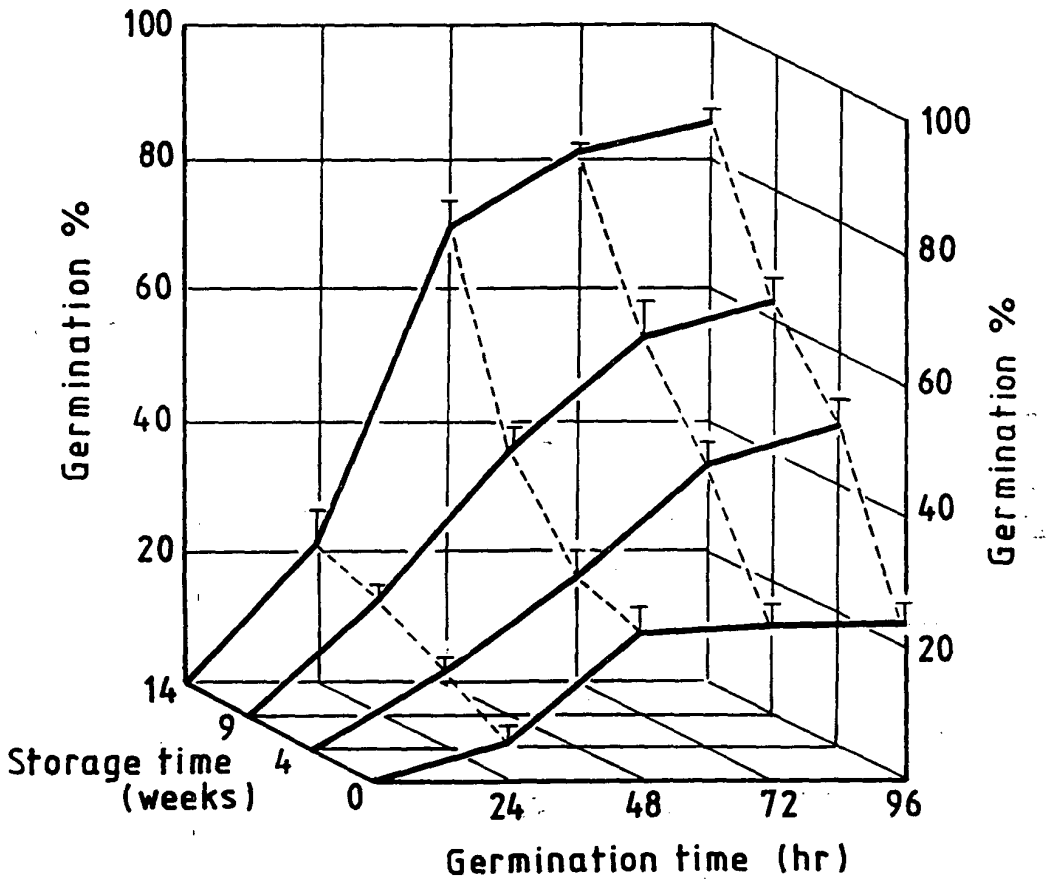


Fig 1. Plots of germination against storage time of: (A) untreated samples in the 1 ml (agar) test; (B) untreated samples in the 3 ml (agar) test; (C) samples treated with sulphuric acid (0.5%) in the 1 ml (agar) test; (D) Samples treated with sulphuric acid (0.5%), in the 3 ml (agar) test.


(B)
100
80
60
40
20
Germination %
14
9
4
0
Storage time
(weeks)
24
48
72
96
Germination time (hr)
100
80
60
40
20
Germination %


(C)
100
80
60
40
20
Germination %
14
9
4
0
Storage time
(weeks)
24
48
72
96
Germination time (hr)
100
80
60
40
20
Germination %

(D)


100
80
60
40
20
Germination %
14
9
4
0
Storage time
(weeks)
24
48
72
96
Germination time (hr)
100
80
60
40
20
Germination %

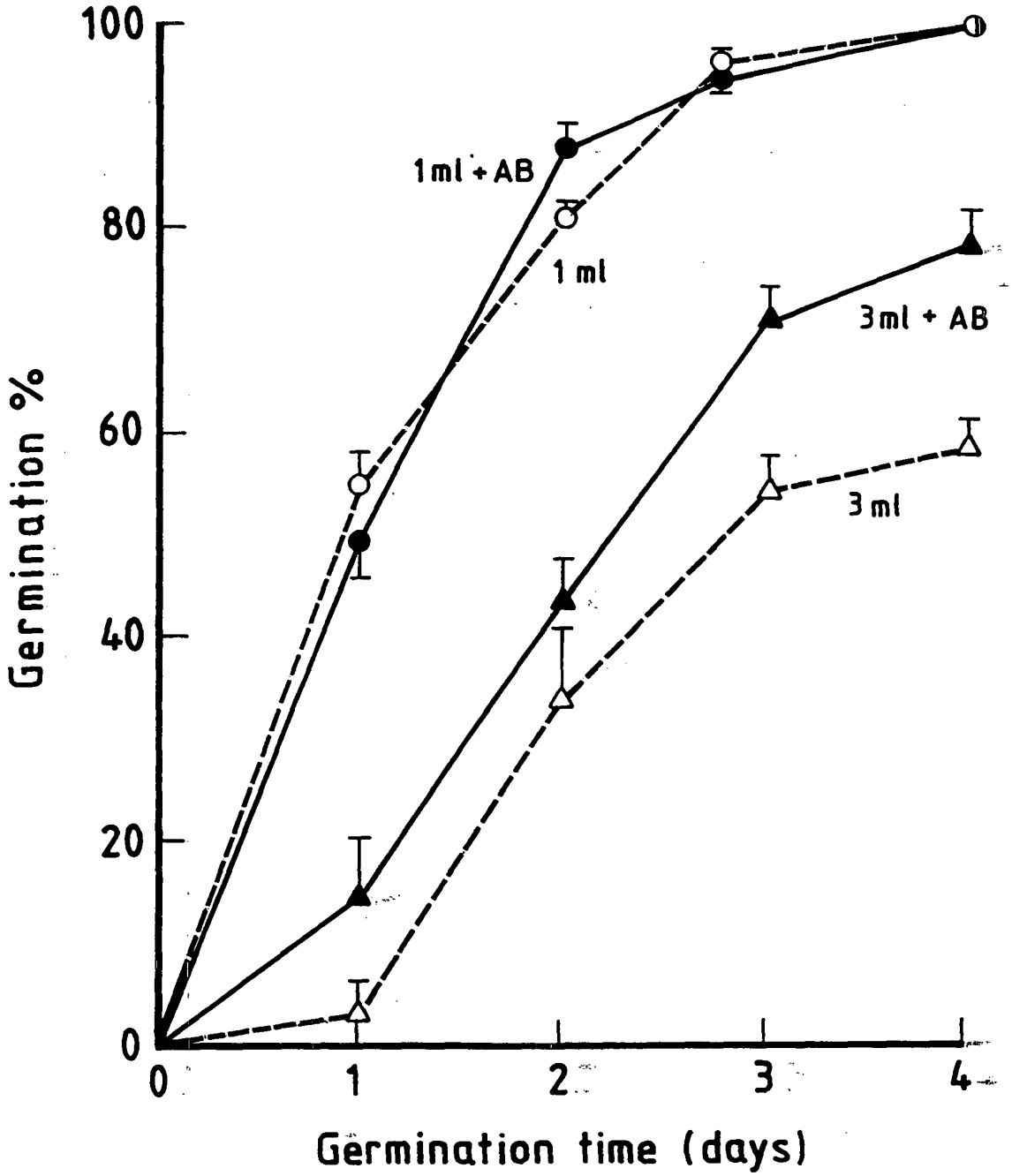


Fig 2. The influences of pre-steeping in water or a mixture of antibiotics (+AB) on the germination of a mature barley (86) in the 1 ml and 3 ml agar tests.

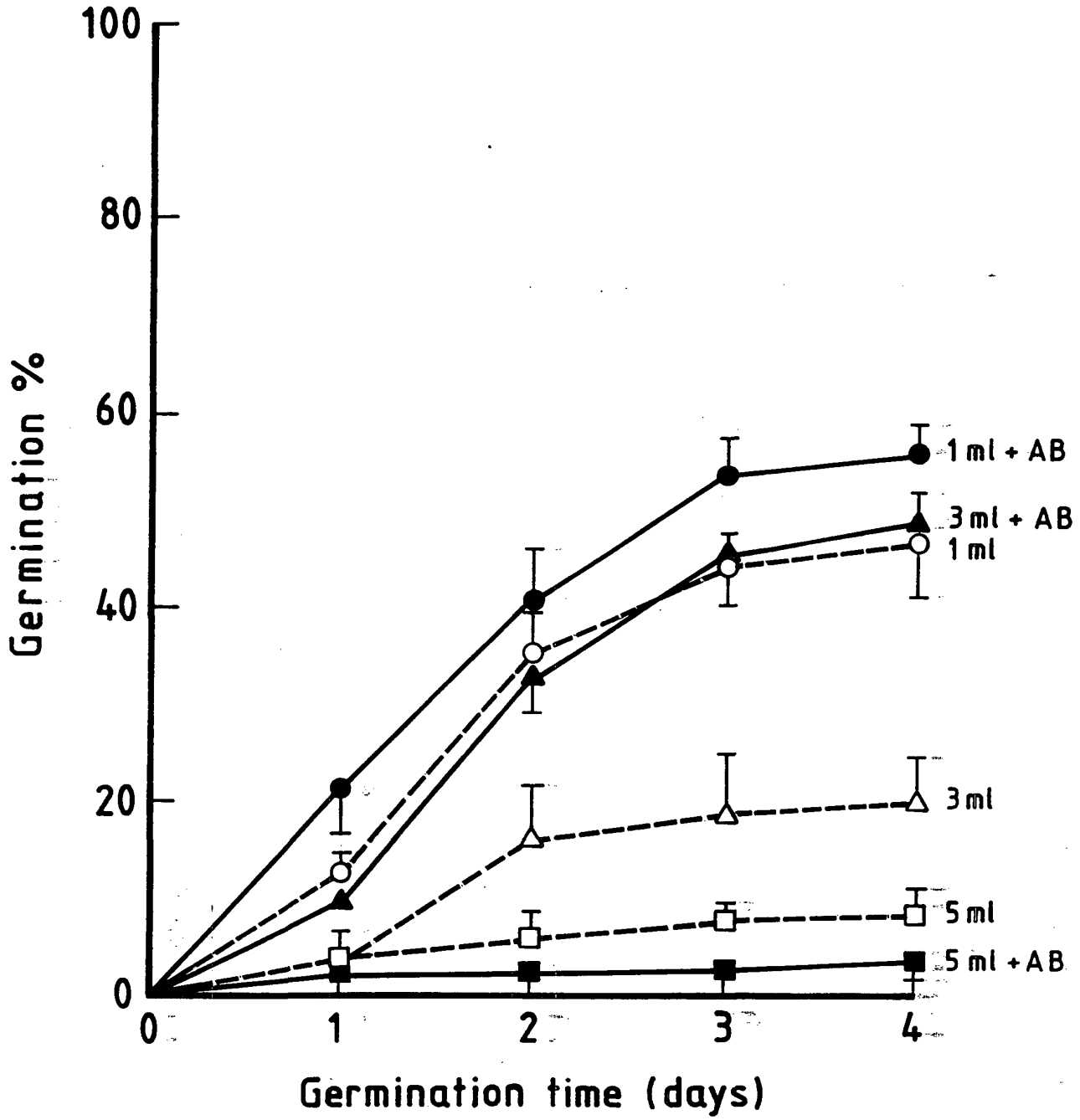


Fig 3. The influences of pre-steeping in water or a mixture of antibiotics (+AB) on the germination of a dormant barley, TDA (87), in the 1ml and 3ml (agar) tests, and in the 5 ml (25°C; agar), high-stress test.

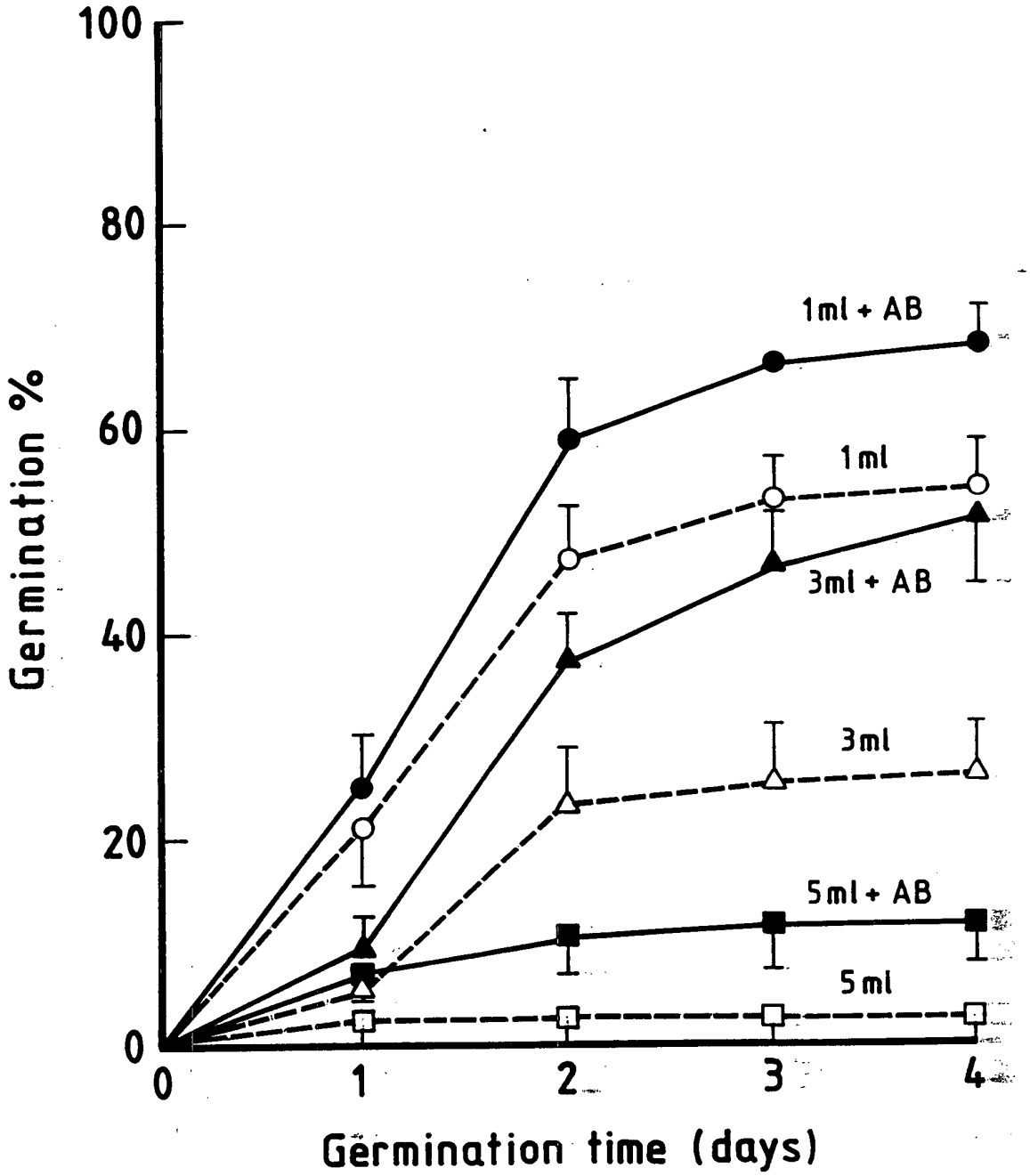


Fig 4. The influences of pre-steeping in water or a mixture of antibiotics (+AB) on the germination of a dormant sample of Triumph barley, TRA(A), in the 1 ml and 3 ml agar tests and in the 5 ml (25°C; agar) high stress test.

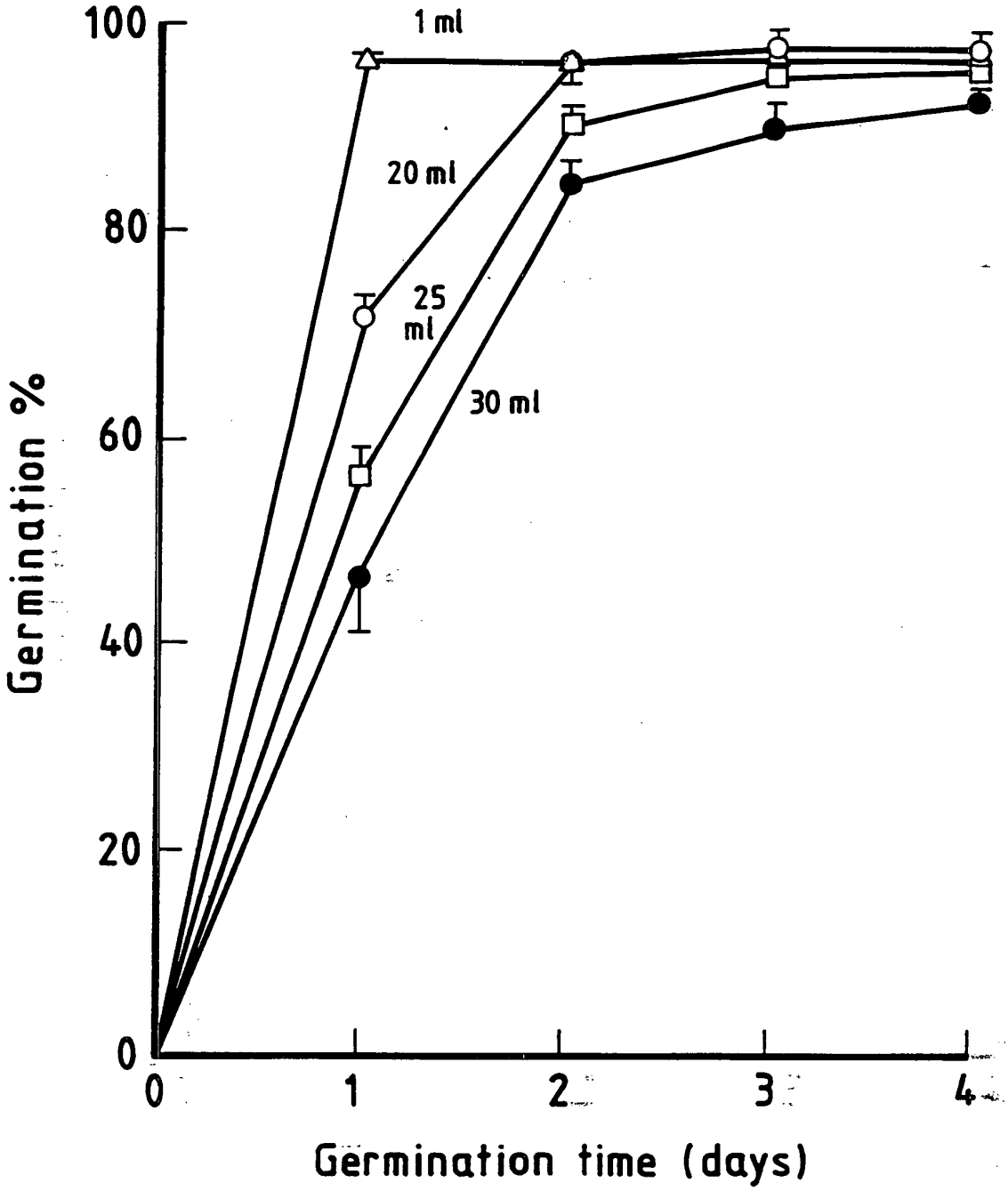


Fig 5 The germination of non-dormant barley (86), decorticated with sulphuric acid, surface sterilised with calcium hypochlorite and put to germinate under various depths of sterile, distilled water.

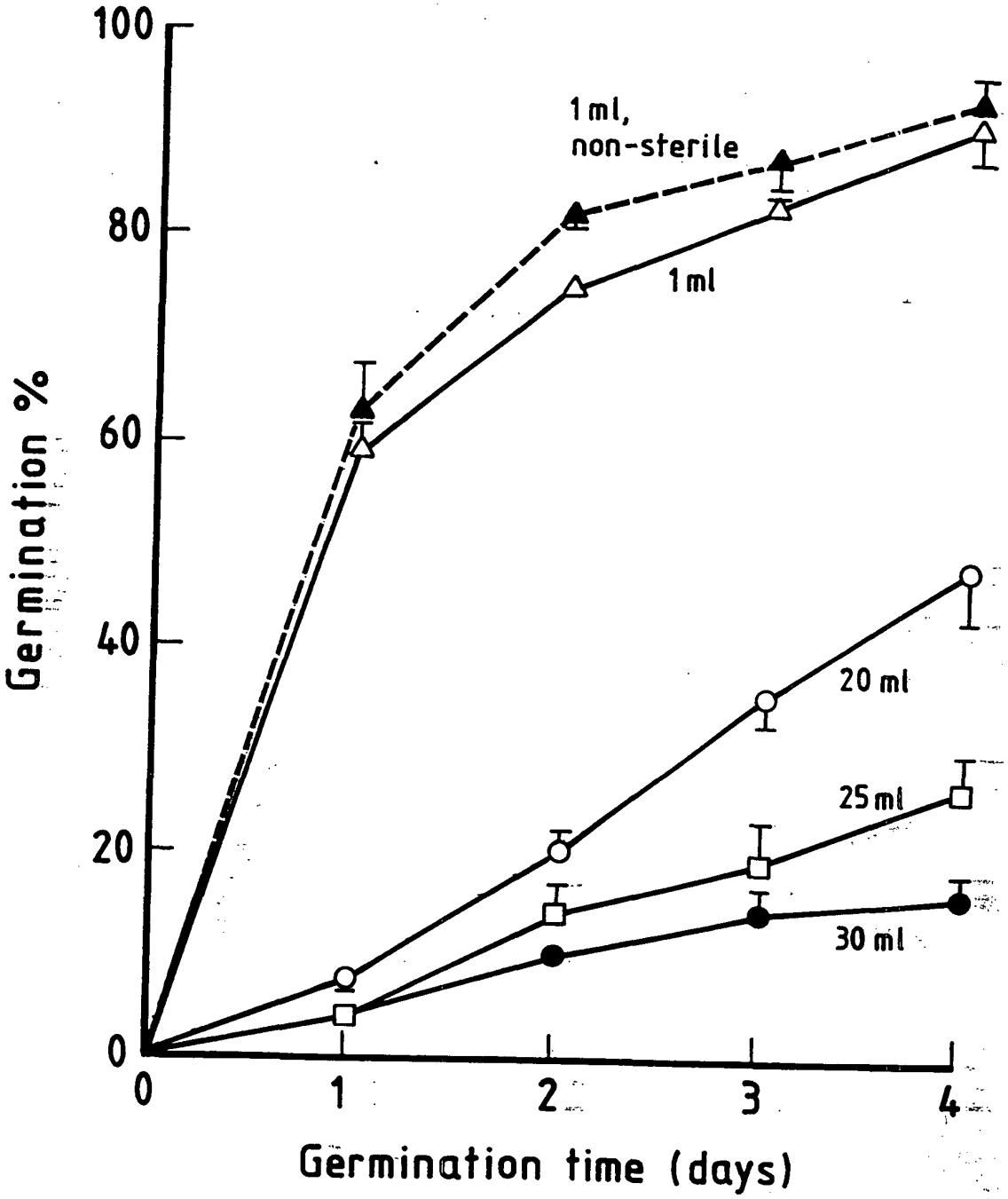


Fig 6. The germination of dormant barley (TDB 87), decorticated with 50% sulphuric acid, surface sterilised with calcium hypochlorite (except for one set of samples; dashed lines) and put to germinate with various volumes of sterile, distilled water.

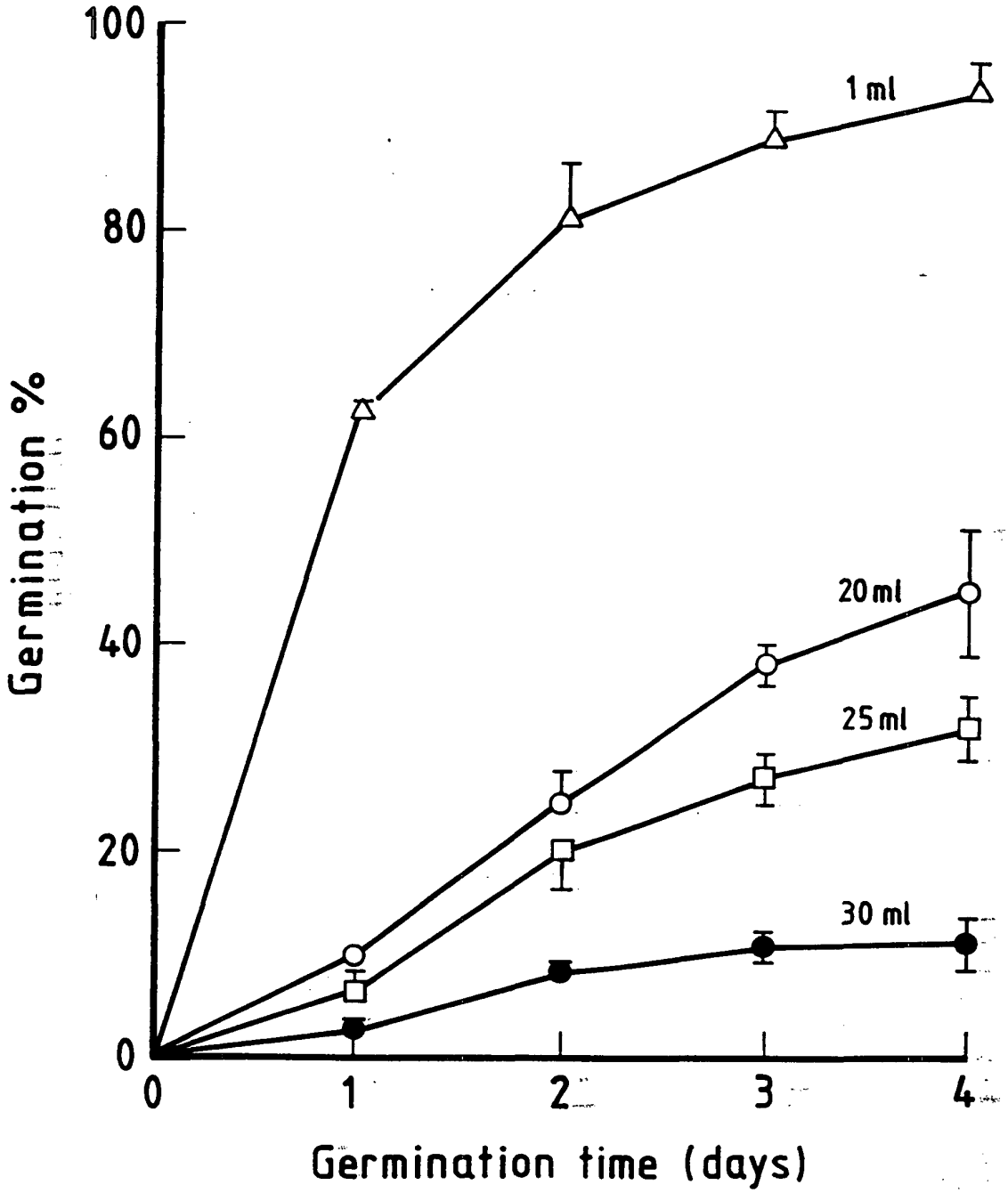


Fig 7. The germination of a dormant barley (TRA (87)), decorticated with sulphuric acid (50%), surface sterilised and put to germinate with various volumes of distilled water.

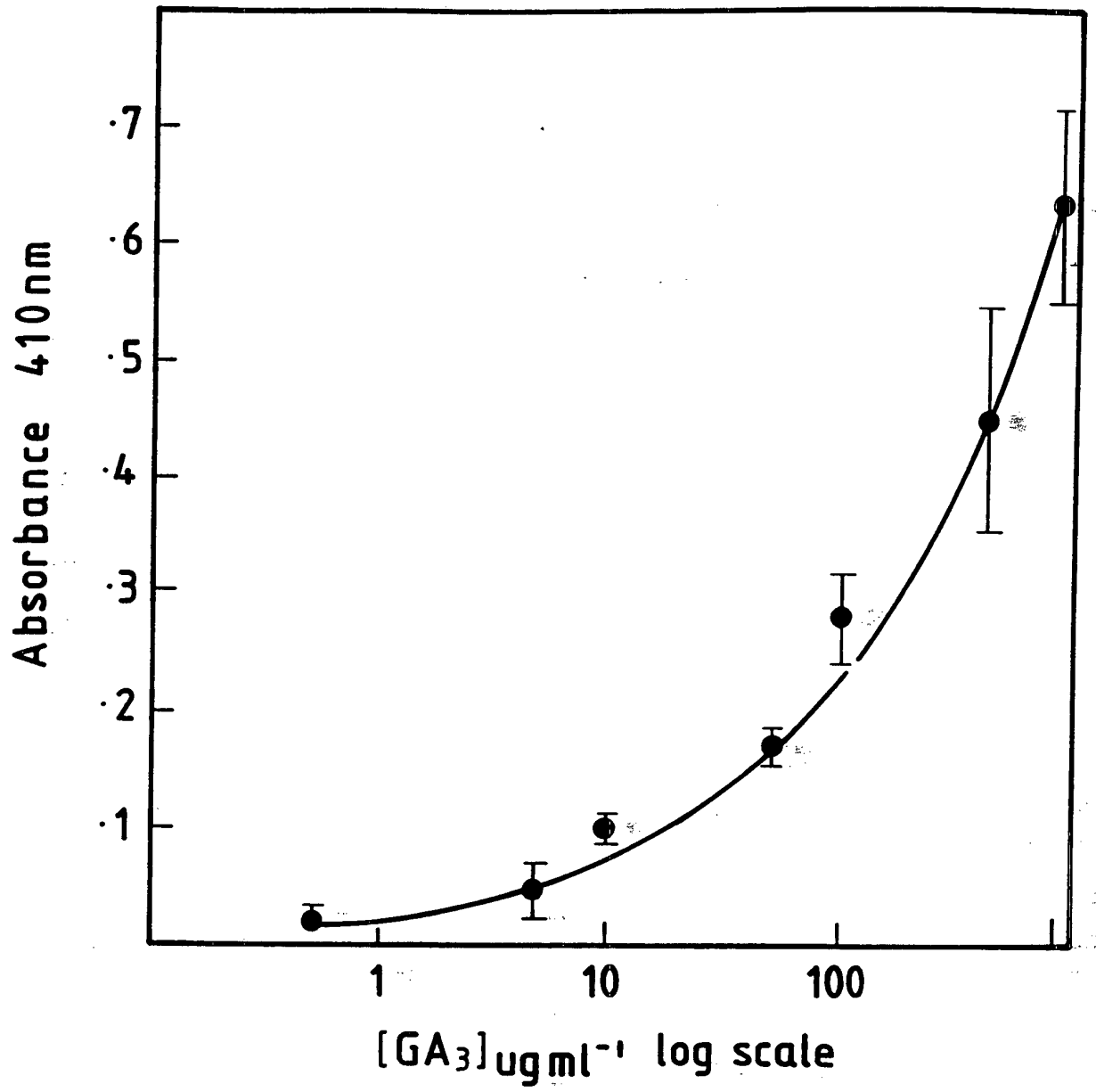


Fig 8. Sugar release from endosperms (4/paper chromatogram section), estimated colorimetrically with PAHBAH reagent, plotted against standard amounts of gibberellic acid (GA_3) originally loaded onto the chromatogram.

Endosperms were incubated (48 h., 25°C; 100% RH) in buffer (0.5 ml; succinate, 20 mM; calcium sulphate, 1 mM; disodium EDTA, 0.1 mM; pH 6.0) on sections of chromatograms (80 x 19 mm; divided into 19 x 19 mm squares). Sugars were extracted with a dilute solution of ammonia (1ml; 0.1M, 8h). Extracts were frozen before the reducing sugars were assayed.

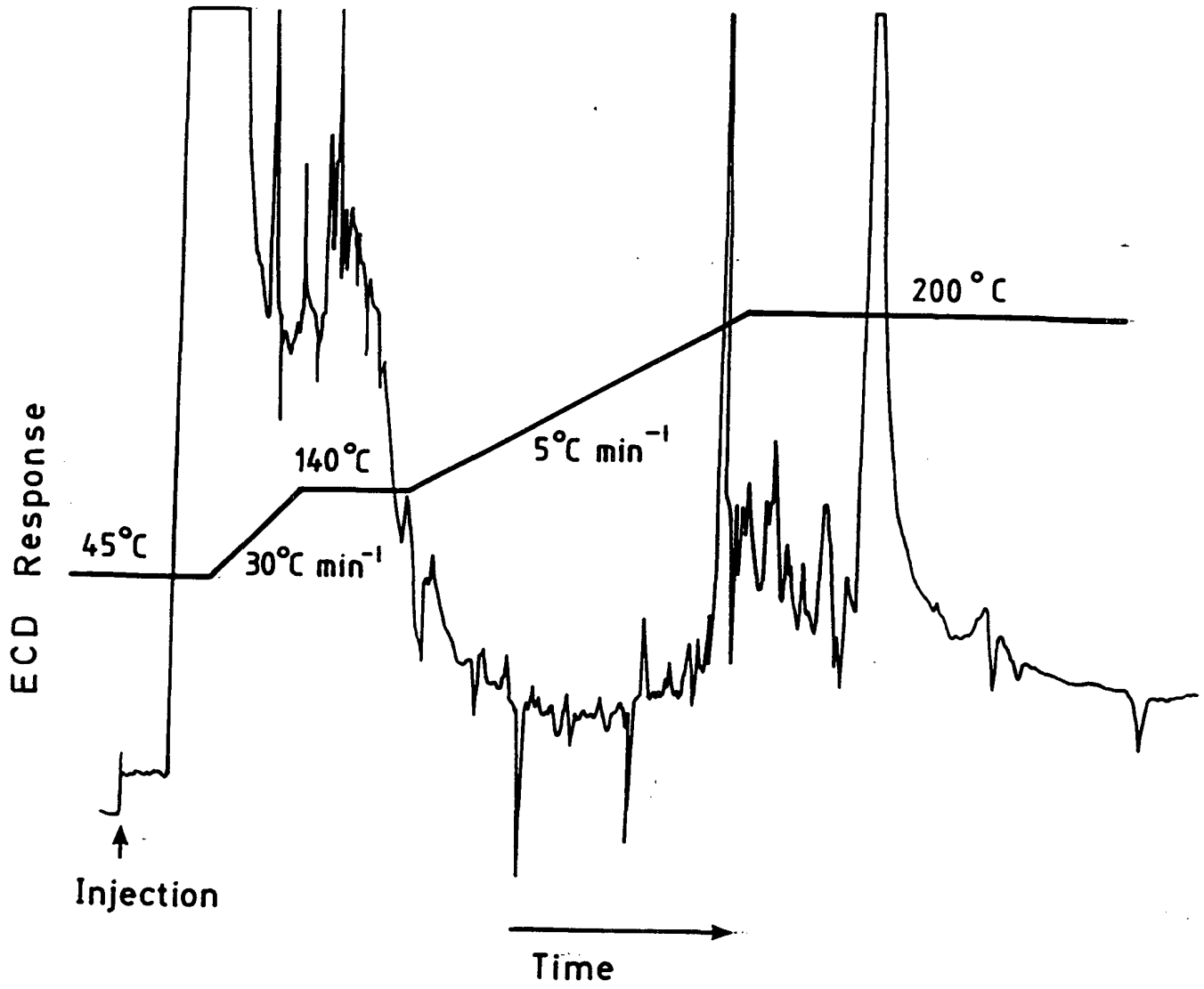


Fig 9. Analysis, by GLC, of standard samples of methyl abscisic acid, (Me-ABA) and methyl-3,5-diiodo-hydroxybenzoic acid (Me -DIHBA). Analysis was on a WCOT capillary column (30 mm x 0.25 mm i.d.), internally coated with SE-30 stationary phase (0.25 μm thick). The temperature programme was an initial 4 min. held at 45°C then a rise to 140°C (at 30°C/min), a 4 min hold, then an increase to 200°C (at 5°C/min). The hold at 200°C lasted for 15 min. The column temperature was then increased to 275°C, at 30°C/min., to purge it, before allowing it to cool to 45°C. The column effluent was monitored using an electron capture detector, ECD.