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SEED MANAGEMENT TECHNIQUES FOR GENE BANKS

Proceedings of a Workshop held at the
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A Report of a Workshop held 6-9th July 1982
at the Royal Botanic Gardens, Kew, U.K.

Sponsored by Eucarpia in association with
IBPGR and UNDP/FAO-ECP/GR

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Seed Health in Relation to the Exchange of Germplasm

P. NEERGAARD

INTRODUCTION

There is general awareness that plant health is essential to effective exchange of plant germplasm. The importance of adequate precautions was expressed in the recommendations of the FAO/UNEP/IBPGR Technical Conference on Crop Genetic Resources, held in April 1981. A practical approach was recently made by a Working Party held in June 1982 at CIAT. This "International Consultation on a System for the Safe and Efficient Movement in Global Germplasm Exchange Networks" provided commentaries on plant health provisions at the International Agricultural Research Centres (IARCs), reports being presented from seven of the centres (CIAT, CIMMYT, CIP, ICRISAT, IITA, and AVRDC). Furthermore, information was given on plant quarantine regulations in some of the host countries of the IARCs, with special reference to the import and export of plant germplasm in these centres. The two meetings presented a number of proposals and recommendations, some of which are discussed in the following review.

RISKS

Any discussion on quarantine matters must focus on two points: risks and safeguards. Obviously, safeguards depend on risks, hence these must be precisely defined.

If infected or contaminated germplasm is used for breeding, pathogens are bound to be involved in the initial process of seed multiplication. Eventually, this means an ever threatening risk of introducing new cultivars with pathogens that may be spread internationally. The risk, however, varies from host to host and

from pathogen to pathogen. This has direct bearing on plant quarantine policy and practical precautions to be adopted by IARCs, genebanks and by national quarantine authorities.

The risks vary from centre to centre according to the crops, hence the quarantine problems are different. Risks are associated with the pathogens, depending on their epidemiological potentials; with the regions where dangerous pathogens occur, or where they may develop dangerously if introduced; and finally, they are associated with the hosts according to the prevalence of dangerous pathogens affecting them.

Pathogens

Previously I have proposed that quarantine objects be categorized according to a combination of the following criteria: (1) pathogens not present in the area to be protected by quarantine precautions or present in limited localities in which they are under effective control (domestic quarantine), (2) pathogens dangerous by their direct pathogenic potential, and (3) pathogens dangerous by their potential for rapid epidemiological spread, often being transmitted from trace amounts of seed-borne inoculum.

Based on these criteria, three categories of quarantine objects are considered (see Neergaard, 1980):

Category A

Definition: Dangerous plant pathogens which are not present in the region of introduction and which have a high or considerable epidemic potential. Many pathogens which belong to this category occur in seed in only trace amounts, this rendering sampling unreliable.

Precautions: Complete prohibition against introduction from areas with infection. Testing based on sampling is not adequate. Particularly valuable material from infested areas may be "filtered" through post-entry control by growing seed-bearing plants from introduced seeds under closed quarantine, i.e. in special glasshouses that exclude escape.

Examples: Arabis mosaic virus, barley stripe mosaic virus, Echtes Ackerbohnenvirus syn. broad bean true mosaic virus, ground stunt virus, peas seed-borne mosaic virus (pea), stone fruit viruses, sunblotch virus of avocado, Corynebacterium flaccumfaciens, Corynebacterium rathayi, Alternaria trititica, Peronospora tabacina, Plasmopara halstedii, Tilletia contraversa.

Category B

Definition: Dangerous plant pathogens not present in the region of introduction (or present in only more or less restricted areas being under effective control) and which have a moderate epidemic potential.

Precautions: The seed may be tested on adequate sampling, if a suitable testing procedure is available. To be suitable for release, the tested samples must be found completely free from infection or contamination.

Examples: Xanthomonas vesicatoria, Colletotrichum lindemuthianum (pathogenic races), Phoma lingam, Pyricularia oryzae (pathogenic races; in rice), Tilletia caries and T. foetida (races; wheat), Ustilago nuda (races; barley), Ustilago tritici (races; wheat), Ditylenchus dipsaci (races).

Category C

Definition: Other plant pathogens of importance to the field planting value of seed. These pathogens are not strict quarantine objects but may be of extreme importance for quality. However, a definite risk pertaining to quarantine is the introduction of new aggressive races into the region under consideration - aggressiveness in relation to the horizontal resistance already established in local cultivars during years of isolation.

Precautions: Testing by adequate procedures of representative samples is advisable. While disease tolerance may be acceptable for ordinary seed trade consignments, according to certification standards of the importing country or according to international standards, it is advisable that material from gene centres be handled with more care in an attempt to prevent the introduction of pathogenic races, including both specific races ("vertical pathotypes") and aggressive races ("horizontal pathotypes") from centres of diversity. Eradicant seed treatment may be considered.

Examples: Alfalfa mosaic virus, soyabean mosaic virus, soyabean stunt virus, Corynebacterium michiganense, Pseudomonas lachrymans, Pseudomonas phaseolicola, Xanthomonas phaseoli, Ascochyta rabiei, Septoria apiicola, Septoria nodorum.

It must be emphasized that, the allocation of any pathogen to any of the above categories depends on whether or not the pathogen (including pathogenic races, if any) is endemic in the region of introduction, and on the effectiveness of the surveys of pathogens present in both the countries and regions involved.

Considering seed-borne pathogens occurring in germplasm of major crops, those referred to "Category A" are of high risk. Among these we may consider pathogens such as loose smuts, Ustilago nuda, blue mould, Peronospora tabacina, dwarf bunt, Tilletia contraversa, many viruses in soyabean and other legumes.

There is special risk involved in pathogens with specific pathogenic races. A pathogenic race introduced into a new region may attack cultivars with established resistance to the local races. This risk is almost totally neglected in quarantine precautions of today and is ignored also by some plant breeders and plant collectors.

Some pathogens that may occur in germplasm and which contain pathogenic races are listed below and the approximate number of known races is given in brackets:

Peronospora manschurica (40) in soyabean, Plasmopora halstedii (4) in sunflower, Alternaria triticina (6) in wheat, Cercospora oryzae (10) in rice, Fusarium oxysporum (69 formae speciales, of which at least 10, each with 2-11 pathogenic races) in numerous hosts including many major crops, Pyricularia oryzae (132 race groups) in rice, Sphacelotheca sorghi (6) in orghum, Tilletia caries/foetida (30) in wheat, T. contraversa (109) in wheat, Ustilago hordei (15) in barley, U. nuda (22 race groups) in barley and U. tritici (20) in wheat, Xanthomonas malvacearum (17) in cotton. Many viruses include strains specific to host ranges of species or cultivars, e.g. bean common mosaic virus, 11 strains.

Destructive pathogens containing pathogenic races should be considered as high risk quarantine objects, also in cases in which the species concerned are present in the importing country.

Regions

High risk regions are, by nature, centres of origin and some are centres of breeding. The centres of origin are not only centres of genetic diversity of hosts but of their pathogens as well. There is a concentration of pathogenic races in the centres of crop plants and their close relatives, the wild forms. Therefore centres of origin of crop plants are high risk areas where especially strict quarantine precautions should be taken.

Some Agricultural Research Centres and Plant Breeding Stations constitute great potential when they deal with pathogens having high epidemiological potential and containing pathogenic races of limited international distribution. In contrast, other such centres may be fairly low risk localities because they are relatively safe in terms of crops free from dangerous pathogens and with low possibility of attack by such pathogens.

Hosts

Some hosts harbour a considerable range of destructive pathogens while others are relatively free from such pathogens. A high risk crop is rice, especially because of blast, Pyricularia oryzae; also legumes are at high risk being attacked by a considerable range of destructive seed-borne fungi, bacteria and viruses - soyabean and Phaseolus bean especially suffer from many seed-borne diseases. In U.S.A. Leppik (1969) found that in 4,500 plant introductions an average of 6 percent were infected with foreign pathogens of potential danger to the agriculture of the country. The highest percentage, however, was detected in large-seeded legumes, about 5 percent of the consignments were infected. Introduced pea seeds produced 17 percent infection and Lathyrus up to 21 percent (Leppik, 1969).

Recent investigations on plant breeding material of pea and soya-bean confirm the danger of uncritical use of seed introductions in breeding programmes, and the need for well-organised quarantine in this connection. Zimmer and Ali-Khan (1976) recorded, in testing the seed only for pea seed-borne mosaic virus, that about 9 percent of 1,235 breeding lines of Pisum arvense were infected, 15 out of 100 commercial seed samples had 1-2 percent infected seed. In U.S.A. Hampton and Braverman (1979) serologically tested 1,835 plant introduction lines of pea for presence of pea seed-borne mosaic virus and found 23 percent of the lines to be infected.

Goodman et al (1979) serologically tested 897 accessions of soya-bean germplasm collections for soyabean mosaic virus. Out of 497 lines of temperate maturity groups only 83 had no seed transmission when a sample of 200 seeds from each was planted. In the remaining 414 lines infections ranged from 1-36 percent. Of 400 tropical germplasm lines similarly tested 294 lines were infected, infection ranging from 1-10 percent.

On the other hand, to some extent it may be permissible to consider some cereals as low risk crops, provided that safe eradicant seed treatment is practised. Such relatively low risk crops are maize, pearl millet and wheat (except for loose smut).

SAFEGUARDS

The first of five recommendations on quarantine precautions that were made by the FAO/UNEP/IBPGR Conference in 1981 was that "all germplasm exchange should take place through national quarantine services", the second that "setting up national and regional laboratories should be considered by governments to expedite the passage of germplasm through quarantine".

What are the realistic possibilities for bringing these recommendations into effect? To answer this, the following facts should be considered:

(1) The number of collected individual portions of germplasm is enormous. Undoubtedly every year tens of thousands of individual accessions of seeds from wild plants and from cultivated "escaped" plants are collected in many different regions throughout the world. These accessions are usually further divided after some processing before the seed is stored. The processing may be very different according to technical capacity and competence. Additional portions of processed germplasm that have been stored for a period of time are distributed from the genebanks.

(2) The size of individual accessions collected varies much, from perhaps a few scores of seeds to some kilos. This entails narrow limitations for proper testing, sampling being virtually impossible in many cases.

(3) Usually no plant pathologist is associated with expeditions which collect germplasm. Often, materials are collected by taking whole heads and putting into bags thus favouring inclusion of pests and pathogens.

(4) The seeds may be partially cleaned, dried and dusted with fungicide or insecticide. The presence of treatment chemicals may seriously hamper quarantine inspection of the seed.

(5) National quarantine services are not always equipped and staffed to handle germplasm material. Most quarantine services are simply not prepared to screen such material because information is not available about the specific pathogens that may be encountered and the risks involved. Also knowledge about adequate

techniques to be adopted, or facilities to carry out tests may not be available for inspection of these small consignments.

(6) Germplasm is distributed, with or without processing including cleaning, to many stations and individuals all over the world. This means that the quarantine services of many countries are involved, hereby implying repetitions of inspections.

(7) Because national quarantine services usually are not adequately equipped technically to screen germplasm materials e.g. for microbiological checking, considerable amounts of such materials are passed, virtually without any control.

(8) The fact that uninspected germplasm thus may enter with the approval of quarantine authorities, inevitably leads to feeling of safety under false pretences. Naturally, this bears undesirable consequences for subsequent further international distribution of the materials.

These remarks suffice to show that handling of germplasm for quarantine is difficult, especially because of the great genetic variation of both the hosts and their pests and pathogens. Hence, a considerable variety of testing techniques is required for quarantine. A further difficulty is the huge number of accessions in international exchange, whether newly collected or delivered after storage in genebanks. Finally, yet another difficulty is that very often the individual consignments are so small that there is little or no material for adequate testing.

All this calls for extremely economical handling of germplasm material. Therefore, international organization of quarantine for seed of germplasm is imperative.

Obviously, certain national plant quarantine services have a special responsibility; namely those in countries, (1) where centres of origin are located, (2) where major genebanks are located, and (3) where major plant breeding centres, particularly the International Agricultural Research Centres, are located. In these countries, adequate facilities and staff must be made available for quarantine seed health testing, if this has not already been done. Testing should be adequate for all four categories of seed-borne pathogens: fungi, bacteria, viruses and nematodes. The actual situation in this respect is far from satisfactory. In some of these countries, however, where high priority should be given to "setting up national and regional laboratories to expedite the passage of germplasm through quarantine", a beginning has been made, in some cases a substantial beginning. Four examples are:

In India a well equipped seed health testing unit is in operation at the Bureau of Plant Genetic Resources in New Delhi. Through this institution annually about 60,000-100,000 seed samples of genetic resources are exchanged. They are tested for the four categories of seed-borne pathogens, a staff of ten plant pathologists and additional technical staff being responsible for the screening.

Mexico is another example of a country where initial steps in quarantine screening have been taken, though still on a very modest scale. All seed samples for exportation from CIMMYT are inspected visually by the quarantine authorities. These seed samples look very clean, I have been informed, in contrast to commercial seeds. Out of a total of perhaps 30,000 seed samples inspected annually in this way, 500-1,000 samples are tested by incubation procedures, i.e. somewhat less than 5 percent of all samples exported by CIMMYT. The samples to be health tested by

incubation methods are selected according to genetic background (personal communication, S.D. Sanchez).

Nigeria is the host country of IITA. About 75 percent of the work of the Nigerian Plant Quarantine Service at Moor Plantation, Ibadan in the period 1975-1981 emanated from IARCs plant inspections (including those of the plant importations of WARDA) (Aluko, 1982). The station consists of a seed health testing laboratory with a staff of about 25 persons, and with a considerable number of small, closed glasshouse units for post-entry quarantine.

In Kenya the quarantine station at Muguga has facilities similar to those of Nigeria, including a well organized post-entry quarantine station with many closed quarantine glasshouse units. Moreover, it has a unit for meristem culture to clean up infected plant materials, thus saving valuable genetic resources that otherwise would have to be discarded.

The responsibility for providing healthy genetic resources in international exchange and for plant breeding evidently must be shared between on the one hand, the official quarantine authorities, and on the other the institutions handling these materials, i.e. the genebanks and the plant breeding stations, especially the IARCs.

Genebanks today are in most cases not adequately equipped and staffed to screen the incoming germplasm for health. The Seedbank of the Royal Botanic Gardens, Kew, U.K. tests the seed for germination on plain agar in small plastic flasks. This enables transplantation of the seedlings without loss of materials. It also enables observation of the health condition of the seedling. During further growth of the plants infections may be detected by regular inspection at all growth stages. I doubt whether any other genebank has a plant pathologist on the staff.

Plant breeding centres usually have several plant pathologists on their staff and well equipped laboratories for plant disease research. Therefore there are great possibilities for production and screening of healthy materials. By agreement with the Government of India an Export Certification Quarantine Laboratory has been established at ICRISAT under the overall authority of the Government Central Plant Protection Training Institute at Hyderabad. Annually on an average about 12,000 seed samples of germplasm are imported and about 50,000 samples exported by ICRISAT. Nirula (1980) has given details on the testing for quarantine carried out by the laboratory which is well equipped and staffed for the purpose.

The idea of establishing laboratories at plant breeding stations as official quarantine substations such as done at ICRISAT in order to expedite quarantine screening should also be considered for genebanks.

SEED HEALTH TESTING PROCEDURES

It must be emphasised that testing methods must be selected according to the pathogens to be considered, hence the specific risks involved in any consignment must be known.

Briefly listed, the standard procedures include:

(1) Inspection of dry seed (before cleaning of the sample). This should be made for all samples tested.

(2) Washing test. This is a quick test that can be widely used. It should be used for seed that may be contaminated with cereal smuts of the covered type, and with seed contaminated with oospores of downy mildews, as for many cereals.

(3) Standard blotter test. A versatile test for many seed-borne fungi; especially those belonging to the Fungi Imperfecti.

(4) Deep-freezing method. This is a modification of the blotter method, sometimes more sensitive than the standard method.

(5) Agar plating method. This test is more sensitive for some fungi, but for others it is less so than the blotter method. It is, however, very useful for specific tests using selective media such as for detection of certain important species of Fusarium.

(6) Embryo count procedure. This is used to detect loose smut in barley and wheat, and downy mildew in pearl millet.

(7) Indicator test. Greenhouse facilities are necessary for testing for viruses by inoculation to indicator plants.

(8) Serological procedures. Antisera are available for a number of seed-borne viruses and the tests are easy and quick. Some of the antisera are available with instructions for routine testing.

(9) Growing-on procedures. Glasshouse facilities are necessary for such tests.

Post-entry quarantine is based on growing-on testing in closed glasshouse units, as described by Sheffield (1968) and Neergaard (1979).

SANITATION OF GERMPASM FOR QUARANTINE

(1) Plant pathologists should be associated with plant collecting expeditions. At plant breeding centres (such as the IARCs) plant pathologists would naturally be associated with "an internal multidisciplinary committee of scientists in charge with over-

seeing the application of phytosanitary standards as they are applied to germplasm destined for international networks" (ref. proposal 3.1 of the International Consultation on a System for Safe and Efficient Movement of Germplasm, Cali, Colombia, June 1982).

(2) The health condition of plants at the first point of collection should be carefully studied. Where plant populations contain different stages of maturity, plants with green leaves and flowers should be carefully inspected for symptoms. Any disease observed on plants even though not taken into the collection should be recorded and included in the description of an accession, even when the mature plant parts actually collected appears healthy to the collector.

(3) Appropriate fumigation of the seed against insects etc. should be carried out in a fumigation chamber which should be available at genebanks and plant breeding centres.

(4) Physical cleaning of seeds whether collected in nature or harvested in experimental fields should be made by using the best technique available. Often use of special laboratory seed cleaners of different types are preferable to hand cleaning. Appropriate precaution against cross-contamination must be taken such as cleaning machinery between samples.

(5) Seeds and other materials discarded from cleaning should be submitted to dry inspection and subsequently to incubation tests.

(6) For accessions with very few seeds economical procedures will have to be used in incubation tests, such as plating on plain water agar in test tubes or flasks thus providing conditions for using symptom-free seedlings for further growth.

(7) Hot water treatment of small seed consignments is used for certain kinds of seed following standard procedures such as those developed by the NBPGR, New Dehli. Treated seed should always be retested for residual infection.

(8) Chemical seed treatment for plant quarantine is a controversial subject. Unless well-tested eradicator chemicals are used, seed treatment usually leaves residual infection not acceptable in quarantine. Furthermore it is difficult to test treated seed for viable seed-borne inoculum. For this reason seed treatment carried out by the exporter should be avoided; this is especially true for seed treatment intended merely to improve field germination and not for the eradication of seed-borne pathogens. Seed treatment for improvement of stand, such as now used for wheat samples dispatched by CIMMYT, should be carried out by the quarantine agency of the importing countries after appropriate inspection of the seed.

The possibilities for using eradicator seed treatment are still under investigation (Maude, 1983, has reviewed this subject). Very few eradicator chemicals effective against specific seed-borne pathogens are available. Some eradicator chemicals may be versatile, others selective, and these are applicable if they are effective against pathogens under quarantine. Treated seeds should be retested.

(9) Meristem culture. The FAO/UNEP/IBPGR Conference, 1981, recommended "that research initiatives should be taken in the use of in vitro techniques for "cleaning up" plant germplasm to meet quarantine requirements especially as regards viruses". As mentioned, meristem cultivation has been introduced at the Plant Quarantine Station, Muguga, Kenya for purification of infected seed material. At CIAT, meristem culture is in use for sweet potato and cassava. Germplasm of both crop plants is now distri-

buted in the form of tissue cultures, and this has substantially simplified passage through quarantine.

INTERNATIONAL ORGANISATION OF QUARANTINE FOR GERmplasm

(1) Training. The FAO/UNEP/IBPGR Conference, 1981, recommended "that consideration should be given by FAO to the organization of training courses dealing with problems of plant quarantine". Such courses should be given for quarantine officials as well as for officers operating seed testing of germplasm at genebanks or elsewhere.

(2) With a view to the phytosanitary activities at IARCs and other institutions, such as genebanks, involved in international germplasm exchange, the issue of Plant Germplasm Health Statements (PGHS) by such institutions has been proposed by Dr. R.P. Kahn. The proposal was considered by the International Consultation at Cali, 1982. This Consultation made the following statements:

- (i) The participants generally endorse the concept of the PGHS being issued by institutions involved in coordinating international germplasm exchange networks. CIMMYT and ICRISAT indicated that they considered the Phytosanitary Certificate satisfactorily covers the movement of germplasm. Both institutes recognized that there may be a need for a statement of additional assurance for high risk crops.
- (ii) The Consultation wishes to clarify that the PGHS in no way replaces a Phytosanitary Certificate, the issue of which remains the exclusive right of each national quarantine service. Any such PGHS should clearly indicate that the document is not an alternative to internationally recognized phytosanitary certificates.

- (iii) The Consultation recommends that the issuing of a PGHS remain entirely voluntary and that each issuing institution will decide independently, based on health risk analysis, whether a statement is necessary and what form the statement would take. Each PGHS will be appropriate to the needs of the particular centre but would include a list of all safeguards which had been utilized to ensure the health of the germplasm included in the germplasm exchange under consideration.
 - (iv) The Consultation recommends that a PGHS be issued to accompany only germplasm which has passed phytosanitary procedures which would be specified in the PGHS.
 - (v) The Consultation recommends that the PGHS in all cases be identified with an accompanying PSC from the national quarantine service of the host country of the institution shipping the germplasm materials.
- (3) Third country plant quarantine control. The Consultation at Cali, made the following general conclusions:

The participants at the Consultation encourage the development of third country plant quarantine activities to facilitate the international movement of germplasm, particularly original germplasm collections.

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DISCUSSION

Chairman: Hawkes

Participants: Hawkes, Williams, Neergaard,
Roberts, others

In summing up Prof. Neergaard's talk and leading the discussion, Prof. Hawkes focussed on the conflict between the needs of plant breeders on the one hand, i.e. unlimited access to material, and on the other, the very real need for quarantine and restriction of movements. There is certainly room for more understanding and compromise between the two, when quarantine officers appear to take delight in preventing introductions and require immediate destruction of certain material. In applying international regulations, it is assumed that all the risks covered by them are real, whereas Prof. Neergaard's talk had shown that this is not always the case. In the talk certain kinds of pathogen were shown to be of low risk, presumably presenting no problems in introductions. However, research into what kinds of pathogens have low risks could be a complicated and time-consuming process.

Dr. Williams was concerned to restrict the discussion to the effects of seed health and quarantine upon the practical running of genebanks. During the past decade there has been dialogue between breeders, collectors, quarantine services and conservers, leading to a series of ideas to improve regulations and international collaboration. This approach is useful with specific programmes such as the CIMMYT breeding programme for wheat in the hot, humid tropics; but when breeders' collections and such like are excluded the time factor becomes vital for many genebank operations. As an example he went on to describe the activities of IBPGR. This body was set up in 1974 and it had been anticipated that all the collecting would have been done in ten years; and while it now seems that 20 years is a better estimate, it is still a limited time scale in which to carry out much work. Indeed it

constitutes an emergency situation where material may be lost forever, if it is not collected and put into storage within the next few years. IBPGR is dealing with 54 crops and as practical examples it is worth considering the 16 species of Aegilops and the micro species of Brassica collected in the Mediterranean. These groups are both endangered and urgent, and yet quarantine bodies could make precious few practical recommendations for dealing with them. Dr. Williams cited another practical example, where the important sub-tropical cereal pearl millet was collected in Africa and needed to be sent to India. As a practical way to avoid quarantine problems the material is being maintained in Canada.

It is useless to expect improvement of post-harvest quarantine through improvement of inefficient national quarantine services, because this will just not happen quickly enough. Unless the material is part of a long-term programme, quick measures are called for. The Technical Conference in Rome, 1981, had maintained the pious hope that the national quarantine systems would be effective, but this can only work if both the material and pathogens are well known. However, there is much conservation material about which little is known with regard to quarantine measures and it is unlikely we can afford to wait for both improved regulations and research. Dr. Williams could see no alternative at present to a working compromise, whereby good scientists are or should be employed in genebanks and every effort is made to maintain good relations between them and the pathologists.

Prof. Neergaard agreed with Dr. Williams and thought useful information may be gathered by investigating the health conditions of material being collected, and went so far as to advocate genebanks having quarantine sections within them, bearing in mind that most quarantine services are not competent to handle conservation

material, and citing instances of incompetent interference with collections. In passing, he also mentioned the possibility of setting up parallel germplasm banks of pathogens, but Prof. Hawkes thought that with their limited resources genebanks may not be too keen to interfere with these collections, or to start quarantine sections within their walls.

Prof. Roberts suggested that as regards seed banking sensu stricto there is no immediate problem; the problems only arrive when it comes to regeneration or exchange. Current figures suggest that with wheat for example there would be no need to regenerate for over 50 years, so there is theoretically no immediate problem. He remarked that the amount of research advocated by Prof. Neergaard is enormous and would be impossible to carry out in the time scales mentioned by Dr. Williams. Prof. Roberts proposed that for material where there is some danger of complete loss, it may be possible to collect material quickly and get it into storage by allowing a by-pass of quarantine regulations for certain genebanks and allowing them no regeneration material except under special conditions. Evaluation and pathogen checks could then be done at a later date, once the material was in the bank.

Prof. Neergaard suggested that it may be helpful for genebanks to have the same kind of arrangement as agricultural centres whereby a phytosanitary statement (PGHS in talk), as opposed to a certificate was issued, e.g. if a seed treatment had been applied, then that information could be made available with the sample. This particular example caused Dr. Ellis to express concern over the problems due to the abuse (misuse) of seed treatments by stations issuing phytosanitary certificates.

Improved Monitoring Tests for Seed-Borne Pathogens and Pests

B.M. GERARD

INTRODUCTION

The risk of introducing plant pathogens and pests to new regions, thereby creating new crop health problems, may have increased with the recent expansion in the number of genebanks in the tropics. Factors that may be crucial are mostly associated with the natural profusion of seed-borne pathogens and pests in crops during the growing season in tropical countries, the greater prevalence of weeds which may be important sources of infection, and the low level of effective control measures. In addition, short-term genebanks (active collections) in the tropics often operate at high temperatures and humidities which favour the development of insect pests of stored seeds. There may also be a shortage of suitable equipment for specific inspections for seed pathogens, insufficient mesh-screened houses for the protected cultivation of plants during multiplication and regeneration, and a lack of suitable trained staff.

Seeds collected for genebanks come from various sources. Seeds from plant breeder's material and commercial crops present a minor risk because frequently they have been given adequate protection from the commonest pests and diseases that infect seeds. Other seeds with rarer genes are obtained by collectors from landraces and wild species, which seldom receive crop protection measures, and thus may be infected with a wide range of pests and diseases, possibly including unusual seed-borne species that have not been carried previously in international commercial trade.

TYPES OF PATHOGENS AND PESTS IN SEED COLLECTIONS

Pathogens (fungi, bacteria, viruses, viroids, spiroplasmas, mycoplasmas, parasitic weeds) (Neergaard, 1977) and pests (insects, mites nematodes) (Gerard, 1979) that are carried in seedlots may be either seed-borne or present as contaminants on the surface of the seed, in plant debris, or infecting weed seeds. Seed-borne species which can survive long periods of storage in dried seeds in a dormant stage usually do not resume activity until the seeds have germinated.

Most of the pathogens and pests which contaminate freshly harvested seedlots do not damage the seeds and require living vegetative tissue for their development, but some saprophytes are capable of damaging seeds that are stored in unsuitable conditions. These contaminants seldom survive long periods of storage in dried seedlots.

CURRENT PROCEDURES FOR GENE BANK SEEDS

IBPGR recommends that seed collections for genebanks should be based on samples of whole inflorescences bearing ripe seeds, taken randomly from about 50 plants in a population that is apparently disease-free and undamaged by pests, to give approximately 2,500-5,000 seeds (Hawkes, 1970). By placing the collected material directly into a paper or cloth bag a complex of pests and pathogens associated with the seeds and other floral parts may be included.

The collector may send the seeds immediately to a genebank, or several of the following procedures (Fig. 1) may be carried out before arrival:

(i) The collected material may be partially cleaned, with small immature seeds, seed pods and other extraneous floral parts removed and discarded, followed by drying of the remaining seeds to suppress the growth of mould fungi. The collector may decide to apply a light dusting of broad spectrum insecticide to kill active stages of insects that might emerge during transmission to the genebank.

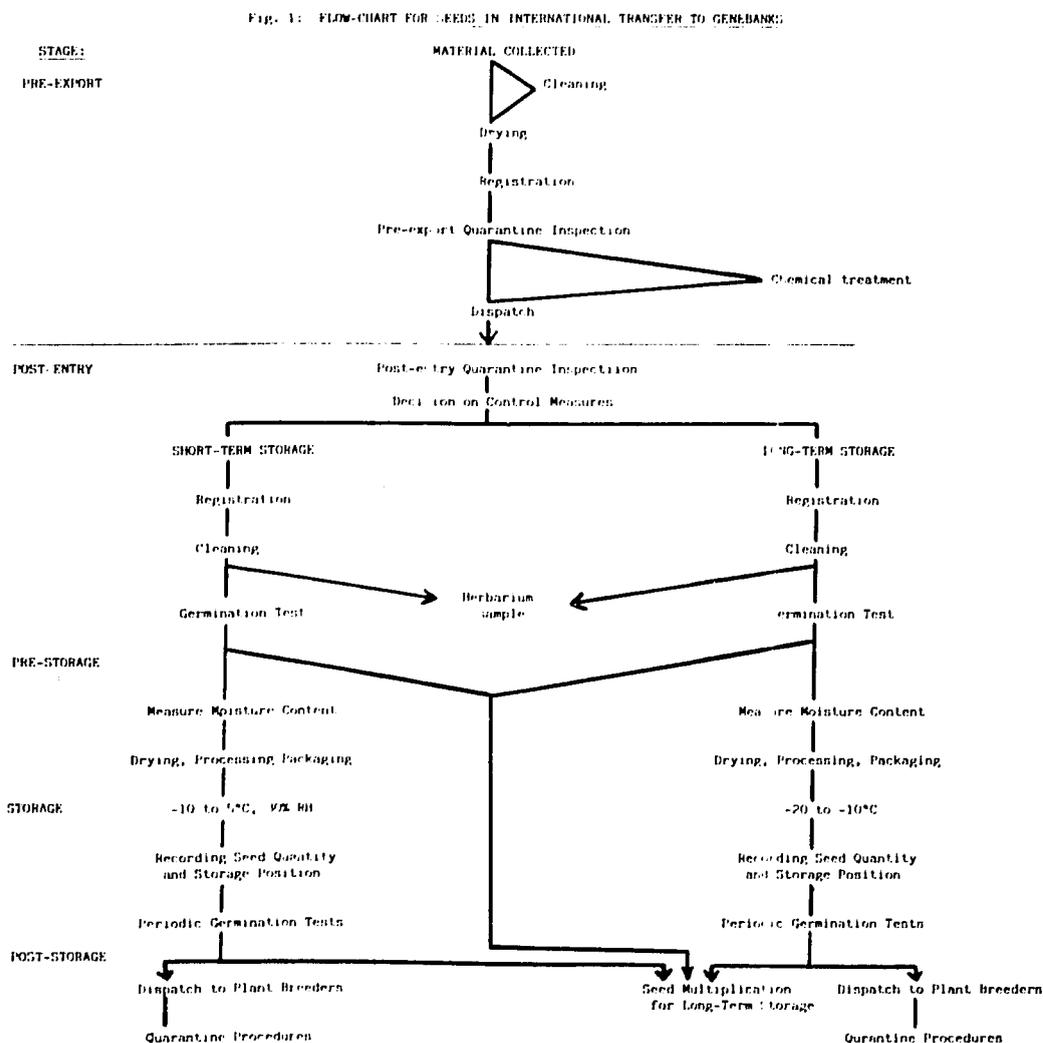
(ii) When seed collections are subjected to quarantine inspection for a phytosanitary certificate before dispatch to a genebank in another country, the standard procedure involves a visual examination, with special inspections performed to satisfy the quarantine requirements of the importing country. The seed may be given a routine treatment with a fungicide or insecticide, or both, by the quarantine service before dispatch; some of these chemicals may greatly reduce germination rates after a few years of storage.

(iii) On arrival in the importing country, and before receipt at the genebank, the seed material may be given another visual inspection or a more detailed post-entry quarantine inspection with particular attention to detecting and removing any listed species of pathogen which has been proscribed to protect the important economic crops. In some countries the need to protect crops of major national importance may cause a considerable delay in the delivery of the seeds from quarantine units to the genebank. If virus diseases are to be screened, it may be necessary to germinate all seeds and test every growing plant before the fresh seeds are harvested and released to the genebank.

(iv) In the genebank, the seed collection is registered as an accession, followed by cleaning to remove low quality seeds and any extraneous material, and then tested for germination, taking a sample of 200-400 seeds. When the accession contains enough

viable seeds, the moisture content is measured in preparation for storage, followed, if necessary, by drying to a safe moisture level before packaging and storage.

Seed samples withdrawn from the genebank and sent to other countries may pass again through pre-export and post-entry quarantine inspections before receipt by plant breeders. Eventually, when periodic germination tests show that the viability of a



genebank seed accession is diminishing, seed is sown for regeneration, and fresh seeds harvested for deposition in the genebank.

The responsibility for controlling any pathogens and pests surviving in the seed samples that are withdrawn from the genebank rests ultimately with the plant breeders and genebank staff who arrange the growing out.

THE PURPOSE OF MONITORING TESTS

The immediate reasons for inspecting seed collections are concerned with protecting the seeds during transit and storage, avoiding interference with germination tests, and preventing damage to the plants grown after storage. Every effort must be taken to avoid the introduction of pathogens and pests that are not already present, which will be able to survive the genebank conditions, and are of potential economic importance in the importing country.

The presence of many types of pathogens and pests cannot be detected unless the correct inspection method is used. Efficient cleaning and drying, exposure to lethal temperatures, and chemical treatments can be effective control measures; they have been used as substitutes for inspection, but the presence of certain harmful species must be detected for the correct control measures or combination of measures to be applied.

OPPORTUNITIES FOR IMPROVING MONITORING TESTS

To ensure the highest levels of seed quality, various changes should be introduced to current monitoring practices at all stages of seed handling from harvest to seed multiplication. High standards are justified for genebank seeds in view of the extra expen-

diture incurred on special measures for harvesting, preparation and storage, and their high potential value for improving agricultural production and quality.

Changes are needed to improve the efficiency of measures to protect the seed collection from active pathogens and pests that might damage the seeds during transit. It is also necessary to control pathogens that might interfere with the interpretation of germination tests, especially in seed collections containing dormant or hard seeds which do not germinate, as accurate measurements are essential for predicting the potential for long-term storage. In addition, changes should be introduced in plant health control practices to minimize the possibility of exotic pathogens and pests spreading to crops in new countries with the germplasm.

Recommendations are required for the treatment of freshly harvested seedheads to ensure the minimum of seed damage by various insects while confined in the collecting bags. Adequate drying may be sufficient to prevent injury to seeds from most pathogens and insects, but in some circumstances the controlled use of hot dry air, or an application of insecticides and fungicides with a wide spectrum of activity against insects, mites, fungi and bacteria may be necessary. The methods recommended must protect the seeds without reducing viability during long periods of storage and the chemicals must not cause allergic reactions in genebank staff during the handling of treated seeds.

Important pathogens and pests should be detected at an early stage so that their spread to crops can be prevented. Disinfection of infected seeds or other effective control measures may be deferred until a later, more appropriate stage. Special treatment of infected seeds may be unnecessary if, for example, the harmful organism cannot tolerate long periods of storage in very dry seeds

and sub-zero temperatures, though it is certain that all stages of seed-borne insects will be killed by prolonged exposure to -20°C (and most cannot survive in this temperature for two weeks).

Detecting low levels of infection

The presence of very low levels of infection may be impossible to monitor and it may be easier and safer to recommend that all susceptible species of seeds originating from certain countries should be given a routine treatment that is guaranteed to eradicate the infection. For example, onion seeds can be treated with methyl bromide to ensure freedom from the harmful seed-borne nematode Ditylenchus dipsaci.

The efficiency of monitoring infections can be improved by testing the seeds that have characters associated with low quality, such as distortion, discolouration, damage and immaturity, which are separated from good quality seeds at several stages between harvest and storage. Seed collections are usually cleaned by sieving to remove broken or undersized seeds, or a vertical aspirator is used to blow away the light material, followed by visual inspection and the removal of discoloured seeds.

A comparison of the efficiency of these three methods for sorting small seed collections infested with seed-borne pests (Table 1) showed that only the nematode Anguina tritici, which turns wheat seeds into small distinctly coloured galls, was separated reliably. Seed infested by the nematode Ditylenchus dipsaci differed from uninfested seeds only when heavily infested, and most lightly infested seeds were not removed. Similarly, seeds infested with the eggs, larvae and pupae of the seed weevil Sitophilus zeamais, which develop inconspicuously within cereal seeds, were not removed efficiently until the weevil's life cycle was completed and the adults had emerged through conspicuous exit

holes making the seeds lighter. Thus, the seed accessions were still lightly infested after cleaning, but most of the infested seeds had been sorted out and normally would be discarded as low quality seeds. Therefore, monitoring for pests in seed collections would be more efficient if the low quality seeds were inspected, because they contain the highest concentration of seed-borne pests. It follows that the low quality seeds must not be discarded, irrespective of the stage of handling at which they are separated from collections, until experienced staff are available to use the appropriate monitoring tests.

Table 1: The efficiency of three methods for separating seeds infested with seed-borne pests from seed collections.

Percentage of infested seeds
separated by method:

Seed-borne pest	Seed	Seiving	Aspiration	Visual
<u>Anguina tritici</u>	wheat	100	98	100
<u>Ditylenchus dipsaci</u>	field bean	87	76	94
<u>Sitophilus zeamais</u>	rice	12	99	95

The specific monitoring tests include inspections after the seeds have been washed or soaked in water, incubated, germinated, grown as plants, or after the embryo has been extracted (Rennie, 1979); following these tests, the seeds become unsuitable for storage.

By using low quality seeds in these destructive tests, the number of storable seeds in the accession is not reduced, which is especially valuable for conserving small seed collections.

Inspecting seedlings from germination tests

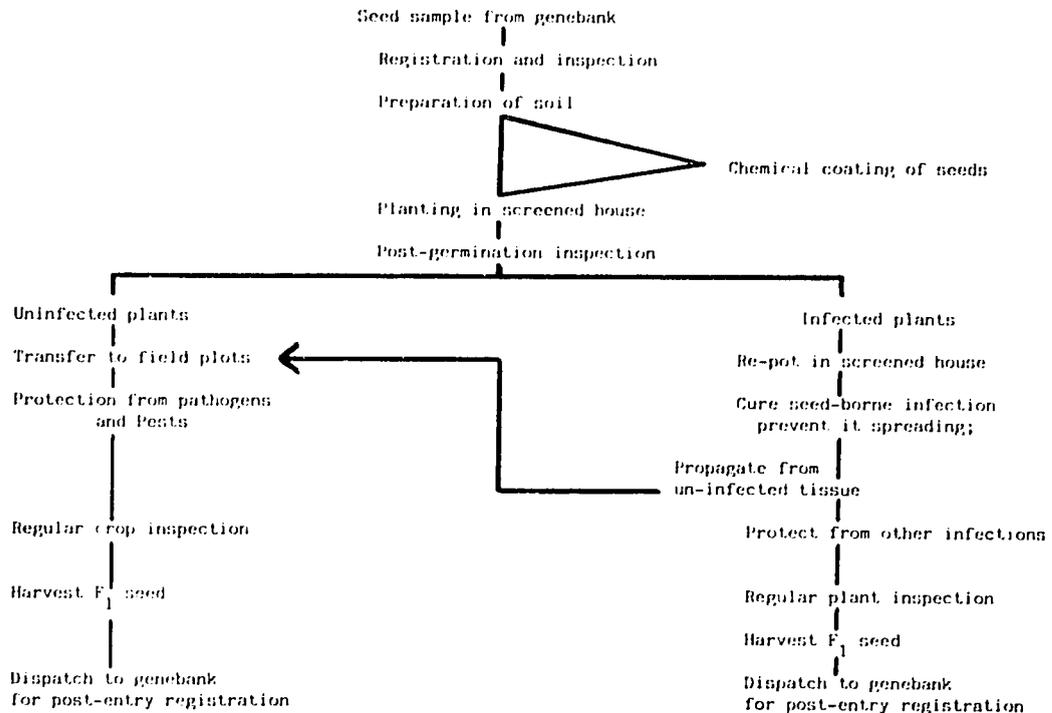
Though 200-400 seeds is the standard for viability tests, fewer are used, with less accuracy, to assess the viability of small accessions. After germination has been recorded, it is suggested that the seedlings should be planted and grown on within a screened glasshouse for plant health examinations. This technique enables the maximum information to be obtained from good seeds, and compensates, to some extent, for their removal and loss from small accessions. If harmful organisms are detected at this stage, it is often possible to apply appropriate control measures later to protect the developing plants from infection by these pathogens.

Multiplication

Strict procedures must be followed when seeds are released from a genebank for breeding purposes or multiplication (Fig. 2). Young plants must be grown in mesh-screened houses, unless it is certain that the seeds are not infected with exotic organisms, to avoid cross-infection by seed-borne organisms to nearby uninfected breeders' plants, and to prevent the escape of exotic pests and pathogens that can spread and become established in local crops. Appropriate chemicals can be applied as coatings to protect the seeds from both seed-borne and indigenous organisms. Further protection can be obtained with chemicals admixed with the soil or applied to developing plants. When viruses are prevalent, plants should be confined in all growth stages in a screened house or glasshouse, or the seedlings may be transferred to isolated field plots far from related plants and potential vectors for planting

during the off-season (Kahn, 1977), and chemicals toxic to vectors may be applied as a further precaution.

Fig. 2: FLOW-CHART FOR SEED MULTIPLICATION IN GENE BANKS



Unless thorough seed health inspections have been carried out, the main opportunity for detecting very low levels of seed-borne infections is during the growth period of the plants. Regular inspections are therefore essential at all growth stages that are likely to present visible disease symptoms. Successful control of seed-borne infections depends to some extent on anticipating which type of organisms may be present, as this influences the choice of inspection methods and the success of detection, and, later, the level of isolation provided for the growing plants and the type of chemical protection that may be applied. Yet few of the genebank staff and plant breeders who are responsible for growing plants from genebank seeds have been formally trained in seed health procedures, which increases the risks that some of the unfamiliar

seed-borne infections will not be detected. It is important that this anomaly is rectified, and three methods which could be used to overcome this problem are suggested.

(i) Seed Health Record

Seeds released from genebanks should be accompanied by a record of the seed-borne pathogens and pests that have been detected in the accession since the seeds were harvested, and additional information about seed health, including recommendations for control measures. This record would be a voluntary compilation.

Initial entries on the seed health record would contain a description of the collection area, symptoms of diseases and pest infestation in the plant population, and any chemical treatments that have been applied to the seeds (Hawkes, 1970). The collector should try to include a list of seed-borne pathogens and pests that have been recorded from the species in the collection area (which sometimes may be obtained from local agriculturalists or botanists), and descriptions of the optimum conditions and procedures for cultivating the plants. This record would accompany the seed collection, and further entries would be added as infections were detected during quarantine inspections and examinations of low quality seeds and seedlings grown on after germination tests, as well as details of any treatments applied to control the pests and pathogens. These accumulated data would be available to assist in the detection and treatment of harmful organisms at all stages, and the seed health record would accompany every sample of seed dispatched from the genebank to plant breeders.

(ii) Handbook

The reliability of monitoring for infections would be improved if genebank staff had a handbook giving guidance on the procedures

recommended for inspecting seeds, detecting and identifying seed-borne pathogens and pests, and the control treatments.

(iii) Training

The Seed Health Record and Handbook would be complementary, but if they are to be fully effective the genebank staff and plant breeders need specific practical training in seed pathology, seed health testing and the principles and procedures used by quarantine services. A training programme for genebank staff such as senior administrative managers, research workers, laboratory superintendents and field planting staff, would be a sound investment for improving the efficiency of monitoring for harmful organisms in seed collections.

Quarantine substations

In countries where genebanks may experience considerable delays in receiving imported seed collections due to the lengthy post-entry quarantine inspection by the national quarantine service, the presence of staff trained in quarantine procedures might enable the genebank to be appointed as a quarantine substation. A high standard would be essential, and periodic checks would be carried out by the quarantine service to ensure that the standard was maintained.

When a genebank has been given responsibility for post-entry quarantine regulation of germplasm, collections of imported seeds could be delivered directly to the genebank on arrival in the country. This policy might reduce delays in which seed packages are sometimes left in the open or in a hot, closed store for several days at the tropical airport with consequent reduction in germination rate and seed longevity. Longer delays occur when the quarantine service grows plants for inspection, sometimes in

unsuitable conditions that may cause the death of seeds, and inevitably results in the erosions of valuable genes before the new seeds are released for storage in the genebank. Provided the genebank operations are reliable, seed quality would be improved without reducing the standard of quarantine inspection if responsibility was transferred to genebank staff, because germplasm material needs a wider range of seed health monitoring.

RECOMMENDATION FOR NEW TESTING PROCEDURES

When seed collections are cleaned and the small, damaged, distorted and discoloured seeds are removed, these low quality seeds should be kept until facilities and experienced staff are available to conduct the appropriate tests for the presence of seed-borne pathogens and pests. This procedure confers two important advantages:

(i) With many seed-borne insects, nematodes, bacteria, and some fungi and viruses the efficiency of detecting is increased by testing the low quality seeds instead of testing samples withdrawn from the seeds selected for the accessions. Useful information is obtained without sacrificing any seeds, which is most valuable with small accessions.

(ii) After recording the viability of seeds in germination tests and testing low quality seeds for infection, the seedlings should be grown on within a screened glasshouse for plant health examinations. This additional testing is most appropriate for species that are commonly infected with seed-borne viruses (such as legumes and solanaceous species), to ensure that special precautions are taken when seeds are used subsequently for plant breeding.

RECOMMENDATIONS FOR NEW ADMINISTRATIVE MEASURES

(i) Seed collected for genebanks should be accompanied by a complete record of plant health which is started by the collector, accumulates information from quarantine inspections, germination tests and specific tests for seed-borne pathogens and pests, and will be dispatched with all samples sent from the genebank to plant breeders. These accumulated data will assist in the detection and treatment of harmful organisms at all stages, and greatly reduce the chances of seed-borne organisms being spread with germplasm.

(ii) Plant health inspection should be improved by expanding the opportunities for genebank staff to receive training in quarantine principles and the procedures for detecting seed-borne pathogens and pests.

(iii) Genebanks and quarantine services should co-operate to avoid unnecessary delays in the post-entry and pre-export movement of seeds.

Where genebanks have appropriate facilities and well-trained and competent staff, there may be advantages in arranging with the national quarantine service to appoint the genebank as the Quarantine Substation. This would permit small collections of imported seeds to be delivered directly to the genebank for post-entry quarantine inspection on arrival in the country, and to be dispatched directly to other countries after pre-export inspections in the genebank. Periodic checks would be made by the quarantine service to ensure that international standards were maintained.

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DISCUSSION

Chairman: Williams

Participants: Roberts, Tyler, Rennie, Boland,
Hawkes, Neergaard, Holly,
Bailey, Ellis, Mumford.

1. Feasibility and Workload

In summing up Dr. Gerard's paper, Dr. Williams noted the validity of many of the recommendations, and also that many regarding high quality, disease free seed, were directed specifically at collectors in the field. Whereas it may be feasible to apply them in Europe for example, in other parts of the world reliance is often placed on relatively untrained people to collect a wide range of crops. Another problem would be logistical: in general genebanks tend to be understaffed.

Dr. Mumford wondered whether it is fair, or wise, to remove the responsibility for pathology testing from the plant breeder and load it onto the genebank manager. Often the plant breeder is an expert on all aspects of the crop he is working on and useful feedback from the plant breeder to the genebank manager could occur. Dr. Williams agreed, but said that such a situation depended on the individual genebank manager to maintain contacts with all his user/breeders, in order to be able to call upon their experience when required. The alternative to making genebanks responsible is a system of 'caveat emptor' plus post-entry control by the user.

2. Insects in Seed Banks

Prof. Roberts questioned the need for monitoring of invertebrate pests, when many genebanks store seed dried to less than 9% moisture content (F.W.), in sealed containers at low temperatures.

Under these conditions insect activity would be minimal and thus storage in a seed bank avoids the problem altogether. Even where higher temperatures are employed, if the seeds are dried to the recommended level and stored in sealed containers, the insect problem would not be great. Dr. Gerard agreed with Prof. Roberts and said that it is important to aim for the 9% moisture content level in this context; but he had simply been trying to cover all eventualities.

Dr. Williams stressed the importance of seed cleaning and removal of debris; and Dr. Gerard admitted that even in the best known insect/seed problem, that of Bruchids in legumes, the insect larvae in the seeds are only a relatively minor part of the problem.

3. Selective Avoidance of Diseased Plants

Mr. Tyler wondered about the usefulness of selectively avoiding diseased plants, although this may be difficult at the time of collection of ripe seeds, when foliar diseases might not be apparent. Such non-random collections may be expedient, especially when breeding for disease resistance is the object. Dr. Williams commented that, despite a divergence of opinion on this topic, in the context of genebanks it is probably necessary to store diseased seeds because disease susceptibility may be in linkage groups with other desirable characters required in the future. Representative variability is rarely achieved, and this is particularly so where there is discarding of material during storage, processes leading to storage, or during regeneration.

4. Removal of small seeds

In response to a comment from Dr. Boland, Dr. Williams encouraged genebank personnel to retain small, light seeds, providing they

can be shown to be viable, for the same reasons as above; i.e. the maintenance of variability.

5. The Role of the Collector

Prof. Hawkes was concerned that collectors cannot be expected to provide all the information that a pathologist needs or would like. At best they can only record symptoms seen, but not assess their value/implications. For instance, a particular plant may be susceptible to one but not other pathogenic races of a particular disease organism. It is obviously important that this information is looked for where possible, but too much emphasis should not be placed upon it; e.g. an epiphytotic may be occurring at the time of collection, but the collector is primarily there to collect germplasm.

6. The Use of Non-Destructive Tests

As opposed to destructive tests on seedlings, such as that shown for Alternaria on Brassica, Prof. Neergaard maintained that it is technically possible to use non-destructive tests; whereby seedlings are grown for observation in plain water agar, and this could be an extension of a germination test when agar is used as the substrate.

Prof. Neergaard also emphasised the importance of isolated trials in order to avoid the spread of diseases.

7. The Record of Genebanks in Plant Health to Date

Prof. Neergaard had mentioned the spread of disease through commercial material and also by use of 'diplomatic bags', which prompted Dr. Gerard to ask whether there are any examples of

diseases escaping from genebanking activities causing large problems.

Prof. Neergaard thought that there are no examples, but there is no need for complacency as there are examples from agricultural research centres, and some of the material held by genebanks has many potential dangers, e.g. rice.

8. IBPGR Manual on Duties of Genebank Personnel

Using the example where genebank personnel cannot approach the appropriate quarantine authorities for co-operation or advice, because the latter are already working to capacity, Dr. Williams stressed the need for a manual outlining the whole range of duties expected of genebank personnel. Such a manual, probably in a series of volumes spelling out exactly the duties of genebanks, is being prepared by IBPGR and should be available in the next few years.

9. The Employment of Plant Pathologists in Genebanks

See also point 1. Following on from 8, Dr. Bailey wondered whether one of the duties of a genebank manager is to employ plant pathologists. Dr. Williams was not sure whether this will ever happen, but Dr. Holly was able to add that there was such an authorised person on site at the Tapioszele genebank in Hungary.

10. The Use of Chemical Treatments

Prof. Roberts expressed concern that chemicals used for eradicating pests and diseases may in fact damage the seeds themselves. Which chemicals do the damage and under what circumstances is not entirely clear; the long-term effects of such chemicals are unknown. He pointed out again that there is no need to treat seed before entry into the bank; such treatment is only really necessary to seed upon leaving the bank. Dr. Gerard replied that he was currently conducting research into the effects of some of these chemicals, adding that it may occasionally be necessary to protect seed in sub-standard banks with high temperature and poor packaging. Apart from the latter situation he could see no real advantage in applying chemical treatments to seeds before long-term storage. Dr. Williams raised the important practical point that despite their being potentially harmful, chemicals are often applied for reasons of importation, transit and other situations where a quarantine officer would only pass seed when he knew it had been treated.

11. Tests on Discarded Material

Dr. Ellis wondered if any conclusions can be drawn from tests on discarded material, with reference to seed actually stored. Dr. Gerard said that if a pathogen is discovered in discarded material, then the stored sample can obviously be treated at a later date. It is certainly all useful information, but worth remembering that discarded material often has a higher level of infection than the stored material which may have a low level of infection anyway.

The Influence of Collecting, Harvesting and Processing on the
Viability of Seed

R.D. SMITH

INTRODUCTION

An initial survey of the literature reveals many reports on these topics. Unfortunately, very few of the studies investigate the effects of these factors on the subsequent storage behaviour of the treated seed; most only compare the germination level before and after treatment. Nonetheless, the direct relationship between the true initial viability " K_i " and absolute lifespan of any seed batch of the same species, Ellis and Roberts (1981a), suggests that these reports should be useful in determining the effects of such factors on subsequent behaviour. However, a detailed consideration of the effect of " K_i " on seed longevity shows the usual estimation of germination level before and after treatment is so imprecise as to prevent this possibility. The arguments presented here concern only this point. A much fuller account of seed viability is presented by Ellis in Chapter 5.

THE VIABILITY OF SEED

When studying loss of viability, a seed lot containing many individual seeds, is held under constant conditions of temperature and seed moisture content. Following a known period of storage, a sample is taken from this lot and the seeds set to germinate under standard controlled conditions. During the germination test, each individual seed is assessed against an arbitrarily defined standard of germination, commonly the emergence of the radicle to a length greater than 1 mm. At the end of the test, those individuals which met the standard, are considered to have germinated. In seed lots without dormancy, the ungerminated seed

are considered to be dead. The sampling of the lot for germination testing is then repeated after further known storage periods until all the seeds are dead. An ideal set of such results is presented in Table 1.

time in arbitrary units	no. of seed sown	no. of seed germinating	no. of seed with life span equivalent to interval	% viability
0	400	400		100
1	400	400	0	100
2	400	399	1	99.7
3	400	396	3	99.0
4	400	388	8	97.0
5	400	367	21	91.7
6	400	328	39	82.0
7	400	268	60	67.0
8	400	200	68	50.0
9	400	128	72	32.0
10	400	68	60	17.0
11	400	32	36	8.0
12	400	12	20	3.0
13	400	4	8	1.0
14	400	1	3	0.2
15	400	0	0	0
16	400	0	0	0

TABLE 1. Idealised data for loss of viability with time of a seed lot held at constant temperature and moisture content.

By subtracting the number of seeds germinating at t_1 from those doing so at t_0 , t_2 from t_1 , t_3 from t_2 ... the number of seeds within the sample will be estimated which have lifespan between t_0 and t_1 , t_1 and t_2 , t_2 and t_3 ... In this way it is possible to build up a picture of the distribution of individual lifespans within the population. When presented as a frequency histogram (Fig. 1), the bell-shaped profile, characteristic of a Normal distribution is found. More usually the results are converted to

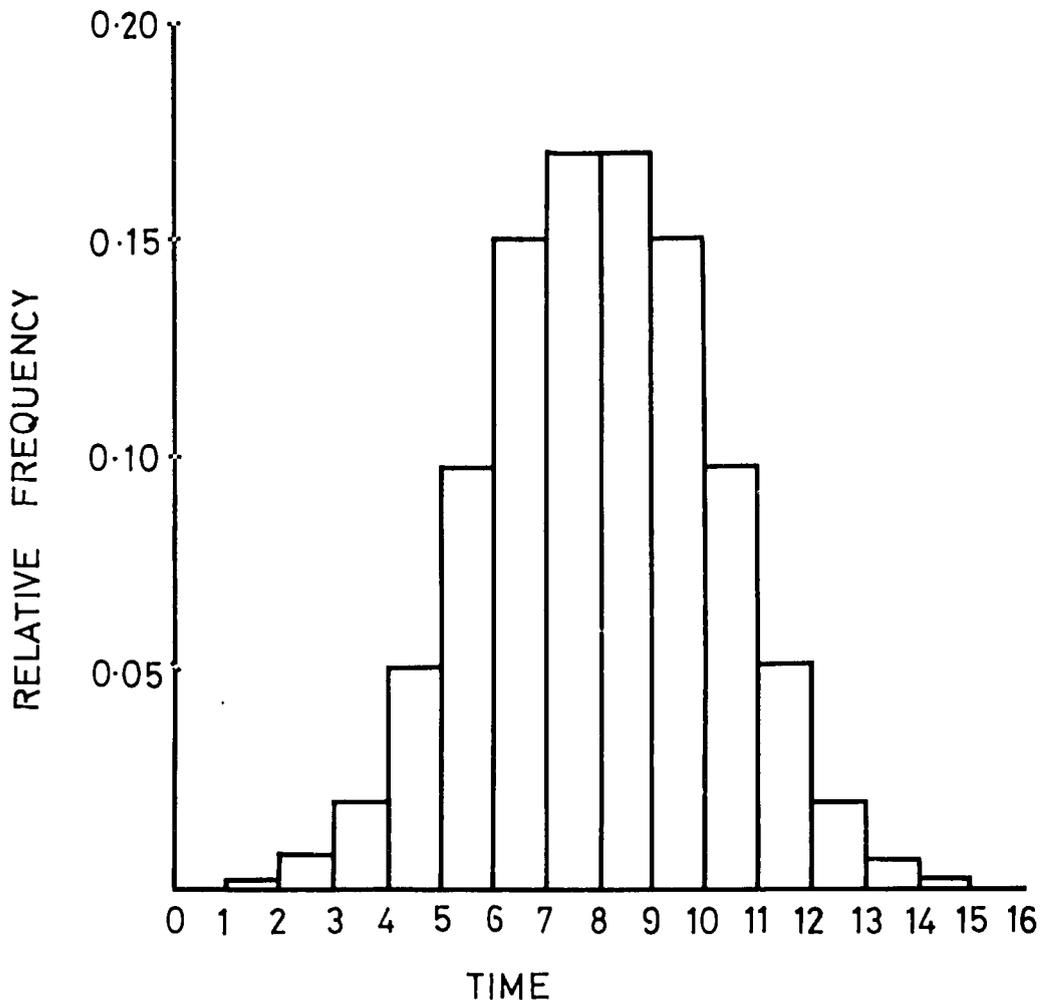


FIG 1. Relative frequency histogram of individual seed lifespans within a seed lot; data taken from the idealised set presented in Table 1.

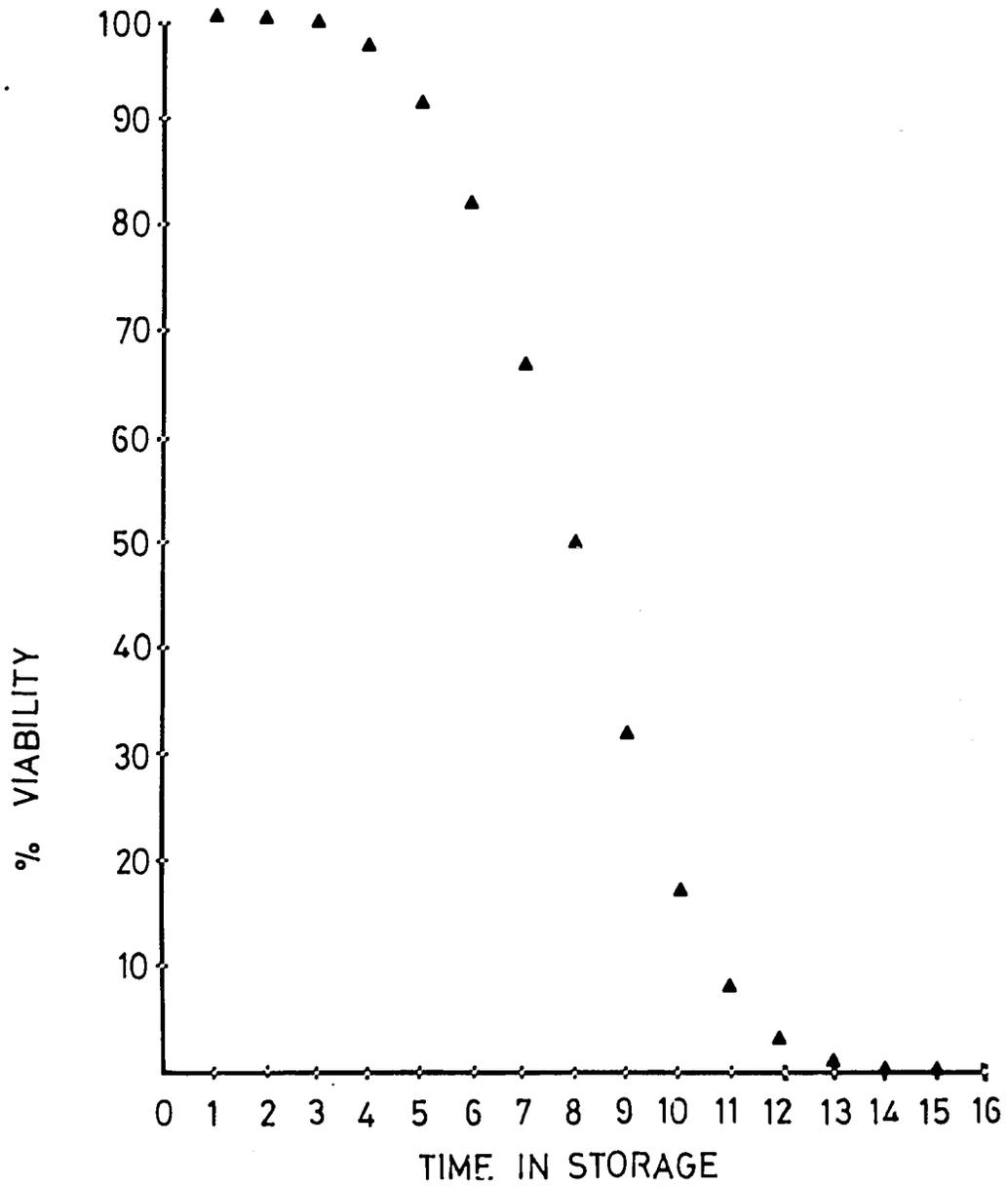


FIG 2. Loss of viability, expressed as percentage germination, of a seed lot with time. Data taken from the idealised set presented in Table 1.

percentage germination values and plotted against time to produce the sigmoid curve normally associated with viability studies. (Fig. 2) In the light of the frequency histogram, this sigmoid curve can now be seen to be a negative cumulative Normal distribution described by the formula:

$$y = \left\{ \frac{1}{\sigma \sqrt{2\pi}} \right\} \exp \left\{ -\frac{(p - \bar{p})^2}{2\sigma^2} \right\}$$

where y is the relative frequency of deaths occurring at time p , \bar{p} is the mean viability period, and σ is the standard deviation of the distribution of deaths in time.

However, closer inspection of this formula shows the controlling parameters to be σ the standard deviation and p the mean; the rest are constants. Thus, the sigmoid curve is made linear when the percentage germination values (cumulative relative frequency x 100) are expressed in standard deviation units¹. (Fig 3). Such standard deviation units are known as Probits. Furthermore, the fixed form of the Normal distribution, when sample sizes are 30 or greater, means that the probit value for a known relative frequency also becomes fixed. Another characteristic of the Normal distribution, important in the following arguments, is that whilst the mean of the distribution is fixed, the upper and lower limits stretch to infinity.

The insensitivity which precludes the use of germination levels before and after treatment as a guide to any effects on longevity, becomes apparent when a further idealised situation is considered. Ellis and Roberts (1981a) have shown that under the same con-

1. For further details see Ellis chapter 5.

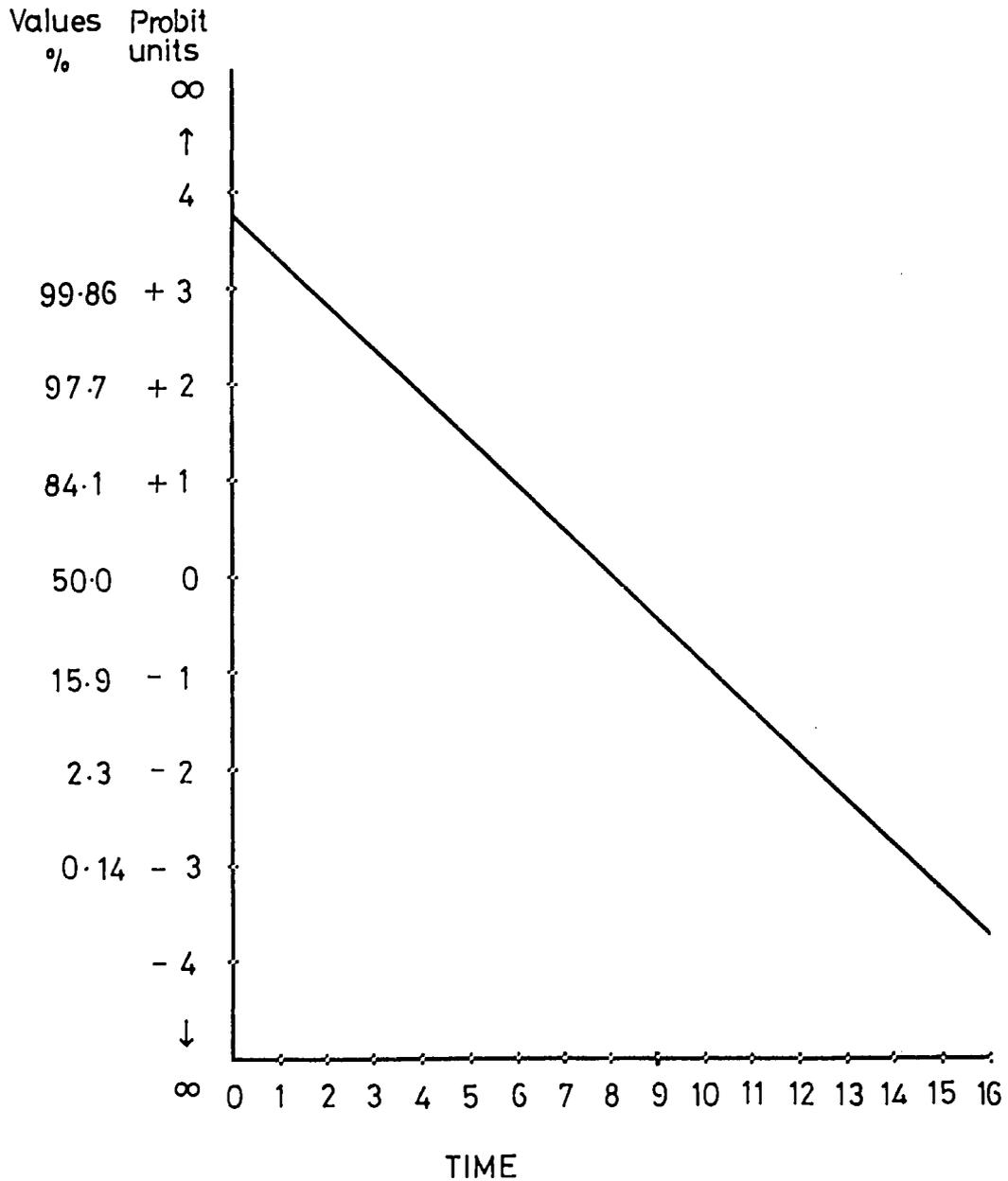


FIG 3. Loss of viability, expressed as probit germination, of a seed lot with time. Data taken from the idealised set presented in Table 1.

ditions, seed lots of the same species show the same rate of loss of viability. When plotted on a probit scale against time this results in straight lines of identical slope. The differences in absolute longevity found between seed lots of the same species are

due to differences in the true initial viability of each seed lot.

The sense of this is more easily seen if the situation is imagined in which one seed lot is kept under constant conditions and the loss of viability monitored. If at intervals, samples are taken from this lot and held under the same conditions then it seems unlikely that the rate of loss of viability will differ in any of the samples. Differences in the initial viability of each of the samples could be expected, corresponding to the viability of the original lot at the time the sample was taken.

Fig. 4 represents the situation which would occur when a sample of the original seed lot is taken at time 1 and observed independently; time 1 for the original lot becoming time 0 for sample 1. Sample 2 is that taken for the original at time 2 and so on. If the time taken for each lot to fall to the same viability level is considered - in this case 84%, the recommended regeneration standard for some crops in genebanks - the original batch will take 6 time units, the sub sample taken at t_1 , 5 time units, the sub sample taken at t_2 , 4 time units etc. Thus an equal decrease in the initial viability of a sample, expressed in probit units brings about a similar reduction in absolute longevity. Yet when expressed as percentage germination values, the differences between the initial viability of each successive sub-lot are 0.01%, 0.12%, 0.46%, 1.70% and 4.40% respectively. It is therefore not surprising that when the germination levels, expressed as percentages, before and after treatment, are statistically tested, the differences found are not greater than those expected due to a random sampling error.

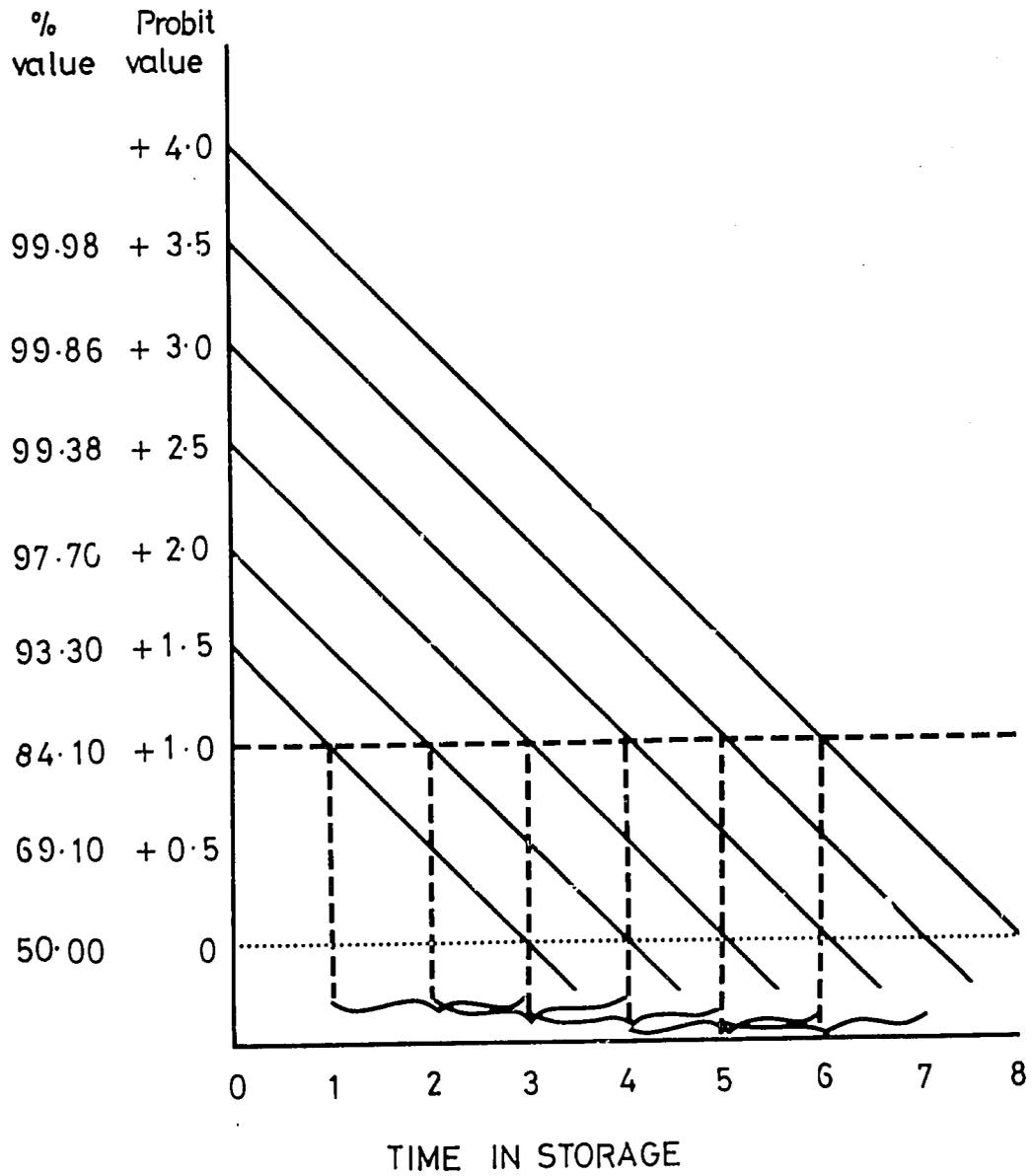


FIG 4. Loss of viability, expressed as probit germination, of a series of sub-samples taken at different times from an original seed lot and held under identical constant storage conditions as the original lot.

Table 2 shows that even when perfect samples are drawn, the size of sample necessary for the usual t-test to detect with equal certainty, differences in initial viability which have equal effects on longevity - depends on the levels of viability under consideration. Tests of 200 seeds could be expected to detect, with a probability of 0.95%, differences of 0.5 probit units at levels of viability up to 93.3%. A 400 seed test would be needed to detect a similar probit difference at initial viability levels up to 99.4%. Above these levels, tests involving thousands of seeds would be required, making them impractical.

True initial viability of seed lot	Size of sample taken							
	100	200	300	400	500	1000	2000	3000
99.99	100	200	300	400	500	1000	2000	3000
1 σ } n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	95
99.86	100	200	300	399	499	999	1997	2996
0.5 σ } n.s	n.s	n.s	n.s	n.s	n.s	n.s	95	99
99.38	99	199	298	398	497	994	1988	2981
0.5 σ } n.s	n.s	n.s	n.s	95	95	99	99.9	99.9
97.70	98	195	293	391	488	977	1954	2931
0.5 σ } n.s	n.s	n.s	95	99	99.9	99.9	99.9	99.9
93.30	93	187	280	373	467	933	1866	2799
0.5 σ } 95	95	99	99.9	99.9	99.9	99.9	99.9	99.9
84.10	84	168	252	336	420	841	1682	2523

TABLE 2. The effect of true initial viability of a seed lot on the size of sample needed to detect an equal loss of viability with equal certainty in a t-test. For this model, it is assumed that an accurate sample was drawn at each level of viability. Thus, the number of seeds germinating in each sample can be calculated by multiplying the true viability of the population from which the sample was drawn by the number of seeds in the sample and rounding to the nearest whole number.

In reality, this situation becomes more complex due to the imperfections of sample taking. Therefore unless a large sample (thousands) of seed is germinated before and after treatment, the effect on longevity of such treatment will not be revealed. Such unsatisfactory evidence has been ignored.

One final point illustrated in Fig. 4 is worthy of note. The time taken to fall from the same known viability level to a second known level is the same for all seed lots, as would be expected for lines of the same slope. In Fig. 4 the time taken for all lots to fall from 84.1% to 50% is two units.

Moving from theoretical considerations to practice, it is proper to ask what experimental evidence is available to support the view that small differences in seed viability, when expressed as percentage values, can result in significant increases in the absolute lifespan of seed lots. Recent work at Wakehurst Place on the viability of seed of Nicandra physoides (Solanceae) supports this view. On harvest, the seeds were found to be dormant; full germination was only achieved in the presence of high levels of gibberellic acid (GA_3) exogenously applied. Whilst the bulk of this seed was held dormant at $-75^{\circ}C$, a sub-sample was after-ripened at $36^{\circ}C$. Increasing periods of after-ripening brought about increased levels of germination in the absence of GA_3 . Following 239 days after-ripening, germination percent levels in the high 90s were obtained without GA_3 .

Fig. 5 shows the viability plots for after-ripened seed held at $52^{\circ}C$ and 7.8% and 6.4% moisture content wet weight basis (WWB). The behaviour of seed held at the same temperature and 5.1% mc was calculated so that comparison could be made with the observed behaviour of dormant seed at the same moisture content. The calculation was made assuming the relationship between longevity and moisture content in Nicandra is the same as has been proposed in

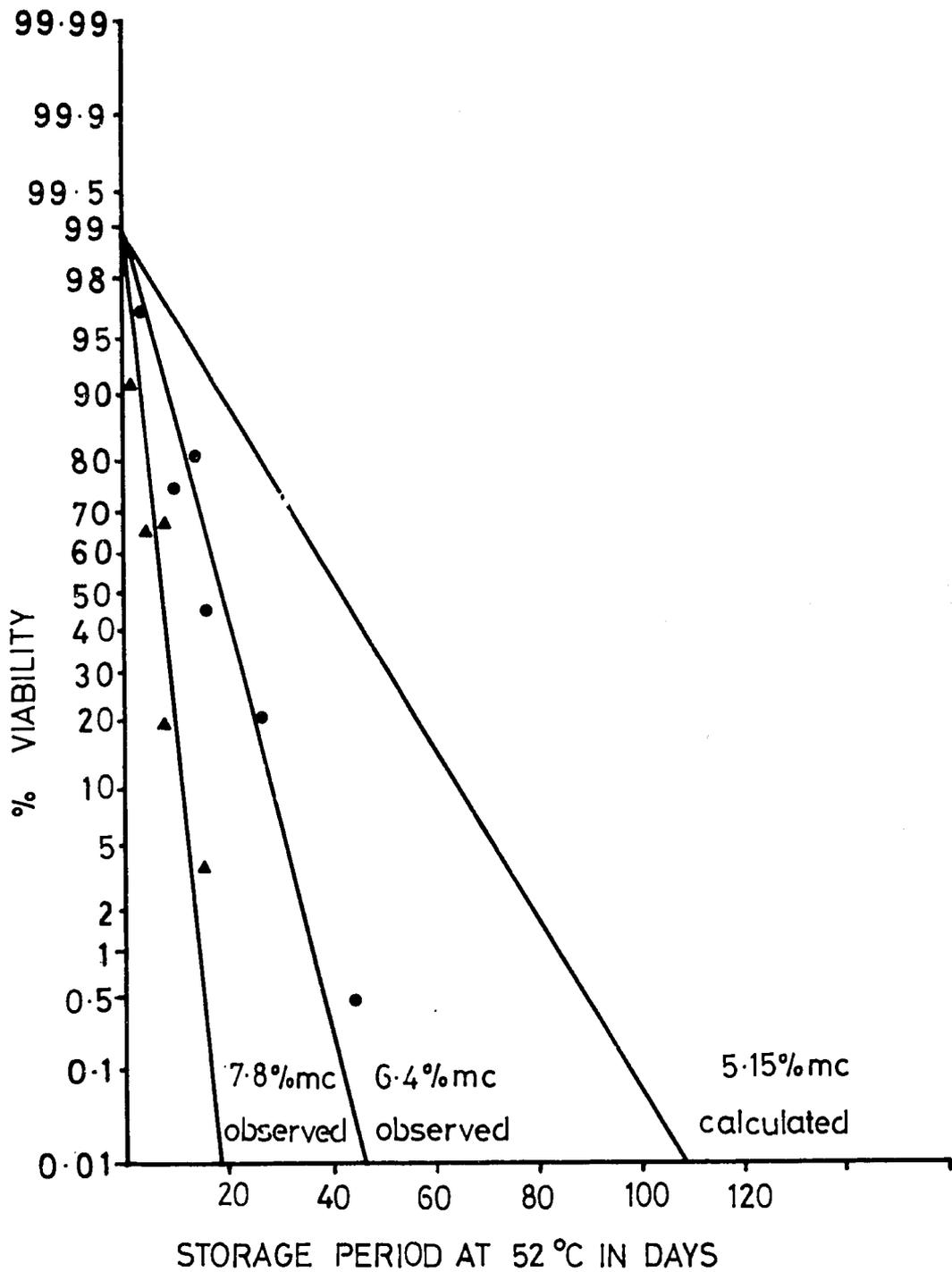


FIG 5. Observed loss of viability at 52° of after-ripened seed of Nicandra physodes held at 7.8% and 6.4% moisture content. (wwb.)

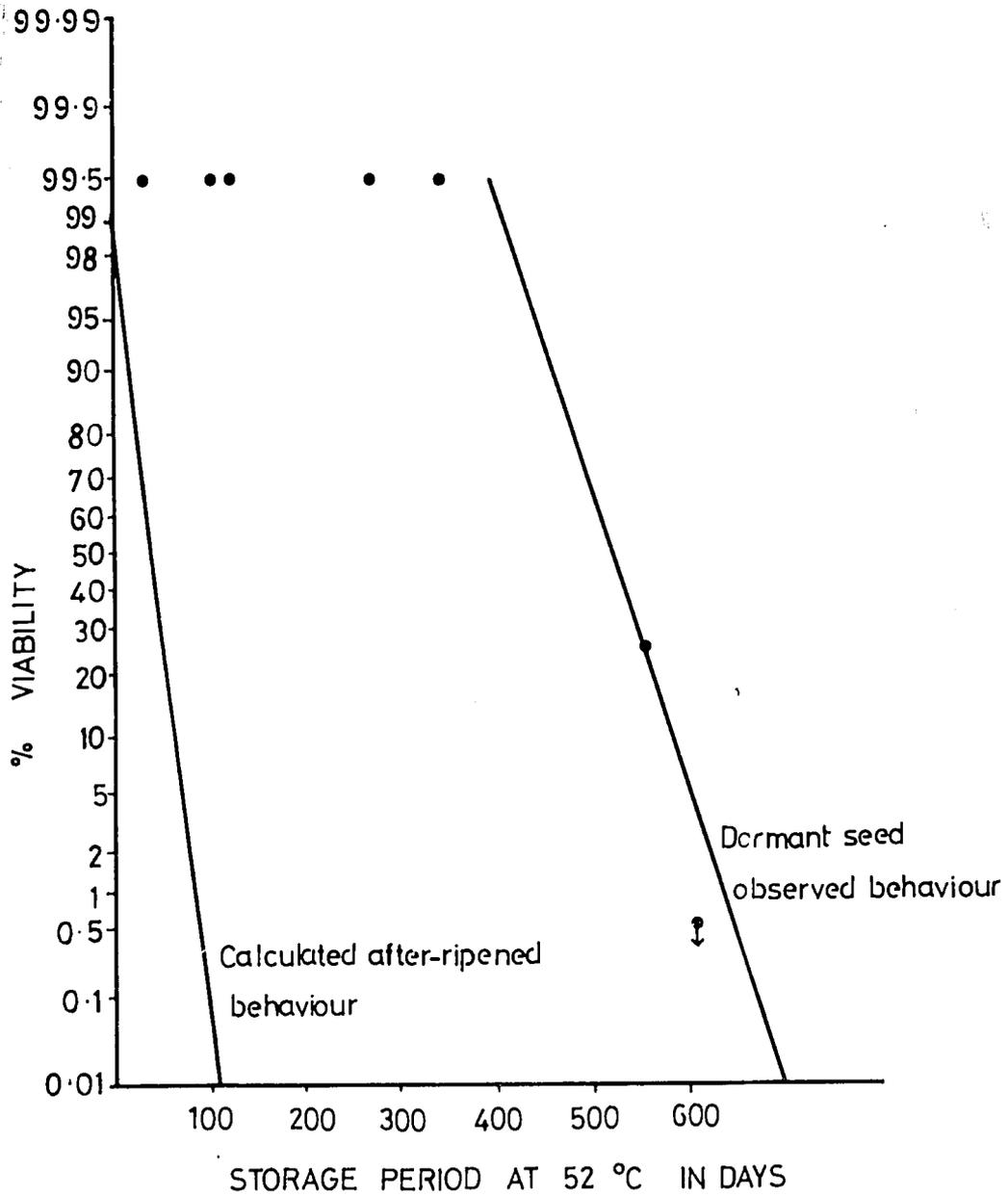


FIG 6. Observed loss of viability of dormant seed of Nicandra physodes held at 52°C and 5.15% moisture content (wwb) compared with the expected behaviour of after-ripened seeds.

the viability equations for other species. The intercept of the two observed after-ripened lines, representing the true initial viability (K_i) is close to 99%. The calculated time for the viability of seed held at 5.1% mc to fall to 84% (the recommended regeneration level) is close to 24 days. However, the observed behaviour for the dormant seed under identical conditions (Fig 6) is considerably different. The time taken for viability to fall to 84% is in excess of 450 days: an 18 fold increase in longevity over the calculation for non-dormant seed. The true initial viability of the dormant seed cannot be estimated from this plot of the data, due to the restricted nature of the y axis. Fig. 7 shows these data replotted, with the y axis greatly extended, so that intercept values can be determined. Viability plots for the same batch of dormant seeds stored under different conditions are

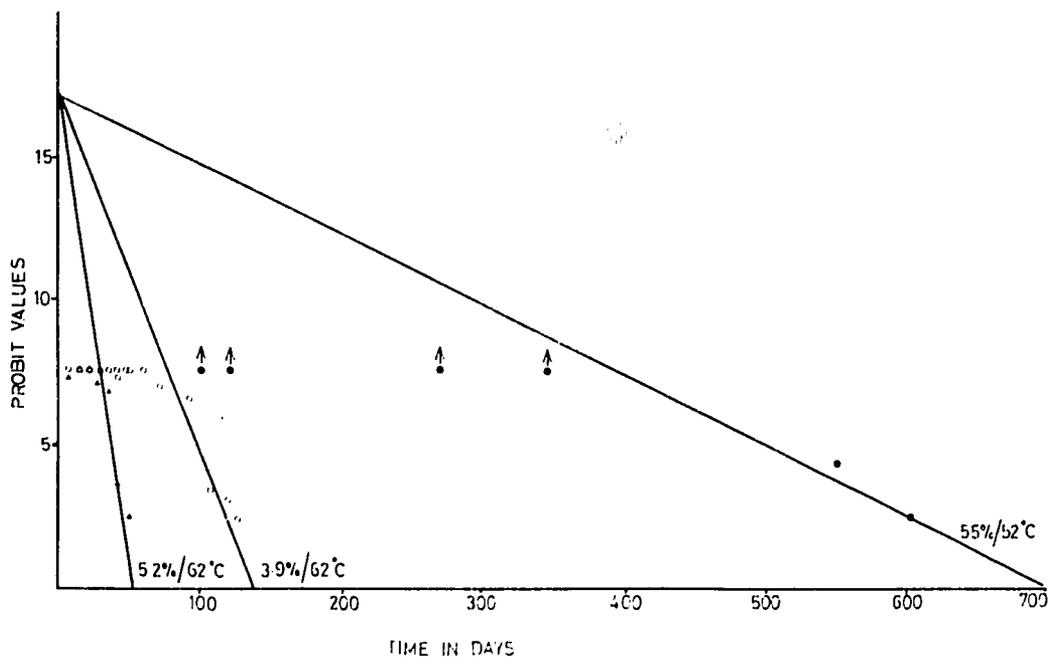


FIG 7. Observed loss of viability of dormant seed of Nicandra physodes held under a variety of constant storage conditions.

also presented for comparison. The common intercept of all the plots suggests that the true initial viability of the dormant seed loss is at least 9 probit units greater than the non-dormant seeds. Unfortunately, insufficient data are presently available to test the other postulate i.e. that the rate of loss of viability is the same in both dormant and after-ripened seed under the same storage conditions.

Hopefully, the data and arguments presented have shown that differences in the initial viability of a seed lot, though small and undetectable when expressed as percentage values, result in differing absolute lifespans when different seed lots of the same species are stored under identical conditions. If the IBPGR recommended conditions for long-term storage are adopted, then all collections held in a genebank will be under identical conditions. Thus the factor controlling the time taken by each lot of the same species to fall to the regeneration level, will be the true initial viability (K_i) of that lot.

The remainder of this paper is devoted to a consideration of those factors, which have been un-equivocally shown to effect seed viability at some stage between its development on the plant and its final banking. No apology is made for considering those factors which are not normally under the control of the Bank Manager and relate to the collection and transfer of the material to the Bank. They are discussed in the hope that this will provide Bank Managers with the understanding to influence such matters; hopefully improving the initial viability of the seed on receipt.

COLLECTING

It seems reasonable to assume that the quantitative effects of moisture content and temperature on seed viability found under controlled laboratory conditions, also occur under field

conditions. Thus, the expected loss of viability in the field could be estimated if the variations with time of seed moisture content and temperature were known. Monthly mean temperature and relative humidity data from meteorological sources offer the prospect of making such estimates, provided the hygroscopic equilibrium relationship between relative humidity and seed moisture content is known.

Such estimates will depend upon the validity of the assumptions implicit in their calculation i.e. that:

(i) the monthly mean meteorological data, based on the arithmetic meaning of observations taken at fixed times, does not differ greatly from means which take into account the daily cycling of temperature and humidity which will be experienced by the seed.

(ii) the seed moisture content equilibrates rapidly with a change in the relative humidity level.

(iii) the effects of temperature on the equilibrium moisture content for seeds at a known relative humidity are sufficiently small they can be ignored.

The meteorological data and calculated viability losses for KJumal Tar Experimental Farm in the Kathmandu Valley, Nepal, are presented in Table 3. The reasons for choosing these data were two-fold.

(i) Nepal is considered to be in a centre of diversity for many grain crops and consequently much genetic resource collection has taken place there. This diversity has led Europeans collecting in Nepal to expect that the problems of maintaining seed viability during their missions will be much the same as in Europe.

(ii) Losses in grain viability under ambient conditions have been studied at this location allowing the estimates to be checked for feasibility.

month	mean daily temp °c	mean rel. humid. at 08.40 %	expected grain moisture content %awwb	expected viability loss probit units
J	9.8	72	14.9	0.07
F	11.6	69	14.4	0.08
M	14.2	64	13.5	0.06
A	19.6	55	12.4	0.08
M	21.0	72	14.9	0.24
J	23.5	78	16.4	0.64
J	23.4	82	17.6	0.96
A	23.2	77	16.0	0.55
S	22.1	78	16.4	0.52
O	18.8	74	15.5	0.25
N	13.8	76	15.8	0.16
D	9.6	66	13.6	0.03
Annual Total				3.62

TABLE 3. Meteorological data for Khumal Tar, Kathmandu Valley, Nepal, 1973, with calculated grain viability behaviour.

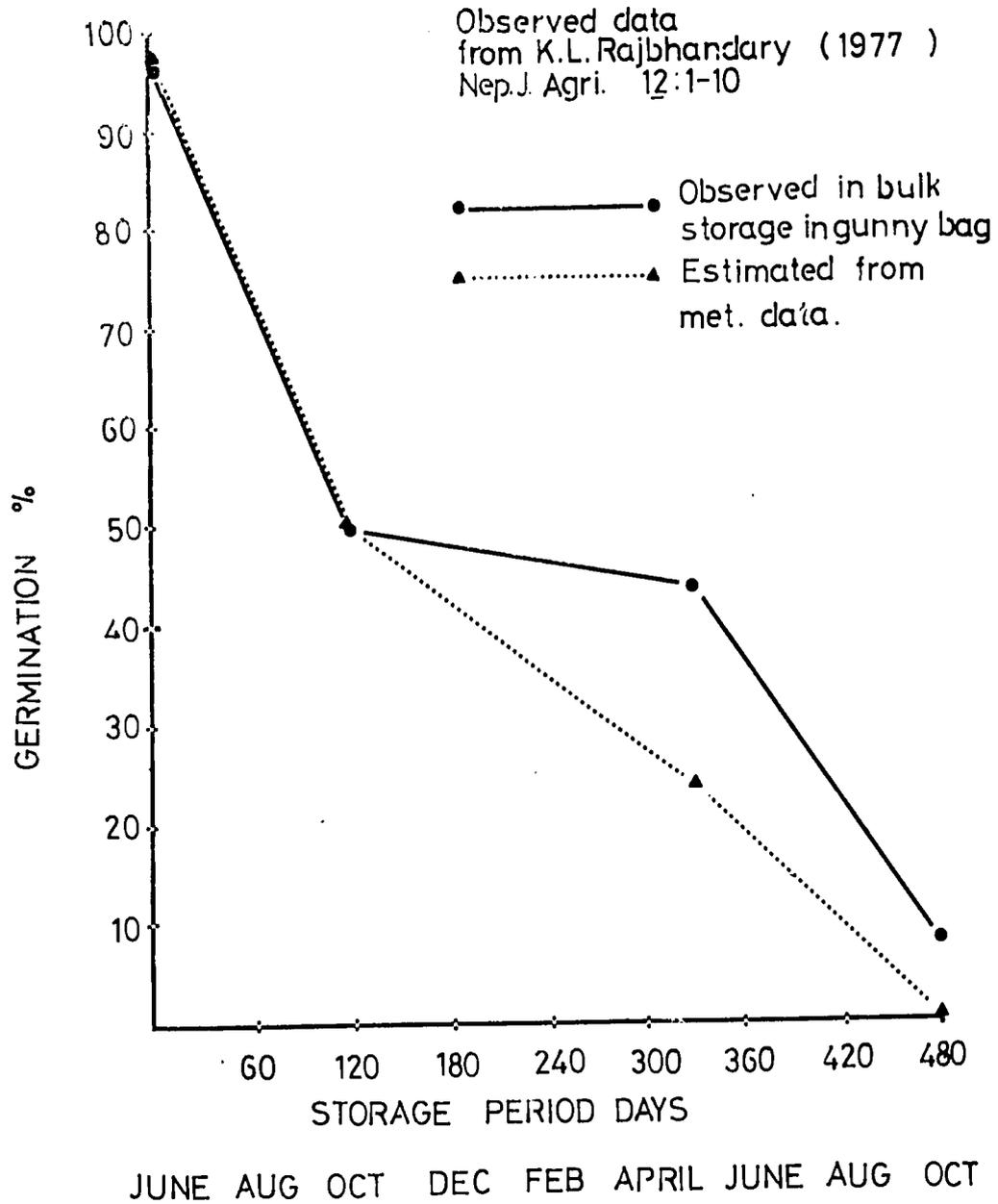


FIG 8. Observed loss of wheat grain viability under ambient Nepalese conditions compared with their behaviour estimated from meteorological data.

Viability losses were calculated using the "improved" viability constants of Ellis and Roberts (1981a) so that the risk of model error confounding the results was reduced. The validity of this approach can be appraised when the predicted viability losses, in both absolute and relative terms, are compared in Fig. 8 with those observed in wheat at the same location. The wheat was harvested in June this being the normal agricultural practice in Nepal. The calculated results show harvest to coincide with the onset of the monsoon when rapid viability losses are to be expected and are found, as shown by Rahjbandhary. Indeed, over 60% of the annual loss of viability occurs in the period June, July and August with 85% of the loss occurring between June and November.

In order to estimate the variation between seasons, similar calculations of expected viability loss were made from the meteorological data for 1972 and 1975 (Table 4). Comparison of the data for each month with its mean value shows that loss of viability can vary by up to 50% above and below the mean. This is also reflected in the totals for the period April to December for each year.

Calculated monthly viability losses for grain held under ambient conditions at Khumal Tar, Nepal.

month	1972	1973	1975	average
J		·07		·07
F		·08	·15	·11
M		·06	·12	·09
A	·09	·08	·09	·09
M	·13	·24	·33	·23
J	·36	·64	1·40	·80
J	·72	·96	1·50	1·09
A	·48	·55	1·50	·84
S	·53	·52	1·40	·82
O	·47	·25	·86	·53
N	·43	·16	·28	·29
D	·06	·03	·18	·09
Total April-Dec	3·27	3·43	7·54	4·75

Average daily losses of viability for each month expressed the period for an equivalent loss of viability to occur under IBPGR recommended long term storage conditions.

21 years storage / day
34 "
27 "
27 "
69 "
240 "
327 "
252 "
246 "
159 "
87 "
27 "

TABLE 4. Calculated monthly viability losses for grain held under ambient conditions at Kunal Tar, Nepal, in 3 seasons.

In an attempt to put these viability losses into perspective, the period for an equivalent loss of viability to occur under Bank conditions is calculated as occurs during each day of that month under ambient Nepalese conditions. These startling figures suggest that when collections are made under adverse environmental conditions, two options are open if viability is to be retained at high levels.

- (i) active drying of the seed in the field. Even though this may often be impractical.

(ii) regular and frequent despatch of the seed collections to less damaging conditions; such as the controlled environment drying facilities currently recommended by IBPGR.

Comparisons between the expected storage behaviour of barley seed held under ambient conditions in Kathmandu, Nepal; Cambridge, U.K.; and Thessaloniki, Greece, are instructive (Table 5). The losses which could be expected in Europe within one year are considerably less than in Nepal. Indeed, these reductions are even greater when a three-month period including and following harvest are considered. Nonetheless, such reductions still result in absolute lifespans under Bank conditions being diminished during this period by an average 80 years per day at Cambridge and 50 years per day at Thessaloniki. In a controlled drying facility operating at 15% R.H. and 15°C the expected losses would be 0.3 years per day. Thus, even in the more benign climates of Oceanic and Mediterranean Europe frequent and regular despatch of any seed collected would still be of advantage.

If the decision to despatch seed is taken, some consideration of the likely conditions to be encountered en route is worthwhile. Our experience at Wakehurst Place suggests that whilst this approach works well on the whole, delays in despatch can occur due to local inefficiency in airline operations. On one occasion seed, awaiting transshipment, was kept for one week under climatic conditions worse than those of the originating country. In view of such problems and the high cost of making field collections, use of air courier services to transport the seed will be justified despite expense. This will be especially so if the expense of regeneration, made necessary by loss of viability in transit, can be avoided.

Mackay and Tonkin (1967) have shown variations in the season in Britain to effect the subsequent longevity (Fig. 9) of seed under constant conditions. The "above average" seasons were those with more sunshine and less rain; those "below average" were characterised by less sunshine and more rain. In both barley and oats, the effect of the "below average" season is to reduce the time taken for the batch to fall to 80%, whilst leaving the time taken for it to fall from 80% to 50% substantially unaffected. This is consistent with the understanding of seed viability presented earlier. The constant time taken for viability to fall from one fixed level to another suggests that the rate of loss of viability under common storage conditions is the same irrespective of season. The differences recorded in the time taken for viability to fall from its initial unknown value to 80% suggests that in different seasons the true initial viabilities found at harvest differ. In above average seasons the greater initial viability which was observed can be expected. The lower rainfall will reduce the relative humidity and thus lower the seed moisture content. The viability equations demonstrate that the beneficial effects of the reduced moisture content will be greater than the adverse effects of above average temperatures which can be

From D.B. MACKAY and
J.H.B. TONKIN (1967)
J. natn. Inst. agric. Bot. 11 209-255

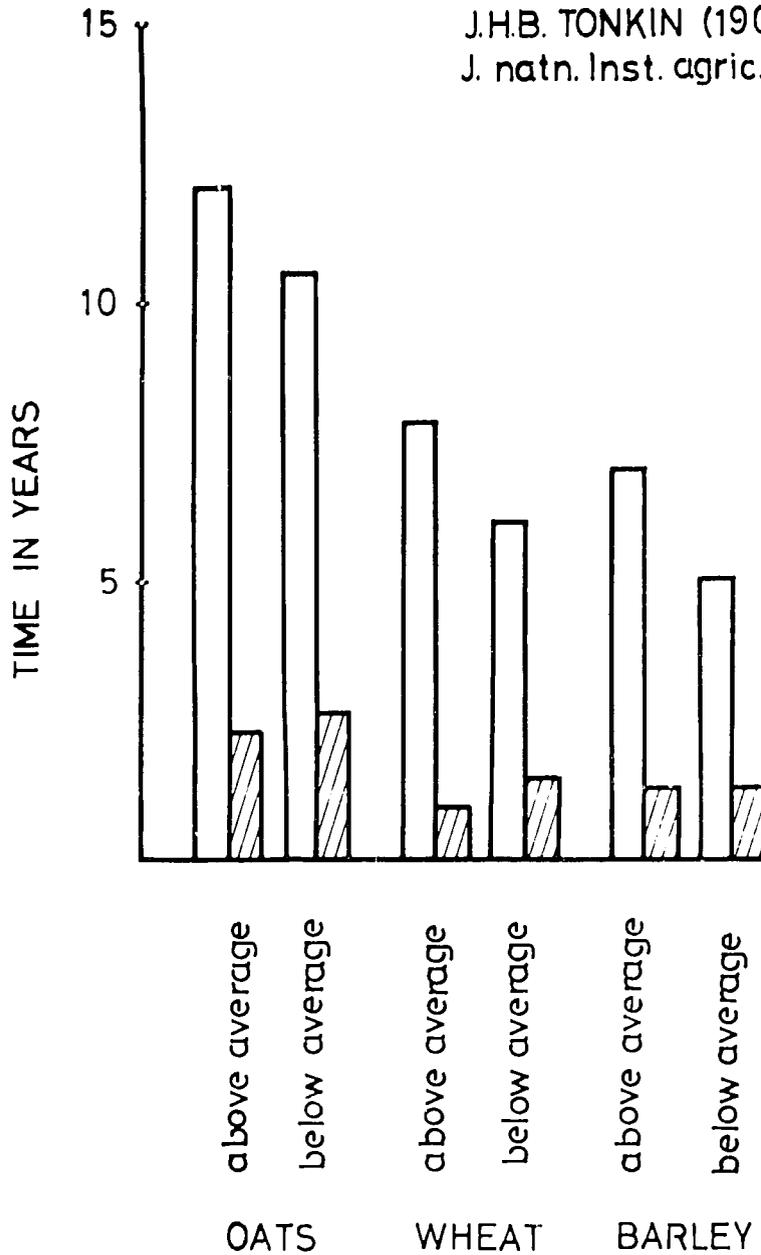


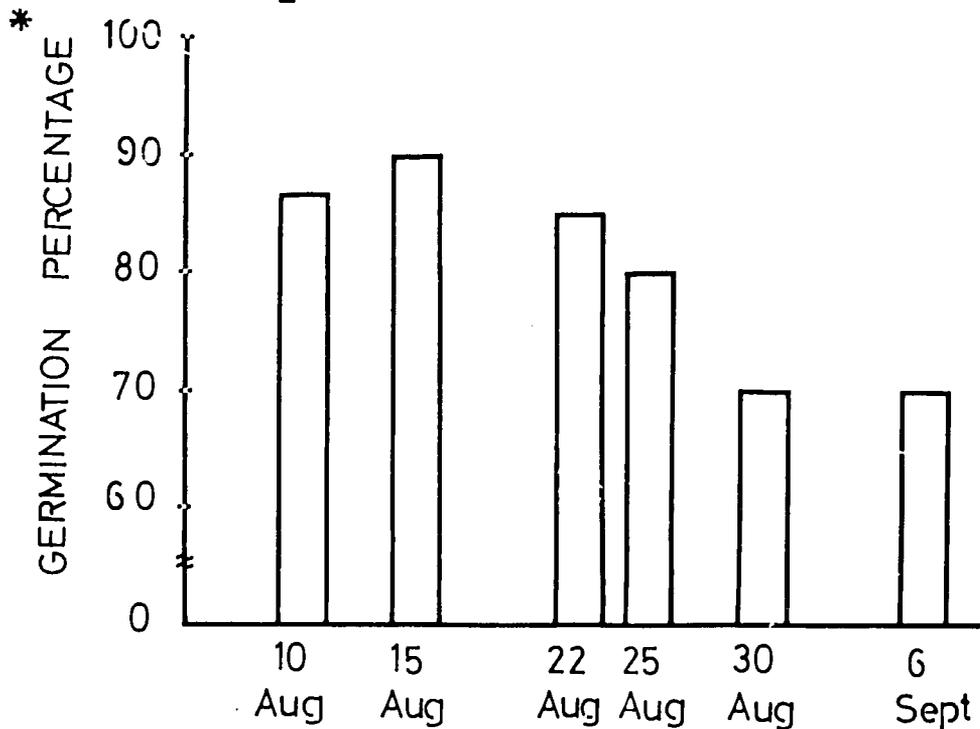
FIG 9. The effect of above and below average ripening and harvesting conditions on the loss of cereal grain stored under common conditions. The open columns represent the time taken for viability to fall to 80%; the hatched columns the time for viability to further fall to 50%.

expected to accompany more sunshine than usual. In below average seasons the reverse will be true.

Equally the relationship between later harvest dates and reduced seed longevity shown by Shands et al (1967) can be expected (Fig.

From Shands, H.L. et al, Crop Sci. (1967)

7: 444-446



HARVEST DATE 1960

* Average value for 3 varieties, each variety being held under constant conditions and tested on 10 different dates.

FIG 10. The effect of early or late harvest date on the viability of barley grains.

10). The increasing period between maturity and harvest will necessarily result in a greater accumulation of damage prior to harvest. This reduction in the initial viability will then be reflected in a reduced lifespan under constant storage conditions. The slight increase in longevity between the first two harvest dates suggests that harvesting grain before it is ripe reduces its longevity. This is in contrast to the reports of Ellis and Roberts (1981b) where harvesting of green immature grains was not found to effect either initial viability levels or viability constants. Thus early harvesting is to be preferred to late harvesting, although whether enough is known for immature seeds to be harvested without risk remains uncertain. In such a case, it is perhaps wise to err on the side of caution.

So far consideration has only been given to losses of viability which can be accounted for by the viability equations. However, many dormant weed seeds are known to survive for many years fully imbibed within the soil. This contradicts the viability equations which indicate that raising the moisture contents to such levels should reduce the viability periods drastically. This discontinuity between the storage behaviour of seeds at low moisture contents and when fully imbibed was first shown by Villiers and his co-workers (1975) who reported dormant lettuce seed held imbibed at 30°C for 15 months did not show measurable losses in viability. Yet assuming fully imbibed seed to have a moisture content close to 33%, the viability constants published for lettuce by Roberts and Ellis (1977) indicate approx. 6,500 probit units of viability should be lost in this period. It seems unlikely that such losses would leave the viability level of the seeds unaffected. Villiers was further able to show that in lettuce seeds, the sub cellular damage which accumulated if the seeds were initially stored at the lower moisture contents normally associated with seed storage, was lost, presumably through repair when the seed was later kept imbibed under conditions which imposed dormancy.

The apparent contradiction between the findings for imbibed and dry storage can be reconciled if a critical seed moisture content exists above which damage is repaired and below which the damage accumulates, resulting in loss of viability as described by the viability equations.

Because seeds of many species (e.g. Malus, Citrus, Cucurbits and many Solanaceae) normally mature and are shed at high moisture contents, it becomes important to know the critical moisture content at which the switch between repair and non-repair occurs. Knowledge of which factors control the loss of viability at the high moisture contents will also be necessary if appropriate collecting techniques are to be developed.

Viability of coffee seed held at a constant temperature (15°C) and maintained over a wide range of moisture contents (11-46% mc) in 500 gauge polythene bags has been studied by Van der Vossen (1979). Transforming these data to a plot of the mean storage period against moisture content (Fig. 11), shows the expected trend of decreasing viability periods with increasing moisture contents at the lower moisture contents (11-21%). However, at higher moisture contents increase in the moisture content increases the mean viability period. This would be consistent with Villier's findings, if increasing seed moisture content increased the level of cellular hydration which in turn increased the rate of the repair processes. As the rate of many physiological processes occurring during germination, have been shown to be dependant on the level of hydration achieved during imbibition, this seems likely. The decreases in longevity at the highest moisture levels will be returned to later.

If this explanation is accepted, then the critical moisture content at which coffee seed switches from one system to the other can be deduced to lie between 13 and 21%.

King et al (1981) have suggested viability for lime (Citrus arantifolia). The moisture content constant c_1 was estimated from the relative effects on storage lives observed at 15.8% and 7.9% mc. If the equation is solved for the higher moisture contents which were also included in the experiments, the estimated mean

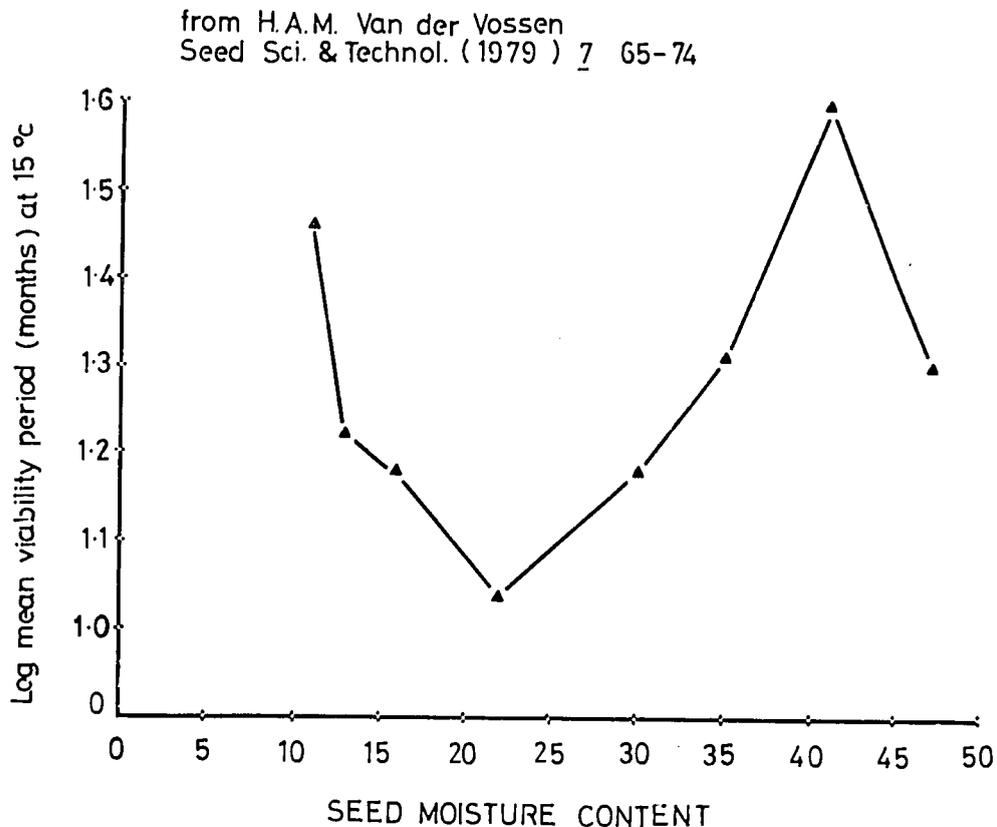


FIG 11. The effect of seed moisture content on the rate of loss of viability of coffee seed held at 15°C.

viability period at 15°C for 37.5% and 30.6% mc are 27 and 51 days respectively. However, the observed mean viabilities under these conditions are 70 days at 37.5% mc and clearly in excess of more than 70 days (when sampling stopped) at 30.6% mc. From the same argument as proposed for Coffea, the critical moisture content can be deduced to lie between 30.6 and 15.8%. As the lime seeds had moisture contents of approximately 45% on extraction from the fruit, the first phase of drying, prior to banking, will take place under conditions when repair can occur but for which the controlling factors are not yet fully understood.

A similar approach can be applied to work carried out at Wakehurst Place on a tropical timber tree, Agathis macrophylla (Araucariaceae) from the Solomon Islands. Laboratory storage studies had quantified the viability constants to be:- $K_L = 3.889$, $c_1 = 0.1481$, $c_2 = 0.052$. In the wild, seeds are shed at about 40% mc when the cone "shatters". Table 6 shows the observed losses in both moisture content and viability when the seed was kept under the ambient humid tropical conditions of the Solomon Islands (27°C and 82% RH). The seeds were sheltered from the direct effects of sun and rain by a simple shelter and supported on chicken wire in a thin layer some 3ft above the ground.

Time since harvest days	Seed moisture content % w w b	Observed viability level %	Observed viability loss Probit units	Calculated viability loss Probit units	Calculated viability level % ₁
0	37.3	100.0			99.999+
2.5	24.4	99.0	1.393	3.03	99.999
4	18.2	100.0	1.393	7.58	99.7
5	14.3	98.5	1.655	0.835	97.0
6	14.5	94.0	0.509	0.445	93.0
7	13.3	87.0	0.429	0.337	87.0
9	18.4	84.0	0.132	0.728	65.5

1 Calculated assuming observed germination level on day 7 to be accurate.

TABLE 6. Comparison of observed and calculated viability losses of Agathis macrophylla seed held under ambient conditions on Santa Cruz, Solomon Islands.

The expected losses were calculated from the viability equation, assuming that the arithmetic mean of the initial and final moisture content were experienced for the intervening period. The reasonable agreement between the observed and expected losses for the two periods between day 7 and day 5 suggest that at these lower moisture contents the model holds. There is also acceptable agreement for the interval between day 5 and day 4 if the true viability on day 4 was 99.9% (probit value 8) and disguised by the small number of seeds used in the germination test. Despite the large differences between the observed and expected results in the interval between day 4 and 2.5 the results are equivocal. The model could hold up to 24% mc if the seed lot had a very high initial viability so that the expected viability losses were occurring at levels unlikely to be revealed by a 200 seed germination test. However, it seems highly improbable that the 300 probit units of viability could be lost between day 0 and 2.5 without effecting seed viability to a greater extent than was observed. In Agathis the critical moisture content can be deduced to lie between 18 and 24.4% mc.

The discrepancy found in the interval between day 9 and day 7 could be explained by the seed rising above the critical moisture content for a time during this interval allowing repair to take place. This seems likely as some 250 mm rain fell in this period suggesting that a considerable proportion of the time would be at 100% RH causing the seed moisture to rise.

So far, the evidence for increased longevity at moisture contents above the "critical" has come from seed naturally shed at high moisture contents. However, seed of many of the crop plants of interest to genebanks reduce to much lower moisture contents during maturation and before dispersal. Nonetheless, the same phenomenon appears to occur in such seeds. A detailed study of the effect of moisture content on seed viability has been under-

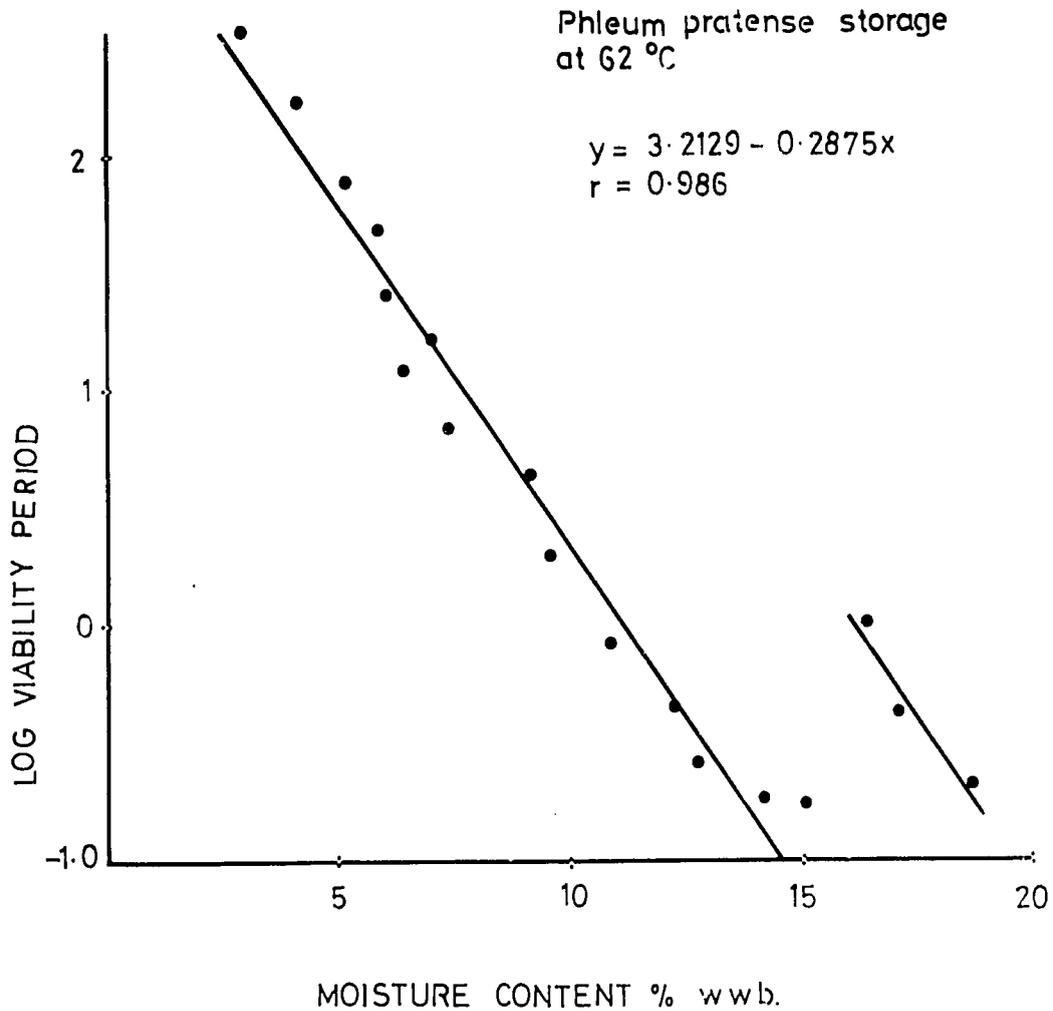


FIG 12. The effect of seed moisture content on the rate of loss of viability of Timothy S48 (Phleum pratense) seed.

taken at Wakehurst Place on Phleum pratense with the intention of quantifying the effects of reducing moisture content levels to below those currently recommended by IBPGR for long-term conservation. Some of these results are presented in Fig. 12. An increase in the moisture content results in a logarithmic reduction in the viability period between 3 and 15%. Between 15 and 16% mc, a break occurs in this relationship. Above 16% mc

the relationship resumes. The similar slope of the two sections of this line suggests that the relative effect of moisture content on viability is the same at the moisture contents either side of the break. However, at moisture contents above 16%, absolute longevity is approximately increased by an order of magnitude.

If, as in all other hydrated biological systems, repair in seeds above the critical moisture content is dependent on processes powered by aerobic respiration, then these results become explainable. In the experiment, seeds were hermetically sealed in glass tubes, in order to keep the differing moisture contents constant. The treatments were immediately begun following this sealing. Thus, the observed behaviour at higher moisture contents is explained if the seeds repaired damage until the finite supply of oxygen was exhausted. After that any damage accumulates and loss of viability occurs in the same manner as at the lower moisture contents. A similar situation has been shown to be true for lettuce seed at high moisture contents. (E.H. Roberts pers. comm.¹) In coffee seed the loss of viability at the highest moisture content may be due to the lack of oxygen as they were sealed in heavy duty polythene which is only partially permeable.

The present understanding of seed storage physiology over the whole range of moisture contents can be tentatively summarised in Fig. 13. At present, very little is known about the factors controlling loss of viability of seeds at high moisture contents under aerobic conditions. Yet these will often be the conditions under which they are collected. Therefore some advice, no matter how tentative, is required. The scheme shows that attention should be paid to ensuring that oxygen supply does not become

1 Now published: Ibrahim, A.E. and Roberts, E.H. (1983) of lettuce seeds. I. Survival in hermetic storage. *J. Exp. Bot.*, 34, 620-630.

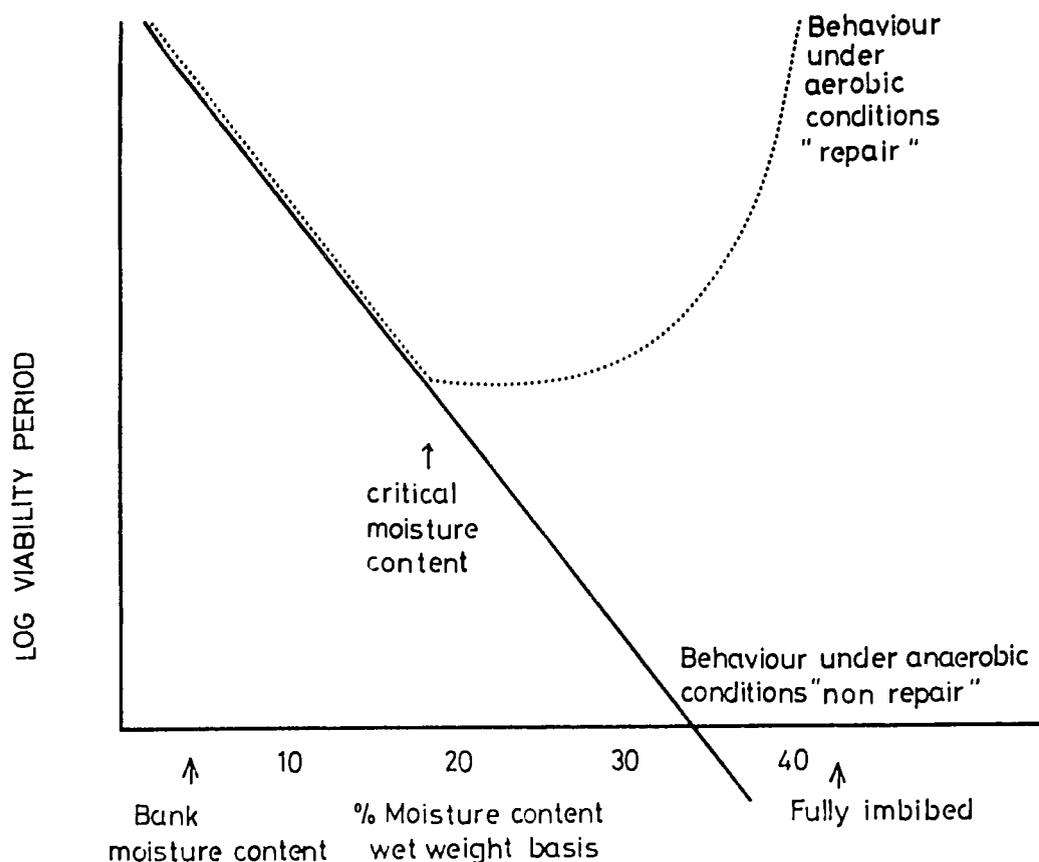


FIG 13. Schematic presentation of the effect of seed moisture content between 3-40% (wwb) on the rate of loss of seed viability under aerobic and anaerobic conditions.

limiting as this will remove the possibility for repair and thus increases the rate of loss of viability dramatically. The seeds should therefore be kept in freely permeable bags and packed in such a way so that oxygen diffusion is not restricted. However, if oxygen can diffuse in, then water can diffuse out and the seed will begin to dry. Here the evidence suggests that reducing the moisture content from levels close to full imbibition will result in increased rates of loss of viability until low moisture contents are reached. For example in Coffea the rate of loss of viability was greater at all the lower moisture contents than that

observed at 41% mc. Extrapolation suggests that seed would need to be dried to 9% moisture content or lower if loss of viability were to be reduced in comparison to that found at 41%. Therefore, if maximum viability is to be retained, then the time spent at these intermediate moisture contents must be reduced to a minimum.

When collecting seeds normally dispersed in fleshy fruits, this can be most easily achieved by trusting evolution and assuming that the environment within the fruit is unlikely to be harmful to seed longevity. Therefore seed should be kept in the fruit during transport and only extracted when drying can be carried out under controlled conditions.

High relative humidities in the ambient environment will greatly reduce the possibility of rapid field drying of the seed to moisture levels where longevity is greater than would result if the seed was held under aerobic conditions at its dispersal moisture content. Thus, care will have to be taken to ensure adequate ventilation of the seed in order to prevent an aerobic condition occurring which would increase the rate of viability loss. As was recommended earlier, frequent despatch of the seed from the collecting region to a location for controlled drying will reduce most of these risks.

DRYING

This topic is dealt with more fully in a later paper by Adam Cromarty, thus two points need only be made here.

The first is that any choice of drying conditions will be a compromise between the beneficial effects of reducing seed moisture content more quickly by increasing the temperature and the deleterious effects raising the temperature will have on the rate of

loss of viability. Thus, the balance will have to be made to ensure that the overall result will be to increase longevity. Simple calculations, based on the viability equations, should enable this balance to be accurately drawn.

The second point concerns the advantages in terms of preserving longevity which can be achieved by the aspiration of a thin layer of seed when compared with drying seed in a bag, even though the temperature and relative humidity are the same in both cases (Fig.

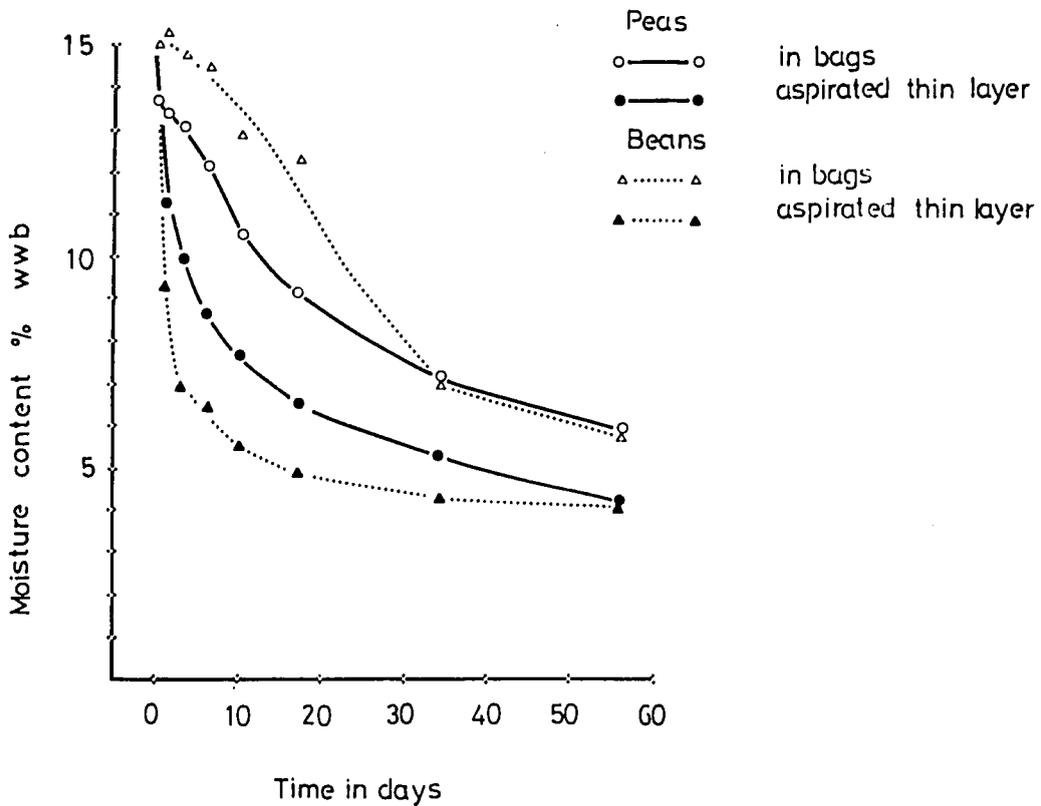


FIG 14. Drying curves of pea and broad bean seeds held at 15% RH and 15°C under different conditions.

14). The considerably greater drying rates obtained over the first 10 days when either the peas or beans were aspirated in thin layers, suggests this may be the appropriate way to ensure that the least time is spent at the unfavourable intermediate moisture levels. Over the moisture content range covered in the experiment the reductions in initial moisture viability expected in peas or beans dried either in bags or in an aspirated condition were nonetheless small. In broad beans, 0.014 probit units of viability would be lost in drying to 5% moisture content in an aspirated thin layer, rising to 0.049 probit units when dried in bags. In peas ventilated drying to 5% m.c. resulted in 0.002 probit units being lost. In bags this value rose to 0.035 probit units. Such losses are insignificant when compared to the damage which can occur in the field. However when converted to storage periods at -20°C and 5% mc the differences suggest that consideration should perhaps be given to aspirated drying of seed lots sufficiently large for banking. In this way, any lots very close to the regeneration level would have sufficient bank storage life preserved to give time for evaluation to take place before regeneration was necessary. Thus the unnecessary regeneration of duplicate material could be avoided.

SEED PROCESSING

One final phase of bank activity known to have some effect on seed viability is cleaning and processing. Here, the evidence is at its most equivocal, presumably due to the differing resistance of seeds of various species to mechanical damage.

Ellis and Roberts (1981b) have demonstrated that repeated threshing of barley in a single drum thresher effects neither initial viability nor any of the storage constants. Barley is known, however, to be relatively resistant to mechanical threshing damage.

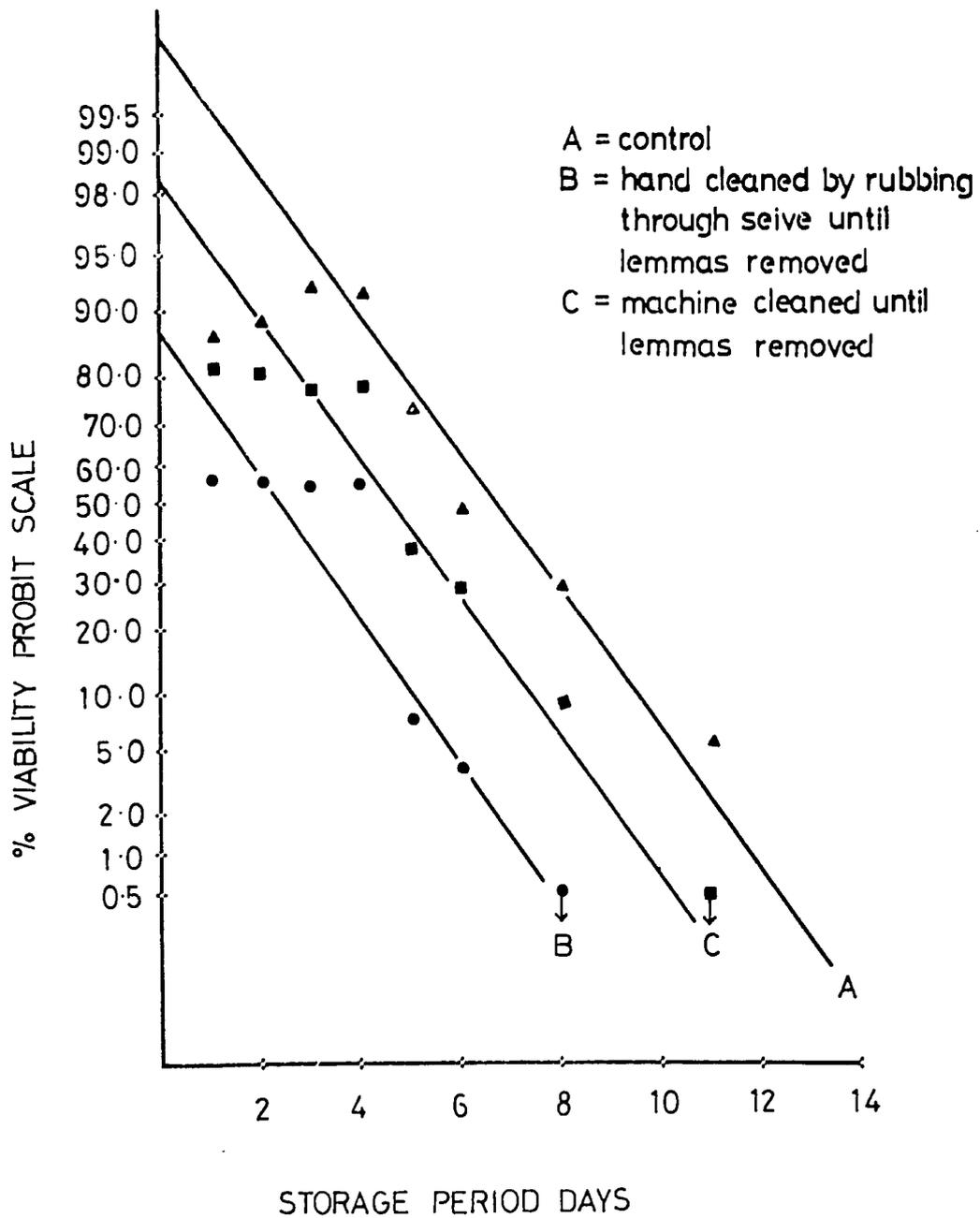


FIG 15. Loss of viability in Timothy (*Phleum pratense*) seed under constant conditions following (62°C and 9.3% moisture content wwb) different seed cleaning methods.

On the other hand, Fig 15 shows a reduction in the initial viability of sub-lots of Timothy seed which had the lemmas removed by hand or by machine. Here the effect seems to be to reduce the initial viability of the lot. In contrast MacKay and Tonkin (1967) show that the milling of Sainfoin and Tall Oatgrass did not reduce their subsequent longevity, indeed the reverse was true.

In the absence of clear experimental evidence, seed processing should be monitored to ensure that no physical damage occurs to the seed. In this way, it seems likely that risk of reducing viability can be avoided. If potentially damaging processes are necessary before the seed can be sown, these should be delayed until after storage. Thus, the risk will only ever be experienced at any one time by part of the bank sample and any fatal errors can be corrected by the simple expedient of taking another sub-sample.

CONCLUSIONS

In conclusion, I hope that the evidence presented has shown that:-

(a) the absolute storage life for each seed lot under bank conditions will depend on its initial viability at the time of banking. Differences in initial viability are often undetectable by simple t-tests carried out on the results of germination trials before and after treatment unless sample sizes of several thousand are used.

(b) the processes which bring about loss of viability exert their influence under field conditions from some as yet unspecified time when maximum seed viability is achieved.

(i) at moisture contents above a critical value, usually in the range 16% - 24% wwb. loss of viability will depend on

the gaseous environment surrounding the seed. Under aerobic conditions, the controlling processes are not yet clearly defined. However such evidence as exists suggests that the closer the moisture content of the seed is to full hydration, the greater will be its longevity. Under anaerobic conditions loss of viability is in accord with the viability equations of Roberts and Ellis. The loss of viability under anaerobic conditions appears to be greater than those under aerobic conditions when held at identical moisture contents.

(ii) below the critical moisture content, loss of viability is more fully understood, being an integration of moisture content, temperature and time according to the viability equations of Roberts and Ellis. Thus, the expected behaviour of any seed lot under a variety of different conditions can be predicted with reasonable accuracy.

(iii) the simplest practical way to minimise the loss of longevity which can occur during collecting missions appears to be frequent and rapid despatch of the seed to a location so that rapid drying can take place under conditions which maximally preserve viability.

(iv) maximal preservation of viability will occur when the time spent between those moisture contents at which damage is fully repaired and those where the accumulation of damage is sufficiently slow to be ignored is kept to an absolute minimum. The aspirated drying of thin layers of seed under controlled environments (15°C and 15% RH is currently recommended) would appear to achieve this aim.

(v) if easily observed physical damage is avoided during cleaning and processing, then it is unlikely that any adverse effect on viability will result.

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DISCUSSION

Chairman: J. Aaronson

Participants: Bekendam, Roberts, Tyler,
Williams, Stoianova, Gerard,
Rennie

1. The Importance of Drying Seeds Immediately

In answer to a question from Dr. Bekendam, Mr. Smith re-stressed the importance of immediately moving freshly harvested seeds to a facility where they can be dried under the optimum artificial conditions of 15°C and 15% RH; and holding them there until cleaning is possible. By way of example, he described two separate lots of collections made in Greece by staff of the RBG Kew Seed Bank. One was dried under ambient conditions in the laboratory for up to six months before banking; the average viability of accessions in that lot was lower than that of the second lot of collections, which were moved directly after collection into the drying room at Wakehurst Place. Immediate drying also has the advantage of making seed cleaning operations easier.

Agreeing with Mr. Smith, Prof. Roberts cited work prepared in his department at Reading, which has shown the dramatic effects a delay in drying can have on the eventual storage life of chickpea seeds, regardless of storage method. Maximum longevity is achieved by harvesting as soon as possible after physiological maturity; even minute drops in percentage viability can have large effects on potential longevity. In addition to doing it quickly, it is important to reduce the moisture content to a reasonably low level, as small differences in moisture content can have large differences on longevity (see P. 167). Prof. Robert's third point was that more research is required into the question of optimum moisture contents for storage. It should be noted that moisture contents between 15% and 25% (fresh weight basis) are the

worst for seed storage; if satisfactory rapid drying cannot be achieved, it may be better in some cases to keep seeds moist until facilities are available, thereby minimising the time seeds are kept at the 'dangerous' moisture contents.

Dr. Aaronson wondered if and how seeds could be best dried en route on an extended collecting expedition. Prof. Roberts thought that it should not be too difficult to devise field drying techniques once the problem is appreciated and suggested that further research would be appropriate. Mr. Tyler said expeditions from WPBS often had to make collections under wet conditions, and they had found both modified fan heaters and portable silica gel drying cabinets quite useful.

2. Harvesting and Post-harvest Movement of Seeds

Leading on from the last point Dr. Aaronson wondered what instructions Mr. Smith gives to his collectors so that seeds get into storage rapidly and in the best condition; bearing in mind that it is probably only possible to visit an area once and then it is not possible to remain there for the whole ripening season. Mr. Smith's attitude was to collect the best available seeds at the time and then get it into stable storage conditions as quickly as possible. If, for example, collection in isolated and difficult country is anticipated, it would be best to make contact with a reliable freight agent in the capital city or other centre with a good air service to the location of the genebank. The collections could then be brought to him for despatch at frequent intervals from the field sites by runners; Airway Bill numbers can be used to trace and chase seed batches by the genebank in receipt of them.

In some cases it may be worthwhile trying an Air Courier Service to process material rapidly. The added cost must be weighed

against the expense of regeneration if due to delays en route, seeds have dropped below the regeneration standard with the concomitant accumulation of genetic damage. Ideally, the aim should be to get material back to the bank in such a condition that it can be stored for 200 years, say, without regeneration. Dr. Aaronson pointed out that there can be problems with the runner/freight agent system. To illustrate this he quoted an example, where he had been collecting in N.W. India and had arranged for people in Jodhpur to despatch material for him, and yet there was still delay, because the authorities had insisted he be present in person for the despatch of the material. So it will be sometimes necessary to get appropriate clearance to operate such systems.

As regards the desirability of harvesting seeds as soon as possible after physiological maturity, Dr. Williams explained that many genebanks collect from markets and farmers' stores. Obviously seeds collected thus would not be at their best; and he wondered whether regeneration should be recommended as a matter of course in such situations. Mr. Smith was against regenerating until the regeneration viability level is reached and pointed out that regeneration of outbreeders may not be easy. In the field it is important to make the collection, whatever the source, followed by application of whatever procedures are possible to check further shortening of the seeds' lifespan.

According to Dr. Williams, on many collecting trips it may be six weeks before material is returned to the institute; and then it may not be dealt with for up to a further two months. In addition to the above there may be administrative problems. By way of example, Dr. Williams quoted the fate of seed collected on a recent expedition in one country; it was not despatched for eleven months due to the stringent requirements by the authorities there for paperwork. Prof. Roberts remarked that the effects of these

problems would be minimised, if seeds could be dried and sealed on the spot; so long as material is of a low moisture content damage is minimised, regardless of the temperatures experienced (see earlier comments on the importance of drying).

Mr. Tyler suggested that selective collection of immature seed heads, though it might minimise losses in viability, is not to be recommended because of the consequent non-randomness of sampling.

3. Introduction of Pests and Diseases

With regard to drying seeds at 15°C and 15% RH, Mrs. Stoianova wondered about the importance of initial pest control, when for example bruchids have been reported to survive at temperatures as low as -10°C. Mr. Smith replied that in practice bruchids and other pests had not been a problem. According to experts (Dr. Southgate et al) at the U.K. Ministry of Agriculture Pest Infestation Control Laboratory, bruchids within seeds arriving at a seed bank are incapable of eating into neighbouring seeds; and bruchids emerging under dry room conditions are generally cold and sluggish. Prof. Roberts added to this by stating that although a large number of insect species may survive at 15°C and 15% RH very few feed under these conditions. Dr. Gerard said that the main problem with bruchids is if they escape and attack other seeds; but as Prof. Roberts pointed out, the problem is limited because once the seeds are out of the drying room they will be in sealed containers.

Dr. Aaronson wondered what measures could be taken against other problems, e.g. nematodes. Mr. Smith replied that with the resources available to them staff at Wakehurst Place don't do as much as they would perhaps like to; but emphasised the Phyto-

sanitary Certificates are not issued, the onus being on the user to arrange for the import of Kew Seed Bank material to his country.

Mr. Smith wondered whether it is safe to assume that after, say 20 years storage at low temperature and low moisture content, spores etc., would be eradicated. Storage studies on spores of the disease organisms are required. Dr. Rennie replied that information on this topic is very limited. While it is possible to say that the seed microflora would change with length of storage, it is impossible to say that no pathogens would be present after long periods of storage.

Techniques for Drying Seeds

A. CROMARTY

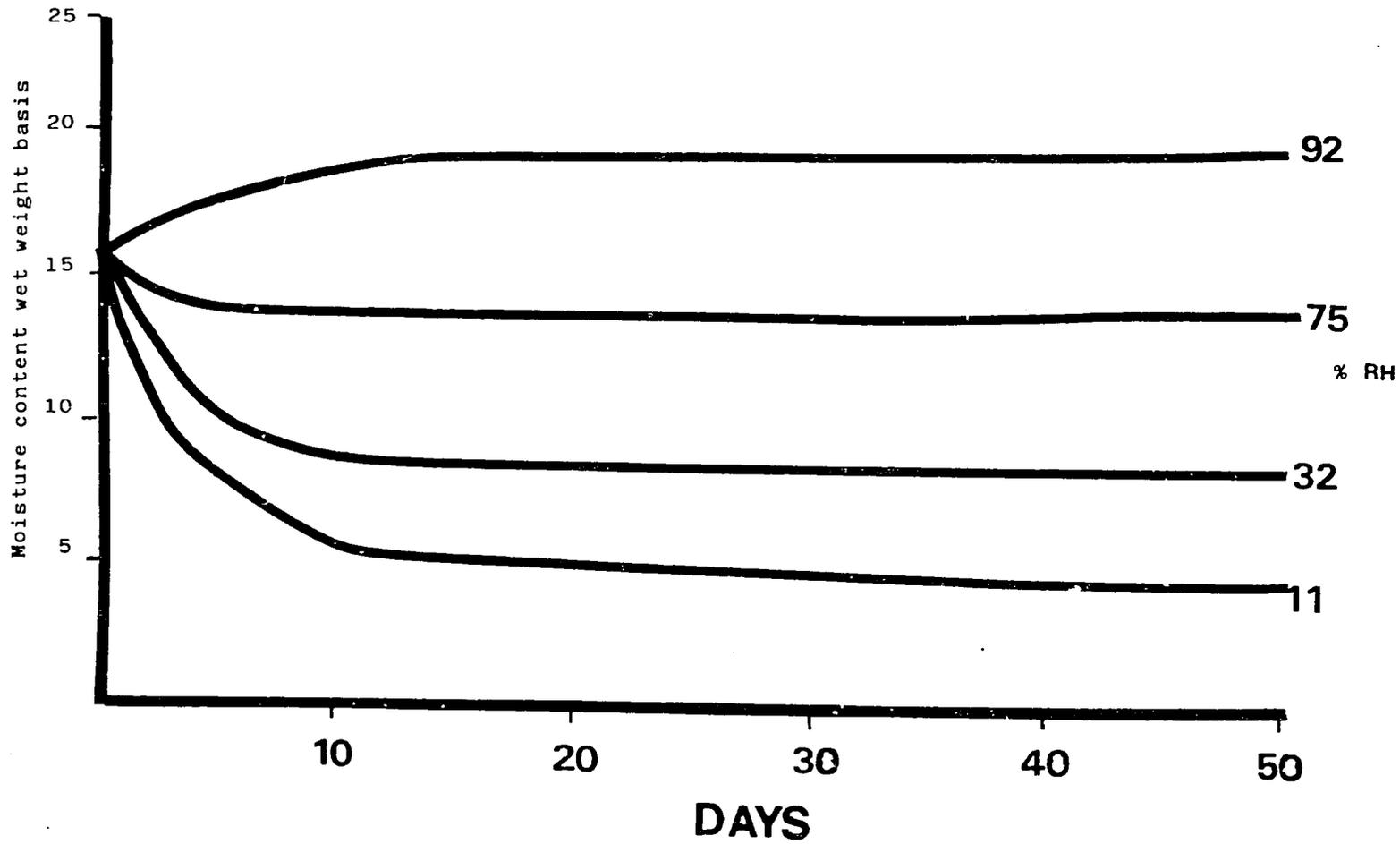
INTRODUCTION

The aim of this paper is to provide a general introduction to the various techniques and equipment available for drying seeds. Inherent seed drying characteristics are reviewed and graphical design methods investigated as a means for coping with present and future operational problems without making complex calculations. A list of useful published papers, which cover drying theory and related subjects are given in Appendix 1 and it is hoped that direct reference will be made to "The Design of Seed Storage Facilities for Genetic Conservation", IBPGR, Rome, which contains relevant information and worked examples.

THE EQUILIBRIUM MOISTURE CONTENT

The equilibrium moisture content is an important factor governing seed viability and drying calculations. It is simply a direct relationship between seed moisture content and the relative humidity of surrounding air. More specifically, free moisture, expressed on a wet or dry weight basis in seeds exerts vapour pressure (related to moisture content and temperature) which is in balance with atmospheric vapour pressure at an equilibrium point. Under all other conditions, water will be lost by desorption or gained by sorption. In practice experimental data are established by allowing seeds to equilibrate under controlled conditions, typically in sealed jars containing saturated salt solutions with known hygroscopic properties and giving known R.H. values; the changing moisture content is monitored at regular intervals by weighing a shallow wire mesh basket suspended from a free running wire which passes through the bung to an external balance. Fig. 1

Fig. 1 Equilibrium data for Rice at 20°C for different relative humidities



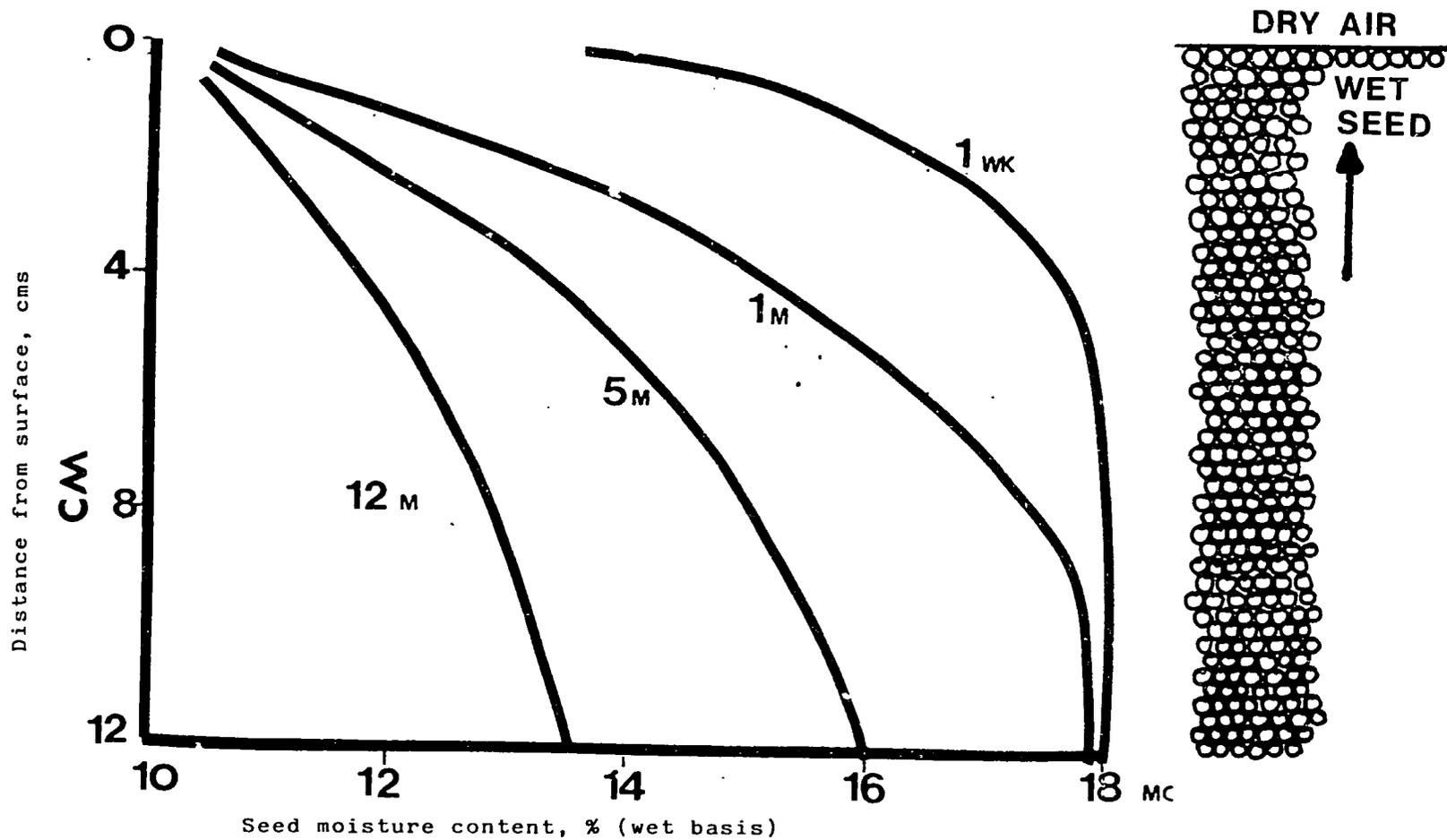
shows time taken for rough rice, kept at 20°C under a range of fixed relative humidities, to equilibrate. For example, at 11% relative humidity the rice takes 10 days to approach 5% moisture content (wet weight basis) but 30 to 40 days to reach equilibrium. (These final losses in weight are difficult to detect because the measuring error is large and the weight change small.)

These characteristics are also relevant to drying room operations because they indicate general drying times under "static" conditions. Both increasing seed size and reducing temperature increase the time necessary to complete the process as does contact between seeds and the lack of air movement within a deep layer.

DRYING IN RELATION TO DEPTH OF SEED

Fig. 2 shows the rate of drying in relation to the depth at which seeds lie in an open bag. Here, seed 4 cm deep takes 1 month to reach 16% moisture content and 5 months to reach one of 14% (wet weight basis). Because seed viability is affected by moisture content, there will be differences in longevity of seed at different depths. Hence, if samples are only taken from the surface layer, misleading viability data for the seed lot as a whole will result. Again the situation is exacerbated when bags are placed on insulated floors because this "seed" behaves as though it is a part of the floor structure. Consider tropical conditions where the sub-floor temperature may be as much as 30°C, the temperature gradient through the floor to the grain becomes a linear relationship. Thus although the air temperature in the room may be 15°C, the floor surface may register 25°C, leading to greater viability losses than would be expected. Periodic mixing can further confuse the issue giving rise to spurious results. Uniformity of equilibrium moisture content and seed viability within a seed lot requires either thin-layer drying or sufficient through venti-

Fig. 2 Drying characteristic of exposed moist grain beds

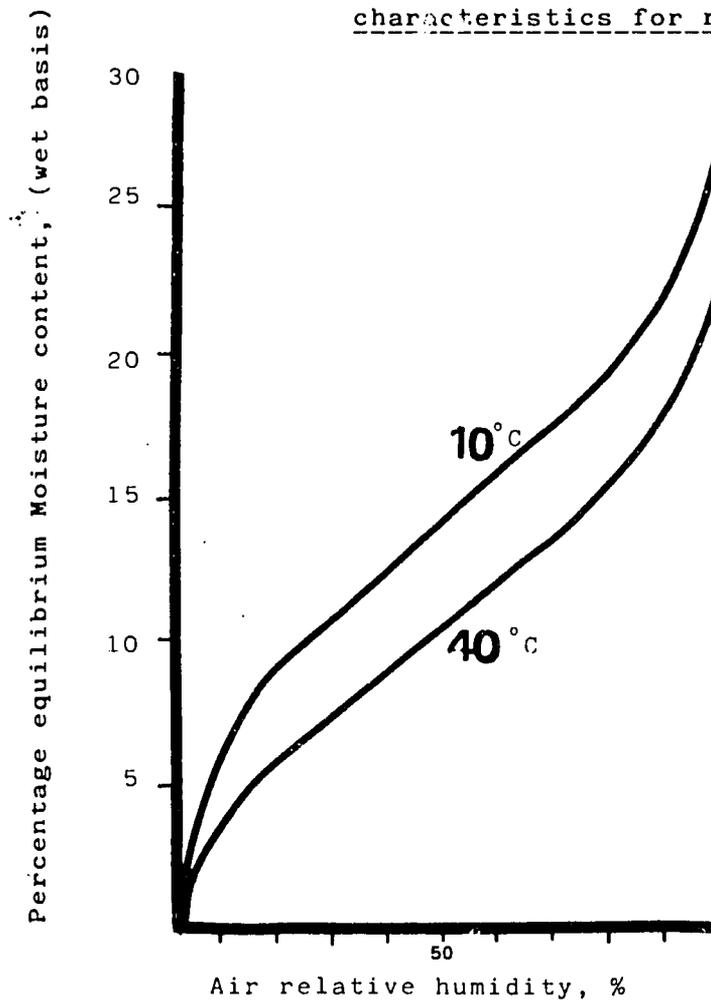


lation to break up the micro climate. At all costs on-floor storage drying should be avoided.

THE INFLUENCE OF TEMPERATURE ON EQUILIBRIUM MOISTURE CONTENT

A plot of equilibrium moisture content versus air relative humidity produces a typical sigmoid curve (isotherm) which displaces downwards as seed temperature increases (Fig. 3). This characteristic becomes important when storage and processing temperature are different; a small rise in seed moisture content may be obser-

Fig. 3 The influence of temperature on equilibrium characteristics for rice.



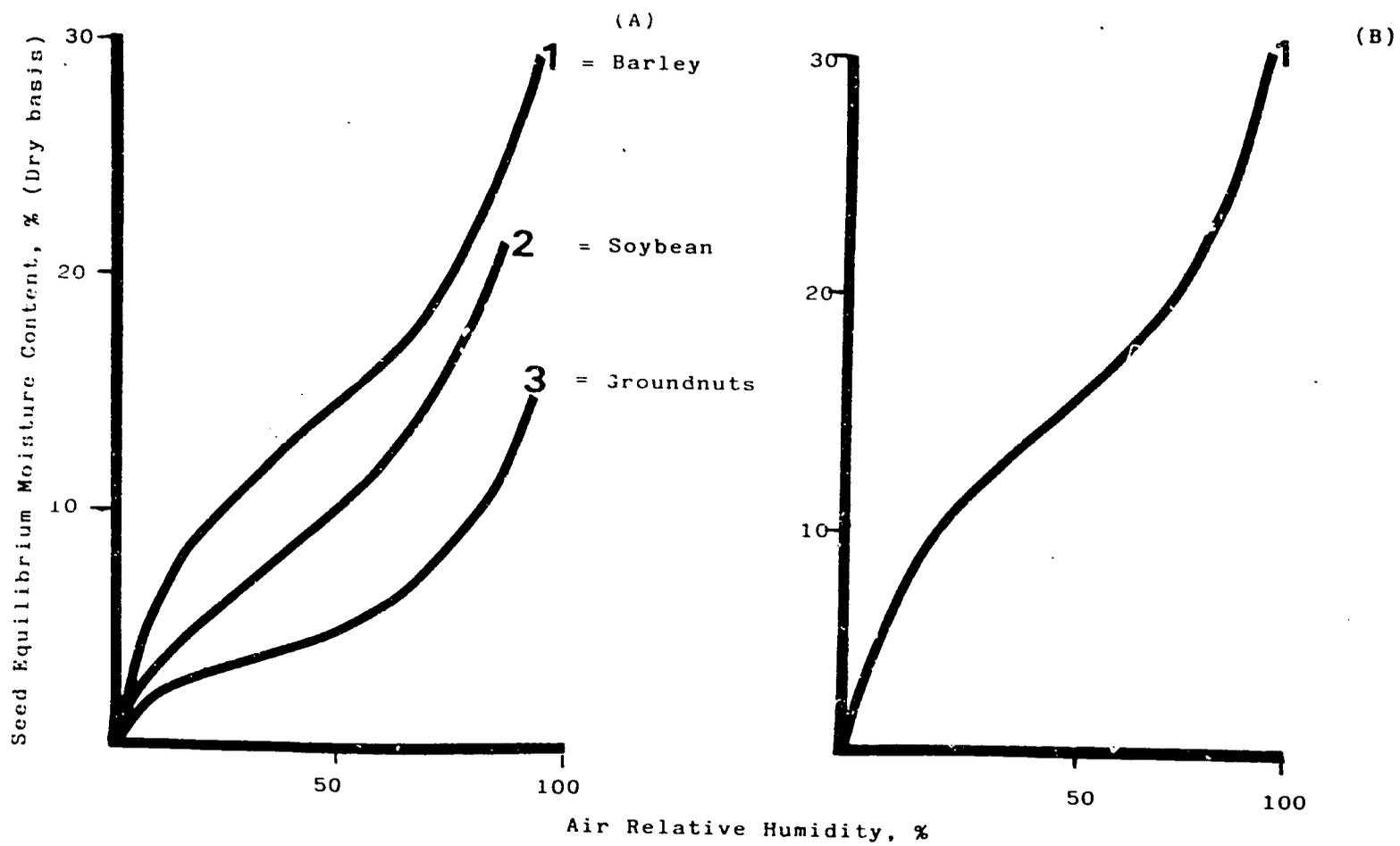
ved under cold storage although the relative humidity is constant.

THE EFFECT OF OIL CONTENT ON EQUILIBRIUM MOISTURE CONTENT

Fig. 4(A) shows how different oil contents in Barley, Soyabean and Groundnut seed can affect the equilibrium moisture content. The curves are depressed as the amount of oil present increases. Making precise moisture content determinations of oily seed can be technically challenging. However, recalculating moisture content, having first disregarded the amount of oil in the seed, produces a substantially uniform result (Fig. 4(B)). In practice, data for true final equilibrium moisture content values are only available for major commercial crops, but a general classification of seed based on seed oil content can serve as a useful guide when drying seeds.

NOTE that the time taken to reach "equilibrium" varies according to the criteria used and secondary dry matter losses (as a result of respiration) adversely affect results particularly when large seeds are dried at low temperatures. Consequently, the limit used in the seed drying Nomogram, which is described later, has been defined by the practical needs of genebank operation rather than an ill defined theoretical equilibrium moisture limit. But the proposed first line can be adjusted to suit individual preferences.

Fig. 4 (A) Influence of oil content on Equilibrium Data, (B) Same data corrected to a common "oilless" base.



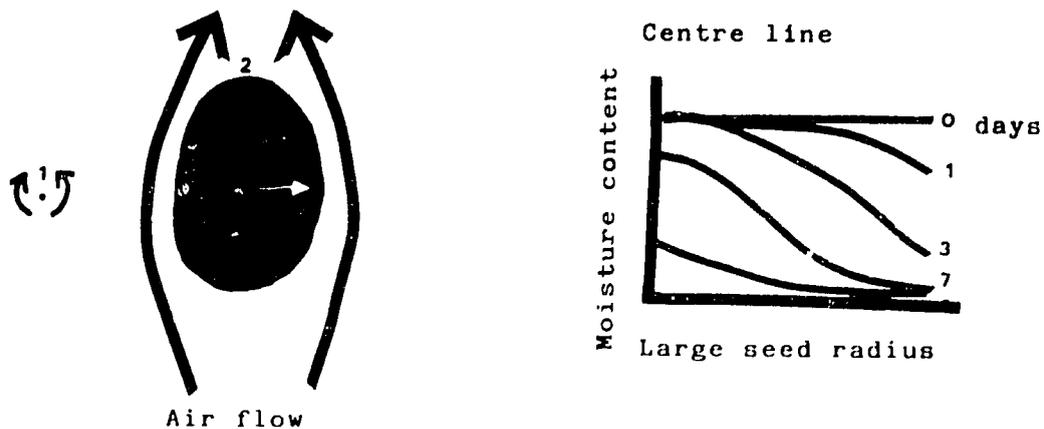
SEED DRYING - THIN-LAYER DRYING OF SEEDS

Drying is a complex continuous dynamic process with changes in seed, local air temperature and moisture content occurring simultaneously. Experimentally thin layers of seed are forced ventilated to ensure that the transfer of moisture vapour from the seed surface to the circulated air is not a limiting factor. Therefore published thin layer test data represent the maximum drying rate that can be achieved for specific conditions and is unlikely to be duplicated in a genebank drying room. The process is "driven" by the so-called "air vapour pressure", which is dependent on the air dry bulb temperature and moisture content. Internal vapour pressures can be estimated for seeds because of their hygroscopic characteristic. A difference between air and seed vapour pressure levels forces water vapour down the pressure gradient to give either drying or moisture regain. However, individual physical characteristics control the observed result.

SEED SIZE IN RELATION TO DRYING RATES

The internal moisture changes occurring in seeds are very similar to the bag drying example described previously with the overall bed depth being replaced by seed diameter. Fig. 5 indicates the size difference between Poppy and Broad Bean seed. The associated graph gives a hypothetical drying characteristic for the larger seed relative to the longest radius in time. Initially seed moisture content is uniformly distributed, but as drying commences, moisture is removed from the seed coat causing internal moisture to migrate outwards to compensate for the surface loss. The drying rate slows down exponentially as equilibrium approaches. Finally the vapour pressures balance. The small seed would have a drying rate similar to the surface layers of the larger seed and dry rapidly. Increasing the diameter causes a disproportionately longer drying time because of the extended

Fig. 5 Internal moisture diffusion



internal moisture flow path. It is uncertain whether these moisture gradients affect viability of large seeds but structural damage does occur. Unfortunately the seed coat of legumes tends to dry and shrink more rapidly than the cotyledons causing the former to split. Multi-stage drying, with descending levels of controlled humidity or a less favourable drier position, must be used to avoid this type of damage.

In many instances seed diameter can be expressed on a corrected seed weight basis and this fact speeds calculations. The overall drying characteristic mirrors internal moisture diffusion. Fig. 6 shows a range of thin-layer drying curves for different species at 70°C. Although not strictly appropriate to genebanks, high temperature/low relative humidity drying does show the effects which size and oil content have on the drying rate. For example, oil rich flax dries rapidly whereas the larger pea seed is the slowest. Isolating the various factors involved can be difficult.

Fig. 6 Typical drying characteristics for various seeds at 70°C

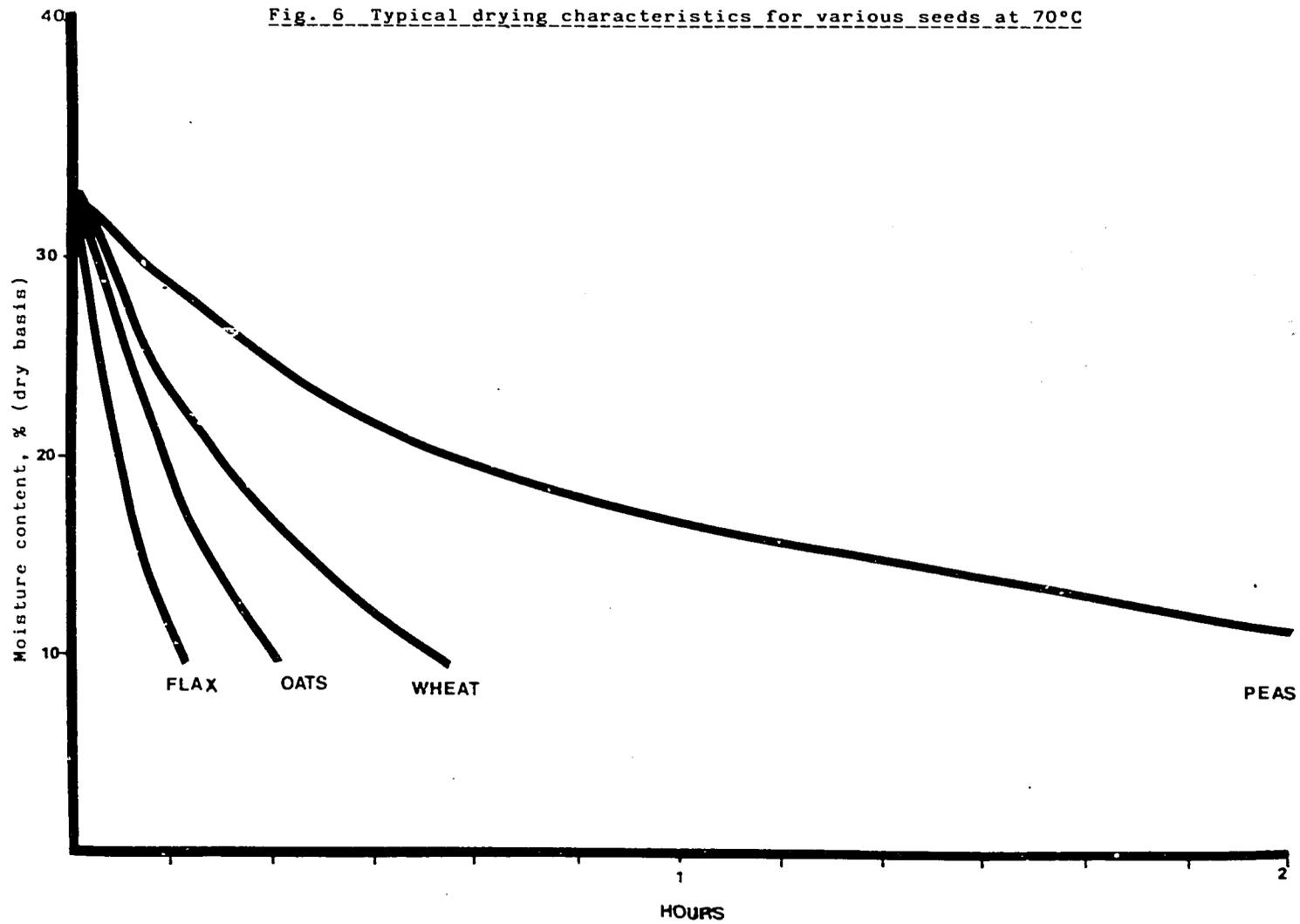
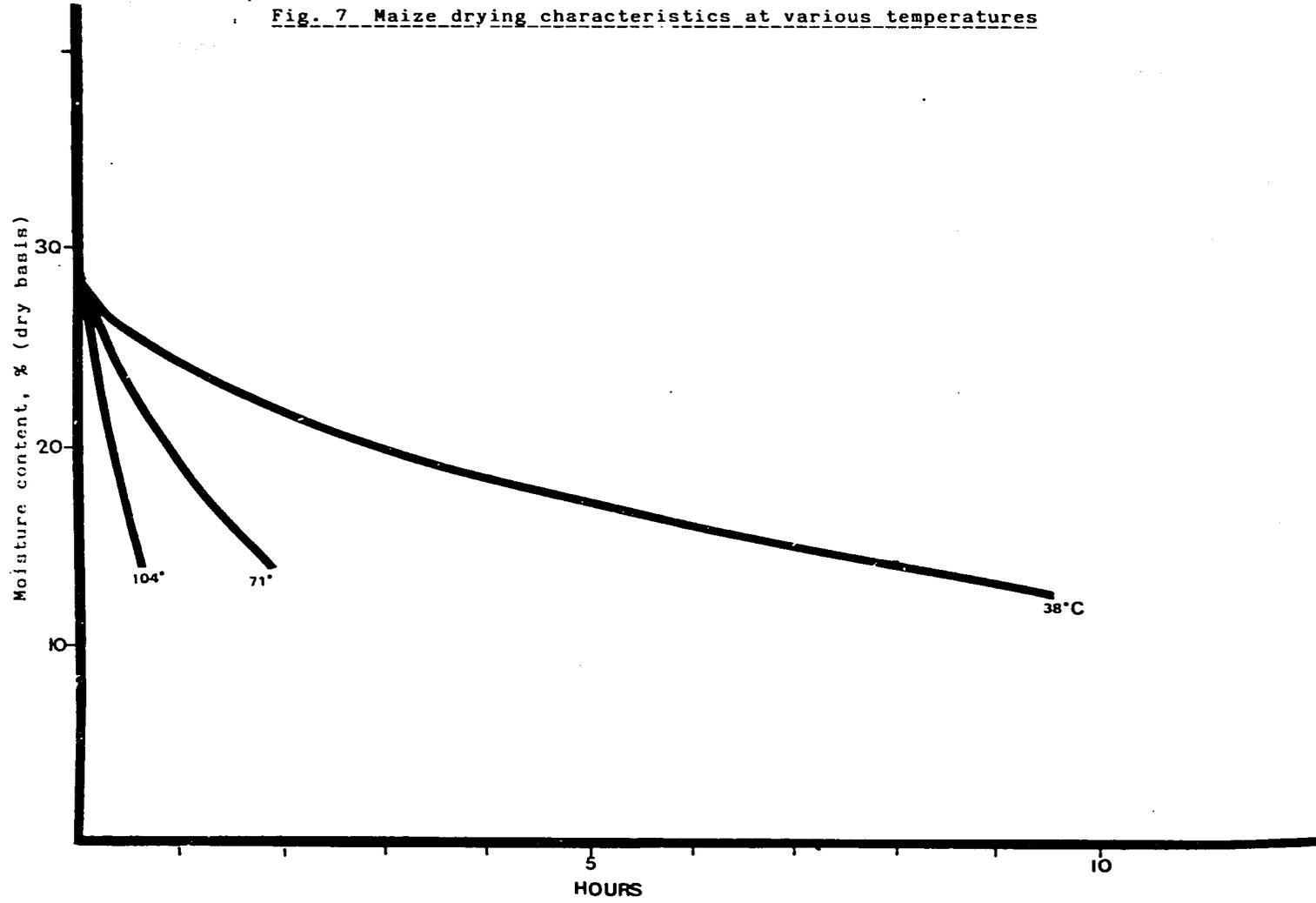


Fig. 7 Maize drying characteristics at various temperatures



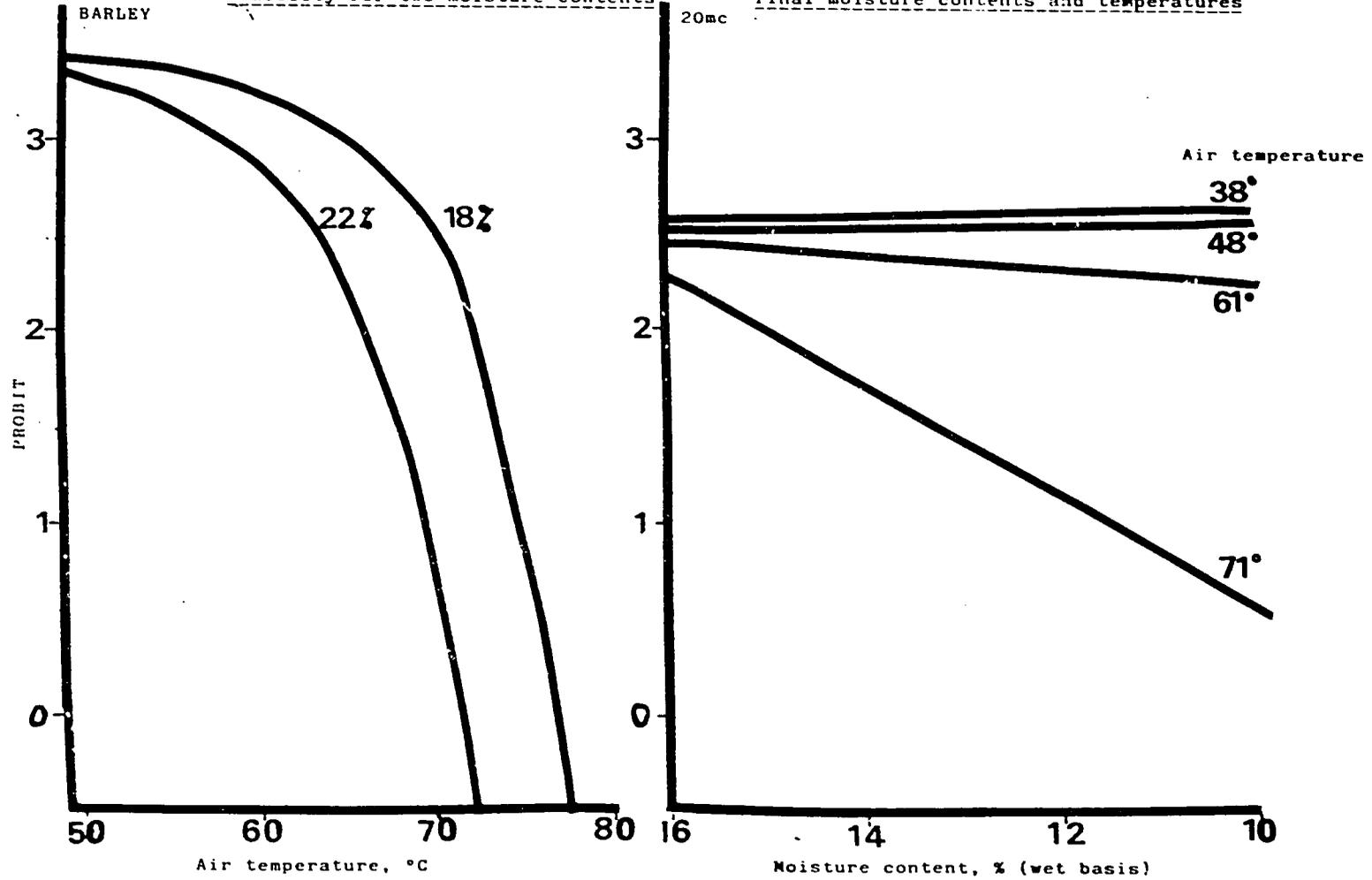
TEMPERATURE IN RELATION TO DRYING TIME

Fig. 7 shows a series of curves of 28% moisture content (dry weight basis) maize dried at 104°C, 71°C and 38°C. The lower the temperature the longer drying takes; once again an exponential relationship is involved. This situation causes a conflict of design requirements because, on one hand the moisture content reduction is rapid but high temperatures cause excessive viability losses while on the other, low temperatures extend the drying time and high moisture contents reduce viability. But a minimum viability loss temperature can be estimated and for a short life crop such as onion it appears to be near 10°C. However, drying at 20°C is almost as effective thus confirming the suitability of existing dry room recommendations. The calculations involved are complex and also depend on the drying system installed. Significant predictive errors occur if dry bulb rather than seed temperature (which may approach the wet bulb temperature for part of the drying period) are employed. It is anticipated that design procedures will be developed to minimise viability loss of individual species, along the lines described.

VIABILITY LOSSES DURING DRYING

Fig. 8(A) gives a plot of viability loss on the probit scale for two seed lots of barley at 22% and 18% moisture content (wet weight basis) dried in a 30 cm bed to 13% under a range of commercially applied temperatures. Note, that a certain proportion of germplasm entering a genebank will have passed through commercial driers and avoidable damage done but not detected because the conventional percentage viability scale is insensitive to seed deterioration close to the the 100% germination level. The figure also shows that the viability losses occurring during drying are related to this original moisture content and drying temperature, as predicted by "steady-state" seed viability studies. The curves

Fig. 8 (A) The effect of temperature on Barley viability for two moisture contents (B) Probit losses for 20% m.c. Barley for different final moisture contents and temperatures



begin to converge back at about the 40°C level. Fig. 8(B) helps to clarify the picture by showing viability losses for 20% moisture content (wet weight basis) barley dried with different air temperatures to final moisture contents ranging between 10-16%. The maximum losses occur, as expected, at the highest temperature. The advantages of low temperature drying are clearly demonstrated. When drying barely seed at a temperature of 38°C there is an insignificant loss of viability.

It is important to look at data for each crop. There are a number of opposing factors. For instance, small seed does not live very long at high temperatures and yet its moisture content may drop quickly under such conditions. Conversely, larger seed with good viability maintenance characteristics may take a long time to dry and large viability losses are recorded due to the time factor.

Although not directly applicable to genebank drying rooms, it is perhaps of passing interest to consider the effect which ultra high commercial drying temperatures have on seed viability. Extending the dry bulb air temperature range upwards would reveal that, having reached a minimum, the probit values begin to climb to reach a second peak indicating a "commercially" acceptable loss. This situation arises by virtue of the fact the temperature of carefully controlled seed, presented as thin-layer, remains comparatively low while the high temperature air stream has an exceptionally low relative humidity promoting local evaporative cooling.

PRESENTATION OF TEST RESULTS

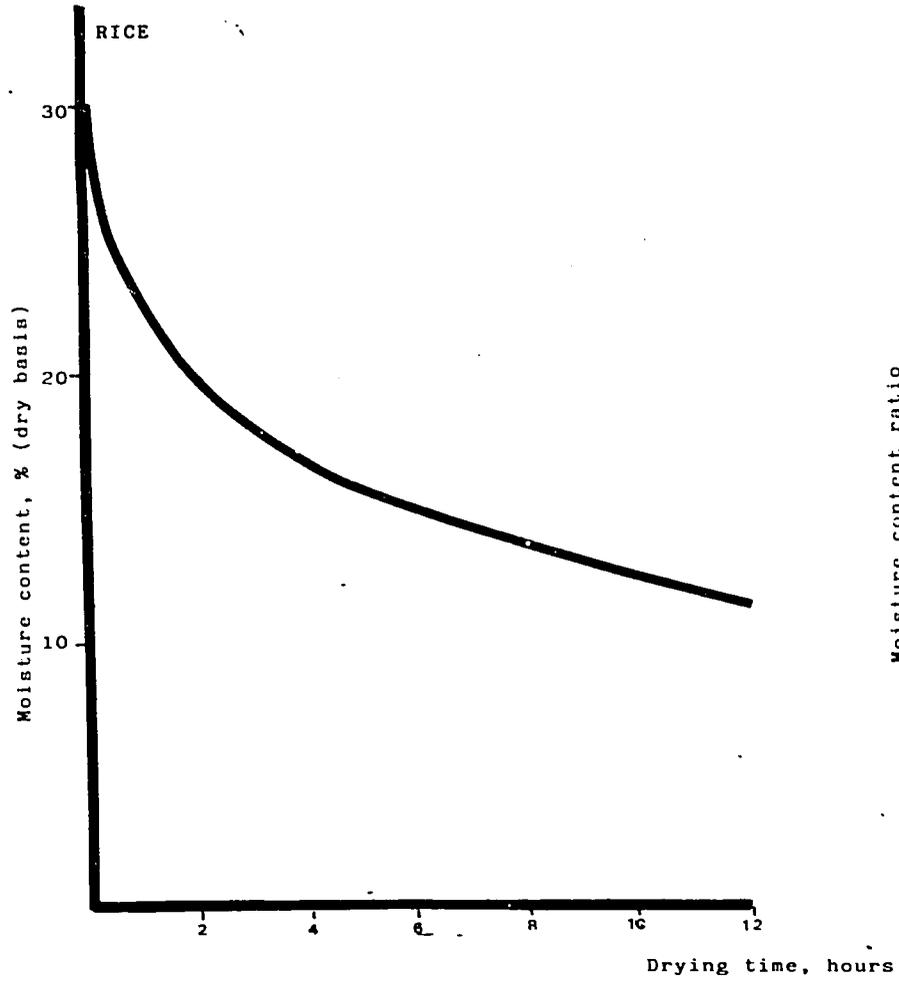
By convention, seed drying test results are expressed on a dry weight basis (i.e. the amount of free moisture in the seed divided by the dry matter content) because this rate directly indicates

the proportion of moisture remaining in the seed, at a point in time, and so greatly simplifies subsequent calculation.

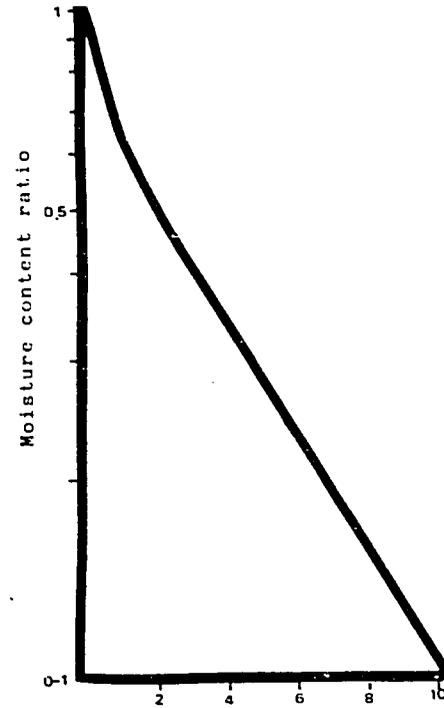
The genebank manager will by now be familiar with the general shape of drying curves for a wide range of environmental conditions. But determining final equilibrium moisture content values or comparing different sets of data can be difficult.

Experience has shown that moisture content versus time data can be replotted on log-linear graph paper to give an almost continuous straight line relationship. This is providing that the moisture content is expressed as a ratio of the total possible moisture reduction under the test conditions i.e. the difference between the observed and equilibrium moisture contents divided by the difference between the original and equilibrium moisture contents. This is called the moisture content ratio (MCR). A typical thin-layer drying characteristic for rice (Fig 9(A)), redrawn on the proposed basis (Fig 9(B)) shows a brief non-linear period which can be ignored when long term trends are under consideration. This form of analysis is only strictly correct when drying rates are nearly as slow as the original "static" equilibrium moisture content test conditions. Empirical "dynamic" equilibrium data, which compensate for internal moisture movement delays, must be used for most high speed commercial drying applications.

Fig. 9(A) Linear scale plot of drying Rice



(B) Same data plotted on Log Linear scale using moisture content ratio.



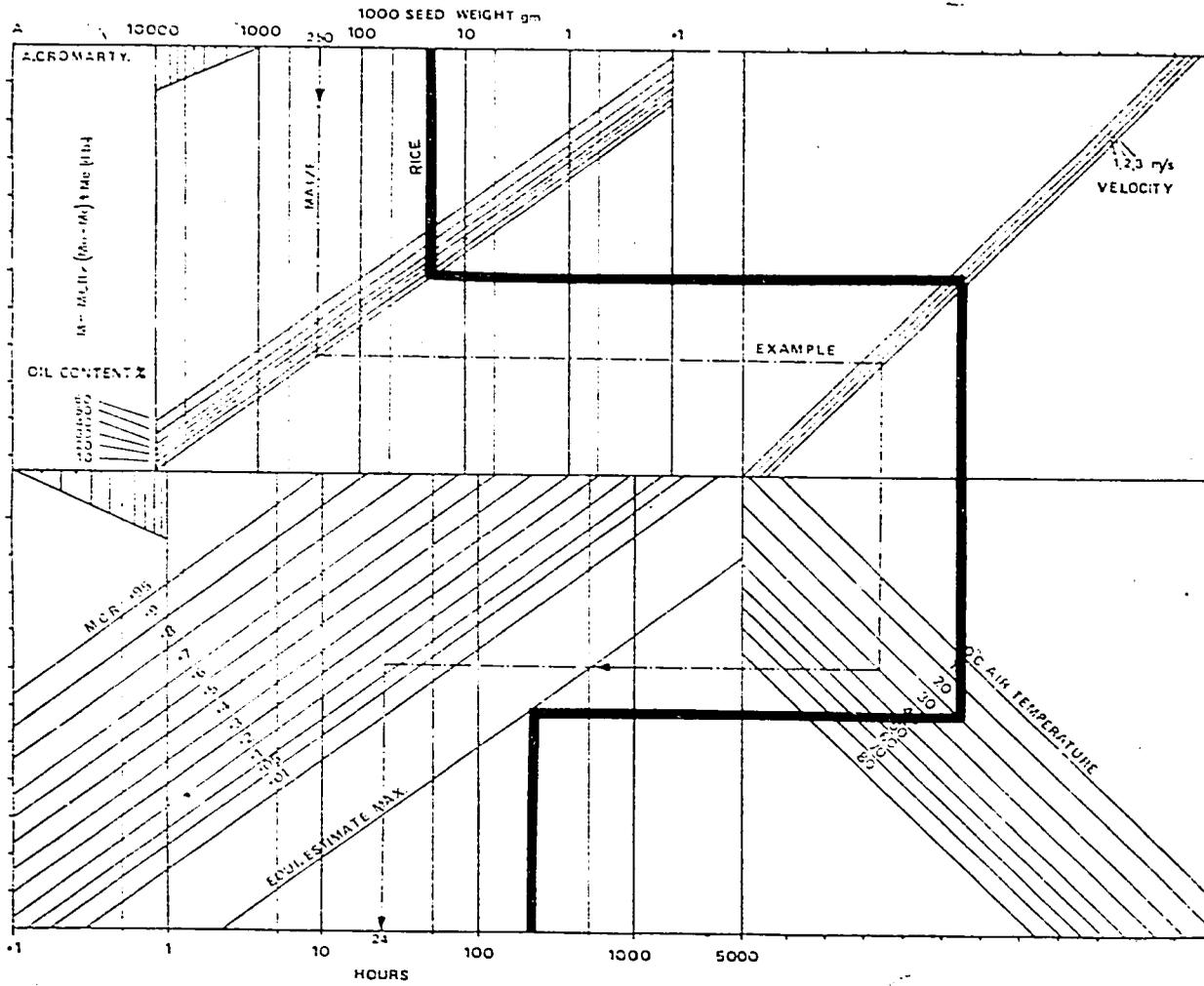
THE SEED DRYING NOMOGRAM

Unlike the seed drying technologist, the genebank manager requires a rapid method for predicting drying times in order to avoid viability losses, limit seed wastage and to help select and operate drying equipment efficiently. But seed weight and a general classification (e.g. oily or non-oily) may be the only information available at the planning stage.

The last decade has seen advances in computer technology and the rationalization of seed drying theory. Even so, only a comparatively small number of major cereal and oil seed crops, with regularly-shaped seeds have been fully investigated. Conversely seeds exhibit great variability both in size, shape and texture. The general solution to this problem has been to prescribe a common drying time irrespective of individual requirements that is both wasteful in terms of drying capacity and delays material entering cold storage. Clearly there is an immediate need for a less daunting approach which sets general performance limits to help drying management. The Seed Drying Nomogram (Fig.10) draws together, within a single chart, the primary factors influencing thin layer drying rate considered earlier. These are size (expressed as the 1000 seed weight), oil content, air velocity and drying temperature relative to the moisture content ratio (MCR); but size and drying temperature dominate the process.

A major difference between commercial and genebank drying procedure is that germplasm is dried to a relatively low equilibrium moisture content (5-7%), just a few percent above the dry air limit, rather than an intermediate level governed by economic forces. Accordingly the displaced final line below MCR 0.01 is presented as a guide to the approximate time taken for seed to equilibrate under still air conditions (0 m/s). The superimposed conditions for rice at 20°C intersect this line to give 200 hours,

Fig. 10 Seed Drying Nomogram



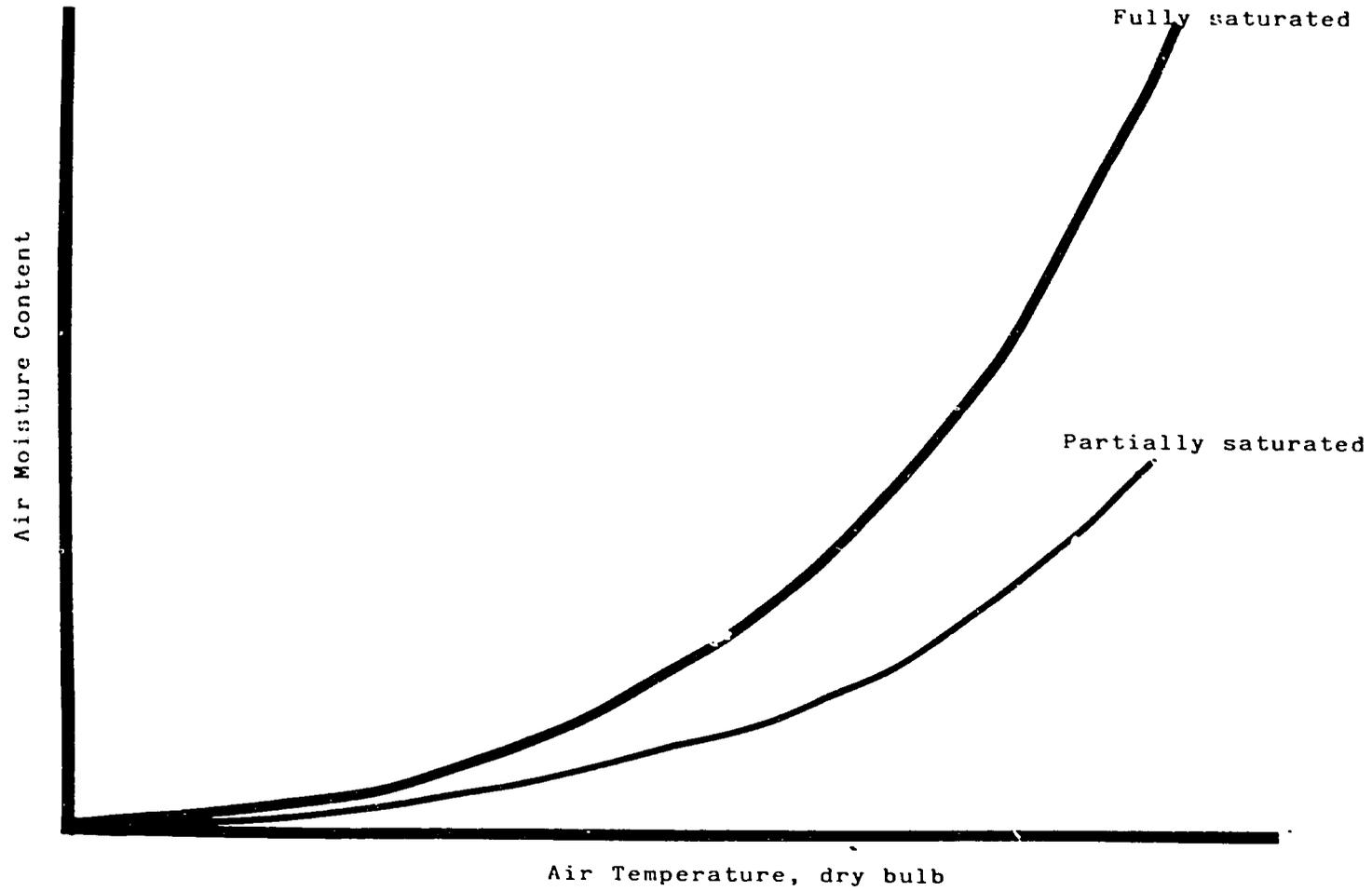
which approaches true equilibrium (Fig. 1) and is similar to the surface layer changes for the bag (Fig.2). Again the process is reversible indicating that small seeds will rapidly absorb some free atmospheric moisture if left exposed to humid air for a few minutes. Consequently dried seed must be handled at a relative humidity giving the storage equilibrium moisture content, otherwise additional drying using Silica Gel as a buffer may be required. Air velocity becomes of paramount importance when seed is dried in deep layers, because the flow rate and air moisture carrying capacity determine the speed at which the "drying front" traverses the bed. Local evaporative cooling depresses seed temperature. Aspects of batch drying are considered later. However, in general it is more satisfactory to undertake a few trial runs with surplus seed rather than to become totally immersed in deep-bed drying theory.

While the seed drying nomogram readily provides an insight into drying characteristics and design criteria, the fact remains that the choice of drying equipment is frequently linked to local climatic conditions which show a wide regional variation. One method of overcoming this problem is to superimpose crop and equipment drying information onto a simple Psychrometric Chart.

USE OF THE PSYCHROMETRIC CHART IN STUDYING DESIGN PHENOMENA

The ability of air to become saturated by water vapour, which is fundamental to man's survival, depends on barometric pressure and the dry bulb temperature. For convenience the former is fixed to a sea level datum. The weight of moisture held, approximately doubles for each 11°C increment within the normal ambient temperature drying range (Fig. 11). Partly saturated air contains proportionally less moisture, giving rise to the relative humidity (R.H.) percentage scale. Vapour pressure is directly related to the air moisture content.

Fig. 11 General Moisture holding characteristic of air

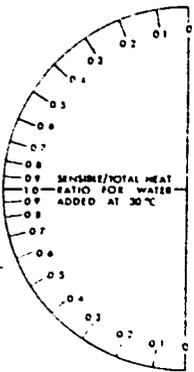
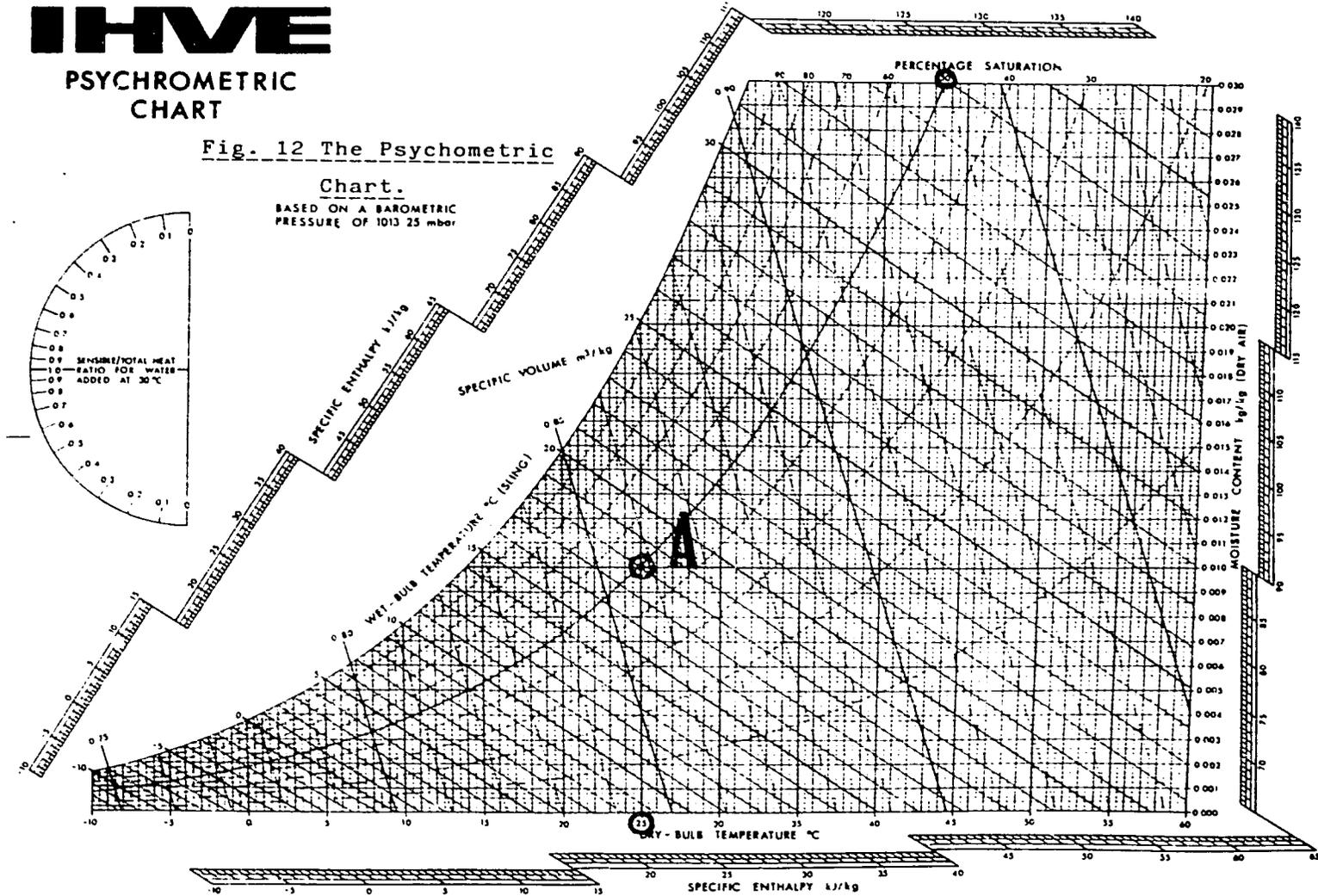


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PSYCHROMETRIC CHART

Fig. 12 The Psychrometric Chart.

BASED ON A BAROMETRIC PRESSURE OF 1013.25 mbar



This type of information can be expanded to produce a comprehensive Psychrometric Chart (Fig. 12) which allows the various properties of air at a given state, to be visualized simultaneously. For instance, air at a dry bulb temperature of 25°C and 50% R.H. (point A on the chart) contains 0.01 kg of moisture per kg of dry air (see intercept of moisture content scale), whereas saturated air (100% R.H.) will hold twice as much. But even so, this value is only 2% by weight of the total air mass and accounts for the large amount of air used for crop drying. Again, small amounts of moisture, say from clothing, will cause significant changes in the genebank relative humidity levels. Next consider what happens to air at 0.01 kg/kg (dry air) moisture content held within a sealed container. Heating from 25°C causes the change of state to follow the horizontal moisture content line progressively reducing the relative humidity. For example at 40°C the relative humidity is 20%. Conversely cooling raises the relative humidity until the air is fully saturated at 14°C. Further cooling causes condensation (i.e. as a liquid or ice) to form on all surfaces below the saturated temperature level. In Seed Banks this is likely to occur when containers of seed are removed from a cold room. The fact that the moisture carrying capacity of very cold air is small is another reason why low temperature drying is slow. However, reheating can produce low relative humidities at moderate temperatures. Thus cooling tropical air causes precipitation in temperate regions while the backflow of cold air is reheated by solar energy to absorb more moisture. Fortunately, local harvest time climatic conditions remain comparatively stable allowing design calculations to be made on a constant air moisture content basis. There are regional differences however.

Fig. 13 shows average monthly meteorological data for central southern England (lower) and South-East Asia (upper) (months are indicated numerically), relative to a 40°C dry bulb drying temperature limit. Clearly the air circulated through English hot air

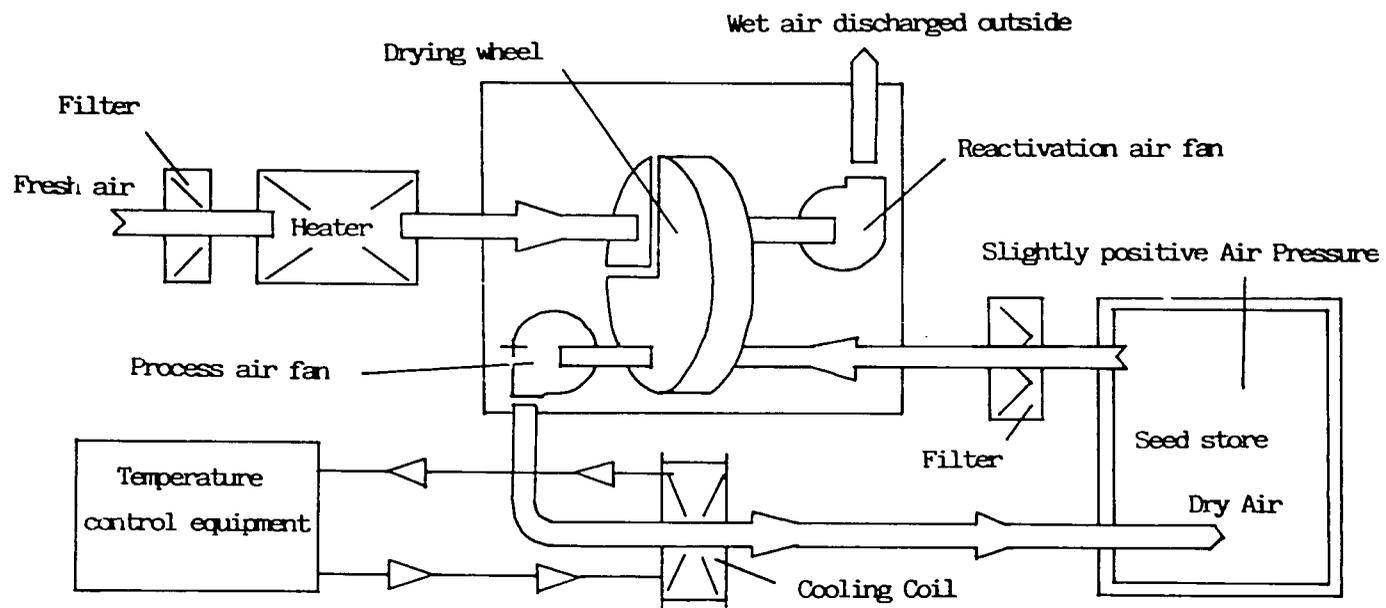
driers can be heated to below 20% R.H. without exceeding the arbitrary limit whereas similar equipment installed under tropical conditions might only achieve slightly less than 50% R.H. unless the air is predried by dehumidification. Adding moisture equilibrium data for, say rice, to the chart (Fig. 14) directly indicates the range of seed moisture content that can be obtained. Clearly such factors have a strong bearing on the limitations of hot air driers under tropical conditions. Various alternative combinations of factors such as acceptable seed viability losses on a probit scale and equipment performance characteristics can also be superimposed on the Psychrometric Chart. By using the viability equation for a given crop, set losses in viability (e.g 1 probit) can be determined for given temperatures and moisture contents. By referring to an equilibrium moisture content versus R.H. graph - the appropriate R.H. values are obtained. These and the temperature values can be plotted on the chart.

Storage and predrying of seeds at Field Stations under tropical ambient conditions, can lead to rapid deterioration of the seed causing substantial losses. For example, traditional sun-drying methods, frequently used for small seed lots, can lead to phenomenal loss of viability through over-heating. Radiant heat drying requires continuous turning. Once again the associated viability losses have largely gone undetected because analysis has been done on a percentage rather than probit basis; as much as half the losses occurring prior to storage can be eliminated by adopting the correct drying procedure.

DRYING EQUIPMENT ALTERNATIVES

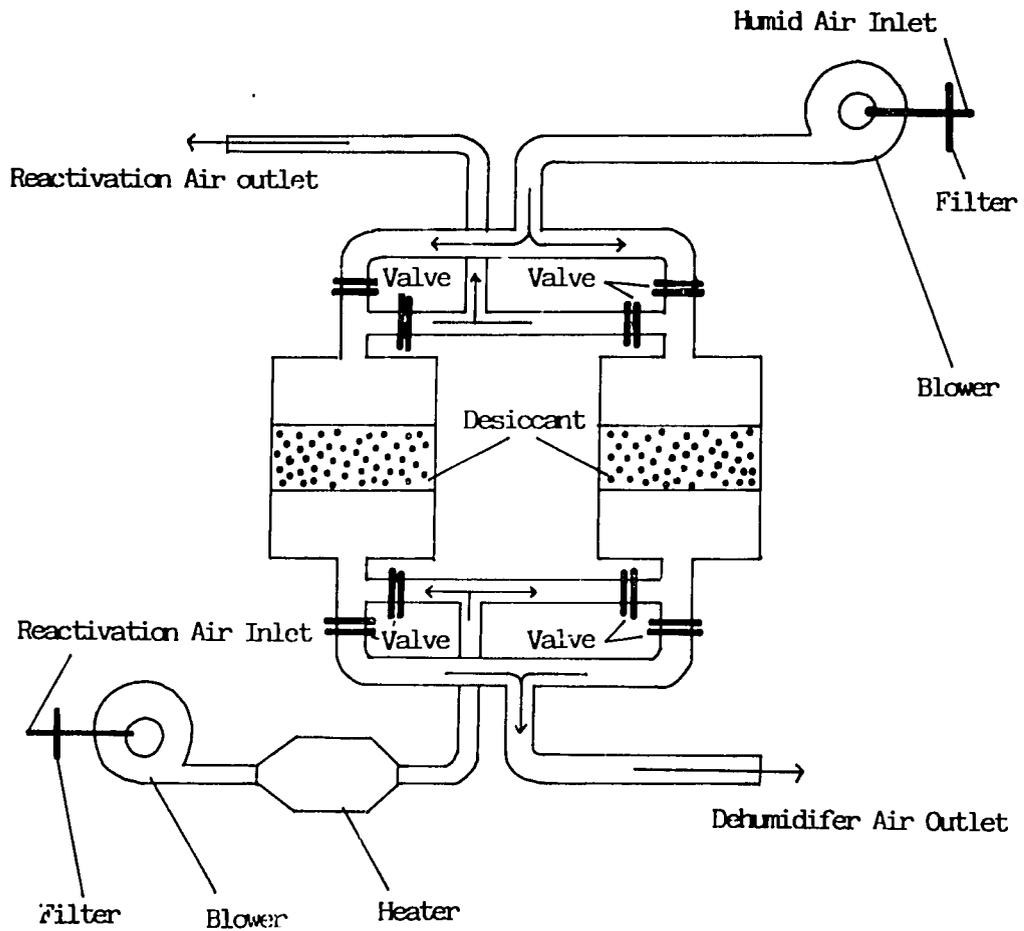
Previous examples, based on the Psychrometric Chart, show that hot air driers have operational limitations. Fortunately moisture can be removed from air to reduce relative humidity by using absorption or refrigeration type dehumidifiers or a combination of both.

FIG. 15. Typical rotary dehumidifier arrangement



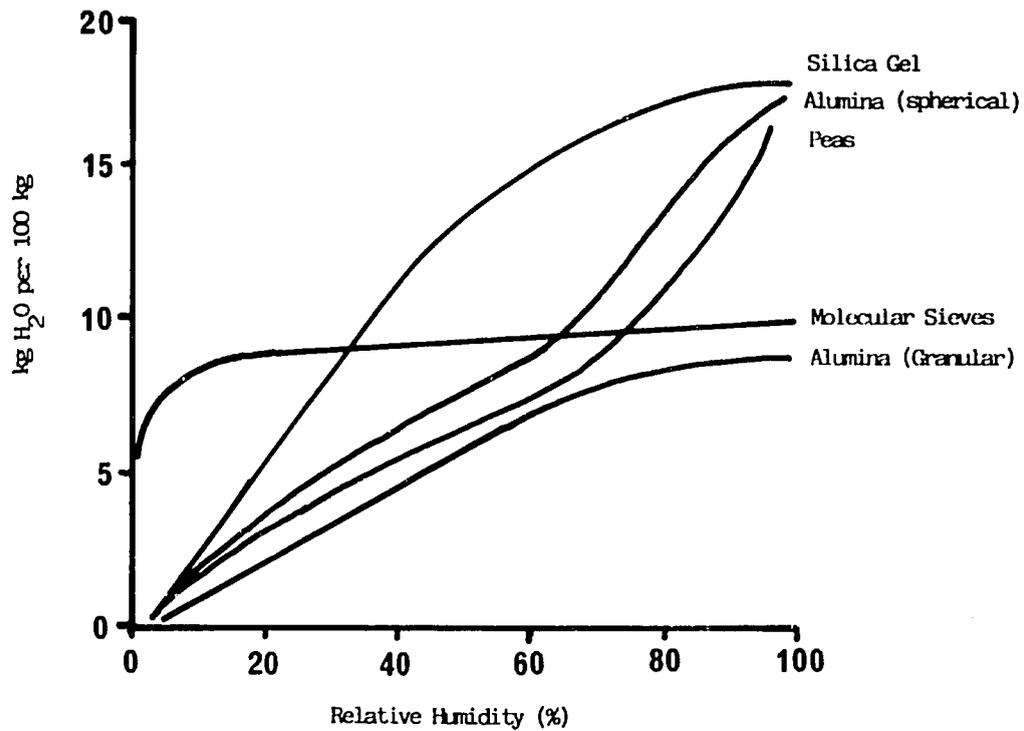
Certain designs of absorption type air driers use hygroscopic salts located in a slowly rotated wheel partly divided to permit counter air flow and continuous reactivation. A typical arrangement, which includes a secondary refrigeration coil, for use in conjunction with a thermally insulated drying room, is shown in Fig. 15. Basically, filtered moist air is drawn from the drying room through the impregnated wheel, where moisture and latent heat exchange occurs, and then is recirculated to the drying chamber. A separate ventilation system draws hot fresh air (at very low relative humidity) through a guarded section of the wheel to receive moisture which is then discharged to the outside atmosphere. The main virtue of this system is that the relative humidity of recirculated air can be progressively reduced to a very low value. This is often expressed in terms of dewpoint temperature (i.e. the temperature at which 100% R.H. occurs and dew forms), which in turn is directly related to a moisture content. Heat exchangers can be fitted to this equipment to reduce operational costs by upwards of 30%. It is common practice to install two such drying units as a precautionary measure against plant failure. However, great care must be exercised to prevent ingress of moisture into the spare unit, which can permanently damage the impregnated wheel through the leaching of active salts. A safety limit as low as 75% R.H. poses certain shipping difficulties particularly when equipment seals are broken during transit. A small, low wattage, heater positioned inside the idle unit or a dry air bleed from the operating machine will overcome this problem. An alternative arrangement involves the use of dual beds (Fig. 16) and various non-return valves. While one bed receives moisture, the other is regenerated by heated ambient air. The duration of absorption and regeneration cycles is automatically controlled by a timer to provide continuous drying.

FIG. 16 Dual bed dehumidifier



A wide range of granulated desiccants can be used with varying efficiency (Fig. 17). Molecular sieves, although costly, are worth considering for certain low relative humidities. A curve for peas is included as a reminder that a wide range of low cost hygroscopic materials can be substituted as short term expedient. Dried air leaving a commercial Silica Gel based dehumidifier has a dewpoint temperature close to -40°C . Operating refrigeration to

FIG. 17 Comparison of relative humidity effect on various desiccants (Equilibrium capacity)



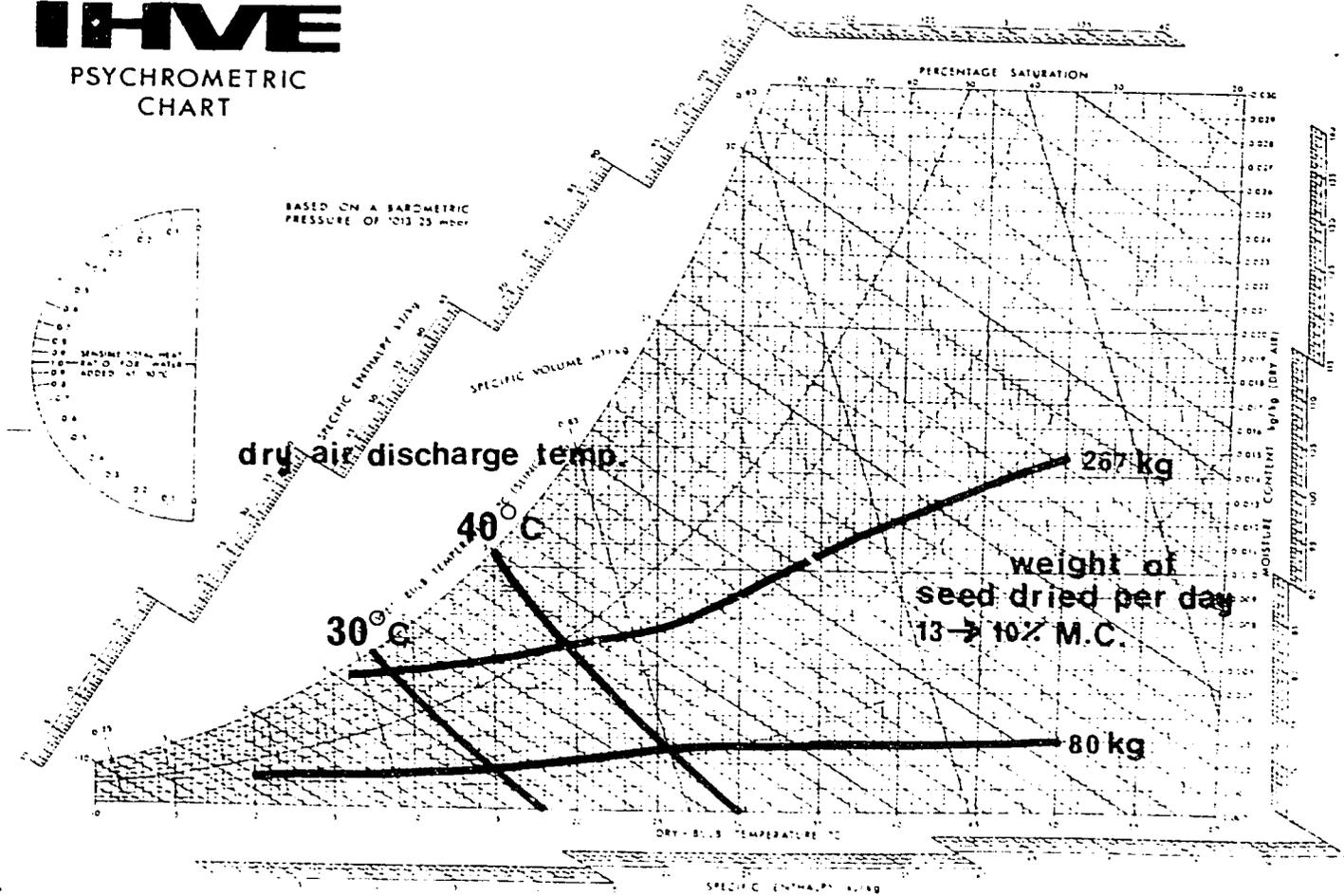
match this state would be prohibitively expensive. As stated, manufacturers type test data can also be plotted on to the Psychrometric chart to define limits of performance in relation to previously listed factors.

Fig. 18 shows a plot of test data for a wheel dehumidifier rated at 4 Kw with the moisture removal rate specified as the weight of seed dried daily from 13% to 10% moisture content (wet weight basis). Two dry air discharge air temperatures are drawn. Examination of the curves show a room (return) dry bulb air temperature of 15°C and 15% R.H. gives a dry air discharge temperature

Fig. 18 Dehumidifier Test Data

IHVE

PSYCHROMETRIC CHART



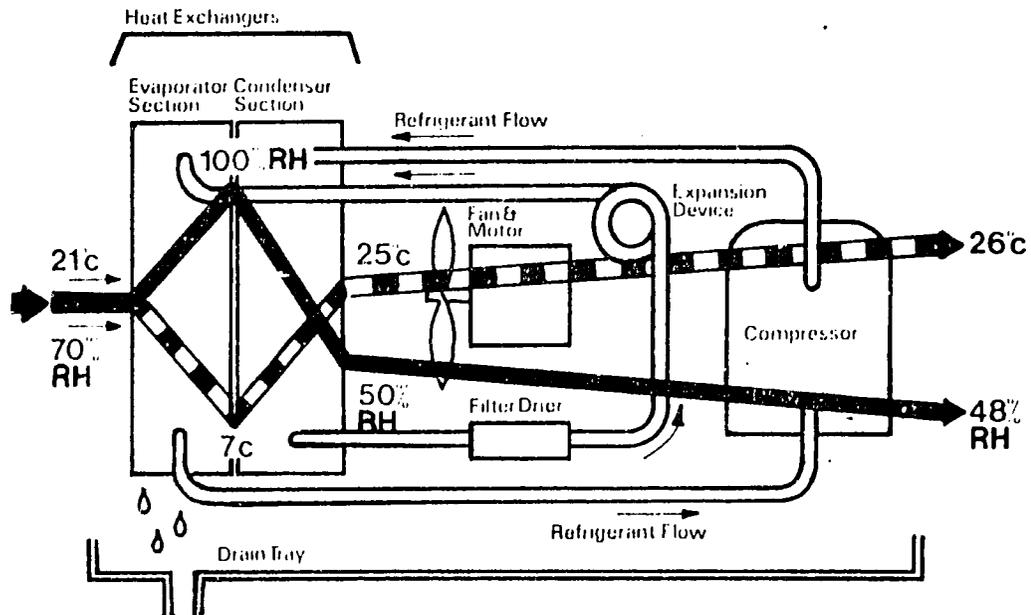
of 30°C, which must be cooled to slightly below 15°C before passing back to the room to give 80 kg/day of drying capacity.

Drying at 50% R.H. would give a reactivated air discharge temperature of 40°C for a room temperature of 18.5°C coupled with a seed drying potential of 267 kg/day. However, the recirculated dried air must be cooled by 21.5°C, to maintain this duty. These figures allow for an appreciable moisture leakage - possibly via clothing etc - into the air condition space. While the chosen seed moisture contents may seem misrepresentative of normal drying room operation, comparatively high values have been selected to cover and avoid internal moisture diffusion restrictions, which limit moisture release and prevent the full drying potential from being achieved.

Simple, single bed, small scale batch driers with or without air recirculation, can be constructed using shallow beds of Silica Gel that can be reactivated in a laboratory oven. But once again the temperature of air leaving the Silica Gel bed is surprisingly high and a cooling system may be necessary to protect the seed. The discharged air temperature condition should be monitored continuously to determine the regenerated point.

Fig. 19 shows a schematic arrangement of a mechanical refrigeration circuit used for dehumidification; air and refrigerant directions of flow are indicated. Broadly speaking the refrigeration circuit comprises of 5 essential elements, the compressor, condenser, expansion valve, evaporator and a recirculated refrigerant. The restrictive action of the expansion device, coupled with compressor operation maintains high and low pressure zones, producing latent heat exchange as the refrigerant is continually condensed (to reject heat) and then re-evaporated provide refrigeration. Therefore, humid air, drawn through the evaporator coil by the air circulation fan, is cooled beyond the inlet dewpoint

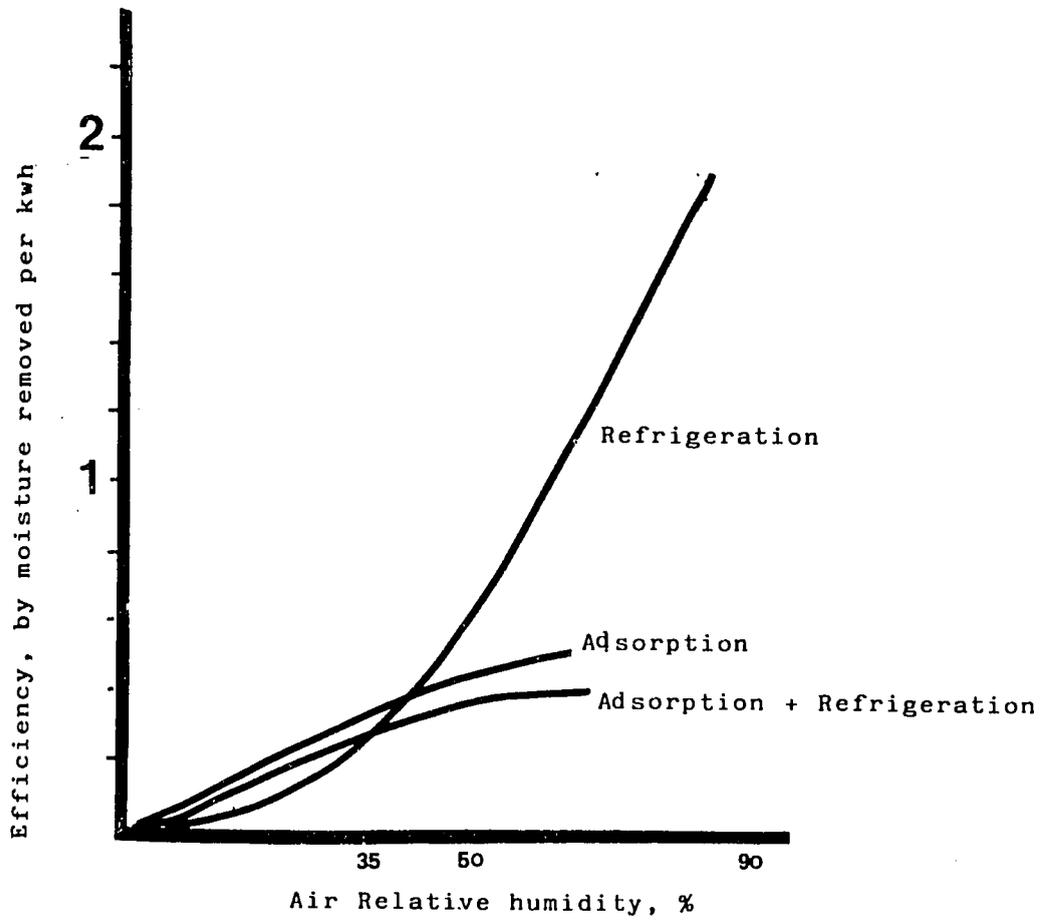
Fig. 19 Refrigeration based dehumidifier



Note: The figures used are for illustrative purposes only

temperature causing surplus moisture to drain away. But the saturated air must be reheated in order to reduce the relative humidity (see psychrometric chart examples discussed previously) and this is done by waste heat rejected from the condenser and compressor motor heat losses. Refrigeration dehumidifiers are economical to operate at ambient temperatures because latent heat removed from the air moisture satisfies a significant part of the reheating load, the purchased electricity accounts for the remainder. Fig. 20 endeavours to show the relative efficiency (expressed in terms of moisture removal) of typical absorption, absorption plus refrigeration after cooling, and refrigeration based dehumidifiers. The break point for refrigeration systems is above the 30-40% R.H. range and could, therefore, be considered for certain seed or air predrying applications particularly under humid tropical conditions, which might otherwise damage certain designs of sorption based dehumidifiers.

Fig. 20 Dehumidifer comparative performance



Conclusion

It is hoped that this paper shows that many crop drying policy decisions, concerning the selection and operating of genebank drying equipment can be undertaken using simple graphical aids presented here.

Seed drying is easy provided that the basic ground rules are followed and performance carefully monitored. Remember:-

Use good quality cleaned seed stocks

Use Forced ventilation

Use appropriate humidity levels to avoid damage

Dry at low temperature avoiding Solar radiation

Present data on a dry weight basis

Use Probit viability scales

Install simple reliable equipment

and Use Common Sense

PSYCHROMETRIC CHART

This chart is reproduced with the permission of the Chartered Institution of Building Services from whom pads of charts sized A3 for permanent records may be obtained.

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DISCUSSION

Chairman: Bekendam

Participants: Hagen, Ellis, Tyler, Bean,
Roberts, Stoianova, Neergaard,
Smith

1. Depth of Seed Lots When Drying Under Forced Ventilation

In answer to a question from Ms. Hagen, Mr. Cromarty explained that with forced ventilation complex drying fronts are involved; and so seed lots should be spread out in shallow layers rather than piled up in deep ones.

Both Dr. Ellis and Mr. Cromarty stressed the difference between a drying character with a throughput of air from say an air-conditioning unit and one with a means to ensure efficient internal circulation of air. It is worth noting that the limiting factor with regard to seed drying is the rate at which moisture can move within the seed rather than the rate at which the air carries the moisture away.

2. Contamination of Seed Samples in Drying Equipment

Mr. Tyler, referring to one of the drying machines illustrated by Mr. Cromarty, wondered if there is a possibility of cross-contamination when several samples are dried at one time. Mr. Cromarty replied that if there was any such worry, it should be a relatively simple matter to replace one system, in which air is blown through a column of trays in series, with another, in which the trays are mounted in parallel and supplied with air from a centralised pressure chamber. Using the latter system, Mr. Cromarty could not envisage any more contamination than would be experienced in adjacent trays in a still air drying room. He maintained that it would be quite straightforward to design such a

system, so long as dry room air is used and the system has a back pressure of at least 2 cm Hg. In response to Mr. Tyler's query, Mr. Cromarty said that the seeds could be contained in sacks or even paper envelopes, so long as air could be blown through them.

3. The Difference Between the Expression of Moisture Content on Fresh Weight or Dry Weight Bases

Dr. Bean drew attention to possible confusion over potential damage to seeds arising from the expression of moisture content on a fresh weight basis and a dry weight basis. For biologists the fresh weight basis has always been more meaningful - the proportion of total weight of an organism consisting of water. Mr. Cromarty replied that engineers have used the dry weight basis for many years and it is now part of the accepted terminology of drying technology. Its chief advantage is that it is possible to tell at a glance how much moisture is being driven off. This is not so easy when using the fresh weight basis. It is worth bearing in mind that 20% moisture content on a f.w. basis is equivalent to 25% on a d.w. basis; and by the time 5% is reached there is only a very slight difference between the two. Prof. Roberts stated that it is important to state the method being used in every case.

4. Safe Minimum Moisture Content

In answer to a question from Mrs. Stoianova regarding drying rates and critical minimum moisture contents in relation to seed chemical composition, Mr. Cromarty referred to his graphs showing the data for barley, soyabean and groundnut. In general, as far as viability is concerned, moisture content should be reduced as low as practically possible.

Regarding the minimum moisture content without damage, Prof. Roberts quoted the IBPGR recommended figure of 5-7%. In fact very few orthodox seeds are damaged at this moisture content; and with many it is possible to go down to 1½% moisture content or lower without apparent damage. Nevertheless, there may be problems with severe cracking at such low moisture contents; and there are some misconceptions regarding the minimum safe moisture content in legume seeds. It is said that large grain legume seeds are killed below 8-9% moisture content, but this is not strictly true. Rather than drying per se, it is rapid water uptake upon imbibition which kills them; and this can largely be overcome by careful humidification in a saturated atmosphere before imbibition proper. Mr. Cromarty added to this that movements of seeds to and from cold storage should be done carefully to avoid the condensation on their surfaces of water, which could be taken up rapidly and cause damage in a similar way. There may also be problems with rice in this respect.

5. Cracking of Seeds During Drying

Prof. Neergaard enquired whether it is the speed or intensity of drying which causes cracking, and wondered how it can be prevented. Mr. Cromarty explained that the rate of water loss from a seed depends very much on the structure of that seed. As examples it is worth looking at some legumes, where the seedcoat can vary in structure. In pea and bean for instance, when dried rapidly, drying occurs initially from the coat, but not from the centre which remains at a higher moisture content. As moisture is removed from the coat and outer areas they contract, setting up tension; and so the unequal drying eventually causes cracking. If there is any worry about cracking, then the larger the seed, the slower should be the drying. In the drying room, despite very low humidity, the temperature is also low, so drying is slow enough to overcome the problem. However, if high temperature is combined

with very low relative humidity, then drying will be very rapid, possibly leading to cracking of the seeds. It is worth noting that the damage is often done in the very early stages of drying.

6. The Use of Ground Material When Measuring Moisture Content

Dr. Bekendam drew attention to the need to grind some seeds for accurate determinations of moisture content, and that perhaps this method should be considered more often, especially when dealing with wild material.

Mr. Cromarty thought that this method might sometimes give spurious results with seeds rich in oils, which being volatile would evaporate leading to apparent moisture loss. This leads to the need for defining temperature in moisture content determinations.

7. The Siting and Efficiency of Driers

Manufacturers of absorption driers usually recommend that driers should be installed in the dry room, with only wet air being exhausted; but Mr. Smith wondered whether this would cause a relative humidity gradient from one end of the room to the other. Mr. Cromarty thought that such a gradient would be inevitable, irrespective of whether the drier is in the room or not, unless there are 20-30 complete changes of air per hour in the room. He surmised that such driers would have been designed primarily for industrial applications under non-insulated conditions, where the heat output would provide a bonus in winter. In the highly insulated conditions of the genebank, it is important that the drier be placed outside, as at Wakehurst Place for example. An absorption drier must have a sizeable refrigeration unit attached to cool the regenerated air; otherwise the temperature of the circulating air will gradually rise. Of course this refrigeration is significant additional cost which must be borne in mind when establishing a

seed bank. It is also possible to recirculate the heat with heat exchangers and thus use it to remove moisture.

Explaining that at Aberystwyth (WPBS) the Rotaire driers are used at 0°C, Dr. Bean wondered whether it is possible to use them at much lower temperatures; if it is possible at -20°C, then seeds could be kept in the bank at low moisture contents without the need for sealing them in packets. Mr. Cromarty said that it is certainly possible to operate at lower temperatures, but there is always the problem of the very high relative humidity at such temperatures. He also pointed out that with a drier outside the drying room, unless it is fully insulated, there might be problems with condensation on the ductwork due to its temperature being below the dew point of air in the building.

Mr. Smith, commenting on experience with driers at Wakehurst Place, said that the engineers at the time could not find a design that met the specifications of the job, so the ones installed are considerably over-specified. The engineers had worked on the assumption that all moisture in the seed was eventually available as free water, and Mr. Smith wondered whether that model has been improved upon. Mr. Cromarty said that on the basis of the moisture content ratio (MCR) it is possible to predict how much moisture needs to be removed in a set time. Thus the drying nomograph chart can be used as a design tool. It should be borne in mind that moisture arriving in the drying room through cracks can considerably raise the drying load, and thus the room should be pressure tested for leaks.

Practical Viability Testing

J. BEKENDAM

Since the latter part of the 19th century, the importance of seed testing for the purpose of safeguarding agricultural yield has become well established. In many countries seed testing became normal practice, often officially regulated, furnishing at the same time the basis for standards of quality to which seed had to comply when distributed. Consequently, we now have in many countries official seed testing, executed at specialized stations or laboratories. Parallel with this development, seed production and seed trading changed to meet official seed testing standards.

However, research has always been carried out to improve seed quality for agriculture. Since the Second World War, seed quality research has been extended to support new developments in agriculture. Especially during the last ten years, seed science and technology research has deepened our understanding of factors which influence seed quality; opened possibilities for further improvements therein, from seed production and processing to storage, packaging and distribution. At the same time the development of new and improved methods for testing seed quality have been developed.

If I have understood the programme of this workshop correctly, this paper should be confined to testing the quality of seed by means of determining the germination capacity. The developments in seed quality testing will be only referred to as far as they give extra support to the methods as used nowadays, in specialized seed testing laboratories. These methods, however improved and adapted have not changed principally since the old days of seed testing.

The International Seed Testing Association (ISTA) has played a very important role in the improvement and application of seed testing methods. The Association was founded in 1924 to standardise seed testing methods internationally because uniformity was lacking, preventing optimal performance of seed quality control and hampering effective functioning of the seed trade.

The primary aim of the Association is to develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures for evaluation of seeds moving in international trade. A secondary purpose of ISTA is to promote research in all areas of seed science and technology, including sampling, testing, storing, processing, and distributing seeds; to participate in conferences and training courses aimed at furthering these objectives; and to establish and maintain liaison with other organisations having common or related interests. At the moment some 62 countries are members. Examples of the most important ISTA activities are listed below.

ISTA issues the International Rules for Seed Testing, comprising amongst others, rules for sampling and for testing purity, germination, use of tetrazolium, seed health and moisture content. The methods prescribed by the Rules have to be applied whenever the international trade makes use of an ISTA International Analysis Certificate. The impact of the Rules on seed testing has become so great that the methods are in general also used for national seed testing purposes. Technical committees in ISTA have the permanent task of updating the Rules. Their proposals must be finally accepted by the ISTA congress which meets every three years. Besides issuing the Rules and certificates, ISTA issues the Proceedings of ISTA: Seed Science and Technology, the ISTA News Bulletin and Handbooks on seed testing subjects (e.g. the Handbook on seedling evaluation, on vigour and on seed health testing). ISTA also organizes regular workshops for ISTA labora-

tories to apply uniformly the methods in the Rules and has recently started regional training courses to introduce seed testing in developing countries.

Viability testing under the Rules, with reference to issuing ISTA certificates, starts with taking a representative sample from the seed lot. As seed lots are never completely homogeneous, sampling should be done with sufficient frequency over the lot. Primary samples are taken by hand or trier from a prescribed number of containers at different depths and horizontal positions. The primary samples are subsequently well mixed to form a composite sample, from which the sample is drawn to be submitted to the laboratory for testing. At the laboratory, the submitted sample is, after thorough mixing, further subdivided to give a smaller, so-called working sample which officially should contain at least 2,500 seeds for executing the purity test.

Reduction of the composite sample to the submitted sample and the submitted sample to the working sample is done by repeated halving with mechanical dividers, by abstracting and subsequently combining small random portions by the random cup method, the modified halving method or by the spoon method. In this respect, with regard to genebank sampling, if only relatively small quantities of seed are available, the working sample for testing purposes should be obtained by one of the abstracting/combining methods or in the case of chaffy seeds by hand (N.B. this method has been taken out of the ISTA Rules, but re-introduction appears necessary).

The purity test is performed to determine the composition (by weight) of the working sample and, by inference, the composition of the seed lot; and to identify the various species of seeds and inert particles constituting the working sample, as follows:

- 1) pure seed, referring to the species stated by the sender or found to predominate in the test.
- 2) other seeds, seeds and seedlike structures of any other plant species (other crop seeds, weed seeds, etc.).
- 3) inert matter, such as pieces of broken or damaged seeds one half the original size or less and other matter, such as soil and sand particles, chaff, nematode galls, fungus bodies and all matter not seeds.

The purity test, is necessary because, seed lot cleaning cannot normally eliminate fully all the non pure fraction. It is performed according to strictly prescribed procedures to guarantee repeatability; which is also of the greatest importance to the viability test, as this test is performed on the pure seed fraction. Consequently, in seed testing one should consider the purity and viability test as one combined test. From an agricultural point of view they determine, together with the seed health test, the quality of the seed lot.

For storage of seeds (especially long-term at -18°C), cleaning must be directed towards eliminating other seeds, inert matter, and all inferior seeds which under ISTA Rules are still considered pure seed (such as immature, undersized, shrivelled, diseased and germinated seeds), in order to improve the initial germination and hence longevity during storage. Therefore, cleaning methods should follow the techniques applied for obtaining high quality seed for precision sowing. Even visual cleaning by hand should be considered, if machine cleaning making use of sieves, wind, centrifugal force, density, resistance or elasticity, does not prove fully satisfactory. In a genebank one should carry out a modified purity test to check whether the cleaning has been successful. Of course, the test need not be performed on the 2,500 seeds, pres-

cribed by ISTA as a minimum. However, the procedures to obtain a representative working sample should be followed. Depending on the results of that test, one can decide to reclean the whole lot again, or, if cleaning equipment is not likely to further improve the lot, to clean the lot by hand making use of aids such as reflected light, sieves and blowers where necessary. If cleaning is performed in this way, giving seed lots of the highest possible quality free of other crop seeds and inert matter, one should not have problems exchanging seed worldwide, across seed legislation barriers.

The viability test, or rather the determination of the germination capacity, has, like other aspects of seed testing, gone through several adaptations to meet agricultural needs. Since the first issue of the ISTA Rules in 1931, there has been increasing stress on detailed examination of seedlings to distinguish critically those which have the potential to produce normal, mature plants under favourable growing conditions (= normal seedlings), from those which are without planting value (= abnormal seedlings). Based on single-plant observation and comparative tests between laboratory and field, the significance of seedling defects have been determined. The defects are detailed in the Rules and seedling evaluation is fully discussed in the ISTA Handbook for Seedling evaluation, giving detailed instructions and illustrations.

The definition of germination has progressed from the stage of defining seed as germinated when only the radicle had ruptured the outer structures, to currently defining germination as the emergence and development of the seedling to a stage where all the essential structures indicate their ability to develop further and to form a satisfactory plant under favourable conditions. Thus, a visible distinction can be made between normal and abnormal seedlings. The percentage of germination is then defined as the pro-

portion of seeds which have produced seedlings classified as normal under optimal conditions in the laboratory. The laboratory test, standardised and carried out under optimum conditions for the species concerned, is repeatable, contrary to germination under practical sowing conditions in the field. Nevertheless the test thus performed shows in general a positive correlation with field emergence, when seed lots contain seeds which are not dormant, well cleaned and of a quality at or above the minimum norms to which seed quality must comply for distribution. It means that under average field conditions, the germination test generally fulfils its most important object; that is to rank seed according to quality as for field emergence. That this relation exists has been substantiated by investigations. In this connection mention may be made of the article by Essenburg and Schoorel (1) and the work of Roberts and Ellis (2). Essenburg and Schoorel found in most experiments they surveyed, (at different places in the world), a high correlation coefficient between the results of germination in soil obtained in greenhouses or in the field. They found large differences between species and between cultivars of any one species. Especially small seeds showed a lower emergence than coarse seeds. However, as we all know, the relation between germination and emergence in the field may not always be exact because of differences in vigour. This fact was also recognised by Essenburg and Schoorel. Roberts and Ellis found a definite relationship between normal and abnormal germination and longevity, a relationship which undoubtedly will be discussed further during this Workshop.

The germination test for the purpose of ISTA certification is performed on 4 x 100 seeds, the seeds being counted at random from the pure seed fraction. Likewise, for the purpose of monitoring seed viability in a genebank, one should take the utmost care that the seed tested is representative of the stored sample. The quantities for monitoring could be prepared beforehand and stored sep-

arately to remove the need to disturb the main batch. Counting of seed, depending on the size and morphological characteristics, is done by hand, vacuum counter or counting board. The seeds are either germinated on or between filter paper or in sand. For smaller seeds usually a filter paper substrate is used and only the larger seeds are germinated in sand (constant temperature). However, in many instances beside testing in sand, such seeds may be also germinated in paper. In other words, the ISTA table of methods allows (in those cases) for alternative substrates. This is not only so for substrate but in many instances also for germination temperature and light. Besides a temperature of constant 20°C, an alternating temperature of 20-30°C may be used, either in light or darkness. In general light is advised, because long etiolated seedlings grown in darkness are less easy to evaluate as normal or abnormal than the sturdy seedlings from germination in light. Besides, the darkgrown seedlings are more liable to be damaged in seed testing, especially when intermittent counts have to be made. Light also has a dormancy breaking effect, but care must be taken that in germination tests light is provided by fluorescent tubes which have a relatively high emission in the red region and not incandescent-filaments which emit strongly in the far red and may inhibit germination in light-sensitive species.

Why is it that ISTA still allows for alternative methods of germination? One of the reasons is the differences in facilities between laboratories. That situation has forced in the past and still forces ISTA to take into account alternative methods for certain seed species. However, alternative methods are only introduced into the Rules as far as they give internationally uniform results. That alternatives, e.g. with regard to temperature, are able to produce uniform results is not so surprising, when recognising the fact that non-dormant seeds usually germinate over a relatively wide range of temperatures, a range which in itself contains a smaller range of optimal and near optimal germ-

ination temperature. Another reason is that the same species grown in different regions or locations may need different methods to germinate optimally because of induced differences in physiological and morphological characteristics, or in seed health. For example, non-dormant seed of a species may germinate best at 20°C constant, while for dormant seed of the same species grown elsewhere the optimum treatment is an alternating temperature regime of 20-30°C.

As mentioned earlier, for evaluation in the germination test, seedlings must have reached the stage where the essential structures have developed sufficiently to permit the recognition of normal and abnormal seedlings. A normal seedling in the ISTA Rules is defined as one which shows the capacity for continued development into a satisfactory plant, when grown in good quality soil under favourable conditions of moisture, temperature and light. This capacity for continued development depends upon the soundness and correct functioning of the developing structures during germination. Experience and comparative tests have shown that not only intact seedlings of which the essential parts are healthy, complete and well balanced, are capable of producing normal plants under favourable conditions; but that certain slight defects usually do not prevent an otherwise sound and balanced seedling from growing into a normal plant. Another exception is made for seedlings which are secondarily diseased or decayed as a result of infection from an outside source, as for example another seed or seedling in the test. So, three categories of seedling are therefore classified as normal: 1) intact seedlings, 2) seedlings with slight defects and 3) seedlings with secondary infection.

An abnormal seedling is defined as one which does not have the capacity to develop into a satisfactory plant when grown in soil under favourable conditions, because one or more of the essential

structures are irreparably defective. Three major classes of abnormal seedlings can be distinguished: 1) damaged seedlings, seedlings with any of the essential structures missing or so badly damaged that balanced development does not occur, 2) deformed or unbalanced seedlings, the unbalanced development may be caused by internal disturbances of a physiological -biochemical nature and 3) primarily decayed seedlings.

Whether the abnormalities listed in the ISTA Rules are all truly related to the inherent quality of the seed can be doubted. Under ISTA, the resulting seedling is considered abnormal because such seeds, when sown, produce in general retarded, but otherwise normal mature plants of relatively low yield. However, for viability testing in genebanks one can avoid this problem by always applying uniformly the same evaluation for normal/abnormal, whether or not the list of abnormalities used is complete. Using the ISTA Rules as at least a guide seems to be good advice to genebank managers.

As usually not all seeds in a test germinate simultaneously, intermittent counts may be necessary depending on the period of testing of the species concerned. The more so, as the seedlings in the germination test are fully dependent for their growth on their reserve nutrition, stored for example in endosperm or cotyledons, while the germination substrate is inert and water is only given for moistening. Consequently, the seedlings must be evaluated (and recorded) before they run out of nutrition, otherwise, they will rot and contaminate the test. For this reason rotten seeds and seedlings must be removed as soon as they occur during the test. Seedlings can usually be evaluated without difficulty in tests on the substrate prescribed for germination. However, when samples produce seedlings which cannot readily be evaluated because of disease, phytotoxic symptoms or other reasons, it may be necessary to deviate from the prescribed substrate, using soil or if appropriate, sand. Under ISTA Rules, the seed must be

investigated as received. For instance, in official testing, disinfection of an untreated sample is not allowed, however heavily that sample is diseased. The only treatments allowed for are a limited number of dormancy breaking methods: prechilling, pre-drying, presoaking, methods to break hard seededness and removing outer structures of seed. As for growth regulators, GA₃ is allowed for only in the cases of Avena, Hordeum, Secale, and Triticum.

It is clear that this limitation does not need to be followed in genebank testing. As with growth regulators any other suitable method could be used when determining the viability of the seed. In general, when assessing the viability of an accession in a genebank, whatever method is used for determining the initial viability, as regards pretreatment, substrate, temperature/light, moisture conditioning and evaluation of seedlings, should be followed in all subsequent monitoring, to ensure comparability. It is apparent that for genebank purposes the ISTA Rules should not be followed blindly, but critically modified towards the particular needs. In other words, the ISTA Rules should be looked upon as an excellent basic guide for genebank work; not only with reference to viability testing, but also with reference to other subjects like sampling, purity - and moisture testing, tetrazolium and seed health testing.

In germination testing, to provide optimum conditions, the equipment used must be reliable and precise as regards temperature regulation. In addition, in so-called wet equipment, moisture conditions must be optimal, even when light is given during the test period. The temperature range between 10° (5°) and 35°C should be within tolerance of $\pm 1^{\circ}\text{C}$ of the set germination temperature. Nowadays, because of initial expense, running and maintenance costs, dry walk-in chambers are very popular in comparison to cabinets and so-called Jacobson tables. In the dry chambers,

many tests can be placed on shelves or trolleys in containers which prevent drying out, while the temperature (constant and alternating) is taken care of by an air-conditioning apparatus. As the chambers are relatively large compared with cabinets or Jacobson tables, dry or wet cabinets or Jacobson tables need to be provided as well, to cover slack times, special temperature requirements and research needs.

From the foregoing, I hope I have convinced you that testing for viability is a specialization. The determination of the quality of seed by viability testing is of the greatest importance for the effective management of a genebank, in relation to the time seeds can withstand storage under specified conditions before reaching an agreed level of deterioration. The assessment of the initial quality of the seed after drying, at the time of packaging for storage, plays an important role in predicting that time; and consequently determines to a large extent the frequency of monitoring the quality of the seed stored in the genebank.

Therefore, it is a necessity for running a genebank efficiently to have the aforesaid specialized knowledge at hand. A genebank can achieve this either by being affiliated to an official recognized seed testing laboratory (which I think is the best possible arrangement), or by somehow acquiring professional expertise, together with the necessary equipment for testing.

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DISCUSSION

Chairman: Smith

Participants: Mumford, Roberts, Cooper,
Neergaard

1. The Need For More Information

Referring to the work done by Ellis and Roberts, Dr. Bekendam made a plea for more nomographs of storage endurance, possibly linked to observations of chromosome aberrations. These nomographs could be done for groups of species, defined on seed composition and/or morphology; and he expressed the willingness of his department at Wageningen to co-operate in such work.

2. The Usefulness of ISTA Rules to Genebank Staff

Mr. Smith wondered if genebank staff could be expected to meet ISTA regulations. In answer Dr. Bekendam stressed that ISTA regulations should only be a guide for viability testing in genebanks; and it was generally agreed that they formed a useful basis, but should be modified or disregarded where inappropriate.

3. Abnormal Seedlings

Leading on from point (2), Dr. Mumford agreed with modified ISTA Rules regarding abnormal seedlings. She felt that these should not be excluded, as there may be particular combinations of genes leading to depressed germination, but which may be valuable to the breeder. Of course, as Prof. Roberts pointed out, if abnormal are removed from a sample, they are not removed from the accession as a whole. Dr. Bekendam pointed out that the abnormal may not necessarily be genetic, but taking beans as an example they may simply be the result of rapid water uptake by dry seeds (imbibition injury - see paper by R.H. Ellis in this volume.)

Dr. Bekendam stressed that it does not matter what rules you apply, so long as there is good reason for them in a particular situation, they are clearly formulated from the beginning and sensibly applied. With regard to abnormal seedlings, Dr. Cooper maintained that with training and experience it is possible to make critical assessments of abnormal seedlings, which can foretell imminent loss in viability (see paper by R.H. Ellis). Dr. Bekendam suggested that a joint workshop between ISTA and genebank staff on cleaning, sampling, purity and germination etc., could be profitable.

4. The Use of Seed Treatment

[See also the discussion following Dr. Gerard's Paper].

Prof. Neegaard enquired as to the general attitude with regard to seed treatments. Agricultural research centres use them, often to ease quarantine problems; but they are not often concerned with losses in germination and, for example, mercury based compounds may affect chromosomes. Dr. Bekendam said that, at least in his own institute, seed is not treated before long-term storage; the material is examined upon sowing. In countries with seed regulations it is important to know the effects of the compounds used and in many instances these are checked. If a breeder uses other compounds, then it is his responsibility. Prof. Neegaard said that the problem highlights the conflict between ensuring the safety of genetic material and preventing the spread of disease; but he thought that the policy of treating after storage and testing is a sound one.

5. The Use of Conductivity Tests

Considering recent research in U.K. and Holland, Dr. Astley wondered whether ISTA had considered conductivity tests for measuring viability and vigour. Dr. Bekendam thought that these tests will

eventually be worthwhile and save much time, but at present more research needs to be done before they can be applied to a wide range of species. At present, the limited application of the test seems to be towards predicting field emergence, rather than viability per se.

The Meaning of Viability

R.H. ELLIS

INTRODUCTION

This paper provides an introduction to the physiology of seed survival in storage for genebank personnel with little or no specialized knowledge of seed physiology. For detailed information on the development and justification of the concepts presented, the reader is referred to the reading list.

A viable seed is one which will germinate and develop into a plant given favourable conditions, provided any dormancy that may be present is removed. We are unable to distinguish a viable seed from a dead seed by visual observation of 'air-dry' seed in storage. We can only distinguish between dead and viable seed by allowing them to imbibe water and observe whether they germinate and develop into seedlings under favourable conditions. However, by this time the seedling is no longer a seed. In this sense therefore, a viability test is destructive.

SEED LONGEVITY

The lifespan of an individual seed is the period until which it is unable to germinate under favourable conditions. It is difficult to define when the lifespan of an individual seed commences. It might be considered as the harvest date, or earlier, or even the point in time when the seed was received. Since the test for viability is destructive, it is impossible to define the end of an individual seed's lifespan. If we test the seed for viability and it germinates then we may only conclude that its lifespan would have ended at some time in the future. If we test the seed for viability and it fails to germinate then we may only conclude that

it's lifespan ended at some time previous to the date of the viability test.

It is rather unusual to test only one seed for germination. Normally a considerable number (e.g. 400) of seeds are tested together for germination. These seeds are normally sampled from a larger bulk of seeds.

If we sample seed from a seed lot (or accession) serially during storage under a constant environment (that is at a constant temperature and a constant seed moisture content) and test each sample for viability by a germination test and then plot the results (normally expressed as a percentage) against the period of storage the results are similar to the two sets of data in Fig. 1, although there will be differences in the time scale - for example as shown by these data for two different constant storage environ-

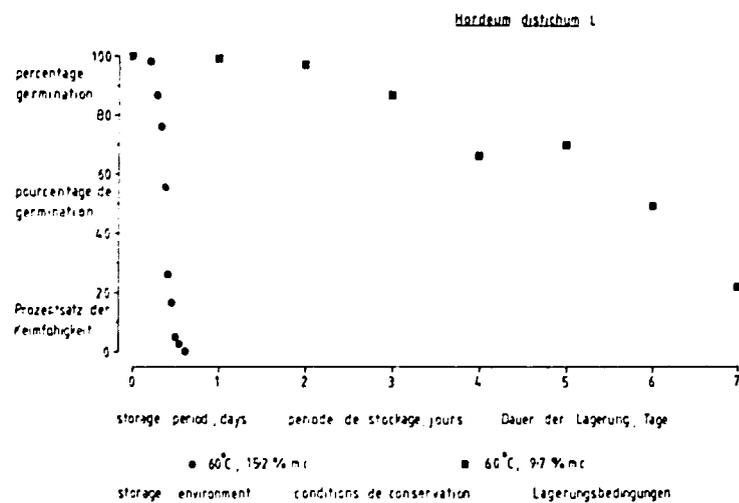


FIG 1. The results of serial germination tests on seed samples drawn from a barley seed lot stored under two different, constant storage environments plotted against storage period.

ments. The question that now arises is - how do we describe the longevity of the population of seeds? In theory we could define the period for, say, each 1% loss in viability to occur. However, this would be very time consuming and difficult to comprehend. In practice only one or two periods are chosen, for example the time taken for viability to fall to 50% - often denoted by the symbol P_{50} - or at the time taken for viability to fall to zero. However, in Fig. 1, to interpolate between two points we must join these up with a straight line. By joining data points up we can detect a pattern - the pattern of sigmoidal seed survival curves.

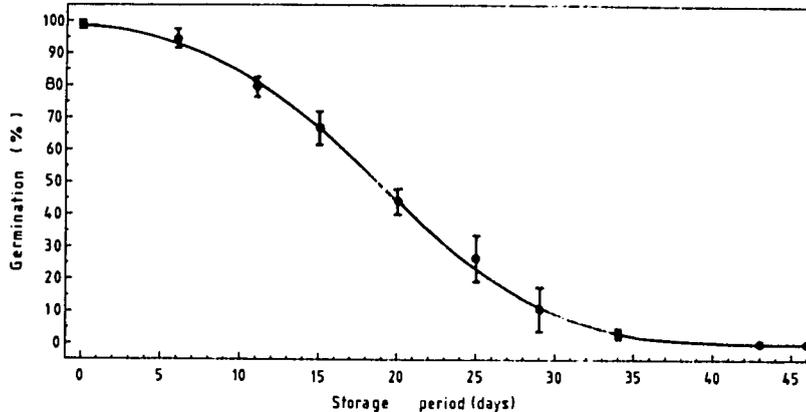


FIG 2. The results and standard deviation (vertical bar) of serial germination tests on seed drawn from a finger millet seed lot (●) plotted against the period of storage at 40°C with 15% moisture content. The solid curve shown is a negative cumulative normal distribution and was fitted to the experimental data by probit analysis.

Rather than join up the adjoining individual sample data points by straight lines we can smooth out the line and draw a sigmoidal survival curve. (Fig. 2) Moreover it is not necessary for this curve to go exactly through each data point, for each data point is subject to sampling errors. In general the form of the decline in germination is similar to that shown in Fig. 2, with the

exception of differences on the time axis. Survival curves of seed lots showing high initial viability often have an initial period during which very few seeds die. This can be seen as a shoulder on the sigmoid. In Fig. 1, for example, this period lasts about two days for seed at 9.7% moisture content. Clearly the initial percentage viability of the seed population is maintained during this period, but it is wrong to assume from this observation that no deteriorative changes have occurred within the surviving seeds. One obvious change is a substantial reduction in the subsequent longevity of the population (Fig. 1).

The Effect of a Failure to Remove Dormancy on Survival Curves

In certain cases germination tests do not provide a full estimate of viability due to post-harvest dormancy of the seed. The confounding effect this has on seed survival curves (Fig. 3). Here

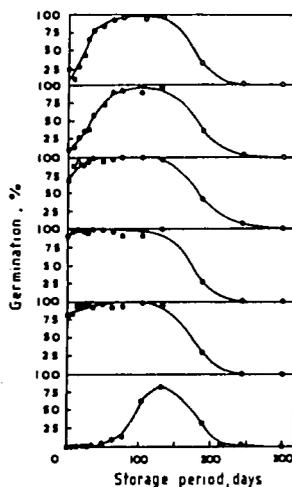


FIG 3. The percentage germination of seed of six rice cultivars plotted against the period of storage at 27°C with 13.5% moisture content. The rising parts of the curves show loss in dormancy; the latter parts of the curves show loss in viability. (Data from E.H. Roberts, 1963 Annals Bot. 27: 365-369.)

the rising parts of the curves indicate a loss in seed dormancy as a result of the after-ripening that occurs during the storage of fresh seed after harvest. However, by ignoring these parts of the curves and looking only at the right hand side of each diagram we can again see the sigmoidal pattern of loss in seed viability.

Fig. 3 shows that there is no casual relationship between the initial proportion of dormant seed in the population and longevity; despite marked differences in the proportion of dormant seeds between the six rice cultivars before storage, the pattern of loss in viability - and thus longevity - was identical in all cases. However, it should be noted that those practices which prolong longevity (immediate harvest of physiologically mature seed, stored at a low temperature and low moisture content) will also result in higher proportions of dormant seeds throughout storage than would otherwise be the case.

Rate of Loss in Viability and Seed-to-Seed Variation in Longevity

The sigmoidal pattern of loss in percentage viability demonstrates that individual seeds within a homogeneous population die after different periods of storage - that is individual seed lifespans differ. Consequently the slope of the sigmoidal survival curve is a measure of the variation in the times at which individual seeds die; the steeper the survival curve the less the seed-to-seed variation. It is often suggested, wrongly, that the slope of the survival curve is the rate of loss of viability. The mean rate of loss of viability of the seed population is the reciprocal of the mean longevity of all seeds in the population. For seed lots which are initially 100% viable the mean longevity can be conveniently estimated as the period taken for viability to fall to 50%. The mean rate of loss in viability is, therefore, the reciprocal of this period.

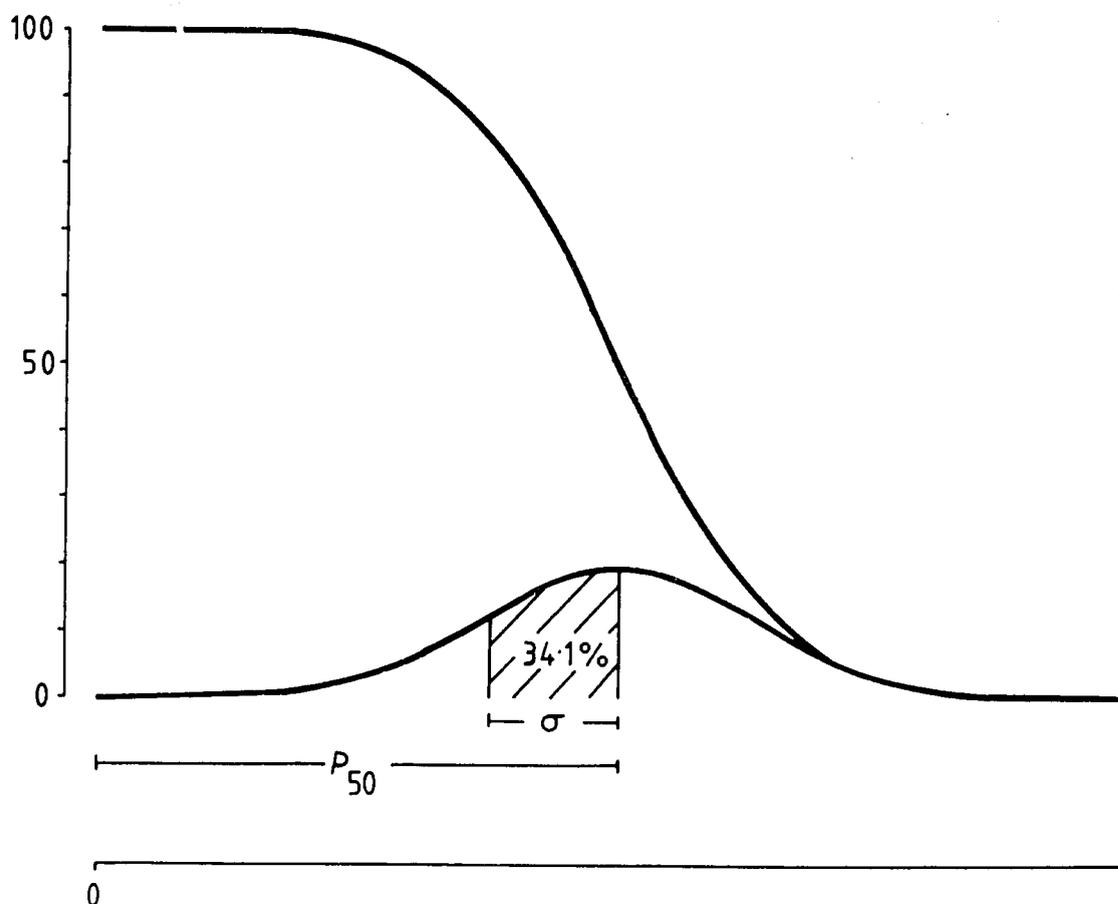


FIG 4. Bell shaped curve showing the normal frequency distribution of seed deaths per unit time, and sigmoid curve showing the corresponding negative cumulative normal distribution. In this and subsequent similar diagrams the vertical axes represent either percentage viability (cumulative distributions) or the percentage of seeds dying per unit time (frequency distributions) and the horizontal axes represent the period of storage. The shaded area is 34.1% of the area under the standard normal curve, that is one standard deviation, σ . The period p_{50} is the time taken for viability to fall to 50%. On the negative cumulative normal distribution percentage germination declines from 84.1% to 50% during the period denoted as one standard deviation.

If we look more closely at the seed-to-seed variation in longevity we can see that only a few seeds die per unit time early on during storage, but that as the storage period increases so the number of seeds dying per unit time increases until a maximum value is reached when 50% of the seeds have died.

Thereafter the number of seeds dying per unit time declines in a manner that mirrors the previous increase; that is the frequency distribution of seed deaths in time is symmetrical about the point in time where 50% of the population has died. The frequency distribution of seed deaths per unit time for a sigmoidal seed survival curve is shown in Fig. 4. In fact the frequency distribution shown is a normal distribution where the times of individual seed deaths in the population are randomly distributed about some mean value. This is convenient since we can describe the complete frequency distribution by only two attributes - the mean longevity (p_{50}) and the standard deviation (σ) both of which are shown in Fig. 4.

Consequently the sigmoid seed survival curve can be described by a negative cumulative normal distribution. The curve shown in Fig. 2 is one such negative cumulative normal distribution which describes this seed survival data very well. It should be noted that both parameters of the negative cumulative normal distribution (σ and p_{50}) are measures of seed longevity. Clearly p_{50} is the period taken for 50% of the seeds in the population to die (Fig. 4). In this example σ is the period during which viability declines from 84.1% to 50%, that is the period during which 34.1% of seeds in the population have died.

THE USE OF PROBABILITY SCALES

To fit a curve by eye to the data in Fig. 2, for example, is not easy. However, one way in which a negative cumulative normal dis-

tribution can be fitted by eye to seed storage survival data is to plot percentage viability on a probability scale against time. (Probability scale graph paper is readily available.) This has been done in Fig. 5. When this is done, a cumulative negative normal distribution results in a straight line of negative slope which can be fitted by eye. In Fig. 5 it can be seen that all the data points except two fit the line very closely. The two points

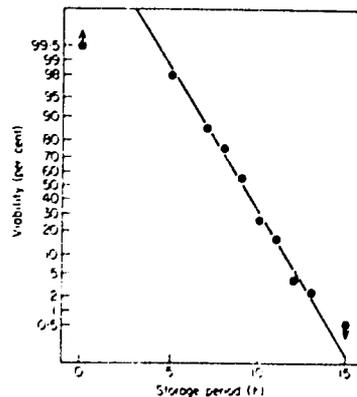


Fig 5. Serial germination test results of a barley seed lot stored at 60°C with 15% moisture content (●). Percentage viability is plotted on a probability scale. The resultant straight line seed survival curve was fitted to the experimental data by probit analysis. (From Ellis and Roberts, 1980a.)

which appear to deviate from the line are the results of the first and last germination tests which showed 100% and 0% germination respectively. However, such values cannot be plotted on the probability scale (for they are on parts of the scale which are approaching infinity) and consequently they have been plotted at 99.5% and 0.5% germination respectively with arrows to indicate their actual values. Such points should be ignored when fitting the straight line.

Linear graph paper can be used to obtain a straight line similar to Fig. 5 if percentage viability is transformed to probit values

and probit percentage viability plotted linearly against storage period. In Fig. 6 two scales are shown: probit percentage viability (v), and percentage viability plotted on a probability scale. Percentage viability has been transformed to the appropriate probit value in Fig. 6 by drawing a horizontal line between the two vertical axes. Once percentage viability has been transformed to probit percentage viability it is extremely simple to describe the seed survival curve since it is a straight line of negative slope. As noted earlier, the slope of seed survival curves is a measure of the seed-to-seed variation in longevity within the population. Now a probit value of 1 is one standard deviation above the mean. Consequently the slope of a transformed seed survival curve is the reciprocal of the standard deviation, i.e. $1/\sigma$ (Fig. 6). Thus the transformed seed survival curve can be described as

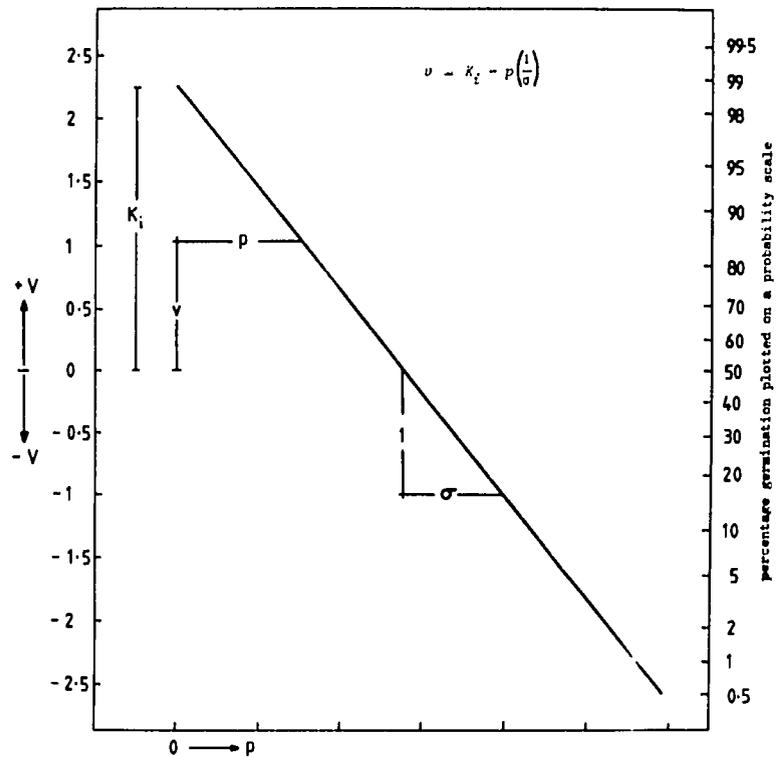


Fig 6. Diagram illustrating the equation to describe the seed survival curve when percentage viability has been transformed to probit percentage viability (v). On the right hand vertical axis percentage viability has been plotted on a probability scale.

$$v = K_i - p(1/\sigma)$$

where v is probit percentage viability at time p

p is the period of storage

K_i is the origin of the transformed survival curve at zero time

and $1/\sigma$ is the slope of the transformed survival curve

Although the normal distribution is symmetrical, substantial loss in viability may have occurred before seed receipt. Consequently only a part of the distribution may be observed. For example in Fig. 7 initial viability was only marginally greater than 50%.

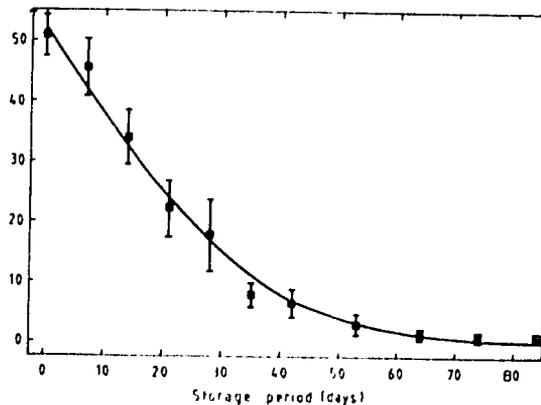


FIG 7. Serial germination test results (■) and standard deviation for a poor quality (i.e. low initial viability) foxtail millet seed lot stored at 20°C with 15% moisture content. The seed survival curve (solid line) was fitted by probit analysis and represents a negative cumulative normal distribution. Note that this seed survival curve is only one half of a complete normal distribution since the initial seed viability was only marginally greater than 50%.

The observed distribution is clearly not symmetrical, but the seed survival curve can be described by a negative cumulative normal distribution.

The most accurate method of fitting a negative cumulative normal distribution to describe loss in seed viability is by probit analysis. This is a weighted transformed regression where greatest weight is placed on the central values of the distribution (between about 85% and 15% germination) and the origin (K_i) is estimated by extrapolation from these central values.

THE INFLUENCE OF STORAGE ENVIRONMENT

The reduction in the proportion of viable seeds in a population is not just a function of time. The temperature and moisture content at which the seeds are stored also influence loss in viability; the greater the temperature and/or moisture content of the seeds the more rapidly loss in viability occurs. In Fig. 1, for example, it can be seen that loss in viability occurs sooner at the higher moisture content. Note in Fig. 1 that both the mean period of longevity and the variation in longevity between seeds are reduced, and that the survival curve at the higher moisture content is steeper.

Theoretically it is difficult to imagine that the origin of a survival curve at zero time can be affected by a subsequent storage environment to which the seeds have not yet been exposed. In Fig. 8 two transformed seed survival curves have been drawn with a common origin at zero time (i.e. with the same K_i value). If this is done it can be seen that if the mean viability in the harsher environment is half that in the better environment, then the standard deviation will also be reduced by half. Actual results for the storage of one seed lot in three different constant environments clearly demonstrates that the origin of the trans-

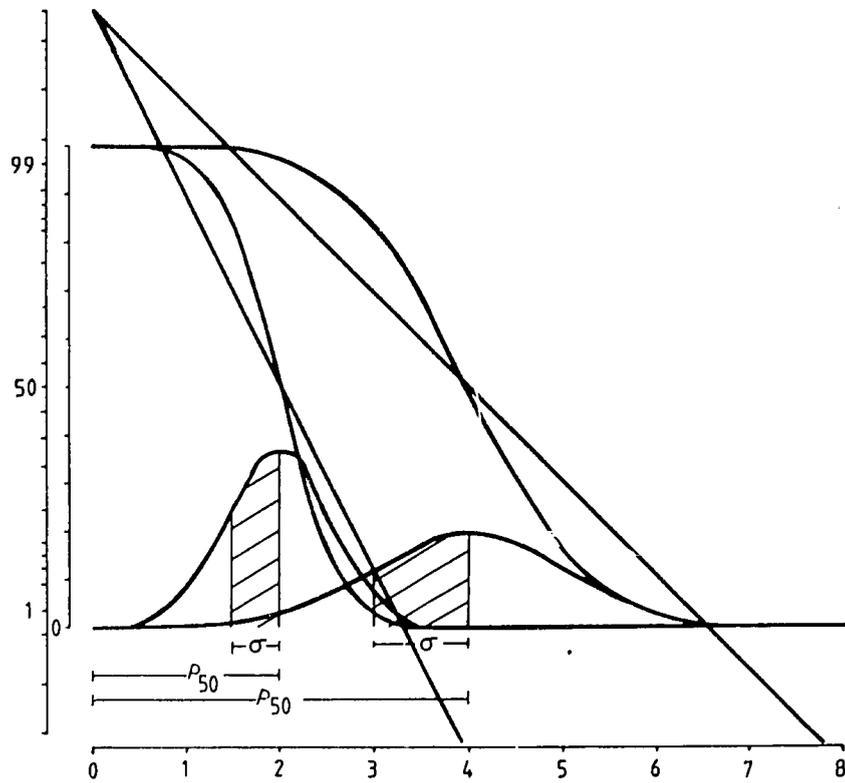


FIG 8. Diagram to illustrate the seed survival curves and frequency distributions of seed deaths in time for one seed lot stored in two different, constant storage environments. Note that the origin of the two transformed survival curves is unaffected by storage environment, and that both σ and p_{50} are reduced by the same proportion in the harsher environment.

formed survival curves is independent of the storage environment and that only the slopes of the three transformed seed survival curves are affected by the storage environment (Fig. 9). Note that there is a discrepancy between the result of a germination test at zero time and the origin of the survival curves at zero time determined by probit analysis. It can be seen that this is the result of dormancy in a small proportion of the population which is lost during the initial stages of storage because germination rises over the first thirty days of storage (Fig. 9)

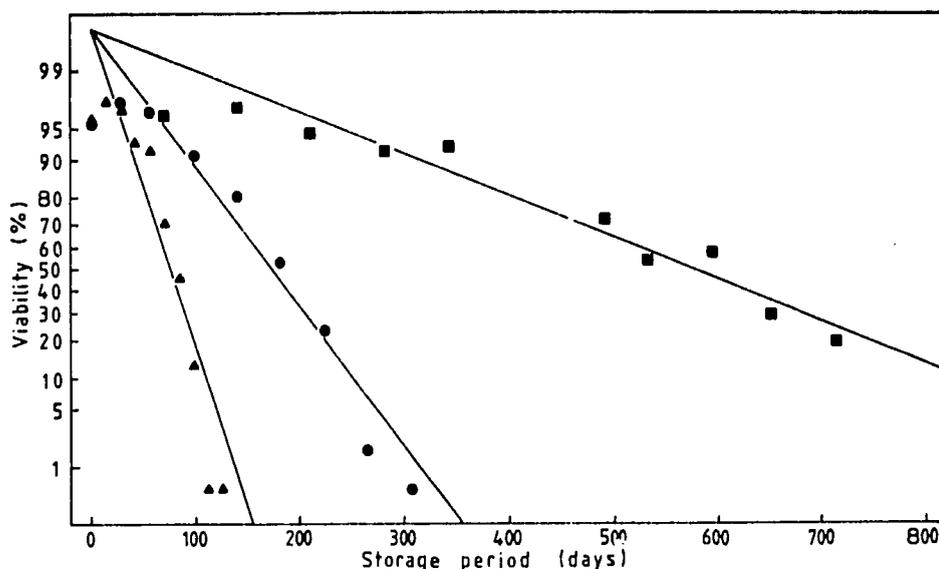


FIG 9. Transformed seed survival curves (percentage viability plotted on a probability scale) fitted by probit analysis for a maize seed lot stored under three different, constant environments (20°C with 15% ■ , 30°C with 15% ▲ , and 40°C with 10% moisture content ●) which show a common origin at zero time.

The general rule that increasing seed moisture content reduces seed longevity (or, conversely, reducing seed moisture content increases longevity) must be qualified. First, at moisture contents between about 20-25% (F.W.) and fully imbibed, seeds survive for considerably longer periods than predicted, provided oxygen is present, fungal deterioration is avoided, and the seeds are prevented from germinating (i.e. by dormancy). This range of moisture contents is of course outside that at which seed would be stored, but is an important area of physiology to consider when collecting and drying seed of species whose seed is harvested at such high moisture contents.

Secondly, low moisture content seed of certain species can be damaged if they are set to germinate in excess water; this imbibition damage can be avoided if the seed moisture content is raised by humidification before the seed are set to germinate. This

phenomenon has been most frequently observed and investigated within the family Leguminosae but it is known to occur across a much wider range of species and genebank staff must be aware of this problem. It should also be noted that the phenomenon can occur at moisture contents as high as 15% (F.W.), but is observed in a greater proportion of seeds the lower the moisture content. The phenomenon can also influence the pattern of staining in tetrazolium tests on very dry seeds.

Thirdly, seed of some species are brittle at low moisture contents. In soyabean this problem is so severe that a cleavage is created between the cotyledons when they are dried below 7% or 8% moisture content (F.W.).

DIFFERENCES IN LONGEVITY BETWEEN POPULATIONS OF SEEDS WITHIN A SPECIES

Frequently, but not always, different seed lots of the same cultivar differ in their longevity in the same environment. Similarly differences in longevity are often observed between seed of different genotypes within a species that have been produced in the same environment and stored in the same environment.

Fig. 10 provides an example of differences observed between seed of different cultivars of a species despite being stored in the same environment. However one similarity can be observed for these three curves. They are of similar form and simply displaced in time from one another. If the survival curves are identical apart from being displaced in time then they must have the same standard deviation and differ only in mean viability (Fig. 11). The transformed survival curves consequently have the same slope and differ only in their origins at zero time, that is they differ only in K_j values (Fig. 11).

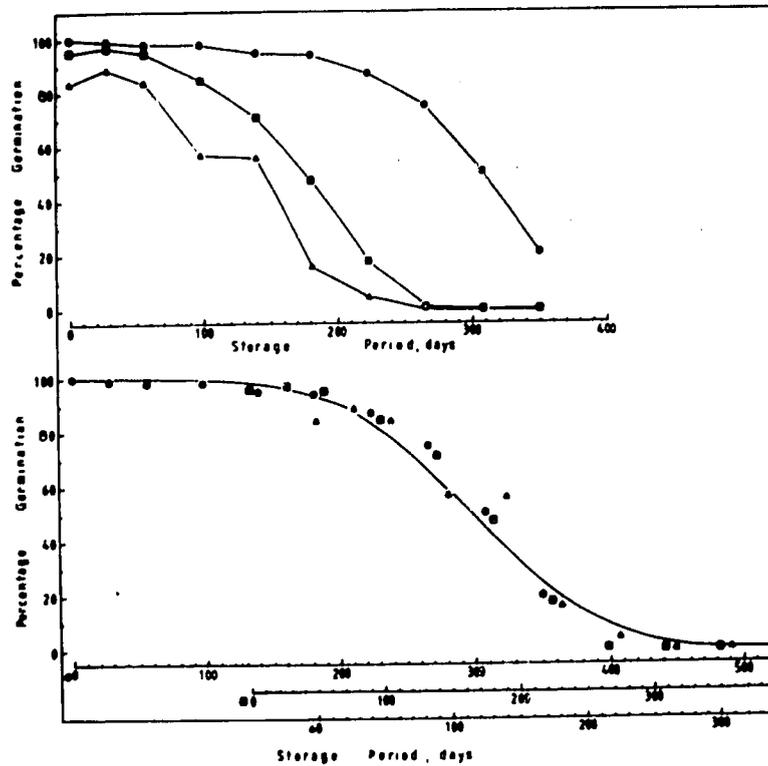


FIG 10. The seed survival curves of seed of three maize cultivars (● , ■ , ▲) stored under identical conditions of 40°C with 10% moisture content. In the upper figure percentage germination is plotted against storage period. In the lower figure a single negative cumulative normal distribution has been fitted to all three cultivars, but the start of storage has been moved along the abscissa for the two poorer cultivars to take into account differences in the value of the seed lot constant K_j (see text) (From Ellis and Roberts, 1981a.)

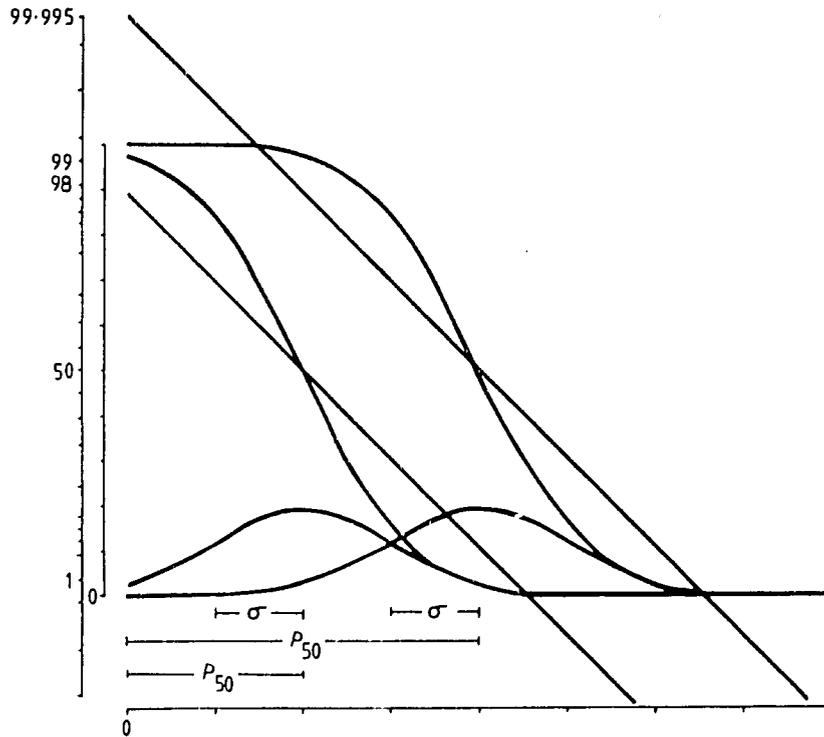


FIG 11. Diagram illustrating the survival curves of two seed lots stored in an identical environment where the frequency distribution of seed deaths in time are identical but displaced. This and the above diagrams illustrate that seed lots of similar initial percentage viability can differ greatly in subsequent longevity even when stored under identical conditions.

The lower half of Fig. 10 demonstrates that the three survival curves can be described by a single negative cumulative normal distribution, provided that the differences in the value of K_i between the three seed lots is taken into account. To do this the start of storage has been moved along the abscissa for the two poorer cultivars to take into account between-cultivar differences in the value of the seed lot constant. The single solid curve fitted is a negative cumulative normal distribution where K_i is 3.87 (the estimate for the best cultivar, ●) and the standard

deviation (σ) has a value of 77 days. The K_i of the intermediate cultivar (■) is 2.14 and thus the storage period is zero for this cultivar after the equivalent of $(3.87 - 2.14) \times 77 = 133$ days storage for the best cultivar. The K_i of the poorest cultivar (▲) is 1.49, and thus the storage period is zero for this cultivar after the equivalent of $(3.87 - 1.49) \times 77 = 183$ days storage for the best cultivar.

The data for seed of six rice cultivars stored in the same, constant environment (Fig. 3) also show similar survival curves. In this case not only do the six curves have the same standard deviation (σ), but the values of K_i are also identical because the six cultivars have the same longevity.

FACTORS OTHER THAN ENVIRONMENT WHICH MAY AFFECT σ

The observation that the standard deviation of different seed lots is the same in a given constant storage environment is only valid for homogeneous seed lots. If two homogeneous seed lots which differ in longevity, that is they have different values of the constant K_i , are mixed to produce a heterogeneous seed lot then the value of the standard deviation in a given environment is inevitably increased (Fig. 12). The consequence of this is that, at any one point, the slope of the survival curve is less steep and thus loss in viability is seen to occur more slowly.

In the example shown two populations are mixed equally to produce the composite population, but note that the proportion of viable seeds in each population changes from 1:1 at the start of storage to 0.8:1 and to 0.5:1 at the points shown in Fig. 12. Such heterogeneity within a seed population may occur within a genotype as a result of the mixing of seed of different provenances (produced in different locations), or may represent genetic heterogeneity. The

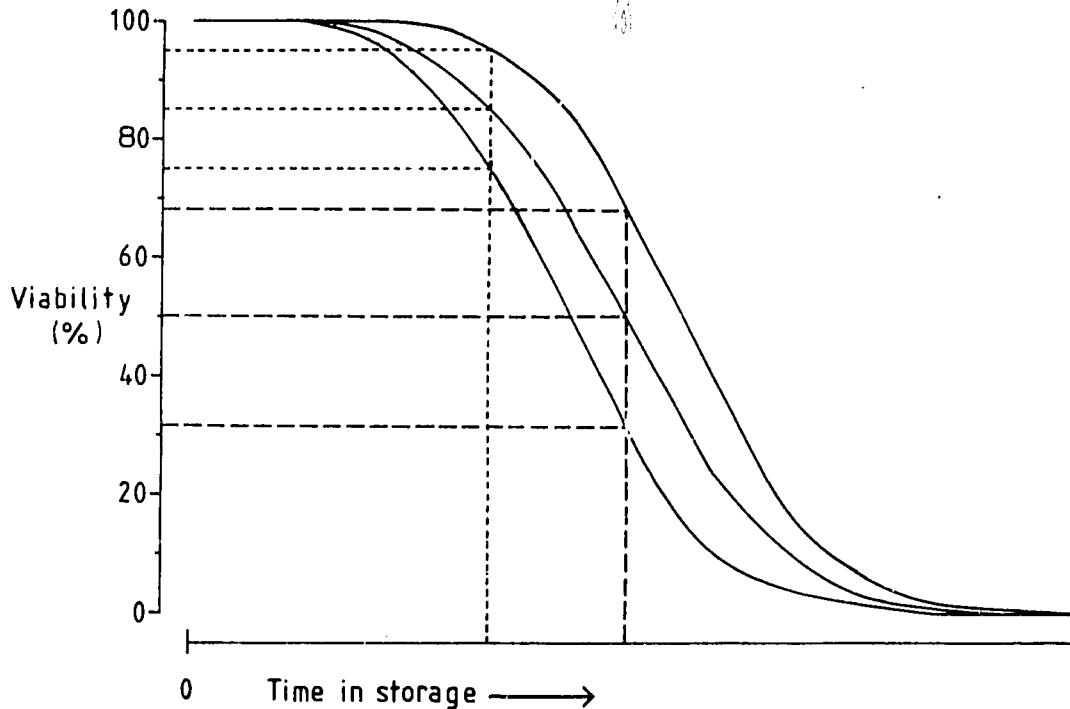


FIG 12. Seed survival curves (percentage germination plotted against time) of two populations of seed stored under the same environment (first and third curves) with an intermediate curve drawn to demonstrate the seed survival curve that would result if the two populations were mixed equally. The proportion of viable seeds in the composite sample derived from the shorter-lived seed lot is reduced during storage.

latter case is of especial concern for genebank personnel and this is discussed again at the end of the article.

A second factor which will affect the value of σ in a given environment is the confounding effect of empty seeds within a population. These should be excluded from any calculations and percentage viability should be expressed as the percentage viability of full seed. For example if there are 10% empty seeds in a population and the result of a germination test is 90%, then the percentage viability of the full seeds within the population is 100%.

In algebraic terms if x is the percentage of empty seeds in a population and y the percentage germination of the sample from that population the,

$$\text{Percentage germination of full seeds} = [y/(100 - x)] \times 100$$

THE QUANTITATIVE EFFECT OF TEMPERATURE ON LONGEVITY

Temperature has a dramatic effect on longevity. As an approximate guide to the extent of the effect of temperature on longevity many authors cite Harrington's rule of thumb;

"Each 5°C reduction in seed temperature doubles the life of the seed".

This phrase describes an exponential relationship. A convenient way of examining such relationships is to plot seed longevity (σ , in this example) on a logarithmic scale against storage temperature on a linear scale. By equation this is then

$$\log \sigma = K - Ct$$

where K is an intercept constant at 0°C, C is the gradient and t the temperature in °C.

Q_{10} , the Temperature Coefficient

The temperature coefficient for change in rate of loss in viability per 10°C rise in temperature for Harrington's rule of thumb (above) is 4, because if an increase of 5°C results in loss of viability occurring twice as rapidly, then an increase of 10°C results in the loss occurring at a rate ($2 \times 2 =$) 4 times as rapidly.

Fig. 13

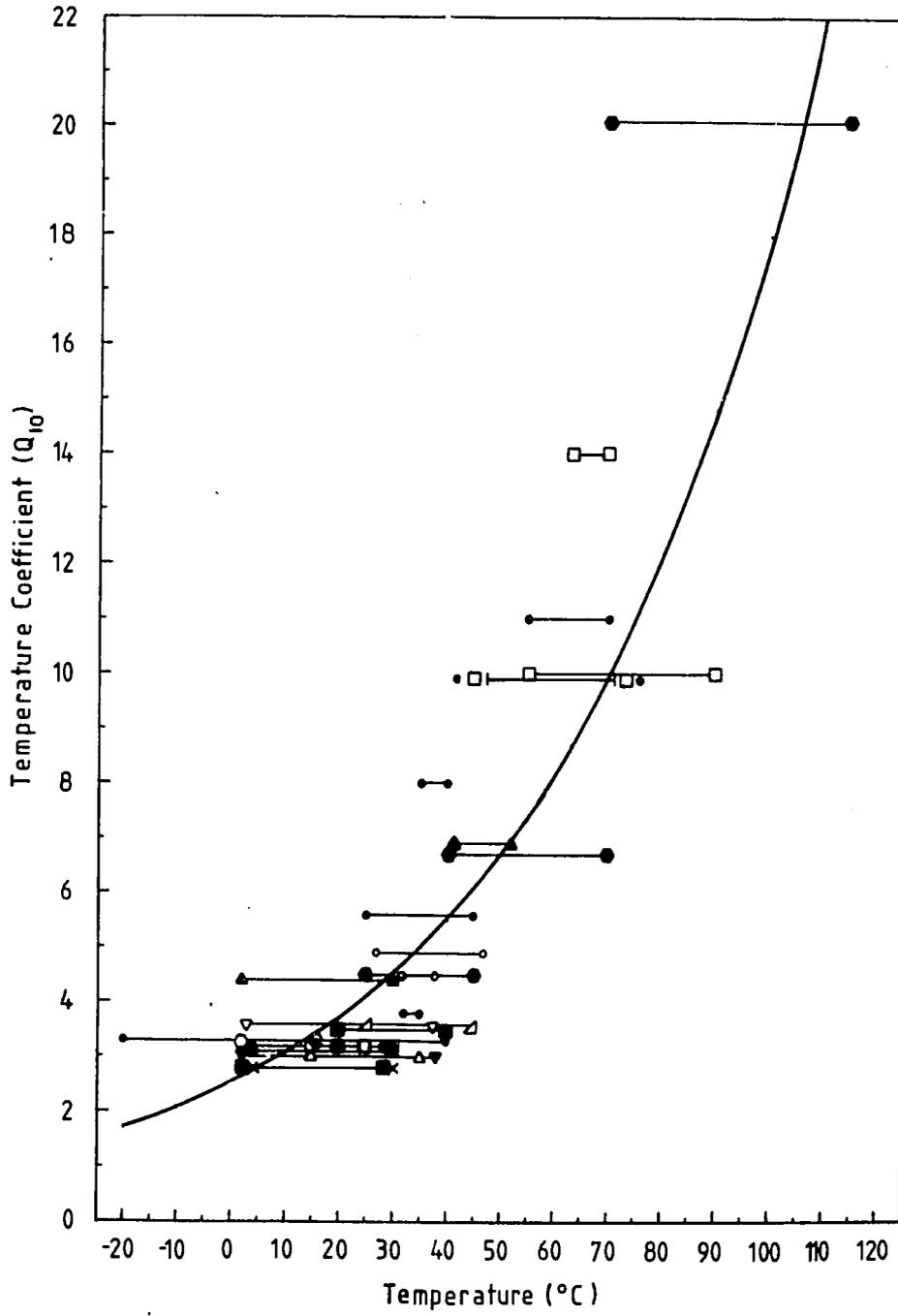


FIG 13. The relationship between the temperature coefficient for change in rate of loss in viability per 10°C rise in temperature, Q_{10} , and temperature determined for barley (solid curve) compared to the stable Q_{10} values reported in the literature for seed of twenty flower, vegetable and arable species over limited temperature ranges (horizontal bars). (From Ellis and Roberts, 1981a.)

Unfortunately the evidence shows that the quantitative effect of temperature on seed longevity is not uniform over all temperature ranges, and that the temperature coefficient rises in value with increase in temperature as denoted by the solid curve in Fig. 13. Consequently Harrington's rule of thumb, which implies a constant Q_{10} value of 4, is only accurate between about 20°-30°C (Fig. 13). The effect of temperature is in fact more complicated than the above, and a more accurate expression is

$$\log \sigma = K - C_H T - C_Q T^2$$

where K is again an intercept constant at 0°C and C_H and C_Q are constants which, when combined as above, describe the gradient.

The value of the temperature coefficient does not differ either within or between species over a given temperature range. Indeed, as Fig. 13 shows, the variable temperature term determined for one species (barley) is able to explain substantial differences in the values of the temperature coefficient determined by many workers over a very wide range of species.

It is worth noting at this point that the Q_{10} denotes the relative effect of a 10°C increase in temperature not the absolute effect. E.g. the p_{50} for one seed lot at a given moisture content might be 100 days at 20°C then at 30°C p_{50} will be 25 days while 200

days at 20°C and 50 days at 30°C for a second lot then the relative effect on longevity of a 10°C rise in temperature is four-fold for both lots but the absolute differences are 75 days and 150 days respectively.

THE QUANTITATIVE EFFECT OF SEED MOISTURE CONTENT ON LONGEVITY

Harrington's rule of thumb - "Each 1 per cent reduction in seed moisture doubles the life of the seed" - indicates the exponential nature of the relationship but, as for his rule for temperature, this rule-of-thumb is only precise over a fairly narrow range of seed moisture content. For barley this range is about 8-9% moisture content (fresh weight basis). The relationship between seed moisture content and seed longevity (σ , in this example) has been quantified by the following form of equation,

$$\text{Log } \sigma = K - C_w \log m$$

where K is an intercept constant at 1% moisture content (because the logarithm of 1 is 0), C_w is the constant for the gradient and m is seed moisture content, % fresh weight basis. Within a species the relative effect of moisture is the same, that is the value of C_w is the same. However there are differences in the relative effect of moisture content between species; that is C_w differs between species. In cereal species C_w has a high value (approximately 6), but in oily seeds the value of this constant is lower (e.g. between 3.5 and 4 for onion and soyabean seed). This indicates that in the latter species the relative effect of moisture content is not as great as in the cereal species. This means that to obtain the same relative increase in longevity it is necessary to dry onion or soyabean seed more than, say, barley seed from a given moisture content.

THE COMBINED INFLUENCE OF TEMPERATURE AND MOISTURE

By combining the two equations for the influence of temperature and moisture content on seed longevity we can describe the combined influence of these two factors on the standard deviation of longevity within homogeneous seed populations:

$$\log \sigma = K_E - C_W \log m - C_H t - C_Q t^2$$

where K_E is a further constant value which denotes the extrapolated value of $\log \sigma$ at 1% moisture content and 0°C. The four constant values (K_E , C_W , C_H and C_Q) do not differ for seed populations within a given species and thus (as we have already seen) σ does not differ between homogeneous seed populations in the same environment.

THE VIABILITY EQUATION AND VIABILITY NOMOGRAPH

In the equation which described the transformed seed survival curve (Fig. 6) the slope is given by the reciprocal of the standard deviation. Consequently σ can be replaced by the anti-logarithm of the above equation to provide a single equation,

$$v = K_i - p/10^{K_E - C_W \log m - C_H t - C_Q t^2}$$

where only the value of K_i differs between seed lots within a species.

As there are five constant values and four variable terms in the above equation, calculations are not easy. The equation can be made easier to apply by constructing a nomograph. Fig. 14 shows a seed viability nomograph for barley, together with instructions for its use.

FIG 14. The seed viability nomograph for barley. The method of calculation is illustrated by the broken straight lines. These lines illustrate a calculation to predict viability after 20 years storage (7,300 days) at 4°C with 10% moisture content for two seed lots, one initially 99.5% viable and another initially 90% viable. To calculate final viability a ruler is placed at 4°C (scale a) and 10% moisture content (scale b). The resultant point on scale c (8,400 days) is then connected with a ruler to 7,300 days on scale d (the storage period) and the corresponding value (0.8) on scale e is noted. The ruler is then placed on the same value of scale f and this point is connected to either 99.5% or 90% initial viability on scale h. The results of final viability are indicated on scale g; the viability of a seed lot initially 99.5% viable would be estimated to fall to 96%, and the viability of a seed lot initially 90% viable would be estimated to fall to 70% after 20 years storage at 4°C with 10% moisture content. (From Ellis and Roberts, 1980a.)

PREDICTING LOSS IN VIABILITY AND THE NEED TO REGENERATE

The improved viability equation and the values of the constants K_E , C_W , C_H and C_Q calculated for both barley and onion seed have been applied in an accompanying paper to estimate the time taken in years for the viability of seed accessions of varying initial viability to fall to 85% in the constant environments maintained at a number of medium and long-term stores for genetic resources conservation (Monitoring Seed Viability in Genebanks, E.H. Roberts, Table 1). Three points should be noted when applying the improved viability equation in this way.

First, although all accessions will be theoretically stored under identical conditions (for example, -20°C with 5% moisture content) there will be small differences in moisture content and possibly store temperature between accessions. Thus maximum con-

ditions of store temperature and seed moisture content (e.g. -19°C and 6% moisture content) should be inserted in the improved viability equation.

Secondly estimates of initial viability of each accession will be subject to error since each is the result of a test on a small sample of seeds taken from the overall population. To limit the possibility of sampling errors resulting in an over-estimate of storage life, it is suggested that the values of the lower confidence limit ($P = 95\%$) of the sample test result is applied in the equation to estimate regeneration interval.

Finally the values of time and temperature derived by or applied in the equation will be extrapolations from observations over shorter periods of storage at higher temperatures. Extrapolation is hazardous. Thus the estimates of regeneration interval derived in this way must be treated with some caution. Nevertheless, it is reassuring to note that attempts have been made to estimate the rate of loss in viability of seed stored at sub-zero temperatures and that the results are consistent with predictions.

The main experimental problem is that, as predicted, loss in viability occurs extremely slowly at sub-zero temperatures and is thus difficult to detect. In Fig. 13 it can be seen that an estimate of Q_{10} derived between -20°C and $+40^{\circ}\text{C}$ is compatible with the value derived by the equation over this temperature range. Moreover, Fig. 15 demonstrates that loss in germination (whether the criterion is normal germination or total germination) declines less rapidly when seed are stored at -20°C than when stored at 0°C or higher (foxtail millet and pearl millet), or less rapidly when stored at -20°C or 0°C than when stored at 10°C higher (finger millet). (In the latter species deterioration occurred at a slower rate and the period of storage was insufficient to detect any differences in germination at -20°C and 0°C .)

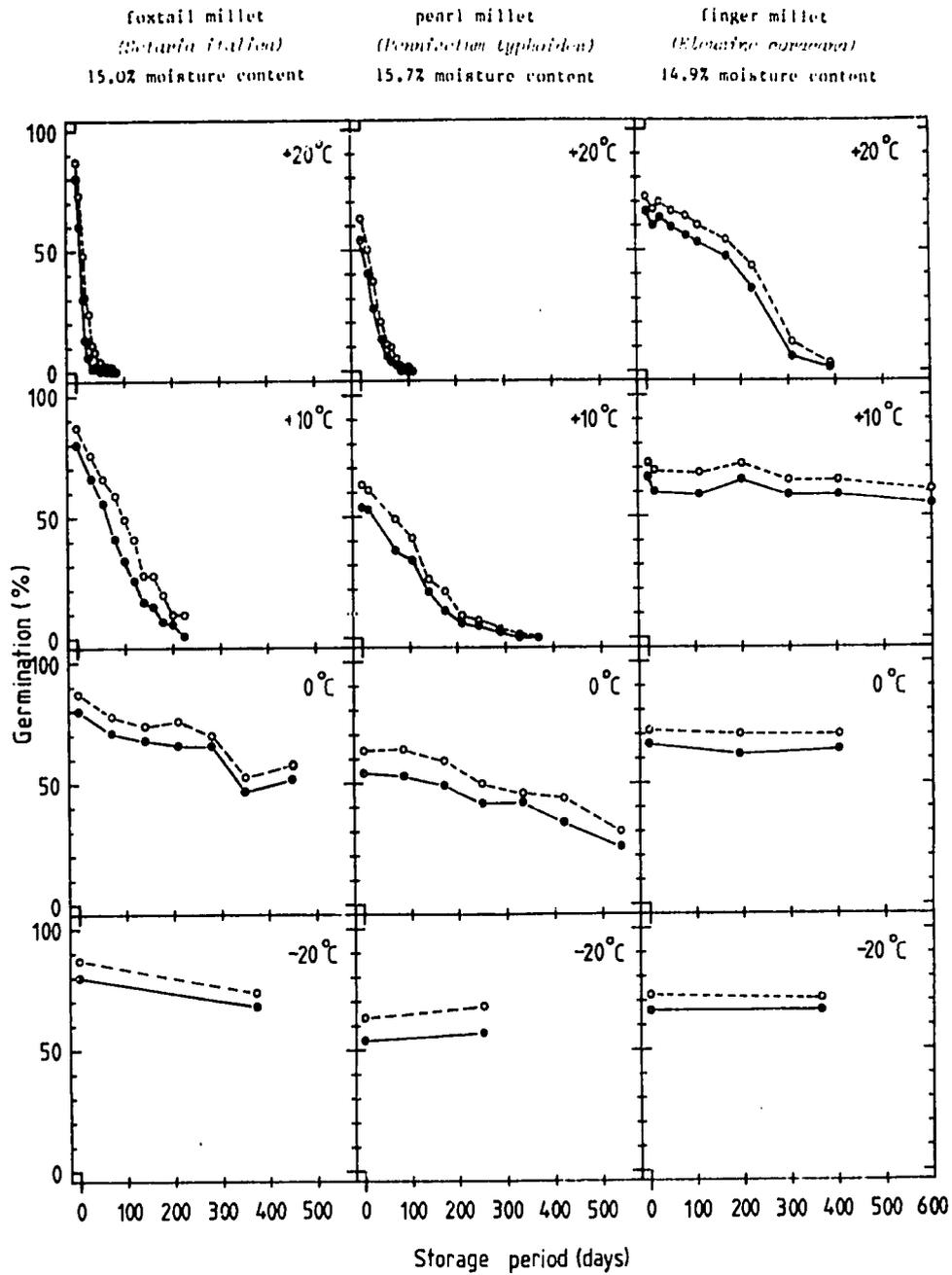


FIG 15. Data (from an uncompleted experiment) which illustrates the benefit to the maintenance of both the percentage total germination (o---o) and normal germination (●---●) of three millet seed lots which results from reducing the storage temperature from +20°C to -20°C.

This question is most difficult to answer. Essentially the level of viability chosen will be a compromise between the costs and difficulties of regeneration - which favour regeneration at a low level of viability - and the effect of ageing on the physiological performance of the surviving seed and heritable damage to succeeding generations - which favour regeneration before substantial loss in viability has occurred.

AGEING

In man the outward signs of ageing are obvious. In seeds, stored dry, there are no such obvious signs of ageing. Thus we must consider whether an individual seed does age before death, or whether seed death is a random, spontaneous event. If an individual seed is tested for germination and it does germinate it is no longer a seed. Thus, as noted earlier, we can only test individual seeds once and we are unable to discern whether any character of the individual seed's performance has altered (i.e. aged) during storage.

Consequently we must examine the performance of samples of seeds drawn from the population. One character of seed performance which is easy to observe, is the time taken by seeds to germinate when tested in a given environment, after different periods of storage. For example in one barley seed lot tested at 20°C 98% of the seeds germinated and all of these seeds germinated between 1 and 3 days after the start of the germination test. But seed of this lot that had been aged showed only 42% germination and these surviving seeds took between 3 and 18 days to germinate when tested. There are many other symptoms of ageing that are observed in the surviving seed of seed population which have been aged.

For genebanks the two most important aspects of ageing which are observed in surviving seeds from populations that have been stored are:

1. the ability to germinate, emerge and establish as plants under less than favourable soil conditions; and
2. the incidence of genetic damage.

As seeds age the proportion of surviving, viable seeds which are able to establish plants under field conditions declines dramatically. For example of one barley seed lot which was 96% viable, 81% of seeds sown produced a plant in the field; but of a sample which was 83% viable only 50% of the seed sown produced a plant. Consequently if 100 plants were required 123 seeds of the 96% viable lot should have been supplied by the genebank, but 200 seeds of the 83% viable lot would have been required.

The incidence of chromosome damage also increases as seeds age. One way in which this can be seen is to examine root tip cells of surviving seeds at anaphase during the first mitotic division. In barley, for example, the proportion of such cells in which chromosome damage is observed increases from between 0-1% for 100% viable seed to 4% for the surviving seed in populations in which 50% of the seeds have died as a result of ageing. In practice this gross chromosome damage is unimportant, but it is indicative of more subtle damage which can be observed in the phenotypes of subsequent generations produced from aged seed.

Rather conveniently the above symptoms of seed ageing that occur in surviving seed have been shown to be correlated with the proportion of seeds in the population that have died. Thus the result of the viability test is also indicative of the performance and genetic integrity of the surviving seeds.

Limiting the Expression of Ageing-Related Phenomena in the Majority of Individuals in a Seed Population

It will be apparent already that if seeds are stored in conditions where the rate of loss in viability is very slow (i.e. at low temperatures and low moisture contents) then the expression of ageing-related phenomena will be limited. That is why IBPGR recommends that seed for genetic resources conservation should be stored at 4-6% moisture content and -18°C or less. Nevertheless loss in viability will occur in this environment, albeit very slowly. To limit the expression of ageing-related phenomena in the majority of individuals in the population we must, therefore, regenerate accessions once loss in viability has been detected, after (say) a 5% loss in viability. But there is little sense in regenerating one accession when viability has fallen from 100 to 95% and another accession when viability has fallen from 90 to 85% since all the available evidence suggests that the important measure of seed quality is the final level to which viability has been reduced, not the percentage loss observed in a single store. This argument suggests that all accessions should be regenerated at a single level of viability. The results of studies on seed vigour and genetic damage would tend to suggest that regeneration should be instituted at about 95% viability, or possibly even higher. This, however, is impractical. Many accessions will show lower levels of viability than this at receipt and would need to be regenerated before storage.

In a generally heterogeneous seed population shorter-lived genotypes will die sooner than longer-lived genotypes (Fig. 12). Thus loss in viability within such populations will result in reduced genetic heterogeneity of the population and the selection of longer-lived genotypes. These changes can only be minimised by regenerating such accessions before substantial loss in viability has occurred.

Resolving these problems has to be a compromise between the above factors and the likely level of accession viability at receipt and after regeneration. A regeneration standard of 85% germination is considered to be suitable for those species in which it is possible to obtain accessions of high quality.

Acknowledgements

The author is grateful to the International Board for Plant Genetic Resources for financial support, to Professor E.H. Roberts for advice, and to Mr. T.D. Hong for assistance.

Further Reading

The following list of books and papers is provided as a comprehensive guide to further reading. It is suggested that they are read in chronological order. To shorten this list read only those papers marked with an asterisk.

Owen, E.B. (1956). The Storage of Seeds for the Maintenance of Viability. Commonwealth Agricultural Bureaux, Farnham Royal, England.

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

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Roberts, E.H. (1961b). The viability of rice seed in relation to temperature, moisture content, and gaseous environment. *Annals Bot*, 25: 381-390

Roberts, E.H. and Abdalla, F.H. (1968). The influence of temperature, moisture and oxygen on period of seed viability in barley, broad beans and peas. *Annals Bot*, 32: 97-117

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

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Roberts, E.H. (1972). Viability of Seeds. Chapman and Hall, London.

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

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

*Ellis, R.H. and Roberts, E.H. (1981a). The quantification of ageing and survival in orthodox seeds. *Seed Sci and Technol*, 9: 373-409

*Ellis, R.H. and Roberts, E.H. (1981b). An investigation into the possible effects of ripeness and repeated threshing on barley seed longevity under six different storage environments. *Annals Bot*, 48: 93-96.

*Ellis, R. H., Osei-Bonsu, K. and Roberts, E. H. (1982). The influence of genotype, temperature and moisture on seed longevity in chickpea, cowpea and soyabean. *Annals Bot*, 50: 69-82.

*Roberts, E.H. and Ellis, R.H. (1983). The implications of the deterioration of orthodox seeds during storage for genetic resources conservation. Proceedings of the FAO/UNEP/IBPGR Technical Conference on Crop Genetic Resources, Rome, 1981 (in press).

DISCUSSION

Chairman:

Participants: Ellis, Roberts, Boland,
Neergaard, Tyler, Hawkes, Bekendam

1. The Relationship Between Laboratory Germination and Field Emergence

In view of the constant relationship between laboratory germination and field emergence, apparent when percentages are transformed to probit values; Dr. Boland asked whether this relationship had been quantified for any species having in mind its commercial importance. In answer Dr. Ellis stated that genebank personnel should be constantly aware of the situation and stressed his remarks regarding the size of seed sample to be sent to users. While not indicating examples where the relationship had been quantified, Prof. Roberts stated that there was no evidence to suggest that any species is immune from the problem, but that it may be worse in some species than others.

2. The Relationship Between 'Slow' Germination and Genetic Damage

If it is true that genetic damage accumulating in storage is restricted to those seeds which germinate most slowly in a germination test; Prof. Neergaard suggested that selecting the most rapidly germinating seeds for onward growth would minimise the incidence of genetic damage. Dr. Ellis stressed that while the above relationship seems likely, there is no firm evidence for it yet. The only evidence is circumstantial, based on the need for an increased incubation period before root tip squashes are performed, while looking for chromosomal aberrations in aged seeds. While more detailed investigations into the phenomenon would be useful, Dr. Ellis was concerned about the possible loss of varia-

bility by removal of slow germinators; and wondered whether this kind of selection should be the responsibility of breeders rather than genebank personnel.

3. Changes of Gene Frequency Due to Differential Longevity of Genotypes in a Mixed Population

Referring to the theoretical model discussed by Dr. Ellis, Mr. Tyler wondered if there was any real evidence for such change in gene frequency with time in mixed genotype populations of out-breeders. The consequent doubt as to whether the original gene frequencies have been restored at regeneration has serious implications for the plant breeder. Though he could cite no evidence for such changes in gene frequencies, Dr. Ellis stated that the behaviour of independent populations (of the same species) supports the model. After all, any loss of viability in storage ipso facto results in selection, emphasising the need for regeneration while the viability of the seed lot is still relatively high.

4. Setting the Regeneration Standard

Prof. Hawkes enquired as to the ideal level of germination in an accession at which regeneration should be performed. Whilst the ideal level is very high, Dr. Ellis stressed the need for a compromise between the ideal and what is actually practicable with limited resources and manpower. If, as it ideally should be, regeneration were to be carried out at the first signs of loss of viability, the workload would render the task impossible.

5. Frequency of Viability Monitoring

Dr. Bekendam suggested that perhaps for certain species stored it may be possible to estimate the viability of all the accessions by checking only a few accessions. While regular viability testing

is essential, Dr. Ellis agreed that it is probably unnecessary to check every accession, but that to be safe the marker accessions to be tested should be those of low initial viability as opposed to random selection of a group of accessions. See Prof. Roberts' paper for comments on cohorts.

Distinguishing Between Dormant and Inviabile Seeds

P. OVERAA

The viability of seeds can be estimated by a germination test. However, if dormancy is present, it may in some cases last more than a year before a result is available. This is critical and limits the value of the germination test considerably.

At the termination of a germination test there may also occur a large number of ungerminated seeds for which it may be difficult to decide whether they are dormant or inviable. Therefore scientists and seed technologists have been looking for methods by which viable and inviable seeds can be distinguished satisfactorily. Different methods using staining agents as indicators to distinguish between viable and non-viable seeds have been developed. In general the methods are referred to as vital staining.

According to Effmann and Specht (1967), Dimitrieviz in 1876 had already found that live and dead embryos were stained differently when they were treated with sulphuric acid; and in 1900 Overton showed that acid dyes did not completely or only slowly penetrate the membranes of living cells, which remained unstained for a long time, while dead cells and tissues stained rather quickly. The dyes that did not completely penetrate into living cells belong to the following groups:

- i Triphenylmethan dyes: Acid fuchsine, acid green, acid violet, watersoluble anilin blue.
- ii Chinonimid dyes: Watersoluble nigrosin, watersoluble indulin.
- iii Azo-dyes: Congo red, Ponceau R., Bordeaux red, and Biebrich red.
- iv Indigocarmine.

According to Issatschenko (1928), Neljuboc reported in 1925 research he had carried out with various dyes mentioned above for the purpose of estimating the viability. He finally found indigocarmine most suitable for the purpose.

Rostovtsev and Lyubich (1978) state that the indigocarmine method has been widely used in the USSR for the assessment of the viability of tree and shrub seeds since 1940 and that the method is also used in other East European countries.

The principles of the indigocarmine method are as follows:

- An 0.05% solution is used for the staining which takes 1 to 2 h at room temperature.
- Before the staining the seed is soaked in water to obtain full imbibition. In some cases for 2 - 5 days.
- The imbibed seeds are then prepared in such a way that the embryo and/or the part of the seed to be examined is in unrestricted contact with the solution when put into it.
- After staining the embryo/seed is rinsed carefully.
- Finally the staining patterns of the embryo/seed are examined and evaluated regarding viable/nonviable.

Another method using 0.05% acid fuchsine as indicator is introduced by Effmann and Specht (1965). In principle the method is just the same as the indigocarmine method, but according to the authors (Effmann and Specht 1967), it is superior to the indigocarmine method and very convenient when used in combination with a sequence analysis.

The superiority is attributed to the fact that the preparation can be simplified considerably as e.g. within cereals, no moistening is necessary if the moisture content of the seed is more than 16%, and the necessary staining time is only 2 minutes.

In this connection it should also be mentioned that nothing seems to go wrong even if the staining period is prolonged considerably e.g. to 1 h or even more than that. It is possible that the seed can be kept in solutions of indigocarmine or fuchsine for a considerable time without adverse effects. The examination and evaluation however, should be carried out as soon as possible after embryos/seeds are rinsed. The embryo/seed should not be allowed to dry out before it is examined, and it seems unwise to keep it in water for a long time as the colour will impair due to leaking of dye from the stained tissue.

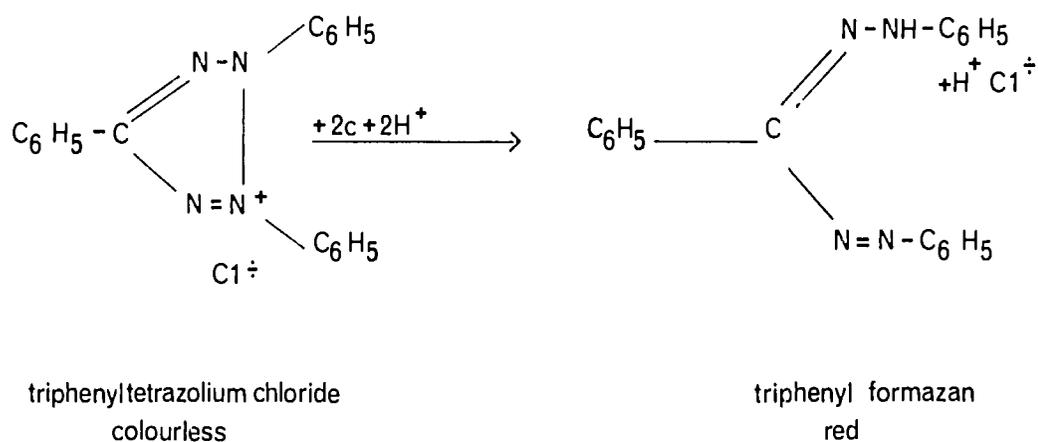
The methods mentioned above may be characterised as passive methods as they do not take part in any process going on in the seed.

Parallel to the development of these methods there has been comprehensive investigation in methods based on the enzyme activity taking place in living cells. James Mackey has already surveyed this area (1949). Most of the methods tried are without interest in routine testing. Only those based on the presence of dehydrogenases in living cells have been found satisfactory. The so called tetrazolium method using 2,3,5, triphenyltetrazolium chloride or bromide as indicators is known and used for different purposes all over the world and has been accepted by ISTA for testing the viability of seeds.

Its origin may, according to Mackey (1949), be traced back to Scheurlen who in 1900 proposed that the reduction of selenic and telluric compounds could be used as indicators to show that metabolism was taking place in cultures of bacteria. Turina in 1922 showed that the same would happen within living plant cells. Hasegawa (1935) probably was the first one to introduce the method in seed testing.

Comprehensive investigations with the method was later reported by Eidmann (1938) and several other scientists.

Lakon (1940) was the first one to bring the topographical aspect into the method. This was a significant step forward. The toxicity of the selenic compounds, however, made them risky to work with. Lakon, looking for less toxic indicators to substitute the selenic compound, was advised by Kuhn and Jerchel to try 2,3,5 triphenyltetrazolium chloride and so Lakon (1942) could report on his new topographical tetrazolium method for the estimation of the viability of seeds. The reaction which takes place when the colourless solution of triphenyltetrazolium chloride is transformed to insoluble red triphenyl formazan, in living tissues, is as follows:



Triphenyl formazan is a red dye which is stable in water, and it does not leak out of stained tissue. In most cases the seed coat is impermeable or only partly permeable to the tetrazolium solution. Therefore it must be opened in some way so that the solution can penetrate into the parts of the seed, which are decisive for germination and development to a normal plant.

Based on this fact the principles of the common procedure for a tetrazolium test are as follows.

- an appropriate solution
- imbibition (moistening) of the seed
- preparation of the imbibed seed
- staining of the prepared seed
- examination and evaluation of the stained seed

From what has been said previously, it may be obvious that the procedures applied for the different methods, are almost identical and may be grouped under the following headings:

Solution - moistening - preparation before staining
-staining and evaluation.

As my experience is mainly gained by working with the tetrazolium method, I shall deal with that in detail, and restrict myself to some brief comments regarding the others.

The Solution

For the tetrazolium test (TZ) an aqueous solution of 2, 3, 5 triphenyltetrazolium chloride (TTC) or bromide (TTB) is used. the pH of the solution should be within 6.5 - 7.0. If necessary the salt must be dissolved in a buffer. The concentration of the solution may be varied according to the species tested and the purpose of the test. Concentrations of 0.1 to 1.0% are common. The salt is readily soluble in both water and buffers. The prepared solution can be stored for months, if kept in darkness and at a reasonably low temperature (20°C). If the solution is exposed directly to sunlight it will be destroyed rather quickly.

To avoid disturbances during staining resulting in a red frothy solution with reddish to blackish precipitate it is advisable to add minimal quantities of a fungicide or antibiotic.

For the indigocarmine test (IC) an aqueous solution of 0.05% indigocarmine is used. The solution is prepared by boiling the dye in water for half an hour and filtering it. The amount of dye and water must be adjusted to give the right concentration. The solution may be stored for 15 days, if in darkness. For the acid fuchsine (AF) an 0.5% aqueous solution is recommended by Effmann and Specht (1967); though lower concentration may be used. Acid fuchsine is readily soluble in water.

The Moistening (imbibition)

Some seeds can be tested by the TZ without moistening before staining, and Lakon recommended that for oats. But in most cases moistening is recommended for different reasons.

- The preparation, e.g. removing the seed coat, cutting etc. is more convenient.
- The different seed structures may be more easily recognized when the seed is imbibed than when dry.
- the staining proceeds more evenly and the evaluation is more convenient; when all seeds are swollen and activated before they are immersed in the solution.

What is said here about the TZ is mainly true for the IC as well as the AF.

Usually the seed is moistened by soaking in water for 16-18 hours at room temperature (20°C); generally that means overnight. Some seeds, particularly those with a starchy endosperm will be sticky if they are soaked too long (Avenae, Dactylis). Such seeds should be imbibed at a lower temperature or for a shorter period. The

moistening period may be shortened by raising the temperature. If a soaking period longer than 24 hours is necessary, the water should be changed.

Seeds that are dry and brittle may be injured by fracturing of the plumule when soaked. Such seeds should be moistened slowly either by keeping the seed in air at high humidity, or by moistening on, or between slightly moistened paper substratum, at least until they reach a moisture level where soaking causes no damage. It should also be noticed that the way of moistening the seed may act upon the staining patterns achieved. This is particularly the case in old legume seeds and others with a high rate of leakage.

Rostovtsev and Lyubich (1978) mention that seeds of Ligustrum spp. Malus, Pyrus and Sorbus spp. are soaked for 2 - 4 days when the IC is applied.

Preparation

TZ - If the seed coat is permeable to TTC no preparation or only moistening is necessary. Most species of the legume family, except hard seeds, have a seed coat which is permeable to TTC. Within most other species we find seed coats which are impermeable to the penetration of TTC to a degree necessitating some kind of preparation. There is also need for some kind of preparation to expose the embryo and other vital tissues for examination after staining. Therefore it is important and time-saving to make the preparation in a way that meets most demands.

All preparation consists of a few main types according to the types of seed and expected staining pattern, but there may be a larger number of variations within each type depending on personal capacity.

Longitudinal Cutting

Through the middle of the embryo axis and $\frac{2}{3}$ the length of the endosperm is common for cereals and all grass seeds the size of the Fescue species. For smaller grass seeds the size of Phleum, Agrostis and so on, a transversel incision in the endosperm near to the embryo is more convenient. All cutting through living tissues which must be examined later, should be carried out as a single stroke sliding cut with a sharp scalpel or razor blade, so that the decisive tissues are not crushed by pressure.

Seed consisting of a slightly differentiated embryo surrounded by living endosperm or gametophyte tissue can be prepared by a longitudinal cut beside the embryo, e.g. all coniferous seeds, seeds of the Umbelliferae (carrot, parsnip etc.) and others.

Transverse cutting - or incision can be used for all grass seeds and for many kinds of dicotyledon seed such as those of the Compositae family and others. The transverse cut should be carried out at a nondecisive part of the seed, e.g. at the distal part of the cotyledons. Transversel cutting can be carried out with different devices such as scalpels, razor blades, dog nail clippers, scissors and whatever else may be found convenient.

Seeds of the coniferous species are preferably cut at both ends, as this facilitates the penetration of TTC, the staining and the evaluation of the embryo and the gametophyte tissue.

Complete removal of seed coat is used for seeds of Brassica and others with more or less the same shape and structure.

It should be noted that removal of the seed coat may be practised for any species, where it is found more convenient than other pro-

cedures. In some cases it is necessary to remove it even before moistening e.g. hazelnuts and others of the same type.

Excision of the Embryo - is a procedure which was applied by Lakon and Bulat for wheat, rye and barley and which was adopted by ISTA. It has been applied in two versions. Lakon's original procedure was to excise the embryo axis without scutellum. As decisive scutellum injuries may be overlooked in that way, the other version should be preferred, namely to excise the embryo axis complete with scutellum.

As I understand it, embryo excision is carried out in coniferous seeds when the IC is applied.

Staining

As far as I know TTC will penetrate into all living cells and there will be converted to formazan, when the obstacles caused by the seed coat are removed. We do, however, examine the staining of tissues or organs and not the single cells.

How rapidly the staining takes place depends on several factors, of which temperature is very important. Prof. Moore says that raising the temperature by 5°C may halve the staining time. Any temperature between 20° and 40°C may be used in routine testing. Where and when the staining takes place is further dependent on the preparation technique applied, the size of the seed (and hence the distance the TTC must pass) and the resistance to the penetration of TTC within the actual tissues. It may also depend on the metabolic activity or the amount of dehydrogenases. Usually the staining proceeds more rapidly in meristems than in other tissues. The concentration of the solution may also influence the staining time and is decisive for the colour intensity obtained.

Thus a high concentration will give a darker colour than a weak one in the same time, regardless of staining time.

The intensity or darkness of the colour is different for different species and it may be influenced by the degree of moistening and the way of moistening. Particularly weak seeds may show a rather greyish colour if moistened by soaking which causes some leakage, while slow moistening may give a far brighter colour. Seeds with green tissues such as Acer spp. may get faintly greyish tinge in a rather dark red colour.

The staining starts where the TTC enters. If you put a bisected cereal seed in TTC and observe the staining you will see it starts at the cut surface in a couple of minutes. The intensity and brightness may reach its maximum in 2 - 3 hours at 30°C. Nevertheless you will see that the embryo may be completely unstained at the distal edge. If seeds of different quality are observed it may be seen that those of a low vigour are stained all through more rapidly than those of high vigour. Some seeds staining rapidly and completely may expose blurred and flaccid tissues throughout. These seeds may be nonviable. Though the cells stain and thus should be expected as living they may be so disorganised as to render the seed non-viable.

Before we leave the discussion about staining I want to emphasize the importance of a standardized staining procedure. If the staining period is too long, the concentration of the solution and the temperature too high, the conversion of TTC to formazan may reach a level where the colour of all live cells will be dark. It will then be difficult to distinguish between sound and weak tissues which may result in mistakes.

The Evaluation

Accuracy in evaluation is highly dependent upon precautions taken during the early phases of the testing to avoid technique injuries that cannot be distinguished from the underlying conditions of the seed.

For adequate evaluation it is necessary to have proper equipment, particularly good light and magnification. When big seeds such as peas and beans are examined, a good handlense may be satisfactory, but in most cases a good stereomicroscope with 5 - 50 times magnification is almost indispensable.

Successful evaluation comes from thorough knowledge, of both the seed of particular species and the full potential of the testing method with that species.

In the seed there are some very decisive tissues and some less decisive. For example, in cereals which develop more seminal roots it is sufficient if two root initials are intact and stain; but only very slight, if any, deficiencies can be tolerated in the plumule tissues.

Well developed and differentiated dicotyledon seeds normally germinate rather quickly while as much as 50% of the distal part of the cotyledons may be unstained, so long as the radicle, the hypocotyl and basal parts of the cotyledons are completely stained.

Small superficial parts of these decisive tissues may be unstained, if the apex and the longer parts of the cotyledons are well stained.

In species with any slightly differentiated embryos surrounded by living endosperm or gametophyte tissues, as in Umbelliferae and coniferous seeds, it is regarded that only the seeds with completely stained embryo and endosperm are viable. It is possible, however, that seeds with slight superficial necroses on the endosperm, not pervading to the embryo may be viable, if the embryo is completely stained.

Application and Benefit of Vital Staining

There is no doubt that TZ and IC and eventually AF are very applicable to the assessment of seed viability.

In his draft of "Handbook for Tetrazolium Testing" Prof. Moore has listed more than 650 species for which the TZ has been applied.

The TZ has been particularly recommended for species that normally germinate slowly and for quick determination of the viability of lots which show dormancy.

Nevertheless it has been more widely used for determining the viability of the more common agricultural and vegetable seeds.

Rostovtsev and Lyubich (1978) report that in the USSR the IC has been applied to 153 species of 42 genera of tree and shrub seeds over recent years, and in comparative tests between TZ and IC no considerable differences between results in both methods have been found.

Effmann and Specht (1965) report about comparisons of AF and germination in 1,172 tests with good agreement. As AF acts in the same way as IC, it is reasonable to assume that they may substitute for each other.

In pilot investigations where I compared all three methods on samples of barley and wheat of known quality (somewhat deteriorated seed) I found no particular difference in results or quality of the tests. As far as I can see at the moment I therefore assume that either method can be used on species where the same preparation procedure is applied.

As TTC penetrates into live as well as dead tissues it may be more appropriate than the others for testing species with decisive embryo tissue surrounded by other living tissue, which may hamper the contact between embryo and the solutions of IC and AF.

It should be acknowledged however, that the reliability of the tests very much depends on the skill and experience of the analyst. Until now there certainly have been mistakes with these methods, as with different germination methods and other biological methods. But as new insight is gained, we will be better qualified to meet the requirements of tomorrow.

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DISCUSSION

Chairman: Jenkins

Participants: Hagen, Tyler, Courty.

1. Practical Application of TZ Techniques by Genebank Staff

Mr. Jenkins wondered how far the technique could be usefully applied in genebanks; and Mr. Tyler shared his caution, bearing in mind that it is a specialized technique and results depend very much on the operative. For many users of the method there remains some difficulty in deciding whether seed is alive or dead; and Mr. Tyler questioned whether its use should be recommended to genebanks, before the standard dormancy-breaking techniques had been exhausted. In answer to the last point Dr. Overaa stated that the test has nothing to do with dormancy; it simply shows whether seed is viable or inviable. He thought that one profitable way in which genebanks might use the test is at the end of a germination test, where the already imbibed ungerminated seeds can be easily subjected to the test, giving some indication of whether they are dead or alive and presumably dormant. (Eds. note - this procedure is included at the end of the germination test. Algorithms are being produced at Wakehurst Place for a number of families).

2. Notes on Numbers of Seeds to be Used in a TZ Test and the Time of Soaking

In answer to a question from Dr. Hagen, Dr. Overaa felt that a sample of 200 seeds, carefully drawn, would be adequate for most purposes, and that something like sequential analysis (see paper by Prof. Roberts) may well be useful in conserving seeds, where accessions are small.

Depending upon the species being tested, the soaking for a TZ test is usually less than 8 h, sometimes as little as 6 h.

3. Interpretation of Staining-shade and Intensity

Mlle Courty commented that when tested with TZ, fresh seeds often stain pink or orange, while upon storage they stain progressively deeper and, until after death they are of course white. She wondered if intensity is important; and whether it might be possible to say something about the age of the seed from the colour or intensity of the staining. Dr. Overaa agreed that this is probably true, despite the fact that colour (intensity) was formerly held to be unimportant. Extending this to cover the rate of staining, he mentioned that with poor quality seeds the TZ generally enters very quickly the whole staining process taking less than 0.5 h; whereas cells in good condition tend to resist the uptake of TZ. This can lead to an apparent negative correlation between intensity of staining and germination result and for example, TZ testing of barley has now been standardized so that if an examination after soaking for just 2 h reveals heavy staining there is a high probability that the seed will not germinate.

Recognition of Dormancy and Definitions of Mechanisms

S.R. COOPER

INTRODUCTION

Seed technologists usually find that they are conducting germination tests at a time when the population of seeds under test is at its most dormant. This is because the tests are, of necessity, carried out very soon after the seed has been shed or harvested. This paper consists of a brief review of seed dormancy, followed by a discussion of how dormancy can be detected and also anticipated so that a dormancy breaking treatment can then be applied.

CLASSIFICATION OF DORMANCY TYPES

The literature on dormancy is extensive and it is only possible to skim the surface in this review. Much of what has been written can be confusing because different classification and terms have been used. To begin with, therefore, some discussion of the terminology used to describe seed dormancy is desirable.

Dormancy in seeds is usually described as the failure of viable seeds to germinate when supplied with conditions of water, oxygen and temperature normally favourable to plant growth. The term "seed" is used here in a loose context to mean the dispersal unit which commonly lodges in the soil and may include other structures in addition to the true seed, e.g. in grasses it may include glumes etc.

Dormancy can occur because of inherent properties of the seed or as a result of external conditions. Various classifications of dormancy types have been made. Koller et al. (1962) used the term primary dormancy to describe the dormancy found in ripe seed as it

is shed and secondary dormancy for the condition which can subsequently develop as a result of environmental interactions. Harper (1957) developed a classification which is useful for seed technologists because it describes the observed behaviour of seeds rather than the physiological processes which might be taking place. He recognised three categories, innate, induced and enforced. Broadly speaking, innate dormancy corresponds to primary dormancy, while induced and enforced dormancy equate with secondary dormancy.

Innate dormancy is found in ripe seed. It develops during maturation on the parent plant and persists for varying lengths of time after the seed has been shed (Nakamura, 1962). It is genetically controlled and the degree to which it is expressed can vary according to the climatic conditions during seed maturation (Vergis, 1964; Belderok, 1971; Wiesmer and Grave, 1972). In Scotland, for example, the experience of the Official Seed Testing Station indicates that cool damp weather immediately preceding harvest normally results in a much higher level of dormancy in cereal seed than is found after a hot, dry period.

Induced dormancy develops as a result of particular environmental or climatic conditions after the seed has been shed and it will persist even after the inductive conditions have passed and the environment becomes favourable for germination. In other words, once dormancy has been induced the seed behaves to all intents and purposes as if it had innate dormancy. For example, Borthwick et al., (1952) found that some lettuce varieties which were not normally light sensitive could be induced to become light requiring by imbibing at 35°C.

Enforced dormancy occurs when the seed is not itself dormant but is prevented from germinating by the ambient conditions but unlike induced dormancy it will not persist after the conditions have

changed. An example of this is water sensitivity in barley where certain samples will not germinate if placed in a slight excess of water. If they are removed and dried and placed in a reduced quantity of water they will germinate normally. Water sensitivity in barley is thought to be due to micro-organisms on the surface of the seed and it can be alleviated by treating the seed with a combination of a fungicide and a bacteriocide (Gaber and Roberts, 1969). Many plant physiologists would argue that enforced dormancy is not dormancy in the true sense, but the seed technologist trying to assess germination is not usually able to make such a fine distinction.

DORMANCY MECHANISMS

The distinction between innate, induced and enforced dormancy is a functional one and as such forms a useful classification for seed technologists. When considering the various mechanisms involved in seed dormancy it is useful to examine these in functional terms too because they often give a clue to the best method to use to break dormancy.

Crocker (1916) proposed a classification of dormancy mechanisms which is widely used up to the present day (e.g. Copeland, 1976). More recently, Nikolaeva (1977) has produced a rather more complex classification which might ultimately allow for easier comparison between the dormancy mechanisms and the methods best suited to break the dormancy. Nikolaeva's classification is just beginning to be taken up by seed technologists but in this review the system outlined by Crocker will be adopted.

1. Immaturity of the Embryo

In a number of species the seeds are shed before the embryo is mature, for example in Fraxinus excelsior, Anemone nemorosa, and

Heracleum sphondylium. Development will normally only take place when the seed is imbibed and kept in a favourable temperature. Even then it may take a long time for the embryo to develop. We have found that we were still getting germination of Heracleum sphondylium after 12-14 weeks on a germinator.

2. Seed Coat Effects

a. Impermeability to water

Hard seed coats are found in a number of families, e.g. Leguminosae, Chenopodiaceae, Malvaceae, Geraniaceae. The coat is impermeable to water and the seeds may stay imbibed for long periods. At the Official Seed Testing Station for Scotland, we kept one sample of white clover on a germinator for about thirty years and were still getting the odd seedling produced from a hard seed that had finally imbibed. Hard seeds are a problem in viability testing because the only way of dealing with them is to mechanically or chemically scarify the seed coats to allow penetration of water. Scarification is specifically excluded from the International Rules for Seed Testing (ISTA, 1976) because of the potential damage it could cause to the embryo.

The way in which hard seededness is lost naturally is not yet fully understood. It has been suggested that the action of micro-organisms may have an effect and in Acacia melanoxylon fire may play an important role (Villiers, 1972). Ballard (1973) concluded that in legumes, at least, softening of the strophiole was the most likely cause of loss of hard seededness.

b. Impermeability to gases

Seed analysts are well aware that the germination of many species of the Graminae can be enhanced by picking or removing the outer coverings such as the lemma and palea. Forward (1958) showed that in addition to piercing or removing the coverings of oat seeds to break dormancy, placing the seed in an atmosphere enriched with

oxygen had a similar effect. Many similar observations have been made by others, and have been integrated by Roberts and his co-workers into their idea that the pentose phosphate pathway plays an important role in the early stages of seed germination (Roberts 1969; Roberts and Smith, 1977). From the practical point of view, though, there is no doubt that in many species the relative impermeability of the seed coat to oxygen is an important dormancy mechanism.

3. Endogenous Dormancy of the Embryo

In many species, the embryo may be morphologically mature but still fail to germinate because of physiological factors. In such seeds a specific stimulus is required to remove dormancy and promote germination.

a. Chilling Requirements

In many species, chilling of the seeds in an imbibed condition breaks dormancy. The length of the prechilling period varies with species from 3-7 days for wheat to 6-9 months for Pinus cembra (ISTA, 1976).

b. Light Sensitivity

Many species have a positive requirement for a light stimulus before they will germinate, the classic example being that of lettuce (Borthwick, et al, 1952). There are, however, a number of species whose germination is inhibited by prolonged exposure to light and others which have a requirement for a periodic exposure of different lengths of time (Koller, 1972). Light sensitivity can be influenced by age of seed, period of imbibition, imbibition temperature, germination temperature and externally applied chemicals (Copeland, 1976).

c. Endogenous Germination Inhibitors

Many species have seeds containing inhibitors. In some cases they are water-soluble and can be washed out, e.g. Beta vulgaris. In others, they require to be oxidized to inactive forms (Roberts, 1969). Applications of growth promoters such as gibberellic acid can often overcome the inhibition and it has been suggested that dormancy depends on the balance between growth inhibitors and promoters in the embryo (Khan, 1977).

4. Combinations of Different Dormancy Types

Combinations of different types of dormancy can be found in the same species. For example, Trifolium subterraneum can show both hard seededness and embryo dormancy (Quinlivan and Nicol, 1971).

Duration of Dormancy

Dormancy offers a mechanism for ensuring that the seed germinates in the most favourable conditions and also for spreading the dispersal of the species in time, thus reducing competition between individuals. Innate dormancy can persist for a considerable time in some species but when it finally disappears the seed will still only germinate if conditions are suitable. In the wrong environmental conditions dormancy may be induced (Koller, 1972) and germination prevented until a favourable stimulus is received. Dormancy may not be an absolute block but rather a limiting of the degree to which a seed will tolerate a particular environment (Vegis, 1964). The phenomenon of thermo-dormancy in lettuce (Toote et al, 1956) is an example of this. In some varieties there is no light requirement if seeds are germinated below 20°C but light must be supplied when germination takes place above 30°C. The possible narrowing of environmental tolerances in seeds is a potential problem in germination testing and can only be satisfactorily dealt with by duplicating tests under different condi-

tions. This in turn makes it imperative that sufficient seed is made available for germination testing.

PATTERNS OF GERMINATION

A population of non-dormant seeds will germinate almost simultaneously although there are considerable differences between species in the rate of germination. In seed tests, Brassica species may germinate sufficiently rapidly to be evaluated after only 3 days while Petroselinum crispum will normally require at least 10 days. A spread in the time it takes for a population of seeds to germinate can be an indication that there is some dormancy in a population. However, some caution is needed in interpreting rates and uniformity of germination since slow growth of seedlings leading to an apparent spread of germination with time can have other causes, e.g. drying damage. Alternatively, a slight change in test conditions may be all that is necessary to ensure rapid and even germination.

Species in the wild tend not to show a very even pattern of germination but frequently germinate in flushes (Roberts, H.A., 1964; Popay and Roberts, E.H., 1970). In some species the explanation for this behaviour is that there are distinct morphological types of seed produced in a population or on one plant which have different germination behaviour (e.g. Chenopodium album, Williams and Harper, 1965). There may also be physiological differences between seeds both from different plants in a population and from the same parent (Cavers and Harper, 1966). Roberts (1972) has concluded that a number of weed seeds have a similar dormancy breaking response, probably light and possibly also the presence of hydrogen acceptors such as nitrate in the seed bed. He also suggested that environmental factors affecting dormancy loss may operate on more than one occasion each year thus causing germination flushes. In general, the seed technologist's response to

these problems has been to try and provide as many dormancy breaking methods as possible simultaneously. Thus in the extreme situation it is possible to provide a pre-chilling period followed by a combination of light, potassium nitrate and alternating temperatures (ISTA, 1976).

DETECTION OF DORMANCY IN GERMINATION TESTS

The commonest way of detecting dormancy is to carry out a germination test. At the end of the test there may be a number of ungerminated seeds which fall into one of the following categories:

- a. empty seeds
- b. hard seeds
- c. fresh seeds
- d. dead seeds

Germination tests mean different things to different groups of people. To the seed technologist it should mean a sophisticated, reproducible test, offering a previously defined baseline for judging seed performance, and using widely agreed techniques and procedures (Mackay, 1978). The International Board for Plant Genetic Resources has recommended that germination tests should be made according to the methods specified in the International Rules of Seed Testing (ISTA, 1976). If these rules are followed, the germination test should be made in two stages. First of all, the seed is purified and then some of the pure seed obtained is germinated to give the final germination result. With this principle in mind, the first of the ungerminated seed categories mentioned above, empty seeds, can be detected before the seed has even been planted.

a. Empty seeds

In seed testing practice, obviously empty seeds should be removed before a germination test is made (ISTA, 1976) and in many species this is relatively easy to do. For example, in flat thin seeds with translucent coverings it is possible to detect the presence or absence of embryo material by shining light through them. This is very easy to do with Festuca, Lolium, Agropyron and similar grasses, and also with species such as Cichorium intybus. It is also possible using this method to detect insect damage and even the presence of pests, e.g. eelworm galls in grass seeds. The instrument used in this technique is called a diaphanoscope and a modern version has been described by Bould and Smith (1981). Diaphanoscopes are not manufactured commercially but are relatively easy to construct.

Many seeds, of course, have totally opaque coverings. In this instance it is possible to detect empty seeds using X-radiography (Simak et al, 1957; Copeland, 1976). The principle is exactly the same as that of the diaphanoscope and similar information is obtained.

It is well worth while considering the installation and use of equipment to detect empty seeds since this is one less problem to be dealt with at the conclusion of a germination test.

b. Hard seeds

Hard seeds are very easy to detect. They do not take in water and so at the end of a germination test they look smaller than those seeds which have imbibed. They also feel hard to the touch whereas imbibed seeds will feel softer and springier.

c. Fresh seeds

Seed analysts use the term fresh seeds to denote those seeds which, at the end of a germination test have imbibed water but

have not yet started to grow, are firm and clean looking, show little sign of mould growth round them and are springy to the touch. They may or may not be dormant because some fresh seeds will never germinate.

For example, the occurrence of embryoless seeds in the family Umbelliferae has been reported (Flemion and Hendrickson, 1949). In this family the embryo is normally quite small and lies embedded in a mass of endosperm tissue which is able to imbibe and swell whether or not there is an embryo present. On the other hand, in onions it is possible to find seeds where the embryo is viable as indicated by tetrazolium staining, but the endosperm does not stain. The seed will imbibe but no growth results.

Seed testers, particularly those dealing with tree seeds, where dormancy is a major problem, have developed strategies to overcome dormancy problems. Thus the tetrazolium test, described by Overaa at this meeting, and the excised embryo test (ISTA, 1976) have been used as alternative viability tests. Such techniques could also be used in genebanks so long as the necessary skills are learned.

d. Dead seeds

Dead seeds appear discoloured and sometimes mouldy at the end of the test. If touched they feel squashy and their contents may be liquid or semi-liquid.

It is not possible to conclude this section without stating that the detection of some of these categories is highly dependent on the skill and expertise of the technician making the test. A wide ranging training in seed analysis is an asset to someone who regularly needs to make germination tests to provide information for comparative purposes. Seed analysts working on agricultural and horticultural species have a lot of experience which has not been widely tapped by those working in genetic conservation.

ANTICIPATING DORMANCY PROBLEMS

A large amount of information on dormancy breaking in agricultural and horticultural species has been incorporated into the ISTA rules (ISTA,1976). It is possible to utilize this information to make an intelligent guess about what kind of dormancy may be present in a species not in the rules by comparison with related species.

Another approach to dealing with potential dormancy problems is that of Atwater (1980). She has compiled a germination methodology for seeds of herbaceous ornamentals in which germination behaviour is predicted on the basis of the anatomy and morphology of the seed. Atwater established a basic division into endospermic and non-endospermic seeds and within each division there are four categories each of which exhibits different patterns of dormancy. Atwater's paper consists of a review of literature supplemented by personal observations and is perhaps open to criticism because of the lack of detailed data to some of the conclusions.

A more fundamental attempt to provide a classification which links the inhibition of germination (dormancy) with the removal of inhibition is that of Nikolaeva (1977). Her approach may ultimately be very useful but does not at present provide enough detailed information to the bench seed analyst. What is really needed is an encyclopaedic list of possible ways of dealing with dormancy in a wide range of species (i.e., a seed technologist's cook book) which can be used as a reference when unfamiliar species are being tested.

CONCLUSION

Although the mechanisms that control dormancy may be relatively few in number, the responses that seeds may show can be very varied. In many cases, dormancy problems can be avoided or satisfactorily dealt with by the blanket application of one or more standard techniques. Nevertheless, there is no substitute for training and experience when it comes to dealing with problems of dormancy in seed tests. The provision of suitable ancillary equipment would also be useful. Finally, more ready access to the information on dormancy already in the published literature would be beneficial.

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DISCUSSION

Chairman: Cooper and Roberts

Participants: Jones, Bekendam, Smith, Tyler,
Hawkes, Bean, Probert, Boland, Overaa,
Williams, Christova, Ellis.

1. Genetic Control of Dormancy

While admitting that it is difficult to define the extent in any specific situation, Prof. Jones proposed that most dormancy is under some kind of genetic control and it may be possible to split this into three types: (i) the genetics of the embryo, which is bi-parental, (ii) the genetics of the endosperm, which is bi-parental but biased towards the maternal parent, and (iii) the genetics of the testa which is maternal. Superimposed on these is developmental control. Prof. Jones suggested that genetic components of control should be susceptible to selection, it thereby being possible to remove "absolute dormancy" but not other types. Dr. Cooper replied that there is undoubtedly some information available, but he himself did not know the answer. There are apparently differences between varieties of wheat, for example, in their sprouting behaviour, thought to be due to differences in mechanical resistance to the protrusion of the radicle. There is obviously a considerable effect of environment, but a wide range of phenotypic variation is based on underlying genetic phenomena, dormancy has to be based on a strong environment x genotype interaction. In cultivated species there has probably been selection away from dormancy.

2. What Level of Expertise in Deciding Dormant From Dead is Required in Seed Banks?

Prof. Roberts wondered if, as Dr. Cooper had advocated, seed bank personnel were able to separate fresh (alive) from dead seed,

there is any point in carrying out dormancy-breaking treatments at all.

Mr. Smith commented that after germination had ceased in a test it is possible to make a visual assesment, in which it can be assumed that fresh, uninfected seeds are dormant, while mouldy ones are dead.

Failing this, he wondered whether it was feasible to carry out a TZ test on the remaining seeds, after they had been imbibed for the duration of the test. Dr. Bekendam thought in practice it would be impossible for seed analysts to distinguish dead from dormant seeds in every instance, by TZ or any other means. He wondered whether a TZ test performed properly could ever be as good as a germination test for assessing viability, although there may be no choice where seeds cannot be made to germinate. Dr. Cooper said that it is difficult to get people, who are trained to perform TZ tests, to perform germination tests at the same time. While admitting that TZ tests are often difficult to perform, Dr. Overaa believed that progress could be made by using them together with germination tests and that comparative studies should be carried out.

Of course, the raison d'etre of a seed bank is to be able to use the material held in it, and Mr. Tyler stressed the importance of being able to germinate seed in addition to deciding whether it is dead or dormant. Dr. Williams augmented this comment by drawing attention to the need to regenerate small seed lots, and how a knowledge of any seed dormancy-breaking requirement could be helpful or even essential in the process.

3. Definitions of Dormancy

Prof. Hawkes gave an account of the types of dormancy he recognises in seeds of Solanum tuberosum. First there is an innate dormancy possibly only a few months after harvest. Secondly, if seeds are not separated by washing from the fruit pulp, the seeds are subject to induced dormancy caused by the latter. Finally, there is another kind of dormancy, which he finds hard to classify. This occurs much later after harvest, and seeds germinate slowly or not at all, but appear to be alive on inspection and respond to GA₃. Dr. Cooper replied that by the fact that germination only occurred in response to an additional stimulus; and simply by the way he had defined induced dormancy in his talk, this third type of dormancy should be referred to as induced. Prof. Hawkes was unhappy about a definition of induced dormancy which did not specify the inducing factor, but reconfirmed that the dormancy in question gets worse with age; the fact that GA₃ released it rules out loss of viability as an explanation. In his experience Dr. Bekendam had observed this type of dormancy quite often, but remarked that there is yet no adequate scientific explanation for it. Prof. Roberts remarked that such mystery is hardly surprising when dormancy in any one species is not yet fully understood, despite a vast literature.

Dr. Bean surmised that implicit in a definition of dormancy is knowledge of the conditions appropriate for germination; and yet it is very difficult to decide whether requirements are specific or not, e.g. where a requirement for alternating temperatures can be replaced by a period of constant temperature chilling.

Dr. Cooper stressed that people should be aware of the problem; but that there are instances, such as in grasses or lettuce varieties, where specific germination requirements exist, movement outside these limits often inducing secondary dormancy. Prof.

Roberts maintained that it is slightly dangerous to use the term germination requirements, because they are so very often not absolute - seeds may be described as possessing dormancy but there may be several acceptable ways of breaking it.

On a related point, Mr. Probert referred to an earlier statement by Prof. Roberts (see earlier paper) in which he recommends that, if seeds could not be dried quickly, they are best kept moist. Mr. Probert thought that with some species this could in fact lead to an unwanted induction of dormancy in seeds held at high moisture contents especially in the dark, where phytochrome would be converted into the inactive form.

4. The Use of Aspirators in Seed Cleaning

When asked by Mrs. Christova for his opinion on the use of aspirators in the cleaning of seed samples of ornamentals, vegetables and grasses, Mr. Cooper said that he had deliberately omitted to mention this method in his comments on seed lot purity; because in the genebank scale of operations it may be possible to remove small, light, though perfectly acceptable seed from an already small sample. It would be possible to use a combination of aspirator and X-ray technique, using the latter to examine the fraction blown off by the former.

5. Links Between Dormancy and Viability

Mrs. Christova described some experiments in which she had established that the period of post-harvest dormancy in Dactylis glomerata is inversely proportional to storage temperature, and wondered whether it would be useful to prolong the period of dormancy of seeds stored in a bank, assuming that the rate of ageing of seeds is reduced with dormancy. Prof. Roberts wanted to treat this point cautiously, because there is currently much argument

whether dormancy and viability are linked, but as yet no conclusive evidence for the relationship.

Dr. Ellis thought that there may be a positive advantage in maintaining dormant seed in a seed bank, if users could then be trained to imbibe seeds under conditions where dormancy remains for a period while repair of any damage incurred pre-or during storage may take place; then the dormancy breaking treatment could be applied and germination would proceed.

Methods Appropriate to Breaking Dormancy

Dr. E. PAPP and L.Gy. SZABO

Seed that does not germinate even when under favourable conditions is either dormant or dead. In the case of larger seeds the two states can be distinguished from one another by the tetrazolium test. As to this test, the ISTA standard (Testing Association Rules 1976) gives directives. If the seed is dormant, then further clues can be obtained from its age. Seeds of some species are known to be in a transitory state of dormancy after maturing; during the following weeks or months this state disappears. In this paper we should like to describe our investigations into the post-maturity (post-harvest) state of dormancy, followed by methods currently available for breaking seed dormancy.

Although the subject of the lecture is the question of breaking the dormancy of seed, we have to speak of seed dormancy itself, since the process cannot be separated from the methods suitable for breaking it.

The seed must be looked at in the context of the mother plant and the environment in which it has developed. When a seed is subjected to examination and its dormancy is to be broken, its past, that is the effects to which it was subjected on the mother plant, is generally not taken into consideration. The seed is considered as a self-contained unit, with the potential for producing a new plant. However, it is possible to follow an examination method that reflects the effects produced on the mother plant and provides a firm basis for the seed examination. The laboratory analyses reflect the results of the environmental effects that have been experienced by the developing seed.

The examinations are based on the pheno-ecological method of examination developed by MANDY (1967). The method consists, essentially, of a series of sequential sowings. Samples of the same seed lot are sown successively every week or ten days. With this serial sowing each plant stand will experience continuously changing soil conditions e.g. in the northern temperate areas average soil temperature will fall throughout Autumn and rise again in Spring. The sowing series also experience varying precipitation and other environmental changes. The pheno-ecological aspects of the sowing series i.e the rate of growth, the length of the flowering and seed maturing period etc. also vary in response to the favourable temperatures and unfavourable environmental effects. Thus, there will be a sowing time which ensures the best conditions for plants; the plants grow at an optimum rate while those sown earlier or later are retarded in development and their phenological phases are delayed. The duration of phenological phases follow an optimum curve.

From a pheno-ecological experiment with barley the effect of sowing time will be manifested in seed development. If in the barley stand the period of the "dough stage" is short, then the period of dormancy will also be short and the percentage germination high (see Fig. 1). The short phenological phases are indicative of a rapid development at optimum rate. With plants sown earlier or later the development will be slower, the phenological phase is longer and the biological value of seed reduced. According to KOLLER (1972) the relationship between plant and seed is under the influence of the environment. In this sowing series the plant stand itself indicated what conditions were the most favourable for it and responded to the favourable effects with accelerated processes of life. (The experiment was carried out in small plots, with ten replications so that the effects of soil heterogeneity could be averaged.)

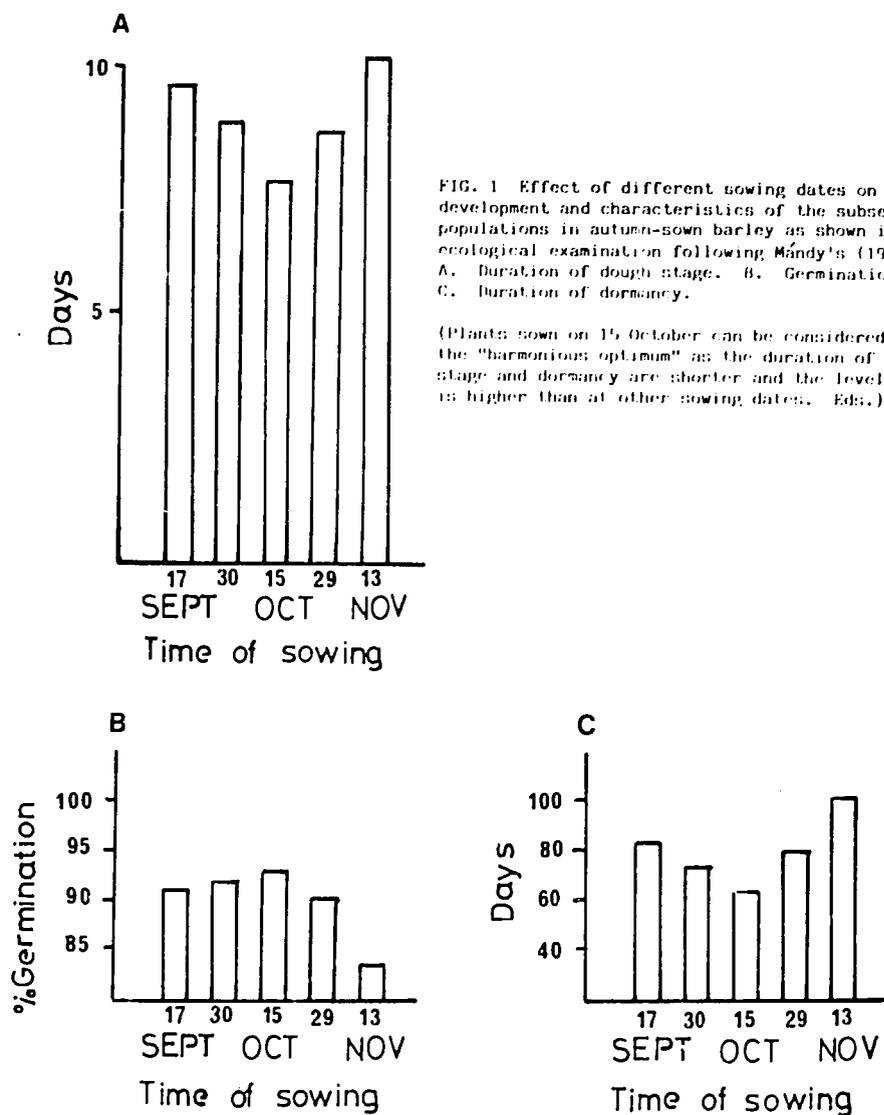


FIG. 1 Effect of different sowing dates on the rate of development and characteristics of the subsequent seed populations in autumn-sown barley as shown in a phenological examination following Mandy's (1947) principles. A. Duration of dough stage. B. Germination level. C. Duration of dormancy.

(Plants sown on 15 October can be considered to develop under the "harmonious optimum" as the duration of the dough stage and dormancy are shorter and the level of germination is higher than at other sowing dates. Eds.)

The phenological stages changed according to an optimum curve. The harmonious development of a plant stand requires in most cases a definitive sequence of changing temperatures rather than a certain optimum temperature. Plants growing in natural surroundings are exposed to maximum and minimum temperatures besides the optimum one, it is therefore not surprising that the cardinal values obtained in the laboratory or growth chamber do not agree perfectly with those shown under natural conditions. The optimum

composed of many factors in natural circumstances may be called the "harmonious optimum" (FRENYO 1959).

Seeds obtained from a seed lot sown successively in accordance with MANDY'S pheno-ecological method were examined and evaluated from physiological points of view.

Changes in moisture content, weight and shape were followed from an early stage of ripening and germination determined for seeds collected on a suitable harvest date for each batch of mother plants derived from the sequential sowing. Germination supplies information on the biological condition of the seed (KOLLER 1972). In MANDY'S opinion "the biological value of the seed, like all other characteristics of the plant, changes in sequential sowing according to an optimum curve."

Seed was sown successively every four days or weekly and monthly. The results of germination gave a "germination curve", or line. As an example we present here the germination lines of wheat, barley, paprika and hemp (Figs. 2, 3, 4, 5), for seed harvested off mother plants sown at the optimal time. The germination lines show the marked interspecific differences. The germination lines obtained during the ripening and dormancy of seed can be classified or typified, in the same way as the plants can be placed in families and genera. These lines reveal certain types of seed dormancy. Studying the seed harvested off mother plants originating from the sequential sowing, we find that seeds obtained from different mother plant sowing times give different germination lines, which show the result of effects produced by the environment on the mother plant. When germinating the seed derived from

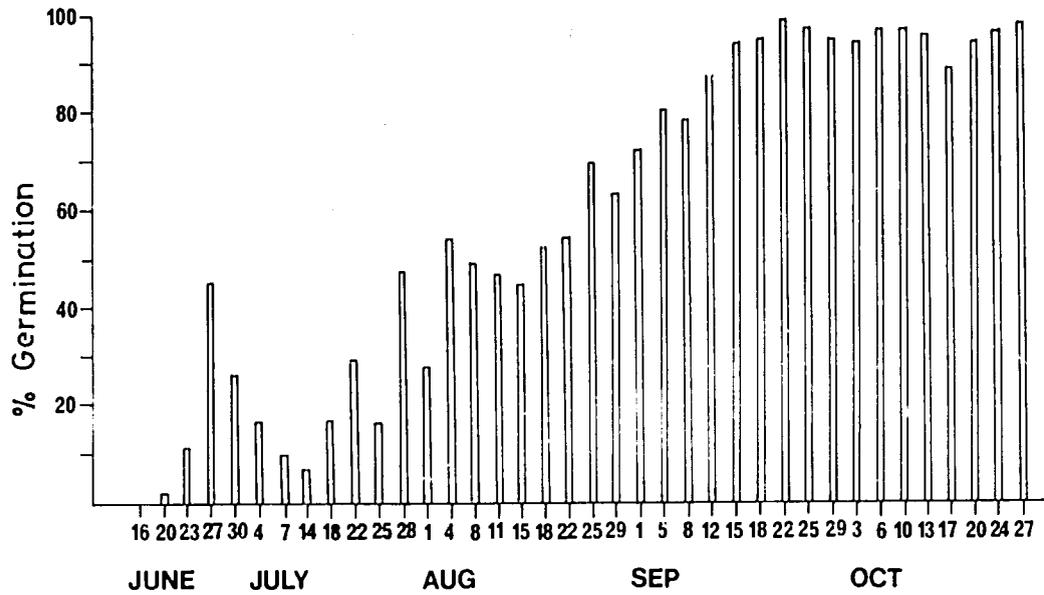


FIG 2. Mean germination of 5 varieties of wheat harvested in 1967, sown at the "harmonious optimum" and sampled at various times during seed ripening and after-ripening.

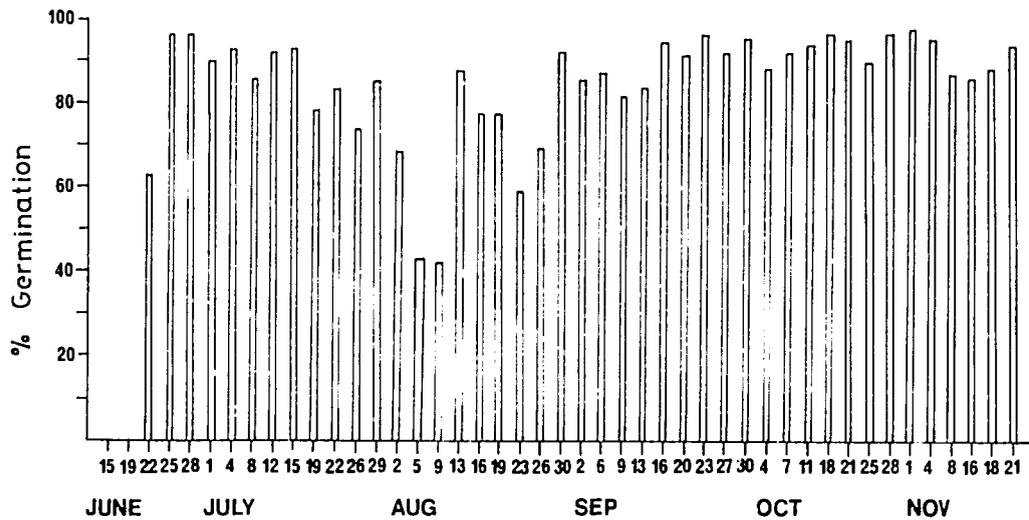


FIG 3. Mean germination of 4 varieties of barley harvested in 1966, sown at the "harmonious optimum" and sampled at various times during seed ripening and after-ripening.

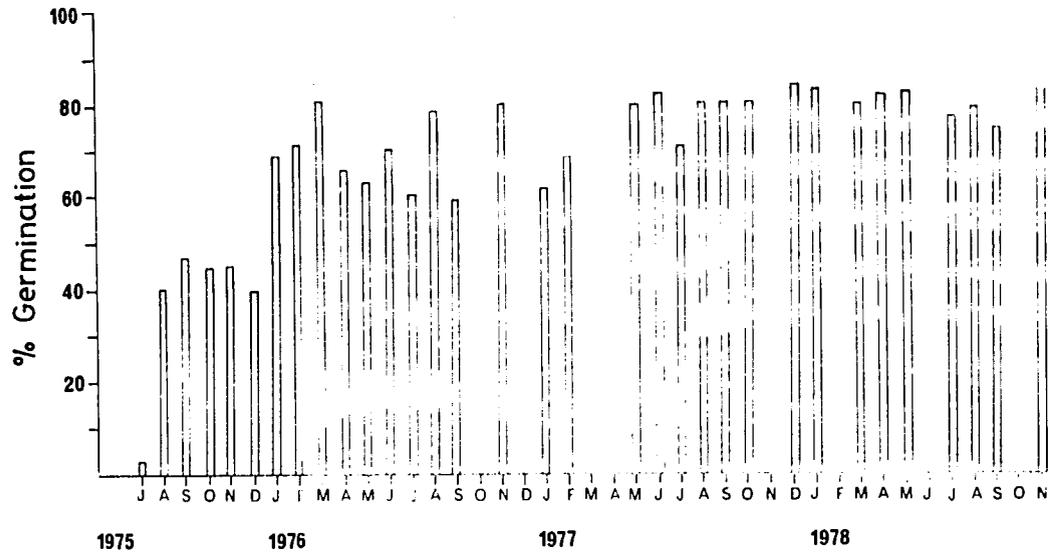


FIG 4. Mean germination of paprika harvested in 1975, sown at the "harmonious optimum" and sampled at various times during seed ripening and after-ripening.

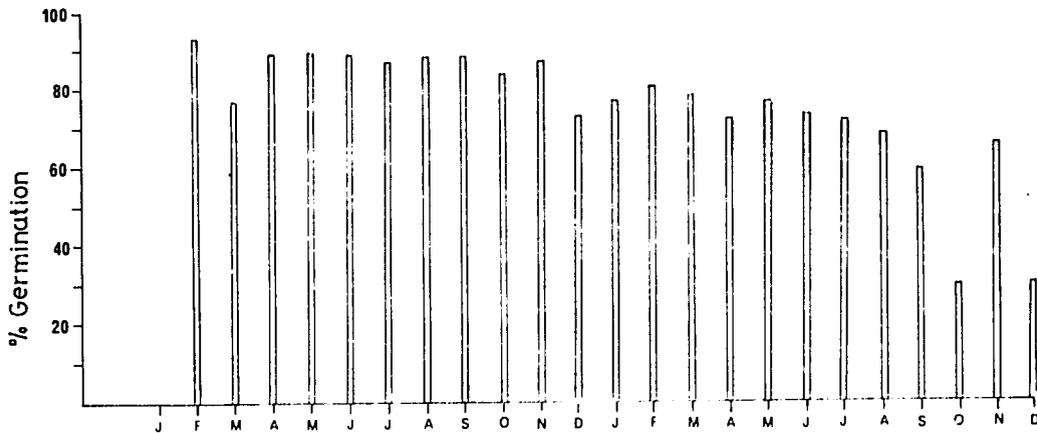


FIG 5. Mean germination of 3 varieties of hemp harvested in 1968, sown at the "harmonious optimum" and sampled at various times during seed ripening and after-ripening.

one or another sowing time we know the history of the seed, that is we have not got out of connection with the environmental effects. As an example we present here the germination results of seeds obtained from millet and wheat sown at different times (Figs. 6 & 7). In future experiments it would be worth taking care to use seeds matured under optimum conditions provided we do not want to conduct some special experiment with poorer seeds.

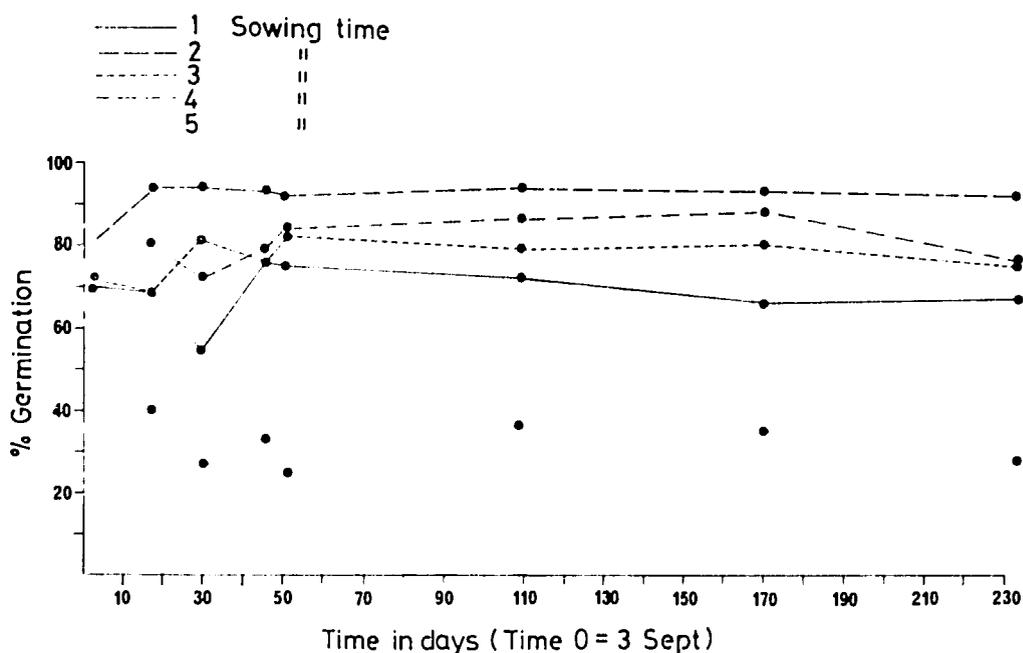


FIG 6. The effect of different sowing times on the germination behaviour of the subsequent seed generation of millet at various sampling dates.

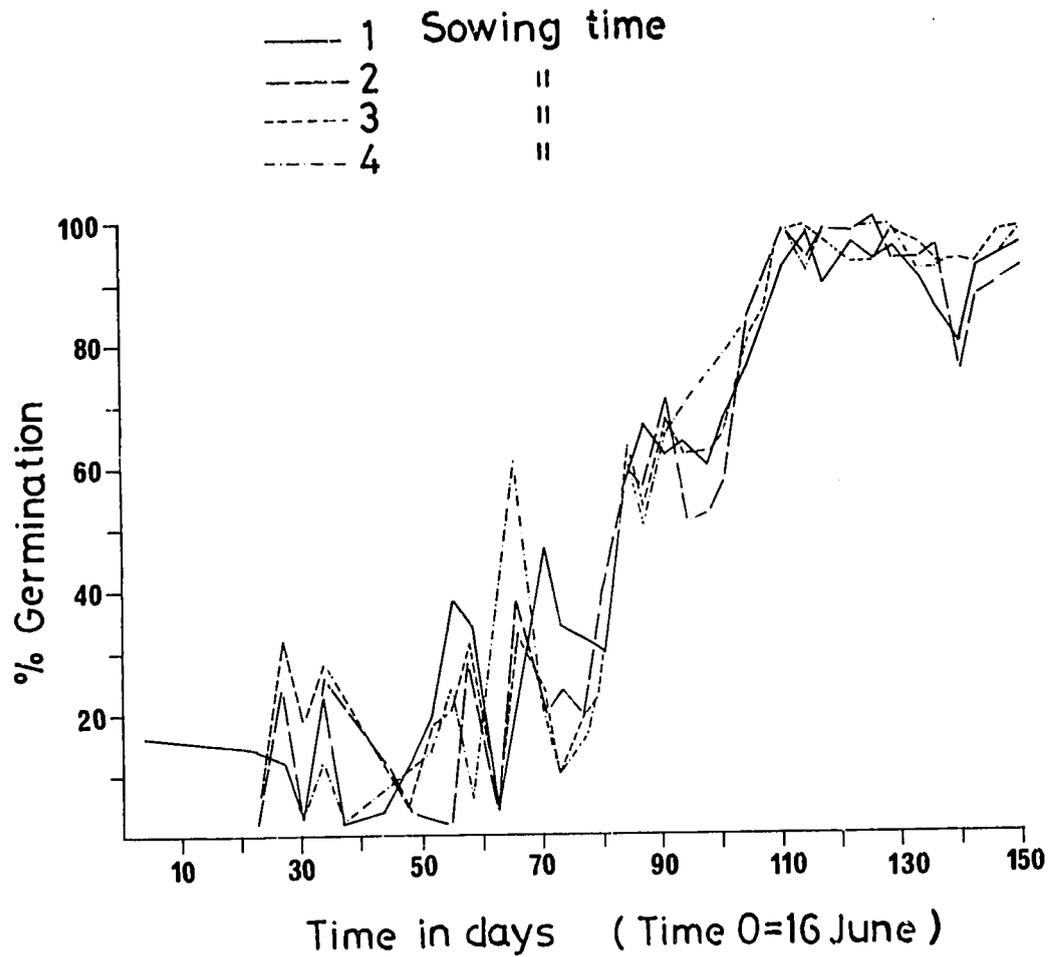


FIG 7. The effect of different sowing times on the germination behaviour of the subsequent seed generation of wheat var. Fertödi 293 sampled at various times from dough stage to the end of dormancy.

Looking at the germination line obtained in the period of after-ripening, we find that following ripening the wheat grains show a gradual increasing percentage of germination. If we perform an experiment with a seed of unknown harvest date we do not know at what stage after-ripening is. It would be well-worth gathering information on the time course of after-ripening for a number of species, and when beginning an experiment the time that has passed since ripening should be indicated so that conclusions can be drawn on the post-maturity stage of the seed.

The Breaking of Post-harvest Dormancy of Seed

The experiments on breaking post-harvest dormancy of seed were fitted in this train of thoughts. Seeds were germinated at intervals during ripening and duration after-ripening, in solutions considered suitable to break the dormancy of seed. The solutions are well-known and generally used. The methods of application and evaluation may be regarded as new by some. The solutions used were:- 2% hydrogen peroxide, 100 ppm gibberellic acid, 100 ppm benzimidazole and 6 methyl uracyl at a similar concentration. Parallel to germination in these solutions, untreated seeds were also germinated and the experiment included a chilling pre-treatment as well. Since the examinations were always run in parallel, the results could continuously be compared with each other and with those of the untreated control. The stage of ripening is indicated by the water content of the seed.

Germination with Gibberellic Acid

Gibberellic acid is present in the seed in various quantities and forms. Its presence suggests that it will have some role in germination. (SEMBDNER et al, 1968). According to DURLEY and MACMILLAN (1971) at an early stage of ripening GA₁ is found in the seed (Phaseolus vulgaris), while in the mature seed the gibberel-

lic acid is present in the form of GA_8 . The former is of high activity, the latter biologically inactive. Barley grains when ripe do not contain free gibberellic acid i.e. GA_1 goes to GA_8 (Groat and Briggs 1969). The same applies to other members of Gramineae - according to STODDART (1965).

Durley's statement may be related to wheat as well, since at an early stage of ripening the wheat grains germinated, indicating that the gibberellic acid presumed to be required for germination was present. At the time of ripening, for about 2/3 weeks, in the period of dormancy, very little germination occurred (it was practically non-existent). Wheat grains showed varying extents of germination until the end of after-ripening. Thus in some seeds no inhibition is present, whilst most of them are unable to germinate during after-ripening i.e. they are inhibited to different extents. The same was pointed out by KETTERING and MORGAN (1970) for peanut seeds. Dormancy is more intense in the basal and less so in the apical seeds. It is presumed they differ in endogenous ethylene formation and consequently starting endogenous GA alike. The varying dormancy periods of individual seeds spread out the germination in time and may thus serve the survival of the species.

If we interfere with this endogenous GA formation by adding exogenous GA, the germination results will change. When germinated in a 100 ppm solution of gibberellic acid, 60-70% of the ripening wheat grains at the dough stage germinated, compared to 20-30% of the untreated grains. The exogenous GA was absorbed by the grains and it induced germination in a certain proportion of them, while the non-germinating 30-40% might have required a higher concentration. (Figs. 8, 9). As KETTERING and MORGAN pointed out for peanut seeds, seeds in a more intense state of dormancy require the stimulus of higher concentrations to start germinating.

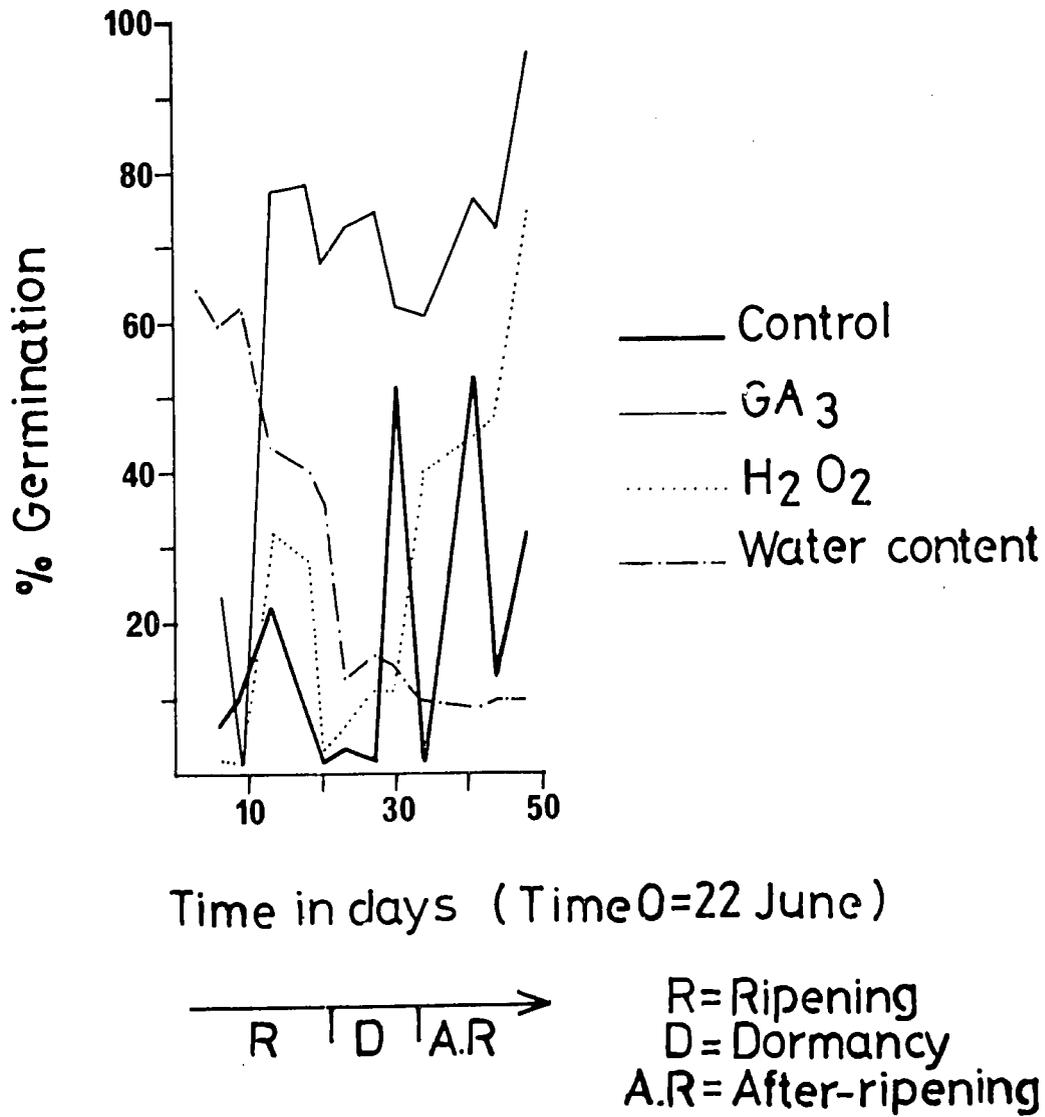


Fig 8. The effect of various dormancy breaking treatments on the germination of wheat var. Bezostaja sampled at different stages of grain development from dough stage onwards.

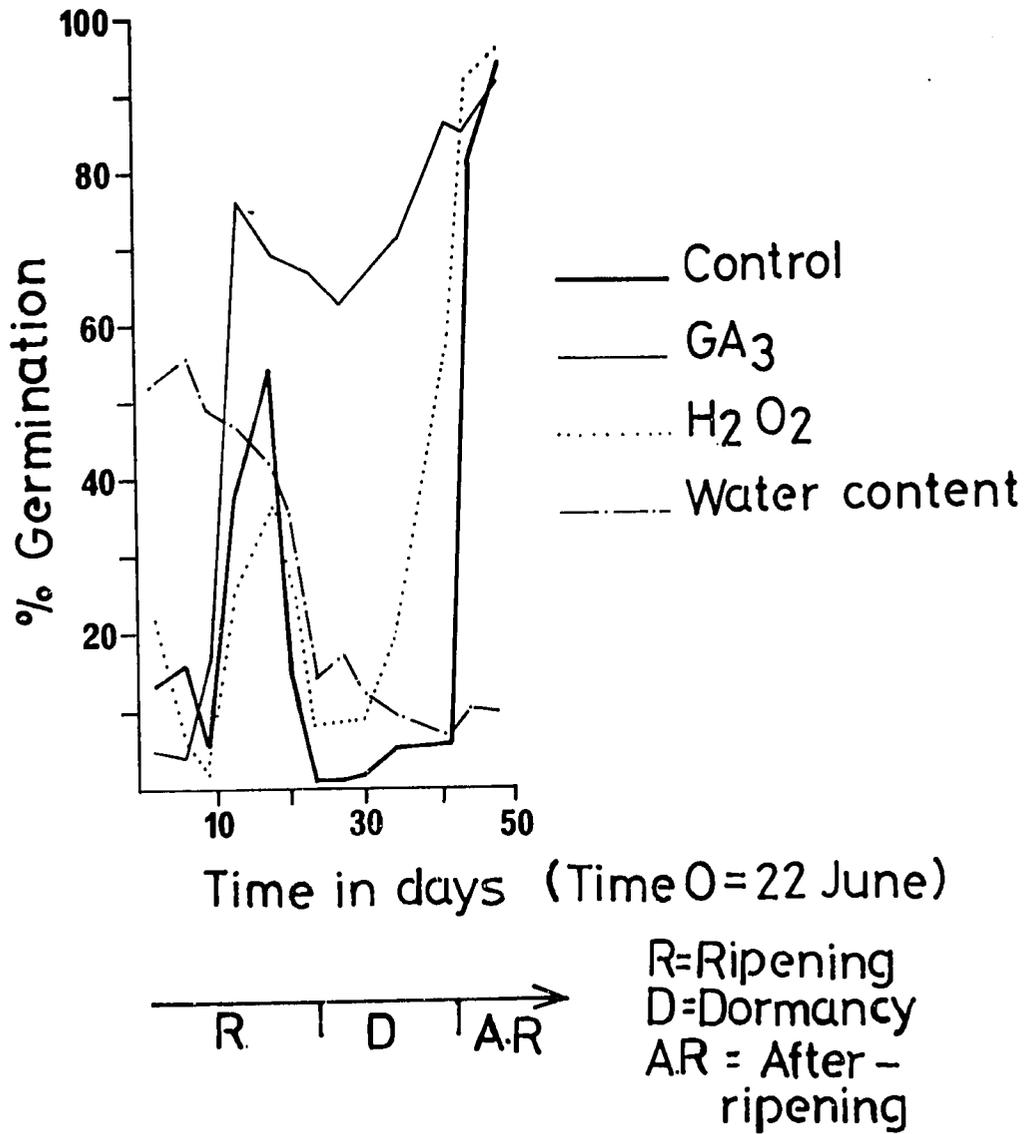


FIG 9. The effect of various dormancy breaking agents on the germination of wheat var. Fertödi 293 sampled at different stages of grain development from dough stage onwards.

When treating ripening and ripe barley grains with a 100 ppm solution of GA_3 we obtained the same results as with wheat grains. The difference was 30-50% in favour of the treated grains.

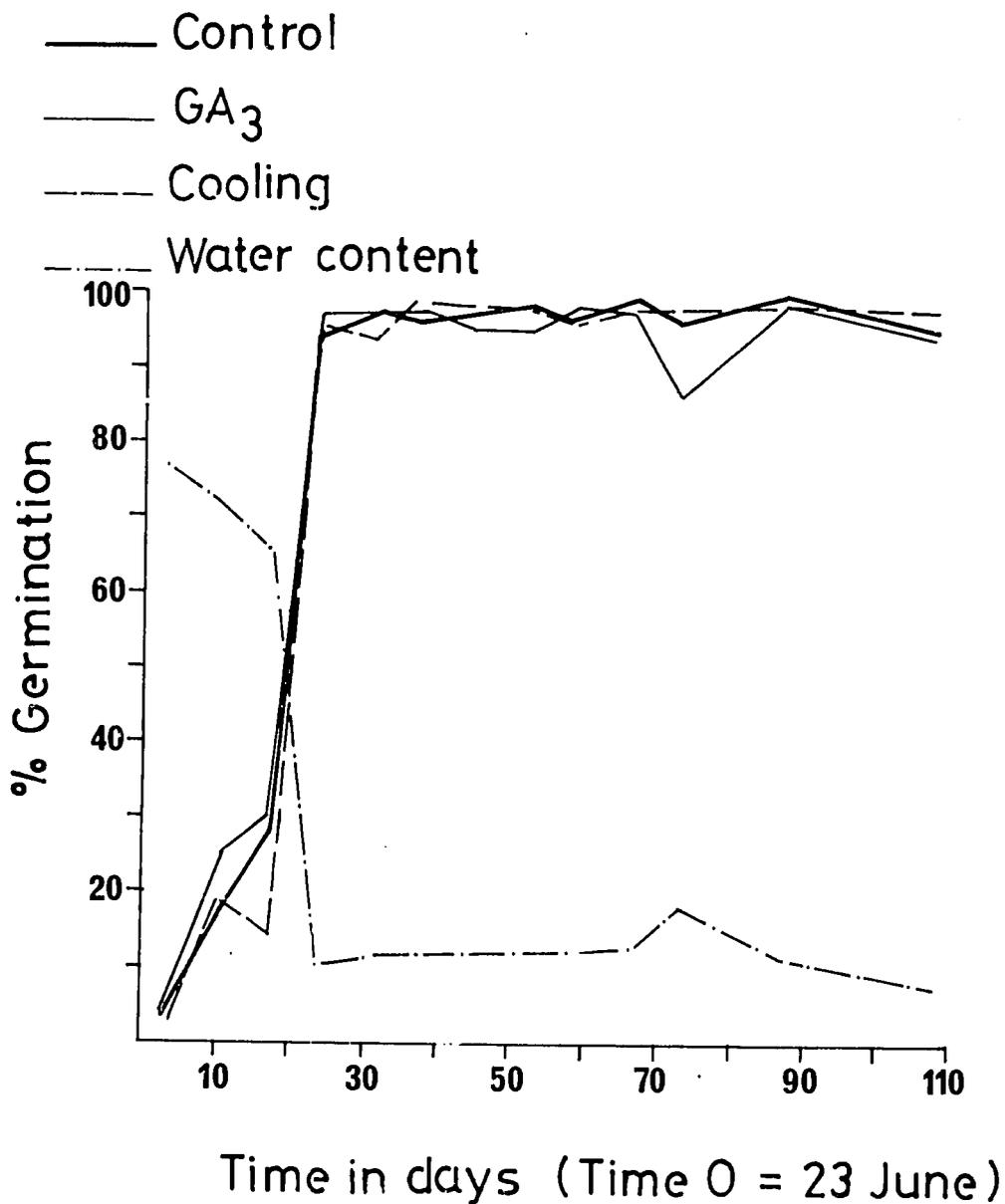


FIG 10. The effect of GA_3 or chilling on the germination of pea seeds at various times during and after development.

Pea seeds have a short 2-3 week period of dormancy after ripening. During that time we tried to make them germinate in GA₃ solution. The seeds were found to germinate only a few percent higher than the control (Fig. 10) i.e. GA₃ is ineffective in this kind of dormancy.

Chilling

A known method of breaking the dormancy of seed is by chilling the imbibed seeds for various lengths of time. According to CHING & CHING (1972) cooling raises and maintains an 'energy level'. Cooling does not directly induce germination, but by raising this energy level starts the activation of GA, cytokinin and ATP synthesis (see SIMMONDS and DUMBROFF 1974). The GA controls the energy level then the hormone synthesis starts (PINFIELD 1968, HAWKER 1976). According to a different opinion, cooling simply creates the possibility for the activation of the GA mechanism, and the synthesis begins only at the germination temperature (WILLIAMS et al. 1974).

Chilling did not increase the germination percentage of all species examined. When applied in the short after-ripening periods of maize, pea and lupin it was almost ineffective, in the case of wheat and barley grains, it substantially improved the germination results.

In an experiment with barley grains we wished to increase the effect of GA by cooling, i.e. grains imbibed in GA₃ were pre-cooled for 48 hours so as to receive a combined dose (Fig. 11). The double treatment raised the germination percentage still higher.

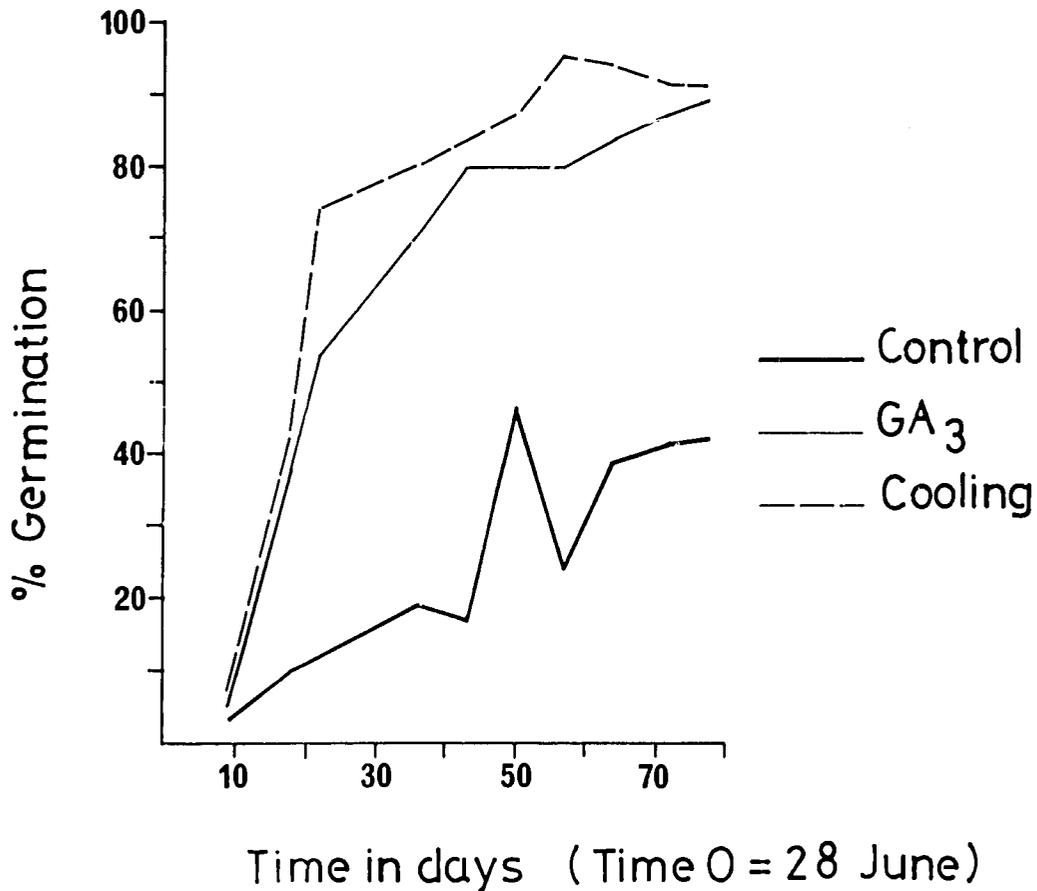


FIG 11. The effect of GA₃ or chilling on the germination of barley grains, var. Ledeci beta at various times during and after development.

To summarise, in mature seeds the level of GA is low compared to the early stage of ripening (CORCORAN and PHINNEY 1962), and germination requires GA synthesis (KHAN *et al* 1967) induced by the apex of the embryo (BRIGGS 1972). Our own experiments suggest that the germination of seeds in the process of ripening and in the period of after-ripening can be stimulated by exogenous GA₃ application. Stimulation of the new GA synthesis and induction by the embryo apex are increased by cooling of seeds.

Hydrogen Peroxide

Ripening and after-ripening wheat grains were germinated in 2% H_2O_2 solution. There is a peroxidase catalysed system active in seeds as one of the energy sources of the glyoxalate cycle, which demands the production of H_2O_2 . The endogenous H_2O_2 is a transitional substance that acts after it has been decomposed. Similarly the exogenous H_2O_2 decomposed but it hardly affected the germination of unripe wheat grains. The germination line of wheat grains germinated in H_2O_2 solutions ran almost parallel to the control line. On the other hand, its effect on grains at the post-maturity stage of dormancy gradually increased in both wheat varieties examined.

Benzimidazole

Benzimidazole shows a cytokinin-like activity (GAYED 1970). According to AMEN (1970) benzimidazole and Kinetin "reverse" the dormancy of seed, considering that dormancy is possibly a state of reversibly suspended metabolism. The stoppage of the pentose phosphate pathway is very similar to the inhibition or lack of metabolism. The complex metabolic process of germination may require nitrate or nitrite to start and the nitrite reductase synthesis can be induced with cytokinin.

Wheat and barley grains were germinated in a 100 ppm solution of benzimidazole. This substance had no effect on the germination of ripening wheat and barley grains. Only in the after-ripening period did the germination increase by an average of 20%. It may be interesting to combine the benzimidazole with a solution of a nitrate salt and examine their joint effect.

When we used benzimidazole in combination with GA_3 (see Fig. 12), we obtained 75-95% germination of barley grains and this level

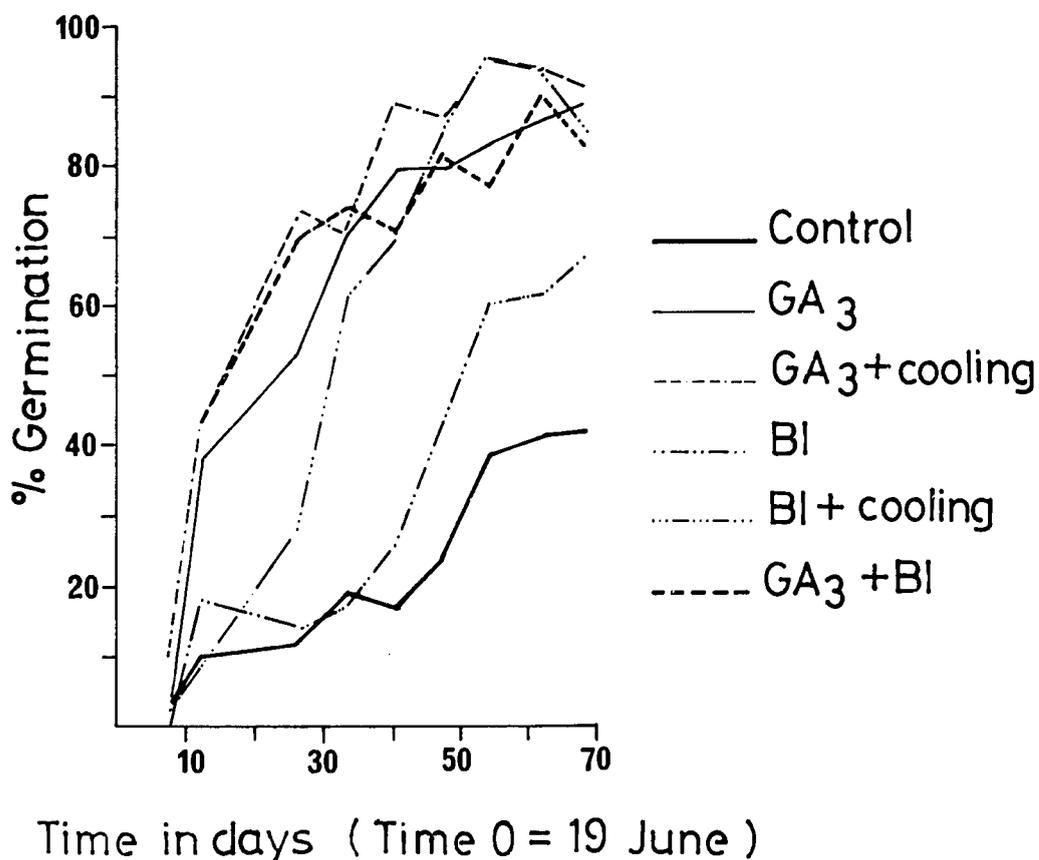


FIG 12. The effect of various dormancy breaking treatments on the germination of barley grains, var. Ledeci beta at various times during and after development.

was maintained or even increased during after-ripening. The effect of benzimidazole on the germination of barley grains, chilled for 48 hours (at 0-5°C) was similar to that of applied GA. Thus benzimidazole while affecting the germination processes of wheat and barley grains at the stage of after-ripening to a certain extent, will be more effective when combined with GA or pre-chilling. THOMAS (1973) emphasises when using benomyl (whose action is similar to that of benzimidazole) a series of concentrations ought to be applied in order to obtain information about its effect. The same is mentioned by KETRING (1970). Since in our experiment only one concentration was used, the ineffectiveness of benzimidazole may actually be a matter of concentration.

6 Methyl uracil

The 6 Methyl uracil shows a cytokinin-like activity. When used by itself at 30 and 100 ppm concentrations to break the dormancy of wheat grain, it had no influence on the germination of ripening grains. Only when applied in combination with a 100 ppm solution of GA₃, or after pre-chilling was it found to be effective. (Fig. 13) This effect closely resembles that with Benzimidazole.

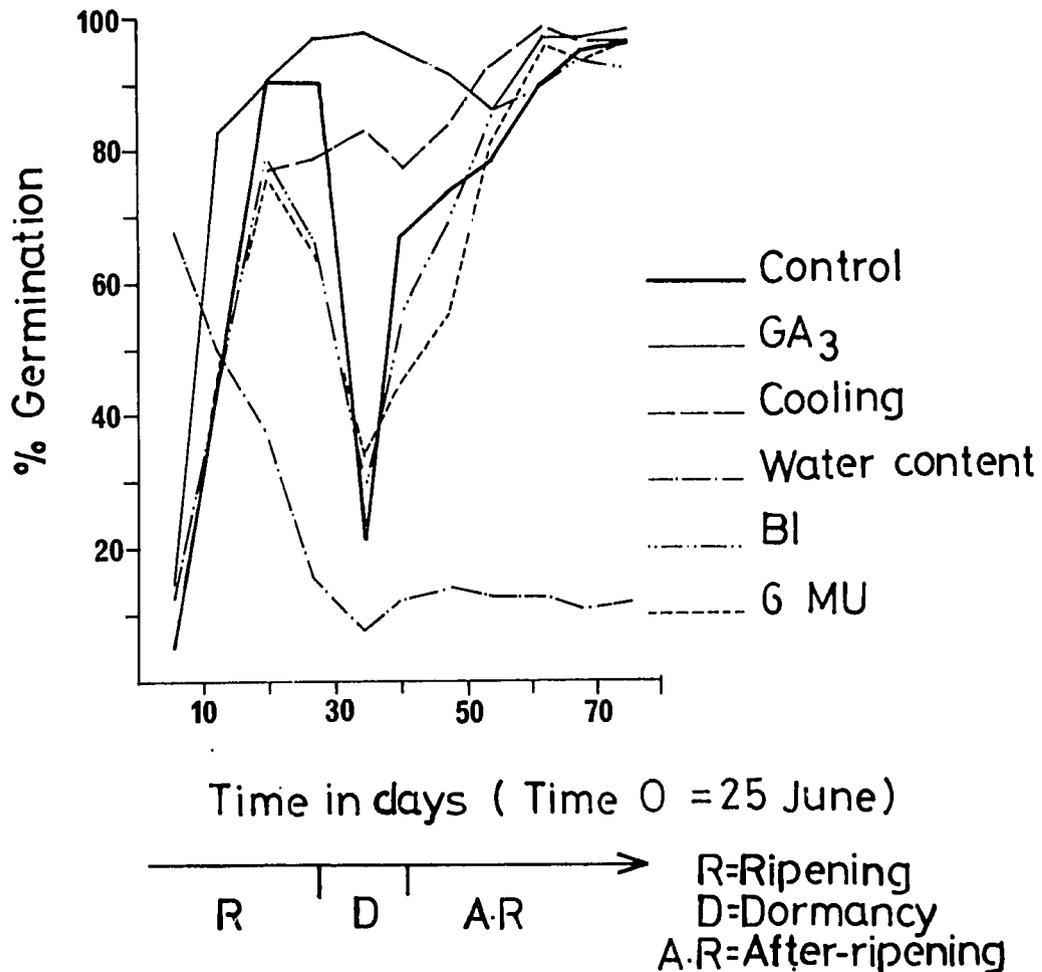


FIG 13. The effect of various dormancy breaking treatments on the germination of wheat grains at various times during and after development.

Possible Modes of Action

The experimental methods mentioned should be regarded as models for further work. With other substances such as ethylene, kinetin, auxin, etc., and by using other temperatures we might have obtained more significant results. Nevertheless, as a result of producing seeds in a pheno-ecological age at harvest with the exogenous factors required to break dormancy can be seen clearly.

NIKOLAEVA (1977) divided dormancy into two main groups: exogenous and endogenous dormancy. The exogenous dormancy can be traced back to physical causes, and can be broken similarly by physical methods. They include:- injuring the seedcoat, heat and light treatments. The endogenous dormancy is related to the biochemical properties of the seed and to break it chemical methods are used. However, the two types of dormancy cannot be sharply separated. (e.g. heat relative to endogenous mechanism.)

An example of exogenous dormancy might be impermeability of the seedcoat to water and/or oxygen. By carefully injuring the seedcoat we make the embryo accessible to water or oxygen, meanwhile the germination inhibitor may be leached out from the seed if present and the latter becomes capable of germinating. Seeds of Papilionaceous species are particularly hardcoated and impermeable. The seedcoats of lucerne and birdsfoot trefoil seeds worked with a rubber roller crack and let the water in. With hardcoated seeds ultrasound treatments may also be successful (MAJOROS 1964). (As also H_2SO_4 , impaction, boiling water, baking.)

The physical treatments include the application of heat, when the seeds are exposed to high or low temperatures. A temperature of 45°C maintained for several days breaks the dormancy of rice and barley grains, although the germination ability will be reduced to

some extent after the heat treatment (Roberts 1965). (It is vital that seeds remain dry.)

Compared with high temperatures, chilling is much more frequently used to break the dormancy of seed. In that case seed swelled in water are kept at 3-5°C for weeks or only for days, according to the demand of the species.

An efficient method is the application of alternating temperature. The seeds are germinated between 15 and 30°C, though the temperature demand may vary with the species. The lower of the temperatures seems to have the greater effect, so its duration is longer. The alternating heat imitates the change in temperature conditions of nature, although such irregularity never occurs.

Physical and chemical effects cannot be sharply separated. By injuring the seedcoat we render it possible for the oxygen to penetrate it. By increasing the partial pressure of the oxygen we can produce a similar effect. The same purpose is served by the application of H_2O_2 .

Light acts through the activation of the phytochrome system and is in interaction with the endogenous regulators (JONES and STODDART 1977), but light either white or red by itself does not break the dormancy of seed. Still, the dormancy is not indifferent to light. Cumin seeds kept in complete dark (in painted glass vessels) lost their germinative ability, while those in the control kept side by side with them germinated at the end of after-ripening (authors's own observation).

The physiological contribution of the seed is determined by the equilibrium of phytohormones and by a dynamic change in their proportions. The effects of cytokinin, benzimidazole, methyl uracil and gibberellins on seed dormancy have been discussed already.

GA₃ has influence on many species, but GA₄ and GA₅ can be used at concentrations of 10⁻²M or 10⁻³M. Gibberellic acid can be applied in combination with cytokinin with a positive effect (KHAN 1978). There are other growth stimulators, such as fusicoccin in the case of certain species, more effective than cytokinin. Its effect on the cell membranes is discussed by CHASTAIN and HANSON (1981).

The stimulatory effects of some respiratory inhibitors on the germination of dormant seed could be the result of their inhibitory action on catalase, which in turn would stimulate the activity of NADPH oxidase and thus the PP pathway (HENDRICKS and TAYLORSON 1975 cit. by ROBERTS and SMITH 1977). (Refer to Fig. 14.)

Conclusion

Seed dormancy and its breaking is a complicated phenomenon not sufficiently understood as yet. The life of seed is influenced by external effects even during its period on the mother plant and the dormancy of seed develops under the influence of a wide range of physical phenomena. The question of seed dormancy can be approached from various sides. In the present paper, the importance of ecological effects acting on the mother plant is emphasised on the one hand and attention is drawn on the other to the fact that during after-ripening gradual changes take place in the seed that can be followed by a series of germinations induced where necessary by exogenous factors. The germination line shows the course of germination characteristics of the species. The after-ripening germination lines of the economically important plant species would be worth being known.

By germinating seeds in solutions suitable to break the dormant stage we can interfere with the course of dormancy. Germinating untreated seeds serves for the control of the results of experiments. Besides those used in our experiments other substances

applied at different concentrations and temperatures may also be suitable for the same purpose.

Seed dormancy might be broken with methods other than those described here. However, experiments on seed dormancy ought to take the age of seed into consideration, since the results of experiments may be related to the stage of dormancy.

We hope that our work will be of some help in solving the problem of breaking the dormancy of seeds.

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DISCUSSION

Chairman: Roberts

Participants: Cooper, Ellis, Mumford, Tyler,
Smith, Probert, Bowling, Overaa

1. The Use of Interactions Between Dormancy-Breaking Agents

In summing up Dr. Papp's paper, Prof. Roberts said how she had emphasised the complexity of the dormancy problem and had examined it from an early stage in the developing seed. From his point of view it was interesting to recall how many different dormancy-breaking treatments had been mentioned e.g. stratification, GA₃, Cytokinins, Fusicoccin and several metabolic inhibitors, and that the range of treatments available widened considerably with the use of interactions between dormancy-breaking agents. Interactions between two or more dormancy-breaking treatments sometimes show reinforced effects e.g. two agents with little effect by themselves, applied together may give a satisfactory result. Dr. Cooper added to this by mentioning that ISTA sometimes use several treatments simultaneously (e.g. KNO₃, fluctuating temperatures and light); this may well be an example of overkill, but it has the required effect.

Prof. Roberts then went on to describe the work of Ellis and Tran Dang Hong at Reading, concerned with the possibilities for routine dormancy-breaking in rice, where interactions between (some or all) of five factors appear to be important. The factors are HNO₃ (in fact two: H⁺ and NO₃⁻); H₂O₂; alternating temperature, the optimum for rice being 34°C alternating with 11°C (N.B. 16 hrs at the higher temperature and 8 at the lower, the opposite way round to ISTA regulations). Finally those four factors are applied in 4-mercaptoethanol, the -SH group being widely noted as active in dormancy removal. This treatment removes dormancy in the most dormant rice cultivars found.

2. Use of Hydrogen Peroxide as a Dormancy-breaking Agent

Prof. Roberts asked Dr. Papp whether she believed that H_2O_2 , for example, affects different species in the same way. She felt that H_2O_2 often appears not very effective because of its instability. Prof. Roberts agreed that care must be taken to use fresh solution. Dr. Ellis stressed that H_2O_2 by itself often shows no effect; it is necessary to rely on its interactions with other treatments. An important safety point is that HNO_3 and H_2O_2 should never be combined together, but applied sequentially, because of the risk of explosion.

3. Fluctuations/Periodicity in Dormancy

Dr. Mumford wondered whether Dr. Papp had observed any periodicity of dormancy with time over the years of her experiments. Dr. Papp confirmed that she had; however Prof. Roberts commented that although such fluctuations exist it is often very difficult to untangle them from the results of environmental fluctuations, and so difficult to interpret them.

4. Depth of Dormancy - Especially in the Gramineae

Dr. Papp felt that there are many types of dormancy of differing depth in a seed lot. Prof. Roberts concurred that within a single seed lot there would appear to be a large seed to seed variation in dormancy, where a low level of treatment will remove dormancy in some individuals, but most need higher levels of a particular dormancy-breaking agent, or even a combination of treatments.. Dr. Tyler was of the opinion that much of the dormancy he encountered in Gramineae at the Welsh Plant Breeding Station is deeper than the types described by Dr. Papp. This dormancy probably associated with survival mechanisms appears to be permanent, as opposed to being restricted to an after-ripening period; and the

treatments required to break dormancy may well imitate ecological conditions. While ecological pointers are certainly useful, Prof. Roberts pointed out that there are some paradoxes e.g. both Oryza sariva ssp indica and O. glaberrima respond to chilling, but as they are both tropical, this condition is unlikely to have been encountered by them often in nature.

Mr. Probert said that by and large temperate grasses respond well to simple 'environmental' treatments such as light, temperature fluctuations or chilling; whereas tropical species tend to be much more difficult, possibly due to the presence of inhibitors. In such species, more complex combinations of dormancy-breaking treatments need to be applied in order to obtain any germination. Prof. Roberts confirmed that his student, Clara Goeddert, had similar problems with dormancy in tropical grasses. Mr. Bowling wondered if it may be possible to imitate the conditions of tropical storms - rapid temperature drop and increased level of soil nitrate - in order to break dormancy in tropical species.

5. The Use of Gibberellic Acid as a Dormancy-breaking Agent

Dr. Papp commented that in deeply dormant seeds where she had failed to get a response to GA, she should have tried higher concentrations. Mr. Smith agreed that when using GA₃ the application of a single standard concentration to every collection of a given species is unlikely to give complete germination all the time. The most efficient way to use GA₃ appears to be by applying a range of concentrations (rising logarithmically probably being more useful). Dr. Overaa amplified this by describing an experiment in which chilling did not break the deep dormancy of oats but by using increasing concentrations of GA₃, i.e. 200, 400, 600, 800, 1000 ppm he obtained corresponding increases in germination. It is also important to note that he obtained better

germination at 400 ppm GA_3 combined with chilling, than at the higher concentrations of GA.

Mr. Smith raised doubt over the use of GA_3 , when its possible effects on seedling growth and establishment are considered: the concentration used in the laboratory may make field establishment difficult. This is important when thinking about passing on germination information to a breeder, who may not be able to grow plants from the seeds. Prof. Hawkes commented that he had used GA_3 for raising potatoes from seed (Solanum tuberosum) without any problems. Prof. Roberts thought that as well as producing abnormal seedlings, the concentrations of GA_3 required to break dormancy may hasten the demise of seeds that are losing vigour.

Containers for Seed Storage

P. M. MUMFORD and M. S. FREIRE

The aim in seed storage is to maintain seed in the best possible physiological state for future use. The environmental conditions which promote this state and therefore seed longevity apply whether the storage period is short or long. Although seeds may survive a short period of poor storage (e.g. three months) their performance with respect to vigour declines compared to seeds stored under optimal conditions and so standards should not be relaxed for 'short term storage'.

Limiting the discussion to seeds which naturally undergo desiccation at physiological maturity (orthodox) it is generally recognised that viability is prolonged by storage at low moisture content and low temperature. Achieving low temperature with normal refrigeration equipment is not a problem. Maintaining seeds at low moisture content however, after they have been dried and prepared for storage depends on the properties of the container used. For a long time the food industry has faced the problems of keeping a large variety of foodstuffs with good shelf-life and from research in this area it is apparent that vacuum sealed metal cans are very durable and a range of laminated materials comprising paper, nylon, ethylene polymers, and aluminium foil are available with varying resistance to moisture transmission. The choice of a suitable container for seeds will be related to the factors itemised, Table 1.

Table 1. Requirements of Storage Containers for Seeds

1. moisture proof
2. able to withstand changes in temperature, -18°C to 40°C , without change in properties
3. easy to handle and label
4. either easy to open and reseal for sampling or available in sample sizes
5. inexpensive

SEED CONTAINERS USED BY GENE BANKS

In 1979 a survey was conducted in which 112 institutions around the world were invited to supply details of the types of containers they used for seed storage and also to send samples for testing. 59 replies were obtained. Unfortunately insufficient quantities of samples of containers were available for testing. The results of the survey are depicted in Fig. 1. Only 16 institutes used one type of container, many using a combination of materials with different aims in view, e.g. paper for short term and metal for long term storage. The methods of sealing the containers was extremely diverse. The temperature and humidity conditions in which storage was carried out were also very variable and many people distinguished between ambient and controlled storage environments.

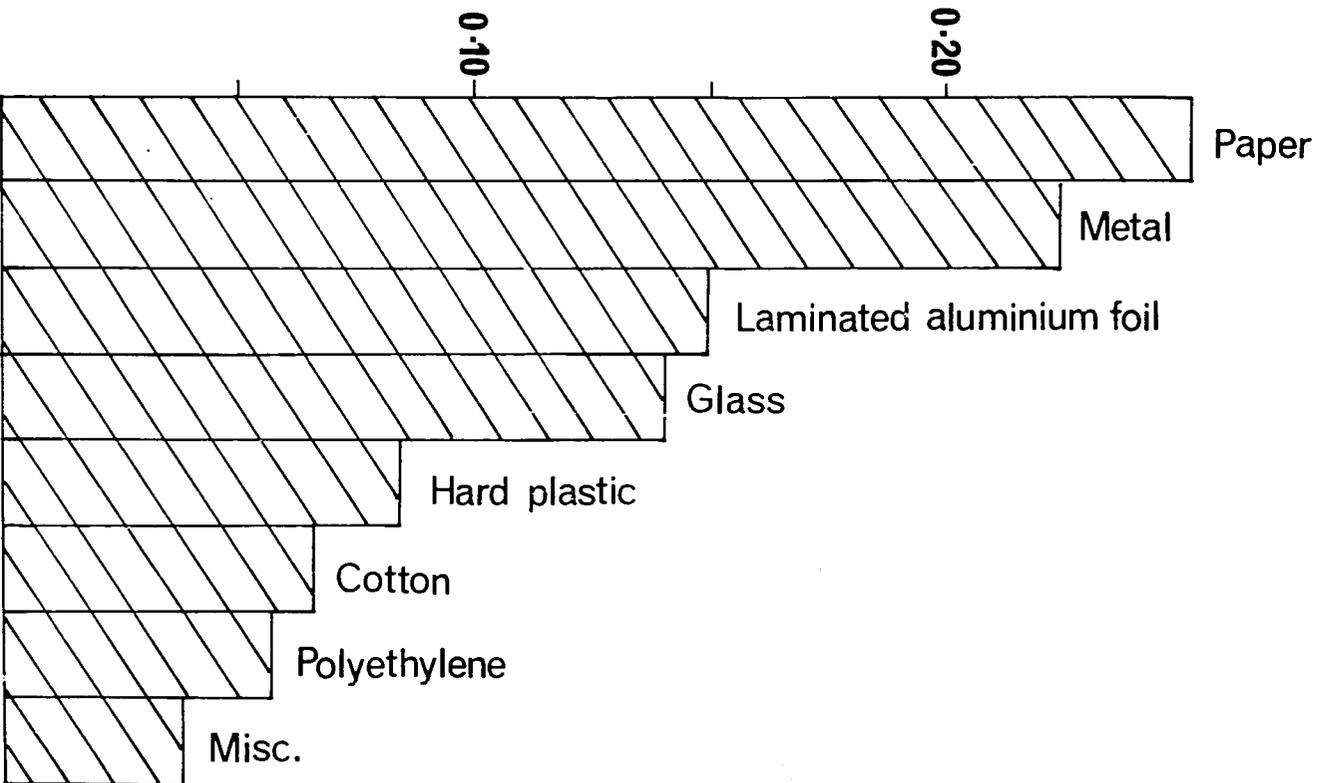


Fig.1 Frequency of use of different container materials for seed storage. (Survey 1979)

TEST CONDITIONS USED TO DETERMINE THE RELATIVE EFFECTIVENESS OF CONTAINERS

In the light of this evidence and the difficulty of comparing reports of longevity of seeds where data were obtained under such variable influences, an experiment was started in 1979 to compare the performance of wheat and rice seeds stored in a variety of types of containers. Details of the storage experiment are given in Table 2. Seeds were stored at two different moisture levels and under three storage environments for a 21 month period. Six types of material were used for constructing containers and these were used in various combinations. Sealing was accomplished in several ways; paper was glued, cotton was sewn, polyethylene and laminated aluminium foil were heat sealed and screw or cork tops were used for metal and glass containers. Where polyethylene and laminated aluminium foil were used inside glass or metal, the inner container was sealed with plastic tape. Seeds were sampled every three months and tested for changes in moisture levels, germination and the vigour of the seedlings was measured using root and seedling lengths.

Table 2. Storage Experiments : Materials and Conditions

MATERIAL.	Upland rice, IAC 25 from Brazil Bread wheat, Maris Huntsman from Britain
MOISTURE LEVELS.	Rice 7.5, 11.5% Wheat 6.4, 13.2%
STORAGE ENVIRONMENTS	32°C, 80-90% relative humidity 22°C, 60-70% relative humidity -18°C

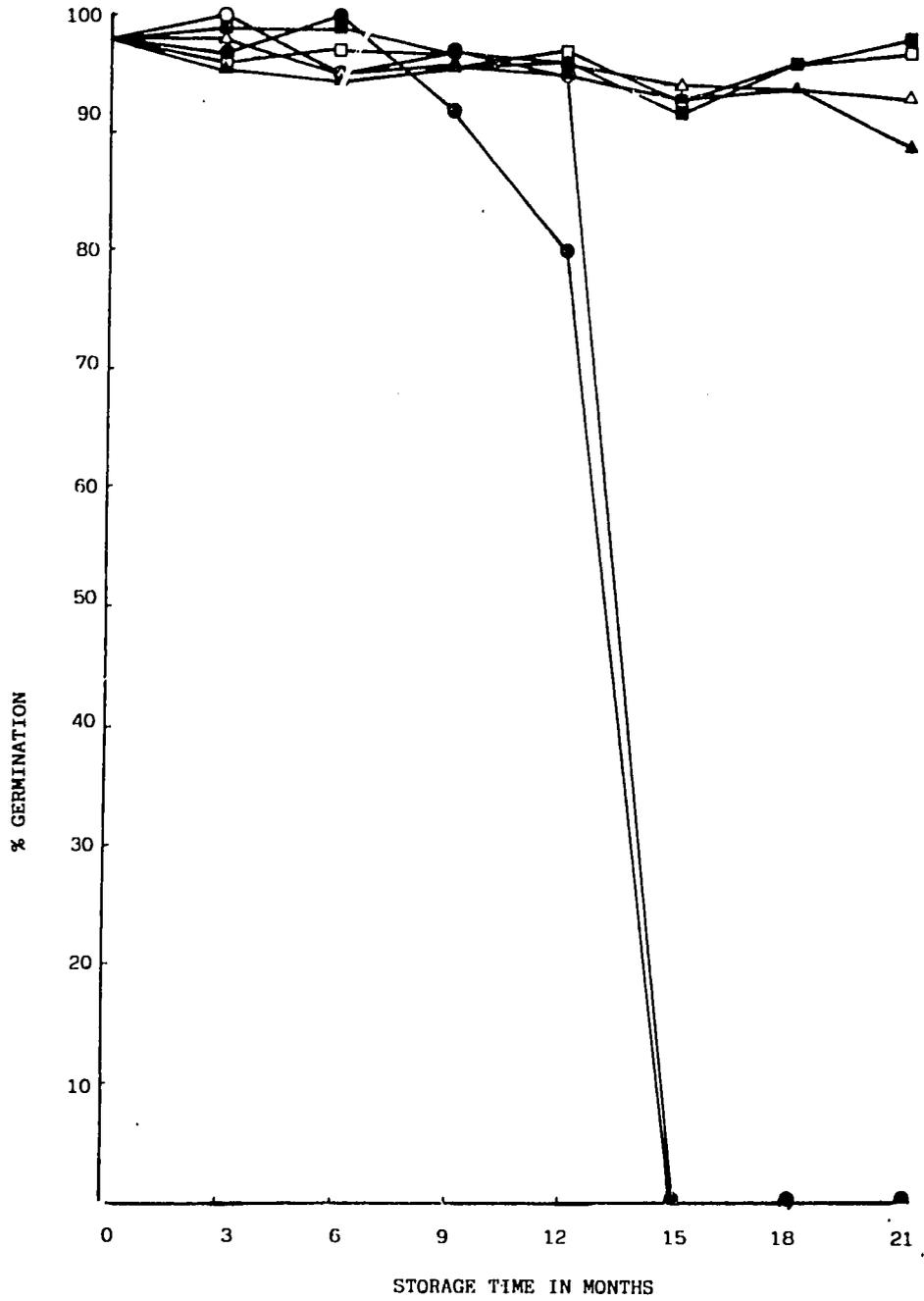


Fig. 2 Germination of rice seeds after storage in cotton bags inside glass bottles with cork stoppers

Storage conditions 11.5% moisture, ● 32°, ▲ 22°, ■ -18°C
7.4% moisture, ○ 32°, △ 22°, □ -18°C

Cont. Storage Experiments : Materials and Conditions

CONTAINER MATERIALS paper, cotton, polyethylene, glass,
 metal, laminated aluminium foil

COMBINATIONS OF MATERIALS paper, cotton polyethylene and lam-
 inated foil inside glass or metal

The general trend expected was a detectable decline in germination and vigour of seeds with time when stored at relatively high moisture levels and the higher temperatures, and an undetectable change in seeds stored dry at low temperature. This trend was seen in many containers and is shown in Fig. 2, for rice packaged in cotton bags stored within glass bottles sealed with corks. The main aim of these experiments however was to compare the effects of containers and an analysis of the data showed there were highly significant differences between containers. To illustrate this fact two extremes are presented, Fig. 3, in which germination of wheat seeds stored in paper and laminated aluminium foil is plotted. Laminated aluminium foil was expected to be better than paper but most important is the magnitude of the difference for dry seeds at the intermediate temperature, 22°C, an environment which pertains in many laboratories, and an environment which the survey indicates is very common for seeds in paper bags not destined for long term storage. Vigour tests were, throughout the tests, rather similar in form to survival curves. Rice and wheat showed similar patterns of behaviour with respect to containers although wheat deteriorated more rapidly than rice.

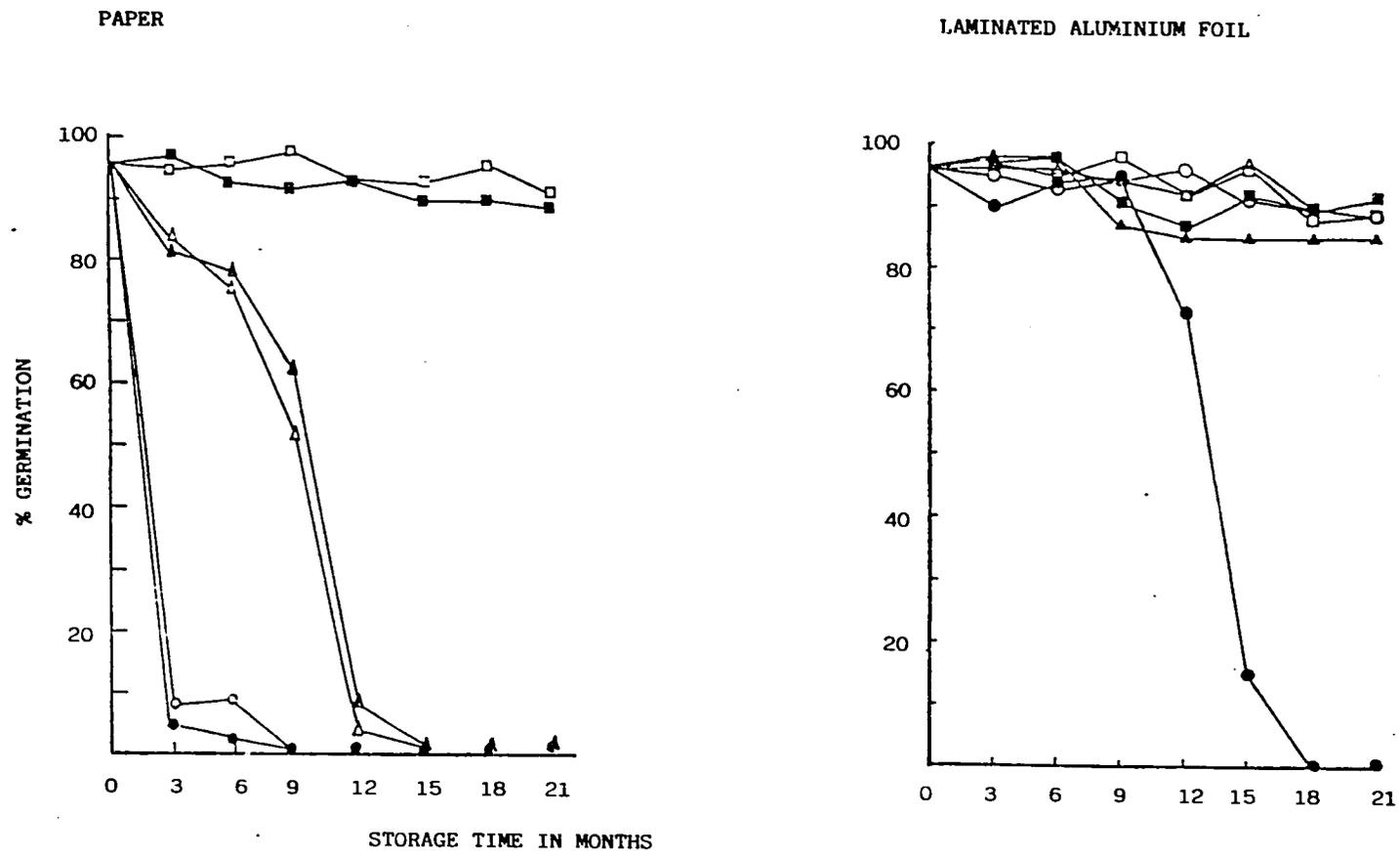


Fig. 3 GERMINATION OF WHEAT SEEDS AFTER STORAGE IN PAPER AND LAMINATED ALUMINIUM FOIL BAGS.

Storage conditions 13.2% moisture, ● 32°, ▲ 22°, ■ -18°C
 6.5% moisture, ○ 32°, △ 22°, □ -18°C

RELATIVE EFFECTIVENESS OF CONTAINERS

From the results an overall assessment of the containers can be made by using the mean germination percentage values for both seed types, over all conditions for the 21 month period, Table 3. With a few exceptions the containers fall into groups according to the material from which they are made and the way in which sealing was achieved. Laminated aluminium foil was best. It is supposed that metal cans heat sealed would be equally effective although they were not tested in these particular experiments. Metal cans with metal screw tops were superior to glass bottles with bakelite screw tops. Sealing glass or metal vessels with cork was less efficient than screw tops and the combination of metal and cork was worse than glass and cork. This is probably because the cork only comes into contact with the grooves in the metal rim of the can while the inside of the glass bottle is smooth and the cork fits flush. Polyethylene bags overall were poor containers with only paper and cotton proving worse.

An obvious reason for the differences in performance of seeds from these packages is the ability of the materials laminated aluminium foil, metal, glass, polyethylene, paper and cotton to prevent or resist moisture entering the container and therefore the seeds. Cotton and paper are porous and unlikely to offer much resistance to moisture transmission while metal and glass are moisture-proof. The extent to which water vapour or water may penetrate polyethylene and laminated aluminium foil was unknown at the start of this experiment. It is essential the efficiency of the sealing process matches the qualities of the materials used and cork being porous reduced the value of glass or metal containers. The overall mean moisture content of seeds from each container over the 21 months was calculated and is shown in Table 3. There is a correlation between mean germination and moisture content, $r=-0.8$, and the uptake of moisture by seeds in storage is considered the

single most important factor in bringing about differences in containers at any one temperature.

Moist seeds subjected to high temperature during storage deteriorated rapidly and were swiftly invaded by micro-organisms. Cotton bags inside other containers showed exceptional behaviour when sealed with cork tops. Cotton was found to absorb moisture very readily and also seemed to be preferred to seeds as a substrate for growth by fungi. In the case of cotton bags inside glass bottles with cork tops it is possible the cotton absorbed more moisture than the seeds but this was not true in metal containers in which moisture content of seeds rose.

Therefore laminated aluminium foil packages can be recommended and so can metal cans with screw tops; and glass bottles with screw tops are reasonably efficient. Each of these containers is convenient to handle and available in a range of sizes to meet sample needs. If for some reason containers must be used which are less moisture resistant than those recommended there is no point in drying seed to very low moisture levels, because during storage moisture will be taken up again. Results showed that in the moderately permeable containers viability decline was a little slower in seeds which went into store at 6-7% as compared to those of 11-13% moisture but in very permeable containers, like paper or cotton, drier seeds absorbed moisture very rapidly and could deteriorate even faster than more moist seeds.

Table 3.

Containers ranked in order according to mean percentage germination of seeds over the 21 months of storage for all storage environments. Mean moisture contents are given.

$r = -0.8$

Container	Germination %	Moisture %
1. laminated Al foil	88.48	9.38
2. lam. Al foil in metal	87.66	9.48
3. metal	87.60	9.62
4. paper in metal	87.07	9.54
5. polyethylene in metal	86.23	9.60
6. cotton in metal	86.23	9.52
7. cotton in glass	86.21	9.60
8. cotton in glass with cork	85.06	9.92
9. glass	84.32	9.86
10. lam. Al foil in glass	79.87	10.38
11. polyethylene in glass	78.91	10.46
12. paper in glass	78.88	10.49
13. polyethylene in glass with cork	78.43	10.30
14. lam. Al foil in glass with cork	78.43	10.21
15. paper in glass with cork	77.07	11.56
16. glass with cork	76.20	10.33
17. lam. Al foil in metal with cork	75.39	10.61
18. polyethylene in metal with cork	75.35	10.45
19. metal with cork	74.18	11.00
20. polyethylene	74.81	10.78
21. cotton in metal with cork	70.68	11.10
22. paper in metal with cork	70.23	10.45
23. paper	54.63	10.78
24. cotton	53.18	15.38

An interaction between temperature and containers was recognised in these experiments. At -18°C only seeds from paper and cotton bags showed a significant uptake of moisture and in germination performance only seeds from paper, cotton and laminated aluminium foil containers were consistently different from the rest. At 22°C the difference between seeds from most containers was small except for those from cotton and paper bags which deteriorated much more rapidly than the rest. There was a significant increase in moisture level of dry seeds from the more permeable containers but no change in seeds higher in moisture content. At 32°C there was the greatest difference between performance of seeds from different containers. Cotton, paper and polyethylene were very poor allowing rapid escalation of moisture levels. The difference between containers sealed by screw or cork tops was most marked and only laminate aluminium foil and metal cans with screw tops proved moisture-proof. Double containers generally proved worse than single ones at 32°C .

Although there was no detectable change in the level of germination of seeds stored in impermeable containers at -18°C , there was however a decline in vigour noticeable in both root and seedling lengths at the 21 month sampling time. Since moisture contents did not change significantly this appears to be an effect of low temperature. Storage for a longer period is required to substantiate this finding.

The behaviour of polyethylene and laminated aluminium foil was selected for special scrutiny and their properties were examined. Seeds in silica gel were placed in packets made of these two materials and the packet edges were heat sealed. They were subjected to environments of very high humidity, very low humidity, submerged in water and exposed to various temperatures. The laminated aluminium foil was a very effective barrier to passage of moisture and the sealing of packets proved quite satisfactory.

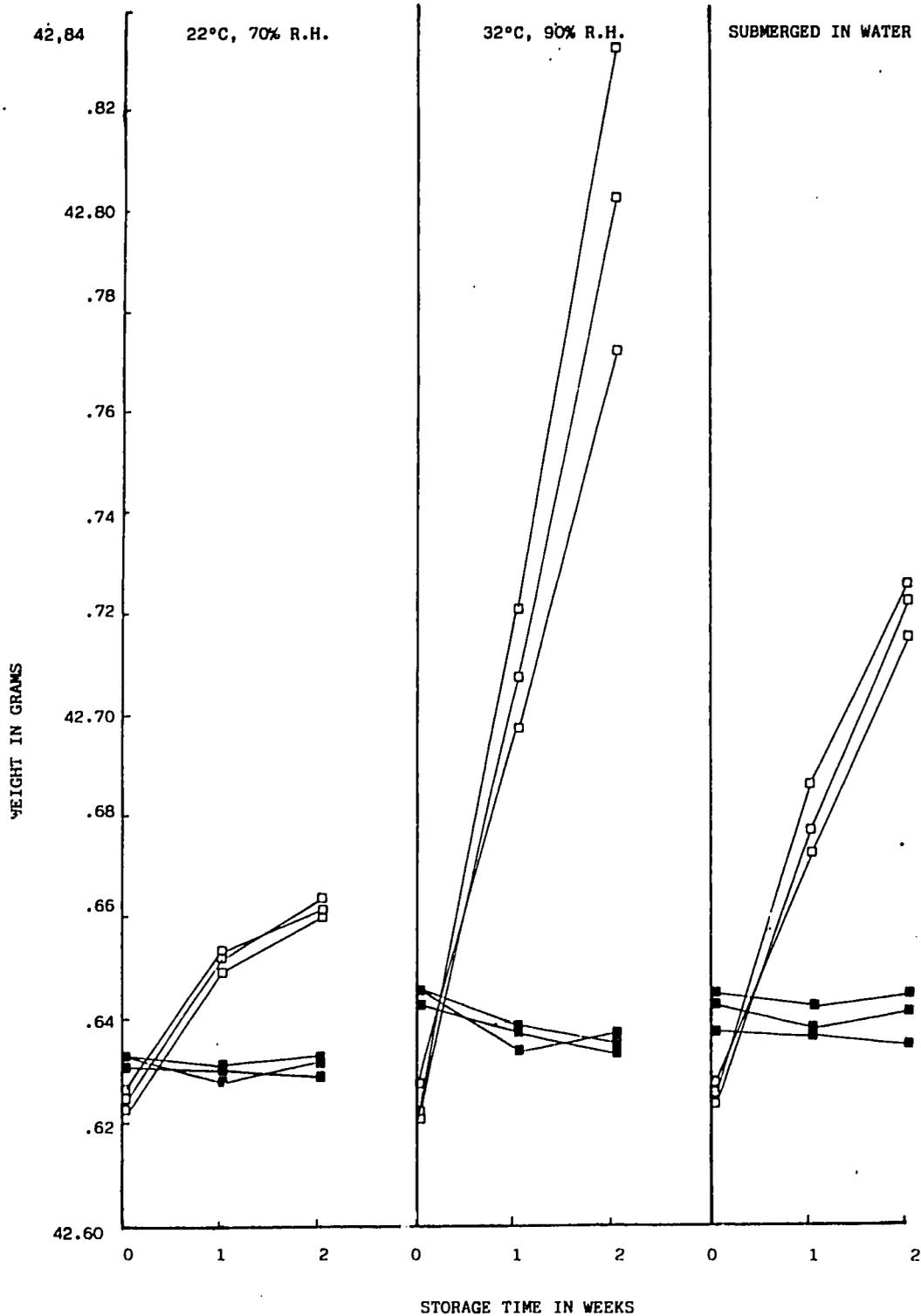


Fig. 4 CHANGE IN WEIGHT OF LAMINATED ALUMINIUM FOIL AND POLYETHYLENE PACKAGES CONTAINING SILICA GEL DURING STORAGE IN HUMID OR WET CONDITIONS

■ laminated aluminium foil □ polyethylene

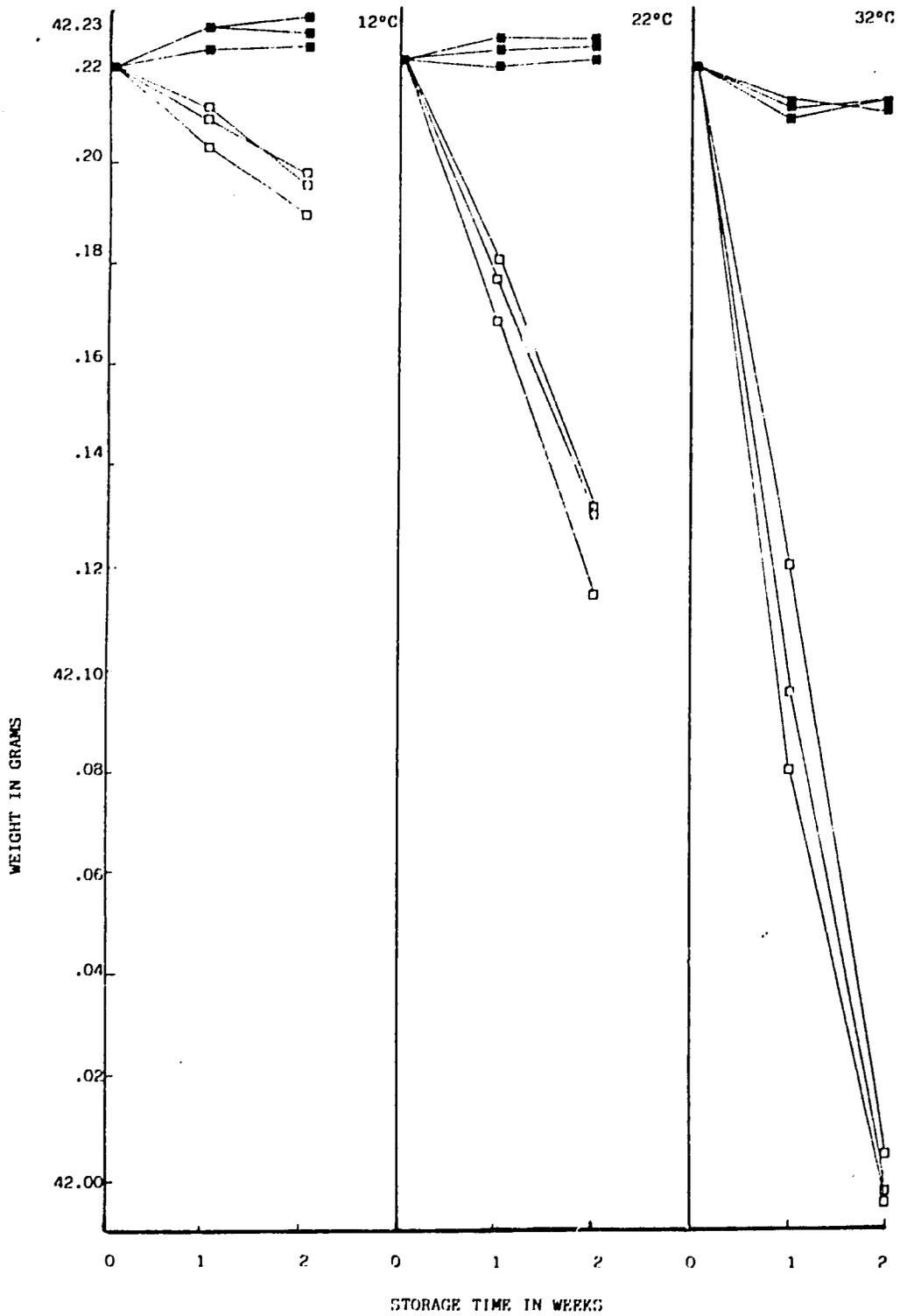


Fig. 5 CHANGE IN WEIGHT OF LAMINATED ALUMINIUM FOIL AND POLYETHYLENE PACKAGES CONTAINING WHEAT SEED DURING STORAGE AT VERY LOW HUMIDITY

■ laminated aluminium foil □ polyethylene

This was in contrast to the properties of polyethylene as illustrated by the uptake of moisture by silica gel when packets were submerged in water or placed in very humid conditions, Fig. 4.

The permeability of polyethylene was dependent upon temperature. This is demonstrated by the loss in weight of packages of wheat seed when placed in conditions of very low humidity for two weeks, Fig 5. There is dramatic increase in permeability with rise in temperature. The physical properties of the polyethylene are also seen and felt to change at the higher temperatures. A number of people at genebanks use polyethylene bags for storing certain species at moderately high moisture content and at around 4°C, e.g. forest species which are sensitive to desiccation. Under these conditions polyethylene probably exhibits quite good resistance to moisture transmission and may prove quite satisfactory.

For general purposes our findings show laminated aluminium foil and metal containers well sealed, to be the best containers for preservation of seed viability. As important as the container is the speed with which seeds are protected from adverse environments, and since deterioration can occur so rapidly it is recommended that seeds from harvest or collection time should be treated with all the attention currently given to seeds destined for long term storage, because just a few months in poor storage conditions can seriously reduce the potential life of any seed sample.

DISCUSSION

Chairman: Smith

Participants: Cooper, Tyler, Roberts,
Cromarty, Ellis, Williams, Astley

1. Concern at The Apparent Loss in Vigour at Low Temperature Storage

Much of the discussion of this paper was centred upon the rather surprising results, contrary to experience elsewhere, suggesting that storage at -20°C has an adverse effect upon seed vigour; and upon possible alternative causes for them.

Dr. Ellis reported that tests done on barley seeds within the preceding month had shown no measurable change in vigour after low temperature storage; and he wondered whether the use of paper towel substrate could have caused problems. Having had considerable experience in the use of paper towel tests, Dr. Cooper said that conditions within them certainly could be critical. If the paper is rolled too tightly, then symptoms similar to those described in the paper are observed, possibly due to lack of oxygen; and this manifests itself especially where many tests are crowded into a small incubator. Dr. Mumford commented that they certainly experienced similar problems initially, due to rolling the paper towels too tightly, but thought they had overcome the problem; and, of course, the same routine was used for all the tests.

Mr. Smith wondered whether anyone running a seed bank had evidence of declining seed vigour in low temperature storage. Mr. Tyler replied that at WPBS he had observed a slightly worrying, but not statistically significant, trend where forage seeds in laminated foil bags showed lower final % germination after storage at -18°C than after storing at 0°C . Mr. Smith wondered if significant differences might have been shown, had the % germination data been

transformed to probits. In the light of extensive experience by his research group at Reading and others, Prof. Roberts was not inclined to believe the presented findings, but agreed that if adverse effects of low temperature storage on vigour and/or final germination percentage could be established, they would have extremely serious consequences with regard to current IBPGR recommendations.

Mr. Smith described some work he had carried out on members of the Araucariaceae, where seeds had been stored in aluminium foil packaging at -18°C without any trace of vigour loss. He wondered whether Dr. Mumford's seeds had been allowed sufficient time for acclimatisation before opening the foil packets upon removal from -18°C storage. Dr. Mumford and Miss Freire replied that the seeds had been kept at room temperature for 2-3 hours before the packets were opened. Dr. Ellis thought that this could be one answer to the problem; he felt that the time allowed may not have been sufficient for the temperature of seeds in the centre of the packet to rise to ambient. Dr. Mumford appreciated that there would be a gradient and while admitting that they may have underestimated the time required for acclimatisation, nevertheless felt that it probably had been long enough in view of the small size of the packets.

In response to a question from Dr. Bekendam, she also confirmed that the seeds had been equilibrated at high RH before imbibition in the tests.

Mr. Cromarty suggested that depending upon the source of the seed, loss in vigour may be a result of faulty drying technique by the seed suppliers. Especially in some commercial driers, there can be steep gradients in moisture content coupled with temperatures of $50-60^{\circ}\text{C}$; and although both the stabilised moisture content after drying and the germination percentage may be acceptable,

this does not rule out trouble later on. Miss Freire wondered if natural drying could raise the same problems, as some of their material was rice, grown in Brazil, harvested by hand and dried naturally. Mr. Cromarty replied that this is so, especially in direct sunlight where seed temperatures may reach 60°C. Both Prof. Roberts and Dr. Ellis re-emphasized that they had never come across this problem in any of the so-called orthodox species upon which they worked; this appears to be supported by the experience of a number of genebanks now operating at -20°C. Susceptibility to chilling in things like Oil palm seeds is a slightly different problem. Bearing in mind some of the possible variations in experimental treatments which may have inadvertently been included in the work under discussion, e.g. inter-batch variation in paper towel quality, Prof. Roberts counselled caution in drawing conclusions. Nevertheless, it is a crucial problem and further examination is urgently required. Prof. Roberts suggested that if they are not already doing so, seed banks and others storing seeds at -20°C should begin testing for vigour as well as viability. Dr. Mumford stressed that she and Ms. Freire were not intending to cause alarm by presenting their data; she agreed that further careful experimentation is necessary to substantiate their observations; and accepted that they may have failed to control all the relevant factors.

2. The Quality of Laminated Foil Packets

Dr. Williams was concerned about the variation in quality of laminated foil packets; in his experience some are no better than polythene bags. As an example Mr. Smith quoted certain packets in use where the supplying company has no research and development facilities and quality control is based pragmatically on economic constraints of the uses, mainly in the fast food industry, where shelf lives of no more than six months are anticipated. Other companies can offer more technical data.

Dr. Ellis remarked that laminated foil packets certainly do leak, but rates of diffusion are extremely low due to the low permeability of the materials. He also commented on the difficulties of efficient sealing, with regard to optimum temperature; also in a well filled packet seeds close to the sealed edge may have experienced high temperatures. Drs. Bekendam and Mumford and Prof. Roberts all agreed that, after exhaustive testing and provided they come from a reliable manufacturer, laminated aluminium foil bags are probably the best available containers for long-term storage. Dr. Mumford explained that the fall in vigour observed in her experiments were due to low temperature alone; seeds in foil packets had shown no significant uptake in moisture.

3. Cap Inserts for Screw-top Containers

In response to enquiry from Dr. Astley, Dr. Mumford stated that the inserts of the metal screw-top containers used in her experiments were of "rubber paint"; while those of the glass bottles (Bakelite lids) were of a thin layer of cork and foil. Dr. Astley commented that natural rubber or silicone synthetic are preferred by many people. Mr. Smith described a bottle system used to maintain aseptic conditions in medical research, in which the lids of glass bottles are provided with a rubber wad as insert. According to Dr. Bekendam, the fit of the lid of cheap plastic containers can be less than effective when placed at low temperature. Dr. Mumford added that this problem is likely to be more evident with glass bottles where the lid is of a different material, than with a metal container, where body and lid are to the same specification.

4. Testing for Moisture Ingress into Containers

Mr. Cromarty wondered whether members could recommend tests for genebank managers to measure the ingress of water into containers,

with a view to some kind of standardisation. Dr. Mumford said there are difficulties with the high RH methods and she prefers submerging containers in a known level of water, as described in her paper. However, Dr. Belendam maintained that he uses 39°C and 95% RH, and that this is easy to use. Prof. Roberts drew attention to measures of the changing properties of plastics especially rates of diffusion with regard to temperature. As temperature is lowered the vapour pressure on either side of the system will change though not to the same extent. Thus changes in rates of diffusion across the material may be due to differences in vapour pressure and not necessarily to changes in the nature of the material.

Mr. Smith commented that container performance may vary in relation to the content of the containers (proportion of stored material to air), e.g. at Wakehurst Place, as at many other seed banks, there is a set container size, but the amount of seed in each container is variable. Dr. Mumford agreed that performances could vary in this way.

Monitoring Seed Viability in Genebanks

E.H. ROBERTS

FACTORS GOVERNING FREQUENCY OF REGENERATION

Even under good storage conditions seeds lose viability and thus it will be necessary to regenerate accessions from time to time (i.e. to grow plants from existing accessions to provide fresh stocks of seed for storage). The frequency of regeneration will depend on (1) the initial viability, (2) the rate of loss of viability and (3) the regeneration standard (i.e. the percentage viability at which it is decided to regenerate the accession). The rate of loss of viability will depend on (1) the characteristics of the species (seeds of some species survive better than others), (2) the initial seed quality (which depends on genotype and the conditions experienced by the seed on the mother plant and during subsequent processing before storage, all of which are reflected in the initial viability), and (3) the storage conditions. The viability of different accessions will therefore decline at different rates. The decision as to what percentage viability to adopt as the regeneration standard is inevitably a compromise between what is theoretically desirable and what is feasible in practice. The lower the regeneration standard the greater the risk of accumulation of mutation and also, in genetically mixed accessions, the greater the risk of genetic selection during storage leading to loss of some component genotypes (Roberts and Ellis, 1983). On the other hand, if the regeneration standard is set too high, both monitoring and regeneration will be more frequent and will increase the work-load and costs of genetic conservation. Taking into account all these problems a regeneration standard of 85% viability is considered suitable for cereals and other arable crops in which it is possible to obtain accessions of high quality which can normally be expected to show

an initial viability of 95% or more. In other species, in which it is difficult to obtain high initial viability percentages, a slightly lower regeneration standard may be more practicable.

METHODOLOGY AND FREQUENCY OF MONITORING TESTS

It is necessary to monitor seed accessions during storage in order to discover whether viability has dropped to the regeneration standard. The most obvious and usually most convenient method is to carry out a germination test in which steps have been taken, where necessary, to remove dormancy which would otherwise interfere with the results. Other papers in this workshop have dealt with methods of treating seeds. Essentially the conditions prescribed by ISTA or AOSA rules provide a useful guide but in some cases these methods will need to be supplemented by additional dormancy-breaking techniques. But having decided on the treatments to be applied and the conditions employed in the germination tests it is necessary to decide how frequently the monitoring tests should be carried out and how many seeds should be used in each test. Clearly the frequency of monitoring tests and the number of seeds used in each test should not be greater than necessary, otherwise there will be an unnecessarily rapid depletion of the accession. On the other hand if the number of seeds used is too small (and thus the tests are unreliable), or the monitoring tests are too infrequent, there is danger of excessive loss of viability leading to either unacceptable genetic change or complete loss of the accession.

In a few species it is now possible to make rough estimates of what loss of viability can be expected after any period under any set of storage conditions for different accessions, e.g. in barley, onion, soyabean, cowpea and chickpea (Ellis and Roberts, 1980; Ellis and Roberts, 1981; Ellis, Osei-Bonsu and Roberts, 1982), and guidance on several more species will soon be avail-

able. In such cases it should be possible to estimate the expected time for the viability of accessions to fall to the regeneration standard and to relate the interval between the initial viability test and subsequent monitoring tests to this estimate. To allow for sampling and other errors, the monitoring interval should be much shorter than the expected regeneration interval (i.e. the time taken for viability to drop to the regeneration standard). It is suggested that the monitoring interval should not be more than about one-third of the expected regeneration interval.

For many species, however, estimates of regeneration interval are currently not possible and in such cases it would be advisable to set the monitoring interval between that which would be appropriate for a species which stores well (e.g. barley) and one which stores poorly (e.g. onion), depending on the general reputation of the storage characteristics of the species. Some examples of estimated regeneration intervals for barley and onion seeds under various typical storage conditions are shown in Table 1. When estimates such as these are used it is clear that the quality of the seed accession, as indicated by its initial viability, has a marked influence on the time taken for viability to fall to the regeneration standard. Because of this it should be remembered that the results of an initial viability test (see later) is only an estimate of the initial true viability of the accession (the true viability may be different), and thus when making estimates of regeneration interval it is prudent to assume a somewhat lower initial viability than that recorded in the initial viability tests. If there is no information at all concerning the storage characteristics of the species, then obviously the first monitoring interval should be shorter than that estimated for onion. But in any case the subsequent monitoring intervals could be adjusted in the light of experience, i.e. according to the results obtained in the first few monitoring tests. One possible approach

Table 1 Estimates of probable regeneration intervals for barley and onion accessions of different initial viability, assuming a regeneration standard of 85% (adapted from Ellis and Roberts, 1983).

Example of organization adopting storage conditions indicated	Temperature °C	Moisture content %	Species	Estimated time (years) for viability to fall to 85% viability from:			
				99%	95%	90%	87%
<u>Long-term stores</u>							
Institut Pflanzenbau FAL Braunschweig- Völkenrode, Fed.Rep.Germany	-10	6	{barley	1990	940	370	140
			{onion	150	72	28	11
Laboratorio del Germoplasma, Bari, Italy	-18	7	{barley	1350	640	250	94
			{onion	150	70	28	10
National Inst. Agric. Sci., Tsukuba, Japan	-10	{7 (5	barley	800	380	150	56
			onion	280	130	53	20
<u>Medium-term stores</u>							
Foundation for Agricultural Plant Breeding, Wageningen, The Netherlands	2	{ 6 (12	barley	730	340	130	51
			barley	12	6	2	<1
	2	{ 6 (12	onion	55	26	10	4
			onion	5	2	1	<1
National Seed Storage Laboratory, Fort Collins, USA (original conditions before upgrading)	4	7	{barley	240	110	45	17
			{onion	27	13	5	2

here is to plot the results of the first few tests on probability paper in order to extrapolate future behaviour (as done, for example, by the Seed Bank at the Royal Botanic Garden, Kew); but such an approach requires some experience and caution in interpretation.

Two approaches are possible with regard to deciding what number of seeds should be used in each monitoring test. The first is to adopt a fixed number of seeds for each test as, for example, in ISTA rules. ISTA prescribes 400 seeds as a compromise between reliability and practicability. Obviously the smaller the sample the less reliable is the estimate of the true viability of the

accession; and the fall off in reliability accelerates as the sample size is decreased. An alternative approach is to adjust the sample size according to the results obtained in the test itself. Clearly if a test carried out on a hundred seeds indicated a viability of, say, 98%, one would have reasonable confidence in concluding that the viability of the accession from which the sample was drawn is greater than the regeneration standard; and thus to use more seeds than this could be considered a waste. On the other hand if the percentage viability of the sample were, say 88%, one would have much less confidence that the true viability of the accession was still above 85%. In this case one could immediately carry out a second test, the results of which, taken together with the first test, would provide a more reliable estimate of whether the regeneration standard had been reached. This type of approach gives rise to the 'sequential probability ratio test' (Ellis, Roberts and Whitehead, 1980). Monitoring tests of this type will detect whether viability has fallen to a given level (the regeneration standard) with an acceptable probability of error, and this approach is much more economical in the use of seed than a test procedure of equivalent reliability based on a fixed sample size.

The statistics of a sequential probability ratio test are complicated, but they can be reduced to a single table which makes analysis very simple. An example (described in more detail in Ellis, Roberts and Whitehead, 1980) for a regeneration standard of 85% viability is as follows. A monitoring test consists of a sequence of sub-tests carried out on multiples of 40 seeds, the first of these is carried out on 80 seeds and the result is compared with those shown in Table 2. So that, for example, if 64 or less seeds germinate in this first sub-test the decision is to regenerate immediately since the viability of the accession is almost certainly below the regeneration standard, and the test

Table 2. Sequential germination test plan for a regeneration standard of 85% viability, using sub-test groups of 40 seeds and probabilities of regeneration shown in Table 3 (from Ellis, Roberts and Whitehead, 1980).

Number of seeds tested	Instruction according to number of viable seeds in test		
	Regenerate if no. of viable seeds is equal to or less than	Carry out further sub-test if no. of viable seeds is between	Regeneration not necessary if no. of seeds is equal to or greater than
80	64	65 - 75	76
120	100	101 - 110	111
160	135	136 - 145	146
200	170	171 - 180	181
240	205	206 - 215	216
280	240	241 - 250	251
320	275	276 - 285	286
360	310	311 - 320	321
400	345	346 - 355	356
440	380	381 - 390	391
480	415	416 - 425	426
520	450	451 - 460	461
560	485	486 - 495	496
600	520	521 - 531	532

therefore ceases at this point. If 76 or more seeds germinate the test also ceases because there is enough information to conclude that percentage viability of the accession is above the regeneration standard, and the accession is maintained in store until the next monitoring test is due many years later. However, if the number of seeds which germinate in the first sub-test is between 65 and 75, there is insufficient information to come to a conclusion and the instruction from the table is to carry out a second sub-test on a further 40 seeds. The result of this second sub-test is then added to the first. If the combined total of seeds germinated is now 100 or less, the test is stopped and the

accession regenerated. If it is 111 or more the test also ceases because one can now conclude with confidence that regeneration is not yet necessary. But if the combined total is between 101 and 110 a further sub-test on 40 seeds is carried out and the result added to the previous two sub-tests. This sequence is continued until a decision is reached. If a decision is still not reached after having tested, say, 520 seeds one would probably decide to regenerate; for it would then be clear that the true viability of the accession is very close to the regeneration standard.

The main advantage of this system is that it uses comparatively few seeds during the initial period of storage and it only begins to use quantities close to or in excess of the numbers used in a standard ISTA test when the true viability of the accession is closely approaching the regeneration standard. This is illustrated in Table 3 which assumes a regeneration standard of 85% and a batch size for the sub-tests of 40 seeds. It shows the average number of seeds which would need to be tested for accessions of various true levels of viability. All test procedures are subject to sampling error. Accordingly there is always some probability that the procedure advocated will call for regeneration when the true viability of the accession is higher than the regeneration standard. Alternatively, it may not call for regeneration when the true viability has in fact fallen below the regeneration standard. The probabilities of calling for regeneration are also shown in Table 3, from which it can be seen that procedure is relatively safe and that unnecessary regeneration (i.e. regeneration when the true viability is greater than the regeneration standard) is minimised. On average, this monitoring procedure will use half the number of seeds compared with a procedure of comparable reliability using a fixed sample size.

Table 3. The average numbers of seeds tested for accessions of different levels of true viability before reaching a decision and the probability of reaching a decision to regenerate assuming sub-tests of 40 seeds and a regeneration standard of 85% when using the test plan shown in Table 2 (adapted from Ellis, Roberts and Whitehead, 1980).

True percentage viability of accession	No. of seeds tested (average)	Probability of initiating regeneration procedure
97.5	80	0.000
95	120	0.000
92.5	160	0.001
90	280	0.05
87.6	400	0.5
85	240	0.95

THE INITIAL VIABILITY TEST AND APPROACHES TO REDUCING THE WORK LOAD WHEN MONITORING ACCESSION

When an accession is registered at a seed bank there is some advantage in knowing not only whether the viability is greater than the regeneration standard but also in estimating what the viability is. Because of this it may be best to adopt a germination test of fixed sample size to estimate initial viability, then to use the sequential probability ratio test for subsequent viability monitoring tests. Thus it may be useful to make a distinction between the initial viability test and subsequent monitoring tests. One advantage of estimating initial viability is that it will give some indication of the initial seed quality of the accession which in turn will give some indication of storage potential and thus guidance as to the frequency of subse-

quent monitoring tests. Furthermore if a large number of accessions of a species are received in a particular year it would be possible to consider whether it is necessary to subject all those accessions to the full programme of monitoring tests. For example it might be decided to monitor only certain representative accessions (including some of relative low initial viability) and assume that the remaining cohort of accessions will behave in a similar fashion. If this policy is adopted then the complete cohort would only be subjected to a monitoring test when the results on the representatives of the cohort indicated that this would be prudent, i.e. when the representatives showed evidence that viability had approached the regeneration standard. But all this would depend on having a tolerably accurate estimate of the initial viability on all members of the cohort, and 400 seeds is probably a minimum for this purpose.

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DISCUSSION

Chairman: Bekendam

Participants: Roberts, Ellis, Smith,
Linington, Astley, Bean,
Mumford, Holly

1. The Practical Usefulness of Sequential Viability Testing in
Different Situations

Much discussion centred around defining the real advantages for various uses of the method of sequential testing. Drs. Bekendam and Astley along with Mr. Linington, could see little advantage in the method where there were sufficient seed to pursue standard (ISTA or similar) tests, or where it would be necessary to have many different sets of tables referring to different species with different regeneration standards and batch sizes.

In answer Prof. Roberts stressed that he didn't propose that genebanks should start using sequential testing immediately. Its major benefits are economy in the numbers of seeds used in testing and a reduction in the chance of erroneous and thus unnecessary regeneration, an expensive process. The method of sequential testing is presented to genebank managers as another method available where these two criteria are important. It was generally agreed that the method is potentially more useful where a limited number of species are encountered such as in a cereals genebank or at NVRS Wellesbourne (Dr. Astley), than at the Kew Seed Bank (Wakehurst Place) for example, which deals with wild species from many widely different families. In the latter situation the "cohort" approach would certainly be inappropriate.

2. Setting the Regeneration Level

In response to a query from Dr. Bean regarding possible changes in genotypic variation in a seed lot with reduction in viability level, Prof. Roberts reconfirmed there is no desire to be dogmatic about setting regeneration standards - at 85% or any other; they need to be set according to the requirement for a particular species. In general they should be as high as is practicable, there being at least three reasons for this:

- a) It is well established in a number of species - that there is a strong correlation between loss in viability and frequency of point mutations arising in storage. There is no threshold for this and it is impossible to restrict it to an acceptable level. As an approximation a loss of 50% viability is more or less equivalent to treating a batch of seeds with 10,000 Roentgen of X-rays. It is up to plant breeders to decide where they are concerned about such an equivalent high dose.
- b) Many accessions will be of cross-pollinating species or mixed batches; primitive accessions even of self-pollinating crops will have mixed genotypes. There is a genetic component to potential longevity. The problem of changes in gene frequency with decline in overall viability has been highlighted by means of a model in the earlier paper by Ellis. (See also discussion of this paper.)
- c) As viability is lost, so also is vigour and so eventually difficulties would be encountered in raising sufficient healthy plants in the field to provide regeneration stock.

3. Monitoring Intervals

When Mr. Smith voiced the fears of many genebank managers about the uncertainty of the length of monitoring intervals, Prof. Roberts admitted that it is indeed a difficult decision to make especially at an institute like Wakehurst Place with its wide

range of accessions, compared with say IRRI, dealing in one crop e.g. rice. It is necessary at present to set many monitoring intervals simply by reputation, rice and wheat for example are known to have very much better longevity than parsnip, carrot and onion, the two Umbellifers often being of comparative low initial viability.

In cases where little is known it is probably best to go for something between the two extremes, with a substantial failsafe margin. Also it is not necessary to fix a monitoring interval and stick rigidly to it; the best policy would seem to be one of initial pessimism followed by a gradual lengthening of the interval should it be warranted. Dr. Ellis pointed out that the Reading group are presently tackling the complex statistics of such a procedure and especially that when material is accessioned into the bank close to the regeneration standard. The monitoring interval of the notably short-lived species carrot, parsnip and onion would be of the order of only a few years, perhaps not even 10. Prof. Roberts amplified the last remark by referring to his quoted figures (from Braunschweig) from his paper to quote the example of onions reaching a genebank with a real viability of 99% and a regeneration level of 88% led to a regeneration period of 28 years; but in reality, with confidence limits involved, monitoring should begin very quickly indeed.

Dr. Mumford wanted to know more of the practical difficulties of regeneration from people actually carrying it out, whether they worked to a standard and whether they regenerated every year or avoided it whenever possible. Dr. Astley replied that as a genebank manager one of his biggest problems is receipt of material which is sub-standard with low quality and quantity at the very outset. This situation is exacerbated by the fact species such as carrots, parsnips and celery grown with great care under the best conditions fail to exceed 70% initial viability. It is thus

necessary to set regeneration standards at Wellesbourne for most of the vegetables very much lower than those for cereals in order to avoid the need for regeneration immediately following accession. As a result of low viability accessions the regeneration facilities at Wellesbourne are already saturated for the next five or six years. Dr. Holly reported on extensive regeneration carried out in Hungary and was able to concur with Astley on the problems of high regeneration standards in relation to low initial viability. An added problem (mentioned by Roberts) with those accessions is low % emergence - to which Mr. Linington suggested transplanting seedlings produced under optimum conditions in the laboratory.

Dr. Holly's observation, that seeds of inbred lines cannot be successfully stored for as long as those for hybrids, prompted Prof. Roberts to mention one of his current projects; the comparison of hybrids vs. inbreds in maize. The results so far suggest that initial longevity is affected by both varietal differences and by differences in conditions during maturation; but that both these manifest themselves by affecting K_i ; i.e. environmental and genotypic effects seem to affect individual seeds by the same amount. If the approach described by Dr. Ellis in his paper (this volume) is followed and the lines are straightened by probit analysis, only two possible changes can be made to those straight lines - either a change in the slope or the intercept. It is the general experience of the Reading group (and others) that genotype only alters intercept, while storage environment alters the slope. There has been no example yet of a third possibility, where both are changed.

4. Use of Germination Rate

Mr. Smith suggested that with the apparent relationship between final % germination and mean germination period, perhaps genebank

personnel should take more account of it. Especially in high viability accessions where final percentage germination may show no apparent drop, an increase in mean germination period may warn more of an impending drop in viability. Both Prof. Roberts and Dr. Ellis agreed that it could perhaps be used as a warning signal, but were wary of introducing it as standard procedure without people being aware of its pitfalls. Even if it is possible to assume that rate of germination (mean germination period) is a good measure of vigour, it is possible that there will be genotypic differences with regard to germination rate in different species which have nothing to do with vigour. Also, rate can be very sensitive to temperature, thus good temperature control in the monitoring test is essential for the accurate measurement of germination rate.

5. Sampling for Monitoring

Dr. Bekendam and Prof. Roberts discussed sampling and concluded that both the approaches available had problems. If sub-samples are pre-packaged for ease of access when the time comes for monitoring, the complication of non-homogeneity of storage environments is always possible. The alternative is to refer back to the batch in the seed bank every time. This raises the question of losses in viability every time the container is brought out to ambient conditions, rapid condensation causing potential increases in moisture content. This problem can be overcome by ensuring that upon removal from the bank temperature equilibration takes place slowly in a dry (air conditioned) room; and that the container is open for the minimum time necessary to remove the sample.

Identification of Future Research Needs

E.H. ROBERTS

The organising committee felt that before proceeding to draft formal resolutions at the end of the conference, a discussion of priorities for future research be initiated by Prof. Roberts, and based on his own list of topics would lead, hopefully, to a consensus view.

Prof. Roberts stressed that his own short list was purely personal and in no particular order of priority. He also drew attention to the fact that subjects such as pathology, pests and diseases for example, are not his particular speciality and because they had occurred earlier in the conference, he may have forgotten the salient points raised. He called on other participants to bring these points forward.

The areas covered by Prof. Roberts were as follows:-

1. Accessions of High Quality Seed

There now seems to be general consensus that it is important to aim to get high quality seed into the bank in the first instance. Nevertheless, this is sometimes easier said than done, as collectors in the field face many difficulties. Significant losses in seed quality can occur before seeds get to the genebank and there are several instances where this has been serious. The main problem seems to be to alleviate the difficulties of the hard-pressed collector, working in the wild, in a landrover (vehicle), on horseback or even on foot. If usefully large collections are to be made and their viability maintained, hard work is involved in both collecting and preliminary processing, before the collector returns to "civilisation". There seem to be possibilities

for alternative approaches to practical techniques, seed drying being a prime example, bearing in mind that the processing must be carried out in a vehicle, say, operated simply and safely, without electricity, etc. There is no need to discuss alternative approaches now, but they definitely constitute a useful area for research and development, the sooner the better.

2. Conditioning of Dry Seeds After Dry Storage

This point refers in particular to damage which may occur on re-imbibition when seeds are placed in contact with free water. The major examples so far described are among the Leguminosae. The consequences of this problem affect both the results of viability tests and germination instructions sent out with samples to the users. There have been no serious studies to establish the range of plant families in which the problem occurs. At least in the Leguminosae it appears possible to overcome the problem by careful "conditioning" at high RH prior to imbibition, but the technique requires development for specific situations. Nonetheless the balance of the advantage still lies strongly in favour of storage at low moisture contents in genebanks, because of the enormous increases in longevity thereby achieved.

3. Techniques for Dormancy Breaking

From the length of discussions after the several papers on the subject, it appears these techniques for breaking dormancy are a serious problem. Being concerned with mechanisms and model systems, much of the available literature is neither helpful nor practical for application by genebank managers. The situation in genebanks is likely to be exacerbated by the fact that (i) the need to move seed quickly from collection site to genebank minimises the chances for loss of short-term dormancy, and (ii) low temperatures in genebanks tend to maintain any dormancy. Coupled

with these problems, ISTA recommendations are sometimes inadequate e.g. in rice, where the recommendations do not work for primitive cultivars and wild species such as Oryza glaberrima.

Related to this, Mr. Cooper suggested that a further extension of the observations of Dr. Atwater who concluded after long experience with seeds of a wide-range of ornamental plants, a relationship exists between morphological structure and the dormancy breaking treatment required. Without denying Dr. Atwater's extensive experience, Prof. Roberts felt that the experimental evidence for such a correlation is meagre.

Dr. Hagen wondered if there was an established correlation between dormancy and viability, raising the possibility of inducing dormancy to prolong viability, as an alternative to a seed store. Prof. Roberts said that there is not much evidence for this kind of correlation but research into the possibilities of increasing or prolonging dormancy by holding seeds in conditions which induce secondary dormancy may be profitable.

4. The Effects of Low Temperature Seed Storage on Seed Vigour

Perhaps the most serious and urgent problem arising from this meeting is the suggestion that low temperature storage may adversely affect seed vigour made by Mumford and Friere. Its urgency stems from the fact that IBPGR recommendations for genebanks are -18°C or -20°C , and most genebanks are now being set up on these standards. Confirmation of the results obtained at Birmingham are needed as rapidly as possible; any laboratory with the facilities available should take this problem seriously and look at it.

In addition to Prof. Roberts' points, two further ones were raised:-

5. Differential Genotypic Elimination With Storage

Dr. Tyler reminded the conference of earlier discussions on the effects of storage on differential genotypic elimination, especially in relation to outbreeding species. He stressed the need for an investigation into any genetic shift and its consequences for regeneration standards.

6. Surveys of Pests and Diseases in Gene Centres

Prof. Neergaard proposed that surveys of pests and diseases in the gene centres of various species are urgently required. For maximum efficiency the expeditions need to be set up with the specific aim in mind, rather than the odd pathologist or entomologist being sent with seed collectors on collecting expeditions/trips.

Workshop Resolutions

J.T. WILLIAMS

1. QUARANTINE FOR PESTS AND DISEASES

The importance of quarantine should be recognised and placed in its true perspective. More genebank staff should be trained in quarantine procedure and where possible genebanks should become registered quarantine stations. In the interim, national quarantine services should play a part.

Because a by-product of genebank procedures are seedlings (from viability testing) and damaged seeds (from cleaning), these should be more often used for pathogen screening of accessions. The information accumulated about each seed lot perhaps should be passed onto the user.

2. SEED VIABILITY (IN RELATION TO COLLECTION)

It is important that high viability seed is placed in genebanks. This should be achieved by taking more care of seed by collectors while on expeditions. The development of field drying techniques will be necessary to this end.

Seeds should be collected at or just slightly before physiological maturity.

On return to the genebank, seeds should be placed as soon as possible into a cool, low humidity room in order to further arrest viability loss.

The problems of collecting seed from markets must be recognised. Such seed is often up to one year old and often of

unknown history. Information on the seed's history can be useful to the genebank manager in helping to make decisions as to its likely lifespan.

3. DRYING OF SEEDS

Drying that is beneficial to the maintenance of viability involves forced ventilation of seed lots with dry air at low temperatures. Optionally these conditions should be 15°C and 15% R.H.

It should be noted that rehydration problems exist particularly with legumes and rehumidification should become more common practice (even for seed lots not at very low moisture content).

4. CONTAINERS AND STORAGE CONDITIONS

The most appropriate containers for long-term storage are food tins, laminated foil packets and certain types of glass and metal containers with reasonably airtight screw tops.

It should be noted from the lectures that low temperature storage may affect seed vigour and high priority needs to be given to research on this topic. Many long-term stores use temperatures in the region of -20°C.

5 VIABILITY TESTING AND DORMANCY

ISTA rules should be used only as a guideline. Genebank managers must accumulate and communicate experience of different species.

Testing must be carried out on representative samples.

More study is required on dormancy breaking techniques. In some cases mixtures of agents might be required e.g. for rice.

Where batches of accessions of a given crop are tested by different genebanks, the dormancy-breaking methods should be standardised to allow for analysis of success rate.

Genebank managers should take note of the usefulness of tetrazolium chloride as a vital dye for distinguishing between dead and dormant seed. They should of course be aware of the problems associated with the method.

6. MONITORING VIABILITY

Sequential testing offers many advantages in viability monitoring to aid decision making and to save valuable germplasm. In the same way, monitoring the viability in a few members of a "cohort" of accessions of the same type from the same area should be contemplated.

Loss in viability should not proceed too far before regeneration takes place. Regeneration standards of 85% for wheat and 75% for maize are acceptable. With regard to other crops there must be more consensus as to sensible regeneration standards. This might be achieved by discussion at the relevant Eucarpia Genebank Committees.

There should be more work on understanding the degree of selective elimination of genotypes during storage and regeneration particularly in outbreeding species.

ABBREVIATIONS

AVRDC: The Asian Vegetable Research and Development Center
(Taiwan, China)

CIAT: Centro Internacional de Agricultura Tropical
(Colombia)

CIP: Centro Internacional de la Papa (Lima, Peru)

FAO: Food and Agriculture Organisation of the United
Nations

IBPGR: International Board for Plant Genetic Resources (Rome,
Italy)

ICRISAT: International Crop Research Institute for the Semi-Arid
Tropics (Hyderabad, India)

IITA: International Institute of Tropical Agriculture
(Ibadan, Nigeria)

IRRI: International Rice Research Institute (Manila (Los
Banos) Philippines)

RBG: Royal Botanic Gardens (Kew, England)

UNEP: United Nations Environment Programme

WPBS: Welsh Plant Breeding Station (Aberystwyth, Wales)

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