CHAPTER 7

The Measurement of Viability

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Viability is usually measured in order to assess the suitability of a seed lot for a particular purpose, most commonly to produce a crop but also for industrial purposes, especially the malting of barley. Techniques for its measurement are also essential in research. Different criteria apply according to the purpose of the examination. In order to judge the suitability of seed for sowing it is necessary to determine its ability to produce plants in the field, so that information is required about the development of the seedling following rupture of the seed coat. But for the assessment of malting quality tests are needed to indicate the number of live grains, the incidence of dormancy and the most suitable steeping procedures (Pollock, 1962); they take no account of seedling development after sprouting (Institute of Brewing, 1967) since this stage is not involved in the malting process. This chapter will deal with the measurement of viability for crop production purposes, and the special circumstances of industrial processing will not be considered further; nor will there be more than very occasional reference to tree seeds.

The results of germination tests are used in two ways: to determine the suitability of a seed lot for sowing and to compare the value of different lots, so providing a basis for trade in seeds. For the former it is essential that they provide a realistic assessment of field planting value; for the latter they must be completely standardised and reproducible within narrow limits when tests are repeated. The most commonly used test demonstrates the ability of a population of seeds to produce plants in the field by exposing it to conditions in the laboratory which are optimal for germination; the capacity of the seedlings produced to develop into normal plants is then judged on the basis of a careful examination of their root and shoot systems. Wellington (1965) has traced the origin and development of this type of test from the principles outlined by Nobbe in 1876, and has shown how the commercial concept of testing, with its emphasis on reproducibility, has been gradually reconciled with the agricultural concept, which stresses provision of the best estimate of planting value.

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This has been achieved largely by the adoption of standard methods of seedling evaluation, including agreed definitions of normal and abnormal seedlings based on the development of essential structures, which are incorporated in International Rules for Seed Testing (International Seed Testing Association, 1966).

A number of indirect methods for measuring viability have also been devised, most of which depend on examination of the metabolic activity of the seed. Their development has been reviewed by Holmes (1951) and Barton (1965). The most widely used of these tests involves treatment of the seed with tetrazolium salts; these are colourless in solution but are reduced in living tissue by enzymes of the dehydrogenase group to the formazan, which is red-coloured, stable and non-diffusible (Bulat, 1961). An assessment of viability is based on examination of the distribution of stained and unstained areas of the embryo (Lakon, 1949).

Sampling and the preparation of seed

Whatever method of measurement is applied it is only practicable to test a very small proportion of the population about which information is required. Crop seed is rarely pure, so that sorting is necessary to obtain the material for test. Thus, if results are to be comparable and reproducible, accurate methods of sampling must be employed and the procedure for selecting the seed must be rigorously standardised.

Procedures for sampling crop seed are prescribed in International Rules for Seed Testing (ISTA, 1966) and embodied in the legislation of many countries. Mullin (1968) has outlined the principles and some of the practical problems which arise; the most commonly used equipment is described in Agriculture Handbook No. 30 (United States Department of Agriculture, 1952). Seed lots are never perfectly homogeneous; the variation inevitably present in a crop is not completely eliminated during harvesting, cleaning and processing. Further segregation of material may occur in bagging and handling, and viability may be differentially affected through mechanical injury, the uneven distribution of moisture during storage or the uneven application of pesticide treatments. In order to obtain a reasonably representative sample for test, primary samples must be drawn from a sufficient number of containers, at different depths and horizontal positions. When seed is sampled from a stream, as in a cleaning machine, the entire cross-section of the stream must be sampled and primary samples must be drawn at intervals throughout the process;

automatic samplers can be built into seed cleaning systems, and are satisfactory provided they fulfil these conditions.

The sample collected from the bulk is too large for testing and special laboratory methods are applied to obtain accurate working samples. A number of mechanical dividers may be used, including soil dividers and centrifugal dividers which are operated on the principle of mixing the seed and then halving it continuously until the appropriately sized working sample is obtained. For laboratory germination tests the final sampling and planting operations can be combined by the use of a vacuum planter. This consists of a flat plate with a series of equally spaced holes drilled in it; it is fitted over a hollow head connected to a vacuum source, with a valve which allows the vacuum to be switched on and off at will. Seed is scattered on the plate so as to cover the surface; the vacuum is then applied so that seeds which happen to rest over a hole are held, while the remainder can be shaken off. By reversing the plate over the seed bed and releasing the vacuum the required number of seeds are positioned on the substrate.

Many seed samples contain impurities such as weed seeds and seeds of other crop species. The viability of this material may be of importance in assessing the extent to which it might contaminate a crop, but it is usually impracticable to test it separately because of the small numbers present. Seed impurities, as well as other material such as chaff, soil, etc., are therefore removed before the pure seed is subsampled for the germination test.

While it is often easy to distinguish impurities there are occasions when decisions are difficult, for example in the case of broken seed, or immature, undeveloped or diseased material. The results of a germination test can be greatly influenced by decisions on whether or not such structures should be included. General definitions are prescribed in International Rules for Seed Testing (ISTA, 1966). These are based on the principle that any material which might conceivably germinate should be tested. Thus undersized, shrivelled, immature and sprouted seeds are included, but grass florets without a caryopsis are not. In order to obtain uniformity of interpretation an element of arbitrariness is inevitable: only broken seeds and caryopses larger than one half the original size are included, no attempt being made to determine the presence or absence of an embryo; clusters or pieces of cluster of *Beta* are tested, regardless of whether they contain fruits, provided they do not pass through a sieve with 1.5 mm × 20 mm slits. Detailed descriptions of structures which are classed as pure seed, and are therefore included in germination tests, have been published for all crop seed genera (MacKay, 1969).

The laboratory germination test

The result of a laboratory germination test indicates the percentage of pure seeds which have produced seedlings, capable of continued development into mature plants, when germinated under standardised conditions of substrate, moisture supply and temperature to ensure that the result is reproducible (Wellington, 1966). Its reliability, both in terms of measurement of agricultural planting value and reproducibility for commercial purposes, depends upon the extent to which the seed environment can be controlled at the optimal levels for rapid and complete emergence, and development of the germinated seedlings to a stage at which their condition can be correctly evaluated.

The principal environmental conditions which must be available for seed germination are an adequate water supply and suitable temperature and composition of gases in the atmosphere. Requirements vary according to species and are determined both by the conditions which prevailed during seed formation and even more by hereditary factors (Mayer and Poljakoff-Mayber, 1963). Populations of crop seeds are likely to be more physiologically and genetically uniform than seeds collected from a series of wild plants; a seed lot will commonly be harvested from a single crop, while the breeding of crop varieties and modern seed production methods favour stability and uniformity. Nevertheless the seeds within a sample may vary in their requirements for germination because of, for example, differences in maturity between seeds from different plants or from different positions on the same plant; they may also have been unequally affected by processing or storage. The optimum conditions for different stages of germination and seedling growth are not identical; while it might be possible to devise a programme for varying the environment to suit particular stages, it could not be applied to a population of seeds since their germination is not synchronised. Thus it is only possible to provide conditions covering the range which is most favourable to the majority of seeds in a sample, probably representing those levels which have the least limiting effect on each of the intermediate stages (Wellington, 1965). In practice it is necessary to determine conditions which are suitable for a particular species, since in routine seed testing it would be out of the question to determine experimentally the conditions to be applied to each sample. The aim has been to find a combination of conditions which will give the most regular, rapid and complete germination for the majority of samples of the same species (Brett, 1939).

SUBSTRATE

Natural soils vary greatly in their structure and in their chemical and biological content. Although it might be argued that tests made in soil would be undertaken in conditions more closely resembling those to which the seed would be exposed when planted in the field, to which the results would then be more relevant, the loss of reproducibility and the inability to obtain comparable figures for different seed lots outweigh any advantage (Saunders, 1923). Artificial media may be much more readily standardised.

Paper is used extensively in seed testing, and is particularly suitable for small seeds and for seeds which may require light for germination. It should have an open, porous formation and be free from defects and impurities which might affect its performance, or toxic substances which might injure the roots of germinating seedlings. It should be free from fungi or bacteria which might interfere with seedling growth; sterilisation may be necessary, but it should not be treated with chemicals which might suppress disease organisms on the seed as well as in the substrate. Its texture should be such that the roots of germinating seedlings grow on, and not into, the paper, so that they are clearly visible for evaluation (Colbry, 1965). Paper should be strong when wet and thick enough to supply adequate moisture. There must be a high degree of uniformity between different sheets and across the surface of individual sheets. Detailed specifications are included in International Rules for Seed Testing (ISTA, 1966).

Light requiring seeds are planted on top of moist paper, and placed either on a Copenhagen tank (Jacobsen apparatus), which provides a supply of water throughout the test period by means of a wick dipping into a reservoir (see later), or in an incubator. Since germination is dependent on water uptake from the substrate exceeding water loss to the atmosphere (Benton, 1965), it is essential to maintain a high relative humidity over the exposed surface of the seed. This is achieved by placing a bell jar or glass cover over tests on Copenhagen tanks, or raising the relative humidity of incubators by evaporation from water trays or by humidifying machinery. Seeds without a specific light requirement may also be planted on top of paper, or they may be placed between folded paper: this method brings a greater proportion of the seed's surface area into contact with the moisture-supplying medium. To prevent drying the folded papers can be inserted into envelopes or between sheets made from polyethylene film. A third method, most frequently used for larger seeds, involves the use of rolled paper. After planting the moist paper containing the seeds is

rolled and placed in an upright or slightly inclined position; this has the advantage of permitting the roots to grow straight downwards in response to geotropism, so avoiding the tangling of roots which can occur when papers are placed horizontally unless the seed is very widely spaced (Munn, 1950).

Paper substrates provide conditions favourable to the development and spread of fungi present on the seeds, which may interfere with seedling growth; seedling evaluation may be difficult when it is not known whether the source of infection is the seed whose viability is being judged or another seed. The problem is usually more severe in folded paper than in top of paper tests; it can be reduced by spacing the seeds widely, by removing infected and clearly dead seeds at intermediate counts, and by transferring ungerminated seeds to clean papers at intervals during longer tests.

Fungi develop less freely on sand; seeds can be buried or pressed into the surface, so that a more effective mechanical barrier is formed between them than is possible on paper. This substrate is not suitable for very small seeds, the manipulation and recovery of which would prove difficult, but it is widely used for larger seeds. Seeds are planted either pressed into the surface of the sand or covered by it to a depth of 1–2 cm, so that a substantial part of their surface area can be brought into close contact with the water in the substrate, thereby increasing the rate of water uptake and germination (Manohar and Heydecker, 1964; Sedgley, 1963).

Sand may be readily sterilised by heat to destroy bacteria, fungi and foreign seeds. Moisture content is standardised by determining the water holding capacity and subsequently adding sufficient water each time the substrate is prepared to bring the content up to the required level: under International Rules for Seed Testing (ISTA, 1966) this is prescribed as 50 per cent of moisture holding capacity for most kinds of seed, but 60 per cent for maize (*Zea mays*) and large-seeded legumes. Moisture holding capacity is determined primarily by the particle size distribution and the corresponding pore size distribution; although there has been little detailed study of the ideal particle distribution (Kåhre and Wiklert, 1965), it is necessary to sieve out particles which are too large or too fine, and a range of 0.8 mm to 0.05 mm is recommended in International Rules. It is essential that toxic chemicals should be absent; these might either be present initially or could accumulate as a result of re-using sand in which seed treated with a pesticide has been previously tested.

Seedlings growing on artificial substrates can usually be readily evaluated, but sometimes they are affected by chemicals or pathogenic

or saprophytic fungi, or exhibit physiological defects the extent of which is not readily apparent. The use of artificial media may exaggerate these conditions, and the substitution of soil or compost as substrate, under standard laboratory conditions, may provide a more accurate reflection of the seed's potential field performance (Tonkin, 1969). Thus seed treatment with fungicides and insecticides often gives rise to phytoxicity in seedlings grown on sand or paper (Brett and Dillon Weston, 1941; Lafferty, 1953; Thomson, 1954), but its incidence may be reduced when the seed is sown in the field or in a laboratory medium containing soil (Wellington, 1957). The tissues of seedlings of broad beans (*Vicia faba* var. *faba*) sometimes develop conspicuous blackened lesions in laboratory tests (Crosier, 1951), but the condition was alleviated when John Innes potting compost (Lawrence and Newell, 1939) or a proprietary soilless compost ('Levington' compost) was substituted for a sand substrate (Official Seed Testing Station for England and Wales, 1964, 1970). When doubts arise about the evaluation of seedlings grown on artificial media, tests can be repeated in compost; the seedlings can then be grown to a more advanced stage when the condition of the structures can be readily assessed.

The principal function of the substrate is to supply moisture. International Rules for Seed Testing prescribe the extent to which sand should be moistened, but there are no precise standards for the amount of water to be added to paper media, only a requirement that they should not be so wet that a film of water forms around the seeds. Collis-George and Sands (1961) have interpreted this as being at an indefinite suction approaching zero (free water), and point out that suction would not necessarily be constant throughout the test; they therefore recommend the use of tension plates and the prescription for each species of suctions optimal for germination.

The supply of oxygen in laboratory germination tests is largely determined by the moisture conditions: seed may not germinate when the water supply is inadequate (Chowings, 1970), but excessive water physically impedes the uptake of oxygen (Chetram and Heydecker, 1967; MacKay and Tonkin, 1965; Orphanos and Heydecker, 1967) which may also be competed for by fungi and bacteria in the covering structures (Gaber and Roberts, 1969; Roberts, 1969).

TEMPERATURE CONTROL AND GERMINATION EQUIPMENT

Mayer and Poljakoff-Mayber (1963) have defined the optimal temperature for germination as that at which the highest percentage of germination is attained in the shortest time, and the minimal and

maximal temperatures as the highest and lowest temperatures at which germination will occur. In laboratory seed testing the temperature at the level of the seed must be controlled near to the optimum. The values are genetically determined, varying for different species, but are also influenced physiologically (Lang, 1965). The range over which complete germination is attained may differ with the age of the seed, usually becoming wider as the seed ages; a regular alternation of temperatures is sometimes more effective than a constant temperature. Both these phenomena, however, are probably due to dormancy, whose importance in germination testing will be discussed later. The temperature regime selected in the laboratory is also influenced by the relative effects on seed germination and fungal development; Fritz (1966) has shown how the use of particular temperatures within the range optimal for the germination of disinfected cereal seed can result in a lowering of germination capacity in untreated seed through the activity of parasitic fungi.

Appropriate temperatures for each of the principal crop and tree species are listed in International Rules for Seed Testing. Precise temperature control demands special equipment, incubators and water baths being the most widely used. Incubators are used for many purposes besides seed testing and innumerable models are available. However, Oomen and Koppe (1969) have prepared a specification for an automatic cabinet incubator to provide standardised germination conditions, eliminating the need for daily attention and watering. This includes air temperature control adjustable between 10° and 35°C, the level for each regime to be uniform within ±1 per cent throughout the controlled area over a number of days. To allow for temperature alternation, the changes from the high to low and low to high phases should be achieved within 30 minutes, a steady temperature being reached in one hour. Air humidity should be as high as possible, but never lower than 90 per cent, and air movement should be low to prevent seeds from drying. A single change of air per hour is considered sufficient. There should be uniform illumination of between 750 and 1,250 lux at the level of the seed. Condensation should not occur. Four prototypes designed to meet these conditions were systematically tested and their adequacy discussed.

The Copenhagen tank (Jacobsen apparatus) (Fig. 7.1) was developed specifically for seed germination (Jacobsen, 1910). It consists of a bath containing water whose temperature may be controlled thermostatically. Seed is planted on top of paper and placed on a metal or glass strip suspended above the bath; the upper end of a paper or cotton wick lies beneath the seed bed and the lower end dips into the

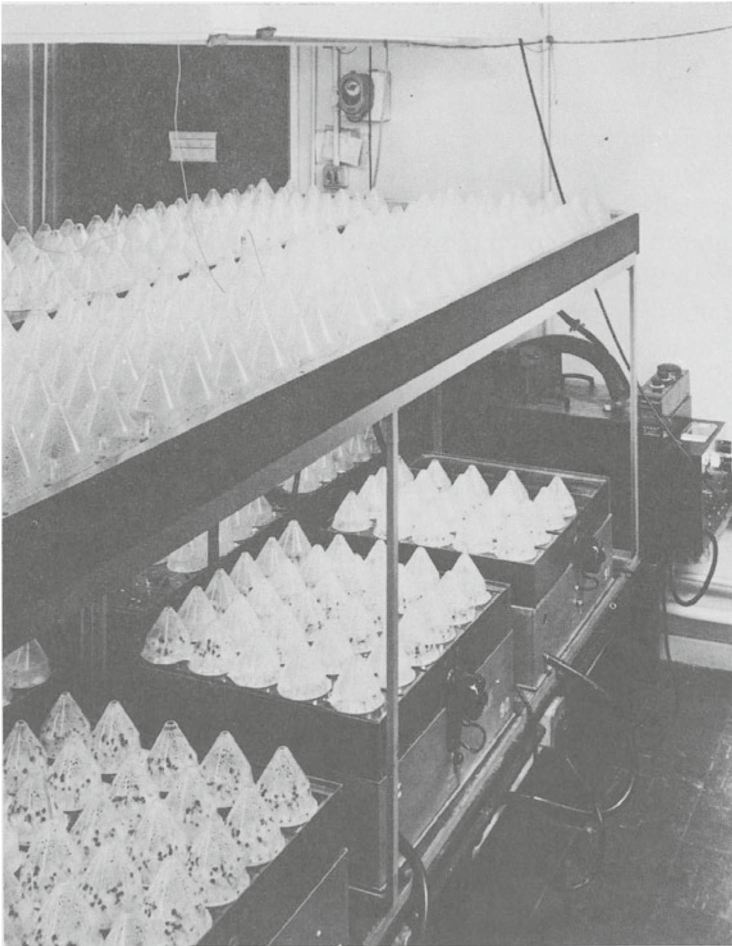

FIGURE 7.1 Copenhagen tanks for seed germination (Jacobsen apparatus). The upper tank is fully automatic, operated by the control box at the rear. (Photograph from the National Institute of Agricultural Botany.)

water. The moisture content of the substrate may be adjusted by altering the water level, and so increasing or decreasing the distance between seed bed and water (Kamra, 1968, 1969a, 1969b). High humidity is maintained around the seed either by covering the entire tank with a transparent lid or by placing bell jars over the individual replicates. The apparatus may be exposed to natural daylight, but artificial light is usually desirable, especially for light-sensitive seeds such as meadow grass (*Poa* spp.) and lettuce (*Lactuca sativa*); fluorescent tubes with a high spectral emission in the germination-promoting red region are recommended (ISTA, 1966).

One of the disadvantages of the standard Copenhagen tank is the absence of direct temperature control at the seed bed; this is influenced by the temperature not only of the water but also of the room, which must be controlled. Improved models which overcome this difficulty and permit rapid temperature alternation at seed level have been developed: Thomson (1962) incorporated panels of reconstituted wood pulp containing internal electric heating elements on which the paper substrate rested; Verhey (1955) and Overaa (1962) used hollow plates, through which water was circulated for heating and cooling, instead of the usual glass or metal strips. Marschall (1969) reduced the quantity of water whose temperate must be adjusted by placing small water trays, each large enough for the four replicates of one test, on standard central heating radiator plates, in order to accelerate heat transfer.

Improved seedling development occurred when the seeds were planted with the primary axis of the embryo vertically aligned, and apparatus has been developed in which they were attached to an inclined plane holding a paper substrate (Cobb and Jones, 1966; Jones and Cobb, 1963). A system for the germination of ryegrass seeds between paper on a vertical glass plate in order to determine the presence of fluorescent substances in their roots has also been described (Official Seed Testing Station for England and Wales, 1952).

When large numbers of tests are made whole rooms may be used as germinators, with temperature, humidity and light controlled. The precise control of large areas presents greater problems than arise with the use of cabinet incubators, and various methods have been applied (Clayton and MacKay, 1962; Verhey, 1955).

A system for the almost complete automation of germination testing has been described by Brandt (1964).

DORMANCY

The germination process can be blocked by various chemical and physical means anywhere along the chain of physiological events leading from imbibition to growth of the embryo (Evenari, 1961). This may be due to the absence of one or more conditions always essential for germination, such as moisture in seed kept under dry storage conditions (enforced dormancy); to incomplete after-ripening, the processes occurring within the seed subsequent to the harvest-ripe stage (innate dormancy); or to the imposition or re-imposition of a block as a result of exposure in the imbibed state to unfavourable conditions, such as excessive temperature (induced dormancy) (Har-

per, 1959). These three categories of dormancy are discussed in greater depth in Chapter 11 (p. 321) and the physiological nature of dormancy is discussed in Chapter 12 (p. 370). In laboratory germination tests enforced dormancy is eliminated by the provision of appropriate moisture, temperature and aeration, but live seeds may still not germinate because of innate dormancy unless the relevant blocks are removed through the use of external agents. Dormancy may also sometimes be induced during laboratory tests if conditions, especially temperature, are not adequately controlled.

A condition which delays or interferes with the full expression of a sample's germination capacity is troublesome in a seed testing laboratory. Since the degree of dormancy changes during after-ripening (Evenari, 1961) the precision with which test results can be reproduced at different times will be reduced unless special measures are taken to overcome the condition. But if the results of germination tests are to yield information on the field planting value of a seed lot removal of dormancy in the laboratory sample can only be justified if this condition is irrelevant to the field establishment of the remainder of the bulk at the normal time of sowing (Wellington, 1965). It has been generally assumed that after-ripening is complete by the time seed is sown in the spring following harvest, or that it will be exposed to conditions in the field, such as low temperature or exposure to light with shallow sowing, which overcome dormancy (Thomson, 1963b; Wellington, 1965); and so special treatments to overcome dormancy are applied in the laboratory whenever necessary (ISTA, 1966). The assumption is probably justified for the majority of traditional temperate crops, whose selection and breeding has tended to eliminate extensive dormancy (Evenari, 1961), but irregular establishment may sometimes occur when, for example, early autumn sowing of cereals closely follows harvest or when seed of pasture species more recently brought into cultivation from the wild is sown (Smith, 1968; Wellington, 1965; Whittet, 1952).

An exception to the general rule that dormancy should be removed in laboratory tests is made in the case of seeds with impermeable seed coats, the so-called 'hard seeds' which occur most frequently in the small seeded legumes. The field planting value of hard seed has been the subject of a number of investigations. After extensive tests Witte (1931, 1934, 1938) recommended that from 50 to 100 per cent, according to species, of the hard seed remaining at the end of a germination test should be added to the percentage germination figure. But Overaa (1960) found great differences between the laboratory germination of scarified hard seeds from different samples of red clover, and sug-

gested that tests after scarification were necessary for a proper judgement of the real value of impermeable seeds. Harrington (1916) concluded that a large proportion of impermeable seeds would germinate in the soil during the first few months after planting, some of them early enough to be of importance to the crop; the remainder would constitute a reserve which, under favourable conditions, might improve thin areas of the stand, although this would depend on the extent to which such areas had been colonised by more rapidly growing weeds. Field emergence has usually been studied in plots sown only with hard seed, so that the effect of competition with seedlings produced from permeable seeds has been excluded. Zaleski (1957) has shown that, in lucerne (*Medicago sativa*), only those seeds which germinate rapidly contribute to the crop, later germinating seedlings, which would include those from hard seeds, being eliminated. In seed testing no attempt is now made to allocate a planting value to impermeable seed; seeds which have not absorbed water at the end of the test period are reported separately as a percentage of hard seeds (ISTA, 1966).

The methods for overcoming dormancy in the laboratory vary according to the nature of the germination block as well as to its intensity. Thus different procedures may be necessary for different species, in different seasons and in seed from different sources. The seed is either specially pre-treated, for example with low temperature, before the germination test, or special conditions, such as light, are applied during the test.

The application of low temperature to seed in the imbibed state in the laboratory (often referred to as 'stratification', after the horticultural practice) is a procedure analagous to the situation in nature where seeds are shed in the autumn and then subjected to low temperatures under moist conditions in the soil, during which after-ripening occurs (Mayer and Poljakoff-Mayber, 1963). The method is effective for many species of agricultural and vegetable seeds (including the cereals), whose seed is first planted on the germination medium and then exposed to a temperature between 5° and 10°C for a period of up to seven days, after which it is transferred to the standard germination conditions for the full test period. It is usually necessary to instal refrigerators or cold rooms for this purpose, although some incubators are capable of operating at a sufficiently low temperature. Care is needed to ensure that the correct temperature is maintained for the full period required, since if it is too high dormancy may not be effectively broken, whereas if it is too low freezing injury may be inflicted (see pp. 36–37, and 140).

In most crop seeds dormancy is lost during after-ripening in dry storage. However, exposure to a short period of relatively high temperature may sometimes accelerate the process (Hite, 1923; Roberts, 1962, 1965; Stapledon and Adams, 1919; Wellington, 1956), although it is not always clear whether the effect is one of desiccation or of temperature (Hewett, 1958, 1959; Stokes, 1965). Seed is usually heated at a temperature not exceeding 40°C, with free air circulation, for up to seven days before being planted, although temperatures up to 47°C can be used satisfactorily for rice (*Oryza sativa*) (Roberts, 1965).

Etiolated seedlings are often difficult to evaluate and light may be applied in laboratory germination tests in order to influence seedling growth. However, this is quite distinct from the application of light to remove a block to germination in light-sensitive seed, for which the conditions are more critical. It is usually necessary to supplement daylight from an artificial source; fluorescent tubes with relatively high emmission in the red region are preferable since, for some seeds, incandescent-filament light and even diffused sunlight may be inhibitory because of their nearly equal red and far-red energies (Borthwick, 1965). An even intensity over the entire testing surface of 750–1,250 lux is prescribed in International Rules for Seed Testing (ISTA, 1966). Photo-dormancy may be induced in imbibed seeds kept in the dark at high temperature (Borthwick, Hendricks, Parker, Toole and Toole, 1952; Borthwick, Hendricks, Toole and Toole, 1954), so that it is necessary to ensure that temperature does not rise excessively during periods of darkness.

Dormancy often narrows the range of temperature over which germination takes place (Lang, 1965; Stokes, 1965). More complete germination may be obtained by exposing the seed to a temperature rather lower than the optimal for non-dormant seed: for example, under International Rules, cereal and clover seed may be tested at 15°C instead of at 20°C. Because germination may be slower at lower temperature the test period is extended. Lang (1965) has suggested that the promotion of germination by introducing a daily fluctuation of temperature does not reflect the removal of specific blocks, but rather an increase in the general physiological activity level of the seed. Cohen (1958) has discussed the possible mechanisms. Whatever its mode of action, the use of alternating temperatures often leads to more complete germination in laboratory tests, especially when a rapid fall from the high to the low phase is introduced. It is widely used, in conjunction with light during the high phase, in the testing of grasses and, with or without light, for many other species (ISTA,

1966). Tests may either be transferred between equipment maintained at the two prescribed temperatures, or the temperature may be varied in individual germinators. The high phase (which ranges from 25° to 35°C according to species) is maintained for eight hours and the low phase (between 10° and 20°C) for sixteen hours of each twenty-four.

A number of chemicals may influence the germination of dormant seeds. Thus potassium nitrate can replace the requirement for, or reinforce the effect of, other dormancy-breaking agents such as light and particular temperature regimes in large numbers of species (Evenari, 1965; Mayer and Poljakoff-Mayber, 1963; Stokes, 1965), and is widely used in seed testing. The substrate is moistened with a 0.2 per cent solution, prepared by dissolving 2 g in 1,000 ml of water, at the beginning of the test, but subsequently only water is used for further moistening (ISTA, 1966). The interactions between light, fluctuating temperatures and nitrate are considered in further detail in Chapter 11. At present potassium nitrate is the only chemical agent approved under International Rules, since until the introduction of gibberellic acid other chemicals were not generally suitable for breaking dormancy in seed testing (Wellington, 1965); but gibberellic acid has been shown to be capable of replacing requirements for light, low temperature and alternating temperatures in a number of species (Evenari, 1965; Nakamura, Watanabe and Ichihara, 1960; Tager and Clarke, 1961). On the basis of experiments with wheat (*Triticum* aestivum) and barley (*Hordeum vulgare*), Bekendam and Bruinsma (1965, 1966) have recommended pre-soaking the seed or treating the substrate with solutions of gibberellic acid at concentrations of from 0.02 to 0.2 per cent according to the intensity of dormancy. Kåhre, Kolk and Fritz (1965) obtained satisfactory germination of freshly harvested cereal seed when the substrate (sand) was moistened with a solution of gibberellic acid at a concentration of 200 ppm.

The dormancy of a range of small-seeded legumes may be broken by treatment of the imbibed seed with concentrations of carbon dioxide as low as 0.3–0.5 per cent by volume (Ballard, 1967; Grant Lipp and Ballard, 1959). If the seed is planted between folded paper, enclosed in a sealed polyethylene film envelope and exposed to standard germination conditions in a cabinet incubator, dormant seed with permeable testas is eliminated, apparently because of the retention of carbon dioxide from the respiration of non-dormant seeds in the sample (Thomson, 1965).

The dispersal unit in *Beta* spp. consists of the hardened receptacle and sepals of the flower cluster, in which the fruits are embedded.

This structure contains inhibitors (Battle and Whittington, 1969; Stout and Tolman, 1941; Tolman and Stout, 1940) which are water-soluble and interfere with germination in the laboratory, although probably not in the soil (MacKay, 1961). Before planting clusters are washed in water at 25°C for ½ to 2 hours; regular changes of the water are desirable during this period to ensure the removal of the inhibitors. If a number of tests are to be made the process can be carried out in a machine consisting of two tanks: in the upper tank water is heated under thermostatic control by an immersion heater, and then flows to the lower tank in which the clusters are placed in small tubes with apertures to allow access to the water; when the water has reached the correct level it is siphoned off and the tank refills. After washing the clusters are dried before planting, since the germination of very wet seed may be reduced through interference with oxygen supply (Bekendam, 1968; Heydecker, Orphanos and Chetram, 1969; MacKay, 1961). Cuddy (1959) reported that pre-washing might be detrimental to germination in some samples which had been processed to produce single-seeded fragments, in which the inhibitor content is reduced through loss of cluster material (MacKay, 1961).

SEEDLING EVALUATION

Seed is exposed to the conditions of the germination test for a prescribed period, the duration of which depends primarily on the species and may vary from as little as 6 days for radish (*Raphanus sativus*) to as much as 35 days for Johnson grass (*Sorghum halepense*), or even longer in the case of certain tree seeds (ISTA, 1966), being fixed at a level where complete germination can be expected for the majority of samples under the prescribed environmental conditions; but the test can be extended for up to seven days if some seed is just beginning to germinate at the end of the period. Seedlings which have developed sufficiently to be correctly assessed are removed at intervals during the test to avoid over-crowding and restrict the spread of fungi. These interim counts were formerly regarded as having a bearing on the relative field performance of different seed lots (Brett, 1939), rapidly germinating lots with high interim counts being of greater value than those germinating more slowly. But the figures can be misleading since they are affected by dormancy and by the need to delay decisions on seedlings of questionable value until further development has taken place, so that the concept has now been generally abandoned (Verhey, 1960). Tests can be concluded before completion of the prescribed period if full assessment of all seeds and seedlings has been possible.

The definition of germination for seed testing purposes differs from the more strictly botanical definition quoted by Evenari (1961) as the processes starting with the imbibition of the dispersal unit and ending with the protrusion of the embryonic root which take place inside the dispersal unit and prepare the embryo for normal growth. Wellington (1965) has explained how it has been evolved in terms of development of the seedling to a stage where it can be inferred that it would have been capable of emerging through the soil and sustaining autotrophic growth, thus involving a degree of seedling growth after completion of the physiological process of germination: in International Rules for Seed Testing (ISTA, 1966) germination in a laboratory test is now defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seed being tested, indicate the ability to develop into a normal plant under favourable conditions in soil. This involves not only the provision of optimal conditions to permit rapid and complete emergence, but also the detailed examination of the structures produced by each seedling and the formation of a judgement on the condition of each of these in terms of its potential influence on field performance. Wellington (1968) has outlined the development and structure of each of the essential structures for the main groups of crop plants, a knowledge of which is essential for the correct and uniform application of the principles of seedling evaluation.

To be classed as normal the seedling requires a well-developed root system. For plants whose commercial product is a swollen tap root, with or without stem tissue, such as turnip (*Brassica rapa*) or carrot (*Daucus carota*), an intact primary root is clearly indispensable to the development of a normal plant. But in a number of genera entirely satisfactory plants can be produced from seedlings with the primary root injured or absent, provided secondary roots have developed sufficiently to support the seedling in soil; these genera are listed in International Rules as *Pisum*, *Vicia*, *Phaseolus*, *Lupinus*, *Vigna*, *Glycine*, *Arachis*, *Gossypium*, *Zea* and all *Cucurbitaceae*. Many of the *Gramineae* species produce seminal roots, in which there is no clearly visible distinction between the primary and secondary roots; such seedlings must have at least two well-developed roots if they are to be classified as normal.

The hypocotyl, that part of the primary axis of the seedling lying immediately below the point of attachment of the cotyledons, represents the region of transition from root to stem. In species with hypogeal germination, such as *Vicia faba*, it remains below ground and is rather short and root-like; but in species with epigeal germina-

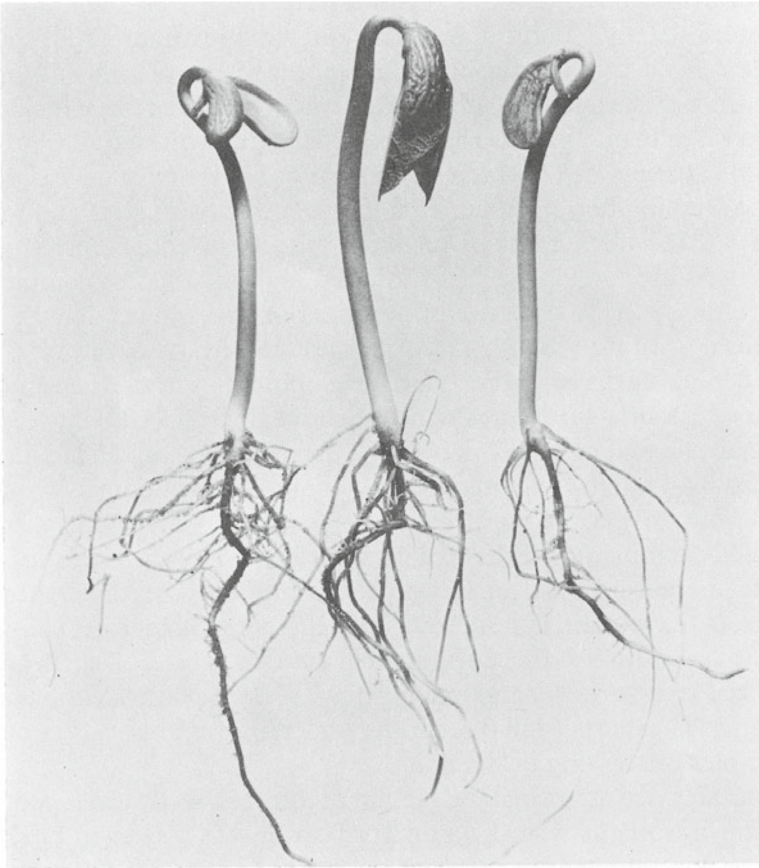

FIGURE 7.2 Dwarf bean seedlings at the conclusion of a germination test. Centre seedling shows normal development; the others show injury to the plumular bud precluding development into normal plants. (Photograph from the National Institute of Agricultural Botany.)

tion, such as *Phaseolus vulgaris*, it extends to carry the cotyledons above ground, and its upper part is stem-like in appearance. In a normal seedling from a laboratory test the hypocotyl should be well-developed and without damage to the conducting tissues, although superficial damage may be present provided it is limited in area.

The epicotyl extends between the point of attachment of the cotyledons and the stem apex. In species with hypogeal germination its lower part is below ground. In a normal seedling it should be intact, apart from limited superficial damage, and terminate in a normal plumular bud. This consists of an apical meristem and leaf primordia enclosed by the developing leaves. In many species detailed examination of the condition of the plumular bud is often impossible during

the period of a germination test, and its ability to develop normally is assumed if, at the conclusion of the test, there is no evidence that the surrounding tissues are damaged or decayed (Wellington, 1970). But in some species, especially large-seeded legumes, the primary leaves may show injury which can affect plant performance (Verhey, 1961), so that the final assessment must not be undertaken until they are clearly visible (Fig. 7.2).

The cotyledons are the first leaves of the plant. In dicotyledons they may emerge and perform a photosynthetic function for a time as foliage leaves or be adapted for storage of food reserves. The single cotyledon of the monocotyledons may protect the emerging shoot during its passage through the soil, at the same time remaining in contact with the endosperm from which the food reserves are absorbed; or it may be substantially modified to form the scutellum of the *Gramineae*, which also functions in the utilisation of food reserves by the developing embryo. The presence of functioning cotyledons is thus necessary for normal seedling development, and must be determined in the germination test. In the case of dicotyledons one cotyledon is considered sufficient to support normal development.

In the *Gramineae* the shoot system arises from a plumule containing leaf primordia and the stem apex, surrounded by a coleoptile formed from the sheath of the first leaf. The coleoptile is negatively geotropic and also protects the developing leaves until they emerge from its tip above the level of the soil. For normal development the plumule must be intact, except for superficial local damage to the coleoptile which does not penetrate to the enclosed leaves; there should be a well-developed green leaf within or emerging through the coleoptile (Fig. 7.3).

In a laboratory test the majority of the normal seedlings are usually removed at interim counts, but the assessment of many of the doubtful and abnormal seedlings must be left until its conclusion to ensure that slower growing but otherwise normal seedlings are not incorrectly classified; but it is desirable to count and remove diseased seedlings as soon as their condition can be assessed, to prevent infection spreading to others. Seedlings with minor defects may still produce normal plants, and it is essential to distinguish between these and seedlings whose condition would preclude satisfactory further development. Essential structures may be physically damaged, deformed on account of weak or unbalanced development, or decayed due to attack by microorganisms, and these defects can arise from a number of causes at various stages during seed formation, ripening, harvesting, processing or storage; some examples are given below.

FIGURE 7.3 Analyst evaluating maize seedlings at the conclusion of a germination test. Both root and shoot systems are checked for normal development. (Photograph from the National Institute of Agricultural Botany.)

Lack of manganese in the parent plant when grown on deficient soils can lead to a condition in pea seedlings, known as 'marsh spot', in which the plumular bud is destroyed (Reynolds, 1955). Although shoots may develop in the axils of the cotyledons the resultant seedlings do not produce normal plants. Less severely affected seedlings exhibit only a necrotic area at the centre of the cotyledons, but these are classed as normal in laboratory tests.

Seed may be infected by pathogenic fungi in the field during its formation and ripening, and seedlings grown from it may be attacked during germination tests. Thus, for example, wheat seedlings in-

fected with *Septoria nodorum* show brown spots, knobs or stripes on the coleoptile (Kietreiber, 1962, 1966), and are classed as abnormal if the discolouration has penetrated to the enclosed leaves (ISTA, 1968). Seedlings infected with *Alternaria*, *Ascochyta* or *Phoma* spp. may show decay or discolouration of the cotyledons; if this affects the area adjacent to the shoot apex, or covers more than half of the total cotyledon area, they are considered abnormal. The treatment of seed-borne disease in germination tests is one of the more controversial aspects of seed testing (Neergaard, 1965); however, the germination test is not designed to establish the presence of seed-borne infection, and special disease tests are available for more accurate determination.

Cereal seed produced in high latitudes is subject to low temperature before harvest and may exhibit freezing injury. Typical symptoms described by Andersen (1950) for wheat, oats and barley include malformed coleoptiles with few or no foliar leaves, spindly seedlings with the tissue of the coleoptile and foliar leaves split longitudinally or desiccated, shrivelled and withered, and seedlings with little or no root development; none of these would develop into normal plants.

Mechanical injury can occur during harvesting and threshing (see Chapter 4). Badly broken seeds are removed in cleaning, but the embryo may be bruised or fractured, when the condition does not become apparent until the seed germinates. Mechanical injury is found in most species, but is especially frequent in the *Leguminosae*. Seriously damaged seedlings are classed as abnormal since, although they may develop adventitious roots or shoots may grow from the axils of the cotyledons, they are unlikely to produce normal plants in a crop. Abnormal seedlings in this category include those with damage to the cotyledons reducing their total area to less than half that of two normal cotyledons (Ching and Pierpoint, 1957) or with the epicotyl or hypocotyl fractured or the plumular bud injured (Andersen, 1954; Verhey, 1961). Essential structures may have open splits or constrictions likely to affect the conducting tissues. Root damage is often found in cereal and grass seed in which only the plumule develops. MacKay and Flood (1968, 1969) have associated injury with embryo structure and the degree of exposure of the radicle in cereal seed and of the radicle, hypocotyl and distal end of the cotyledons in seed of red clover (*Trifolium pratense*). Cereal seed may also show injury to the coleoptile, which splits so that the leaves emerge from near the base instead of from the tip; although the leaves may be normal in a laboratory test their ability to reach the surface when released below ground level is restricted in the absence of the geotropic response supplied by the coleoptile.

It is often necessary to dry seed artificially after harvest in order to reduce its moisture content to a safe level for storage, but this may affect germination if the temperature is too high. Wellington and Bradnock (1964) have described the deformed seedlings produced in barley. In some only the coleorhiza emerged and there was no extension of the seminal roots; in others elongation of the seminal roots was restricted. Some seedlings showed normal development of the plumule, although in others the coleoptile elongated but the leaf was delayed, or the plumule did not elongate and failed to emerge from the covering layers. It is important to ensure that tests are not assessed too early, since some of these categories resemble early stages of normal germination.

Abnormal seedlings can also arise as the result of processing as when, for example, essential structures are fractured during the abrasive treatment used to produce single seeded units from sugar beet clusters (Tolman and Stout, 1944) or to overcome the hard-seeded condition in clover (Witte, 1928). Seedlings produced by seed treated with chemical fungicides or insecticides may be deformed, with roots and shoots stunted and swollen. The condition in cereals has been described by Brett and Dillon Weston (1941), Lafferty (1953) and Thomson (1954). The effect of artificial media in exaggerating these symptoms and the need to re-test affected samples in a medium containing soil has been discussed in the section dealing with substrates.

Germination capacity declines as seed ages during storage, but complete death is usually preceded by the production of abnormal seedlings whose development is weak or unbalanced because the loss of vital functions does not occur simultaneously in the different tissues. Typical symptoms which have been described include stunting of the plumule or failure of the first leaf to develop within the coleoptile in *Gramineae* (Griffiths and Pegler, 1964; Kearns and Toole, 1939; MacKay and Flood, 1969), breakdown of hypocotyl tissue giving a glassy or watery appearance and restricted root and shoot development in *Leguminosae* and *Cruciferae* (MacKay and Flood, 1969, 1970), and failure of the characteristic bend or 'knee' to develop in the cotyledon of onion (*Allium cepa*) (Clark, 1948). Under high moisture conditions seedlings in which the essential structures are decayed may be frequent (MacKay and Flood, 1968, 1969).

At the conclusion of the test dead seeds must be separated from those which are dormant. The most reliable criterion is the condition of the embryo, which is firm to the touch in dormant seed but soft and watery in dead seed, usually but not always accompanied by

mould development. 'Hard' seeds are easily recognised because they have not imbibed.

RELEVANCE TO FIELD ESTABLISHMENT

The concept of germination capacity embodied in International Rules (ISTA, 1966) has been evolved in response to an appreciation of the function of seed testing in providing information on sowing quality. The principles of seedling evaluation have been steadily refined in an attempt to improve the relationship between the results of a laboratory test and the production of plants in the field.

Seed sown in the field is subject to hazards to which it is not exposed in the laboratory, so that precise reproduction of laboratory results cannot be expected; and because of the great differences in soil conditions depression of the laboratory figure is unlikely to be the same at different sites or seasons. On the basis of a review of data from many experiments in the USA and Northern Europe, Essenburg and Schoorel (1962) concluded that there were generally very high correlations between laboratory germination and field emergence but, whereas in some species the emergence percentage usually shows a fairly constant relationship to the germination capacity, others are much more sensitive to differences in soil conditions. Gadd (1932) reported a very high correlation between laboratory and field germination for both autumn and spring wheat, regardless of the variable climatic conditions from year to year. When seed rates were adjusted on the basis of the germination test to give equal numbers of viable seeds Abdalla and Roberts (1969) obtained almost identical densities with barley from storage treatments which produced germination percentages over the range 100 to 15 per cent; but Valle and Mela (1965) found better emergence from samples of wheat, barley and oats with higher germination (88–93 per cent) than with lower germination (63–70 per cent). MacKay and Tonkin (1965) reported that laboratory tests placed samples of sugar beet in the same order as their percentage field emergence, although tests in the greenhouse discriminated rather more clearly between samples of different field planting value.

In modern farming practice the establishment of plant populations at pre-determined densities, without the intervention of transplanting or hand thinning, is increasingly sought in order to obtain, at the lowest cost, maximum yields of produce of the precise quality demanded by the food processing industry. To achieve this, precision drills are used to place the seed accurately in the soil, and the seed itself may be mechanically processed or pelletted within an envelope

of inert material in order to produce units nearer to the spherical shape which is ideal for this purpose. Seed rates are calculated on the basis of formulae incorporating the laboratory germination figure (Austin, 1963; Bleasdale, 1965). Since the majority of samples show high correlations in individual series of comparisons between laboratory germination and field emergence it should be possible to apply a correction to allow for the expected depression; but since the extent of the depression in soil varies, the 'field factor' to be included in the seed rate formula must be based on knowledge of local climatic and soil conditions.

There are some samples which do not follow the general pattern because, although they perform similarly under optimal conditions, they vary in their reaction to environmental factors such as soil moisture or pathogen content (Heydecker, 1960, 1962), and this situation is particularly apparent in certain species. When peas are sown under the adverse conditions of soil moisture and temperature encountered in early spring in the United Kingdom the laboratory test gives little indication of emergence (Matthews and Bradnock, 1967, 1968), though the relationship improves with later sowing (Perry, 1970). A similar situation arises with maize, in which differences in pre-emergence mortality are associated with differences in the ability to germinate rapidly and to grow at low temperatures (Harper, Landragin and Ludwig, 1955a). Mortality in both peas and maize may be reduced, but not eliminated, by treating the seed with a fungicidal dressing (Harper, Landragin and Ludwig, 1955b; Matthews and Bradnock, 1967), and it is necessary to supplement the laboratory germination test with a special test to indicate susceptibility to pre-emergence failure, such as the electro-conductivity test for peas (Matthews and Bradnock, 1967), or the cold test for maize (Harper and Landragin, 1955; Wernham, 1951).

The procedure by which total germination is reported, even when accompanied by an interim figure, has been criticised because it does not provide reliable information on the speed and evenness of germination which is of particular importance in precision drilled crops or in connection with the use of pre-emergence herbicides. Timson (1965) proposed the recording of germinated seedlings each day and the expression of the test result as the sum of each day's totals, but Heydecker (1966) showed that in some circumstances this would obscure important differences between samples. Nichols and Heydecker (1968) considered that the use of quartiles (the times to 25, 50 and 75 per cent of the ultimate number germinating), supplemented by the final germination percentage, would be valuable because it

would yield information on the mean time to emergence and also the scatter around this time; but they found the data were not adequate for predicting establishment in the soil.

The laboratory germination test provides an appraisal of seed quality based on a knowledge of what proportion is incapable of growing and therefore worthless under all circumstances (MacKay, 1966). It seems probable that the additional information required for particular circumstances will have to come from specially devised supplementary tests.

REPRODUCIBILITY OF TEST RESULTS

The reproducibility of germination test results within fairly narrow limits is necessary because the figures are used not only to judge the suitability of a seed lot for a particular agricultural situation, but also for price determination and in the enforcement of seed quality legislation. However, unlike for example physical purity, viability is a dynamic property liable to change with time (Wellington, 1965); the speed and extent of this variation is determined by a series of genetic, physiological and environmental factors both before (Chapters 4 and 5) and during storage (Chapter 2) (see also Barton, 1961; MacKay and Flood, 1968, 1969, 1970; MacKay and Tonkin, 1967; Owen, 1956). Since storage conditions can usually be more readily controlled at a level favourable for the retention of viability in laboratory samples than in seed bulks, a change in percentage germination after a given period is likely to be less when both tests have been made on the same sample than when a second sample has been newly drawn from the bulk.

The extent to which test results vary is also influenced by the uniformity of the bulk. Sampling techniques are designed to produce representative samples from reasonably uniform lots, but they cannot take account of excessive variation (Chowings, 1968). Many of the factors causing loss of viability do not affect all parts of a bulk equally: initial differences in the condition of the standing crop may be reflected in uneven deterioration of the seed; variation in the severity of mechanical, heat or chemical injury and the uneven distribution of moisture taken up during storage may result in the level of viability being higher in some parts of the bulk than in others. Unless the seed is thoroughly mixed successive samples may then show wide differences in percentage germination. Miles (1962) proposed a procedure for measuring the heterogeneity of seed lots, and this has subsequently been incorporated in International Rules for Seed Testing (ISTA, 1966). It involves the separate analysis of a series of samples

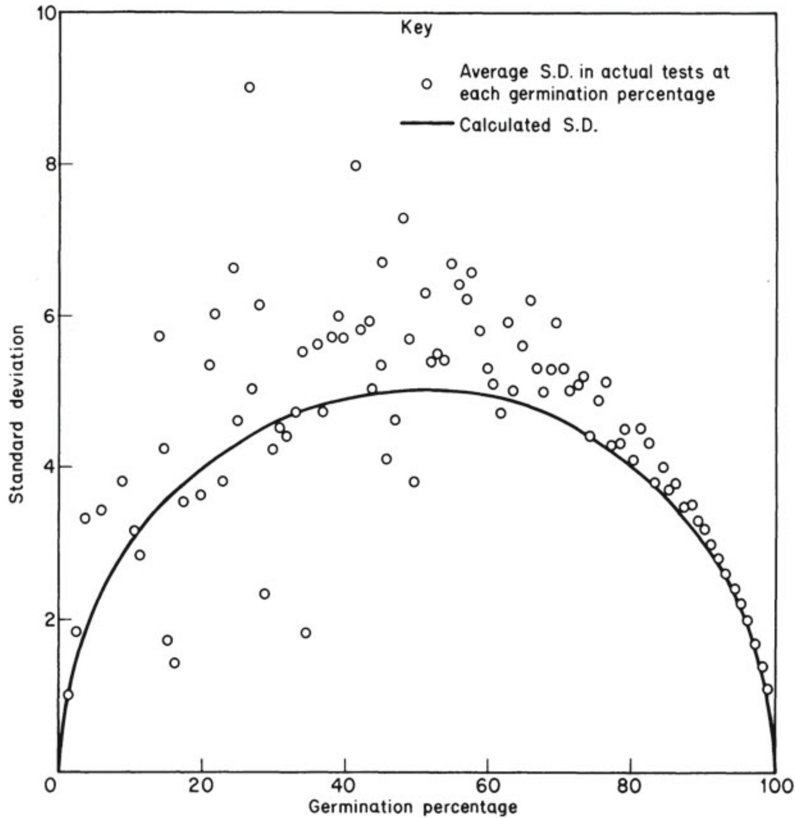

FIGURE 7.4 Average standard deviations of over 20,000 germination tests at the Official Seed Testing Station for Scotland compared with standard deviations calculated on the basis of random selection of seeds. (From Thomson, 1963.)

and the calculation of a 'heterogeneity value' (H) from the results based on the equation:

$$H = \frac{\text{actual variance}}{\text{theoretical variance}} - 1$$

The higher the value of H the greater is the variability of the bulk. Chowings (1968) has applied the test to 83 seed lots, showing that in crops where cleaning processes are highly effective, such as cereals, cleaning can remove evidence of heterogeneity, so that low H values for purity can be achieved without mixing, but the values for germination can remain high.

Variability may also be introduced as a result of imperfect sampling, or of differences in the conditions to which seed is exposed during the test or in seedling evaluation. On the basis of an analysis of standard deviations for over 20,000 tests Thomson (1963a) concluded that, in a single laboratory, at germination levels above 80 per cent, variability

was due almost entirely to the random selection of seeds; but at lower levels variation exceeded values calculated on this basis because the seed was more sensitive to slight differences in test conditions and also produced more seedlings whose evaluation as normal or abnormal presented difficulties (Fig. 7.4). Variation between laboratories exceeds that accounted for by random sampling (Miles, 1961), since there is a greater likelihood of results being affected through differences in techniques, even when uniform rules are applied. Variability between laboratories has been measured and forms the basis of tables by means of which the compatibility of test results can be judged (Miles, 1963); but because in practice variability within laboratories rarely exceeds random variation, figures allowing for random sampling variation only are used to check the consistency of the results of the replicates of a single test or the compatibility of different tests in the same laboratory. Tables for all these purposes are incorporated in International Rules for Seed Testing (ISTA, 1966).

The tetrazolium test

In the laboratory germination test viability is measured by stimulating germination and judging the capacity of the resultant seedlings to produce plants. Although by controlling the environment to provide optimal conditions the time taken to complete the test is reduced to a minimum, it may nevertheless take several weeks, and the period may be prolonged if measures have to be introduced to overcome dormancy. Any deviation from ideal test conditions can affect results, either by depressing emergence, by influencing the development of the essential structures or by favouring the spread of microorganisms. These disadvantages are eliminated if viability can be measured by means of a biochemical assessment of the metabolic activity of the resting seed.

This is the principle of the tetrazolium test, developed by Lakon (1949), in which tetrazolium salts are used to indicate the activity of enzymes of the dehydrogenase group, which are responsible for reduction processes in living tissue. The chemical is imbibed by the seed as a colourless solution and is reduced by the enzymes to a red-coloured, stable, non-diffusible substance, the formazan (Bulat, 1961). In the absence of active enzymes dead tissues remain unstained, and the distribution of living and dead areas of the embryo can be studied. A 1 per cent aqueous solution of 2,3,5-triphenyl-tetrazolium chloride or bromide is used; pH should be between 6 and 7, since the reaction occurs satisfactorily only in neutral solutions; the chemical is light-

sensitive and undue exposure to light must therefore be avoided.

The most satisfactory method of preparing the seeds depends on the species, and there are at present no internationally agreed procedures, except for certain tree seeds (ISTA, 1966). Techniques for very many species have been worked out and published by Lakon and Bulat at Stuttgart-Hohenheim since 1942 (Lindenbein, 1965) and an extensive list of references to these is given by Bulat (1969): they form the basis for most methods used or described subsequently.

Cereal seed may be soaked in water at 30°C for about 16 hours, after which the embryos are excised, together with a thin layer of endosperm; they are then submerged in tetrazolium chloride at 30°C for 24 hours, followed by rinsing in water and removal of the endosperm layer (MacKay and Flood, 1968) (Fig. 7.5). Alternatively, after over-

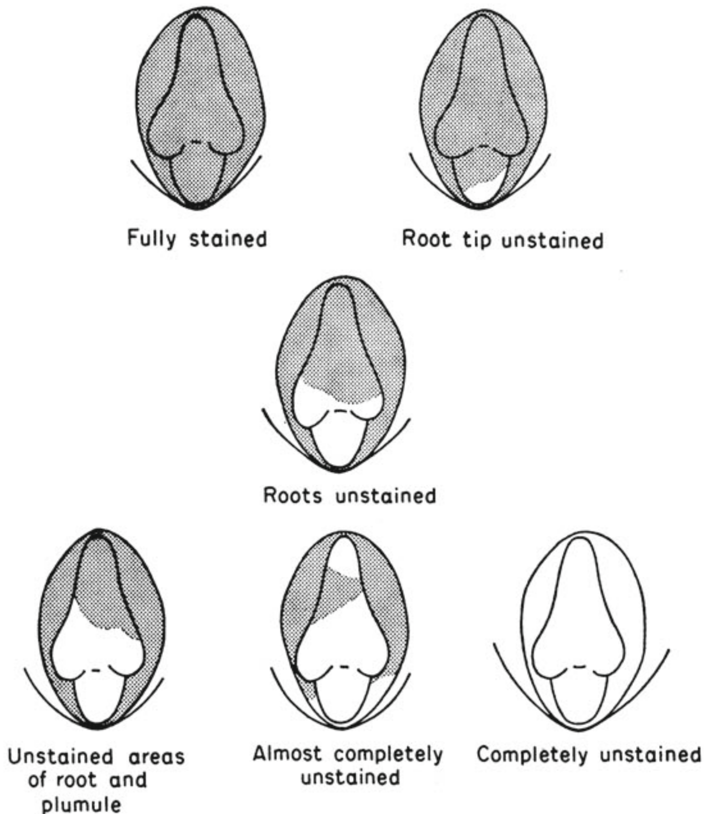

FIGURE 7.5 Distribution of stained (live) and unstained (dead) areas in the rye embryo after treatment with tetrazolium chloride at different stages of storage. The two upper seeds are viable; the remainder are not. (From MacKay and Flood, 1968.)

night soaking in water the grains may be split longitudinally, bisecting the embryo, and soaked at 20°C for four hours (Cottrell, 1947, 1948). Although the latter method is somewhat quicker, complete evaluation of all parts of the embryo is impossible and defects in the scutellum and secondary roots may be missed. The larger seeded grasses can be soaked for 30 minutes in water at room temperature, after which the lemmas and paleas are removed and the caryopses soaked for a further five hours at 30°C; they are then halved transversely and the

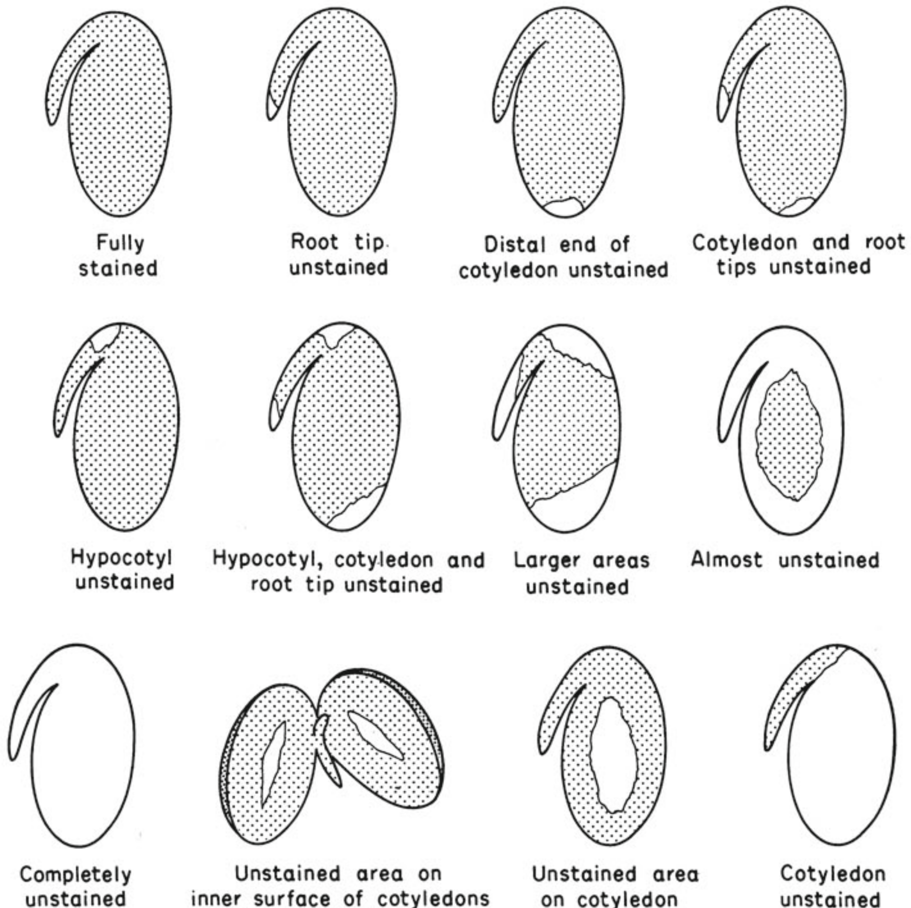

FIGURE 7.6 Distribution of stained (live) and unstained (dead) areas in the red clover embryo after treatment with tetrazolium chloride after different periods of storage. During deterioration there is usually an extension of dead tissue from points on the periphery corresponding with the corners of the seed. Seeds in the top row are viable; of the remainder only the seed showing the inner surface of the cotyledons unstained (bottom row) would germinate normally. (From MacKay and Flood, 1969.)

halves containing the embryos immersed in tetrazolium chloride solution for five hours (MacKay and Flood, 1969). Clover seed may be soaked in water for about 16 hours at 30°C; hard seeds are separated and the imbibed seeds immersed in tetrazolium chloride solution at 30°C for 24 hours, the seeds being assessed after rinsing in water and removal of the testas (MacKay and Flood, 1969) (Fig. 7.6). Brassica seed may be treated similarly, but the testas are removed before immersion in the tetrazolium chloride solution (MacKay and Flood, 1970).

Judgement of the ability of a seed to germinate is based on the degree to which the embryo is stained in the areas essential to growth. Complete staining of all parts is not necessarily essential for germination, but both the position and extent of necroses are critical. Thus a quite large unstained area at the distal end of the cotyledons of a clover embryo would not prevent germination, but a much smaller necrosis on the hypocotyl, by interfering with the movement of nutrients to the root, would result in the production of an abnormal seedling. Since the structure of the embryo varies in different species different systems of evaluation are essential for particular plant groups, and these have been described in the publications of the Hohenheim school (Bulat, 1969).

In the hands of a skilled technician the tetrazolium test can provide results agreeing closely with those of the laboratory germination test over a wide range of samples. But it fails to detect phytotoxicity caused by seed dressings, so that affected samples of no value for planting may be classed as of high viability. Heat injury caused by the use of excessive temperatures during artificial drying is also readily missed, although the presence of an unstained area at the centre of the scutellum is a valuable diagnostic feature of this condition in cereals (Wellington and Bradnock, 1964). The presence of living microorganisms, which are also stained by tetrazolium, may prove misleading if not recognised (Lakon, 1949).

Although the tetrazolium test cannot be regarded as a complete substitute for the laboratory germination test in all circumstances, it can be used in conjunction with it to provide additional information on the condition of a seed lot which may be valuable in assessing its potential planting or storage behaviour (MacKay, Tonkin and Flood, 1970; Moore, 1962) (See also pp. 96, and 228–229).

Other tests

While tetrazolium salts have proved the most successful chemical

indicators of viability several other substances, such as indigo carmine and salts of selenium and tellurium, have also been used as vital stains (Moore, 1969). The level of viability in samples of seed of a number of species has been successfully deduced from X-ray photography. Solutions of salts of heavy metals, such as barium chloride, penetrate dead cells, but not living cells because of their semi-permeability: thus dead parts of the embryo and endosperm show up clearly as contrasted areas on X-ray photographs (Simak, Gustafsson and Granström, 1957). The seed is soaked at room temperature for 16 hours and then, after removal of excess water, transferred to a 20 or 30 per cent solution of barium chloride, usually for one hour (Kamra, 1964, 1966; Nakamura, 1968). After drying the seeds are radiographed using soft X-rays. The photographs are evaluated according to the extent to which the tissues have been impregnated; account has also to be taken of seeds which are without an embryo or have poorly developed embryos, since these would also be incapable of normal germination. An advantage of the X-ray method over the tetrazolium method is that evaluation can be confirmed by subsequent germination of the actual seeds examined, although some damage by the chemical has been reported (Nakamura, 1968) which might interfere with the validity of conclusions unless untreated seed was also tested. In certain tree seeds impregnation of live seeds by barium chloride has been observed, and for these the use of organic contrast agents, such as Urografin and Umbradil, has proved more reliable (Kamra, 1963).

Takayanagi and Murakami (1968) reported that the most noticeable biochemical characteristic for distinguishing between dead and viable seeds lay in the much greater exudation of sugars from the former when they were soaked aseptically in distilled water. They developed a test on this basis in which the germination capacity of rape, barley and rice is judged by the colour changes induced in urine sugar analysis papers by the exudates from either single seeds or a given weight of seeds (Takayanagi and Murakami, 1969a and b). The test is unsuitable for species in which the carboyhydrate exuded is not primarily in the form of glucose, although other chemical analyses could be applied. However, a satisfactory correlation between exudate concentration and laboratory germination does not always hold good; Matthews and Bradnock (1967) found substantial differences in the exudation of soluble carbohydrates from samples of wrinkle-seeded peas of similar germination in the laboratory, although high exudation was associated with poor field emergence under early sowing conditions. This test is discussed further in Chapter 8 (pp. 232–233).

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