SEED BED ECOLOGY, SEED PHYSIOLOGY: PREDICTING THE INTERACTION

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1. Introduction

1.1 Seed vigour expression

The proportion of seeds which emerge under field conditions to give viable autotrophic seedlings is considered to be the major effect of seed vigour (Perry, 1969). Heydecker (1969) stated that for a seed lot to display a vigorous phenoptype requires the inherited potential to be realised by the production and maintenance of a good physiological biochemical and cytotogical state. Desirable qualities of vigorous seeds include that they should not age rapidly when stored and when sown should germinate simultaneously and without delay. Rate of seedling emergence and growth, even under unfavourable conditions, should also be rapid and uniform. Freedom from seedborne disease and resistance to subsequent infection is also important in the production of seedlings mechanically strong enough to penetrate soil layers which may be compacted as the result of cohesion forces (if wet) or crusting (if dry). Before photosynthesis is initiated the seedling must rapidly mobilise its reserves in order to produce metabilites and tissues necessary to attain the autotrophic state. The following rapid increases in dry weights to fill the allocated field area ensure high yields within the minimum period. Such expression of vigour is the product of the innate physiological quality of the seed and its interaction with the prevailing seed bed environment.

Major physiological components which are associated with low vigour levels include depression of initial rate of respiration and dehydrogenase activity and impairment of cell membrane structure which is manifested by leakage of electrolytes and carbohydrates. Levels of vigour can vary widely between the seed lots of a single cultivar. Perry (1969) investigated the hypothesis that reduced vigour in peas was the result of inherited genetic abnormalities due to inbreeding, by comparing the performance of the parent and progeny seed in field trials. Since no correlation of the progeny with parental status could be found, he concluded that variation in relative vigour levels was caused by external conditions acting on the seed at either of two phases of life

(i) during seed development on parent plant

(ii) after seed shed/harvest, during storage

Subsequently during germination, a series of feedback systems with alternative pathways exist so that environmental conditions during the first few minutes of germination are also determinative in whether a seed lot displays good or bad germination and whether seedling growth is vigorous or poor (Woodstock and Pollock, 1965).

1.2 Predicting seed vigour

Measuring the quality of a seed lot in terms of predicting how successfully it will establish a vigorous seedling population under an undefined set of field conditions is of great importance in order to achieve efficiency in modern agriculture. Establishment of a predetermined plant population is especially critical for commercial growers of direct-drilled vegetable crops in order to minimise costs, maximise yields (vegetables cannot yield compensate as readily as crops with a tillering habit) and achieve the precise quality required by the food-processing industry (MacKay, 1970). The actual population and spacing achieved is determined by seed rate, efficiency of the drilling procedure, viability, size and quality of the seeds and seed bed conditions at drilling and during the pre-emergence stage. This includes competition with pests, weeds and adjoining seedlings.

Kraak (1987) concluded that the emergence problems (at least in such crops as sugar beet, onion and flax, where the correlation of vigour test results with field data is particularly unreliable) are unlikely to be avoided by the application of the standard tests currently available. She therefore suggested that the emphasis in research should turn to the factors in the seed bed environment which are limiting.

2. Seed bed ecology

2.1 Introduction

The conditions provided by a soil environment are far from uniform even within a restricted area. The observed level of germination and seedling establishment may be determined by the microheterogeneity of the soil. The micro-climate which actually interacts with the germinating seed is the product of the overall climate and the nature of the soil with respect to chemical composition (eg salt content which can cause osmotic retardation or the source of leaf litter which determines whether inhibitory substances are present) and physical structure which relates to aeration and water content and influences the ability of a seedling's plumule to emerge above, or roots to penetrate, the surface.

The requirement for, or sensitivity to, various environmental factors varies according to the genotype of the seed and its physiological quality. Frequently, ecological adaptations are observed which serve to optimise the emergence response within a particular habitat where individual or combinations of factors may be limiting.

2.2 Water

The water content of soil varies widely depending on climatic conditions, including seasonal fluctuations, and coverage by plants. In terms of germination, water availability at particular periods is the critical factor and this is determined by various osmotic factors, binding by soil colloids, capillary forces and soil compaction/texture as well as competition from other organisms. Temperature is also associated, especially with seasonal fluctuations eg frozen water is inaccessible to plants and the rate of evaporation with which germinating seeds must compete for the moisture content of the surface layer, will depend on the temperature and humidity of the atmosphere.

Soil moisture can be considered in three catagories (Burgess and Raw, 1967).

(i) gravitational - moving through the soil under the infuence of gravity

(ii) ground water - held below the water-table

(iii) held water - retained in the soil after gravitational movement has ceased.

Following heavy rain, initial drainage of gravitational water will return the soil moisture to a relatively stable condition in which the gravitational pull is almost balanced by the capillary and adsorption forces exerted by the interstices and colloidal materials. This situation is known as "field - capacity". Gradual drying out will eventually lead to the "permanent wilting point" at which stage plants are unable to extract water from the soil.

The actual soil water potential required for germination is influenced by the seed-soil contact which, if poor, results in a lowered germination percentage even at high soil water potentials (Collis-George and Hector, 1969). Hunter and Erickson (1952) showed that the critical water potential for germination is characteristic of each species. Some species are highly specific with respect to moisture tension while others can withstand a wide range. With some exceptions, eg seeds of the willow, severely waterlogged soils tend to inhibit germination and the seed of most species becomes non-viable after prolonged periods. However this effect is primarily due to anaerobiosis and increased pathogen activity, and insufficient moisture is more commonly the limiting factor to a germinating seed of crop species.

Doveen and Macgillivray (1943) reported high sensitivity of germination percentages of vegetable seeds to soil moisture conditions. For lettuce seeds, maximum rates of 91% germination were achieved at 12% moisture but fell to 81% in response to a 1% reduction in water content. Gray (1978) also concluded that lack of sufficient moisture in the seeds' immediate neighbourhood was a common limitation on germination and showed an interaction between osmotic potential and temperature such that high temperature augmented the effect of moisture stress.

In the field, moisture conditions at seed-depth are constantly changing so that drilled seed may experience a series of hydration/dehydration cycles before radicle growth starts. Wurr and Fellows (1987) studied the laboratory germination of lettuce seeds placed in solutions of polyethylene glycol (PEG 6000) which imposed artifical moisture stress ranging from 0 to -8 bars. Although a decrease in osmotic potential brought about a corresponding reduction in germination, transfer back to a PEG free medium allowed a normal germinaton response. This led the authors to conclude that even major fluctuations in soil water potential on the seed bed are unlikely to influence seed germination per se provided that 24-48 hours of conditions at 0 bar become available at some point. Therefore the predominant effect of fluctuating soil moisture is on time to emergence. This paper confirmed the work of Reynolds (1975) who stated that failure to emerge must therefore be the result of dehydration of seedlings after germination, pathogen activity, or physical constraints such as soil-capping. He suggested however, that certain combinations of osmotic stress may serve to decrease the ability of seedlings to deal with such biological and physical hazards.

2.3. Temperature

Soil temperatures are subject to diurnal and seasonal fluctuations, and the extent of the changes depends on the soil-type; ie light/heavy and with or without leaf-litter. Very steep gradients exist across depth depending on soil texture, water content and plant cover, but becomes more uniform with increasing depth. Temperature effects alone are difficult to isolate from moisture, but a clear example of ecological adaptation is shown by a single species of Mimulus. Various populations of this species show adaptations of germination requirements with respect to the temperature conditions prevailing in each area (Vickery, 1967).

No simple relationship between temperature and germinaion rate exists as temperature influences several constitutive processes. The range tolerated and the sensitivity to changes varies widely between the species but, in general, the maximum point lies below 48°C (eg for Cucumis sativa); the minimum is less well-defined as the rate of germination becomes so slow, it is difficult to judge when to terminate the experiment.

The temperature range is also subject to modification, eg the external application of Abscissic Acid to lettuce seed not only decreases the rate of germination but also depresses the minimum temperature by 3-4°C while kinetin raises it by 10°C (Reynolds and Thompson, 1971).

In some cases, eg Nicotiana tabacum periodic fluctuations (often diurnal) are required for germinaton to occur. The temperature change itself rather than the actual range appears to be the critical factor, perhaps by acting upon a macromolecular structure in the seed (Mayer and Poljakoff-Mayber, 1987).

The combination of moisture and temperature conditions is frequently critical, temperature often postponing germination immediately after shedding until a more favourable time. This is illustrated by the plants of the desert region of Western Australia in which summer rains initiate germination of mainly grasses, while winter rains are specific for dicotyledonous species (Mott, 1972). That this is a temperature effect is confirmed by reproducing the differential response under laboratory conditions.

2.4 Gases

The volume of the gaseous phase varies with the compactness of the soil. The composition may also be variable, but the usual components are oxygen, nitrogen and carbon dioxide. Micro-organisms may contribute other gases such as methane, hydrogen-sulphate, hydrogen, nitrous oxide and trace amounts of carbon monoxide and ammonia.

There may be significant differences in composition compared to the atmosphere as equilibrium is attained only very slowly and the number and activity of the micro-organism within the soil will influence the relative amounts of CO_2 and O_2. In relatively dry soil, the CO_2 level seldom exceeds 1%, but in water-logged soils especially, the O_2 concentration may fall significantly below that of the atmosphere and the CO_2 level can rise to 3 or 4%; levels as high as 10% have been recorded briefly after heavy rain.

The normal requirement for germination is aerobic conditions (ie 20% O_2) and if this is lowered to any great extent the germination of cereal seeds is prevented. However notable exceptions to this include aquatic plants such as Typha latifolia and watercress (Rorippa nasturtium) which show optimum germination at 8% O_2. This has obvious links with the ecology. Many seeds continue to germinate even in the presence of CO_2 concentrations of 40-80% provided a 20% level of O_2 is maintained. In some cases (eg lettuce) there is a minimum requirement for CO_2 and slight increases in CO_2 levels have been shown to promote germination in Phleum pratense, and is responsible for dormancy breaks in Medicago and Trifolium. This may be due to agricultural selection as sensitivity to small amounts of CO_2 will help bring about rapid and uniform germination.

2.5 Light

In light-textured soils, light can penetrate the surface but its intensity falls rapidly. Light can penetrate water considerable distances provided it is clear, but quickly diminishes under vegetative cover and this is accompanied by spectral composition changes due to differential absorption of red light compared to far-red light.

The germination response of seeds to light varies from stimulation through indifference to inhibition. Light sensitivity is often associated with small seed and this is popularly explained as the need for quick initiation of photosynthesis to supplement limited reserves. However Mayer and Poljakoff-Mayber (1978) observed that this view is an over simplification as seed size is not a true measure of the amount of reserves compared to seedling requirements.

Light sensitivity may be confined to limited areas of the seed eg the micropylar end in lettuce. The ecological significance of this may be to allow germination only if the seed is in the right orientation with respect to the soil. Light sensitivity can be induced in certain seed species. For example Spergala arvensis only becomes light-dependent after burial. This may serve to prevent germination until the seed has reached the surface again. In others, eg Salvia pratensis, the light requirement exists only immediately after harvesting, or, as in the case of Salvia verticillara, can persist for a year or more.

The seed germination response to light is sensitive to spectral differences. A typical example is that of lettuce seed which respond optimally to a wavelength of 670nm (red), whereas illumination at 760nm (far-red) results in inhibition of germination Blue light is another influential wavelength but whether it serves to inhibit or stimulate germination is not clear. Evenari, et al (1957) showed that the seed response depended on the period of illumination with respect to the beginning of imbibition.

External factors eg osmotic stress can modify the seed response to light growth promotors/inhibitors and oxygen tension can alter duration and/or intensity requirements. There is even some evidence that the spectrum delivered to the parent plant during seed formation influences the seed's response as in Arabidopsis thaliana. The effect of light on germination is also subject to influence by the ambient temperature. This interaction is difficult to interpret in ecological terms as little is known about the micro-climate in the immediate vicinity of the seed. For example in lettuce no germination will occur in freshly harvested seeds in the dark. However after a period of storage at 18°C, dark conditions will permit germination whereas seed at 26°C still requires a light stimulus.

2.6 Physical state of soil

For seedlings to establish themselves, the plumule needs to pierce the soil surface in order to start photosynthesis and the roots must penetrate down for water supply and anchorage. Barley and Farrell (1965) studied the growth of pea and wheat roots with respect to soil density, matric potential and apparent soil cohesion. Increased values of these factors provided physical resistance which delayed root growth, increased root diameter and repressed the production of lateral roots. Water flow or splash, and sedimentation of disturbed soil particles (especially in cultivated land) tend to form compact layers ("seals"). This diminishes the infiltration rate and gaseous exchanges and also presents a significant mechanical barrier to seedling emergence (Arndt, 1965).

Such edaphic factors were also cited as influential in the preferential establishment of barley grass (Hordeum glaucum) compared to annual ryegrass (Lolium rigidum) in certain areas. Kloot (1987) showed that the awned florets of Hordeum gave it an advantage in terms of anchorage and leverage abilities in soils that were smooth or compacted.

2.7 Mineral composition

The physical properties of a soil profile are influenced strongly by the amount and nature of the clay minerals present. These are also responsible for much of the base exchange capacity; cation exchange is primarily associated with the negetive charges held on the surface of clay particles (Burgess and Raw, 1967). The importance of the cation content of soil to seedling emergence is highly species dependent, for example the superior tolerance of barley (compared to wheat, triticale and rye) of soil manganese deficiences during seedling growth was shown to be due to highly efficient uptake and utilisation. Differences in emergence between wheat varieties when grown in the absence of manganese were associated with differences in the Mn content of the sown seed (Marcar and Graham, 1987).

Similar regulation of vigour by the mineral composition of the seed is shown by barley cultivars, which differ in nitrogen, phosphorous and molybdenum (Szirtes _et al_, 1981). Also seeds of silver grass and cape-weed, having little potassium, could only penetrate deficient sand by 3/4 cm compared to over 90 cm by lupin seed (Ozanne and Asha, 1965). The primary cause of variation in total seed K+ was seed size rather than concentration of the mineral. However germination of mineral-rich seeds in difficient soils will not necessarily lead to establishment unless, the phenotype is also for efficient uptake/use once reserves have been exhausted.

The ionic constituents of soil can have a role in germination other than nutritive. The diffusion of solutes towards the growing root of a seedling in fairly dry soil can lead to concentrations around the root surface which are ten-fold higher than the average for the soil solution. This contributes to the osmotic problem of water absorption. Alkaline soils are often associated with high concentrations of salt (NaCl) which tend to inhibit germination primarily through an osmotic effect. However some plants are tolerant of high salt conditions at various developmental stages including germination, and halophytic species exist which show a preference for a certain degree of salinity eg _Atriplex halimus_. But even in these plants, tolerances of NaCl are several fold higher during germination and seedling growth. This may be related to the fact that in undisturbed soils the top 3mm or so is usually drier due to evaporation and this results in leaching upwards of the salt to give a concentration higher than the soil profile in general. In cultivated soils regular ploughing or irrigation counteracts this effect. In natural ecosystems, seasonal heavy rains can create an annual fluctuation in salinity. Barley grass has an annual habit that enables it to avoid the peak in late summer and hence can flourish where perennial types fail (Popay and Sander, 1982).

2.8 Organic fraction

The total amount of organic matter in soil varies from 10-12% (dry weight) in good grassland to less than 1% in impoverished soil such as that cultivated for many years. A large proportion (up to 89/90%) of the total amorphous organic fraction consists of humic acids. This is in contrast to the amino-acids which contribute only 0.1% and the simple carbohydrates (eg glucose) which are in even more negligible quantities.

The leaf-litter on the soil surface does not constitute, as yet, part of the amorphous fraction but it is a biotic factor which can influence germination and seedling emergence. The litter of the Valonia oak acts as a mechanical obstacle to seedling establishment, impeding the penetration of the soil by the roots. Leaf-litter may also contain certain compounds which act on some species as germination inhibitors. This can have profound effects on the distribution of species at least in the immediate vicinity of plants such as Brassica nigra. An ecological facet of this phenomenon in the beech tree, in order to allow germination of its own seed which develop soon after shedding, is that the inhibitory properties of the litter only appear after exposure to the winter period.

2.9 Herbicides

Modern agricultural practise frequently involves the use of herbicides to control weed populations. Herbicides constitute a very heterogeneous group of compounds and are generally used to kill freshly emerged weeds, while the deeper sown crop seeds are unaffected. Hence, the basis of selectivity is not physiological and little is known about their effect on the metabolism of seeds. The most widely studied is 2,4 dichlorophenoxyacetic acid (2,4-D). This is a synthetic auxin which causes seedling exhaustion by over-rapid use of carbohydrates reservoirs associated with increased respiration and decreased P/O ratios in the mitochondria. Other herbicides are based on substituted phenols and cresols which inhibit germination due to general phytotoxicity.

2.10 Fungal pathogens

The fungal pathogen Pythuim ultimum which causes the rot of seeds and seedlings has been cited as the predominant cause of emergence failure in peas (Perry, 1969) and is a major limitation to winter wheat production in the wetter areas of America (Hering et al, 1987). In wheat embryos, transplanting seedlings germinated 48 hours previously in a pathogen-free environment to Pythuim infected soil gave normal plants as from seed maintained in sterilised soil (Hering et al, 1987). This indicates that the critical period of susceptibility to Pythuim is early during the germination/emergence stages. The incidence and severity of infection is influenced by the quality and age of the seed and a low soil moisture discourages development of infection.

3. Seed physiology

3.1 Introduction

The definition of seed vigour adopted by the International Seed Testing Association (1977) states:

> "seed vigour is the sum total of those properties of the seed
> which determine the potential level of activity and performance
> of the seed or seed lot during germination and seedling emergence".

This implies that there are several physiological processes which exert some degree of influence on the overall performance of that seed and which can be acted upon by external factors. A greater appreciation of the key limiting reactions and their interactions with each other and the environment should enable a more precise definition of vigour to be made. This would facilitate the evaluation of seed quality by identifying which quantitative properties were directly correlated to field performance and hence allow appropriate tests to be devised.

The physiological quality of seeds is not a genetic property (Perry, 1969) but is the product of the conditions to which the seed was subjected both during development on the parent plant and following harvest. Ovcharov (1969) observed the variation in the physical and physiological qualities of seeds arising from the same maternal plant and related it to different sites and lines of development. He considered that supply of metabolites and nutrients from the leaves and roots was unlikely to be equal to all parts of the generative organs and that this could account for examples such as the characteristic variation in amounts of protein in wheat grain, depending on whether the spikelets originated from the outer (16.6%) or middle (19.1%) caryopsis of the lower zone or inner caryopsis (16.4%) of the middle zone.

The degree of maturity of the seed at time of harvest will also influence its physiological fitness. For example, in pearl millet (Pennisetum americanum) viability and vigour was found to be greatly reduced if the seed had only developed for 33% of the potential grain-filling period. Seed that had reached the middle or beyond of this period was not adversely affected (Fussell and Pearson, 1980). In the same study it was reported that while temperature at time of development did not alter viability the resulting seedlings were superior in height and dry weight if grown at 21/16°C day/night temperature as opposed to higher levels.

Grain damage is related to moisture content at harvest and therefore annual variations in climatic conditions may be expected to be influential on seed quality. Rainfall just prior to harvest appears to encourage pre-emergence rotting in peas (Flentje, 1964). This is possibly because of changes in atmospheric humidity resulting in an alteration of seed volume which causes "crushing".

3.2. Viability

3.2.1 Storage: The viability of a seed (ie its basic ability to germinate given favourable conditions) is a function of the time and conditions of storage. Little is known about what determines the differential storage periods and environments that various species can withstand. The viability period appears especially long for seeds with hard coats eg leguminosae, which can survive several decades. In general, longevity is increased if storage conditions are maintained at low temperatures and relative humidity. Roberts (1972) formulated the equation:

$$\log p = k_v - C_1 m - C_2 t$$

log p = mean viability period

m = moisture %

t = temperature (°C)

K & C = constants

which predicts the lifespan and viability over a range of conditions.

However the optimum set of conditions varies between seed species and, in contrast to the majority which are best stored in a dessicated condition, some show enhanced longevity in the imbibed state eg lettuce seed (Lactuce sativa) and Chenopodium album, the latter having been found viable after hundreds of years (Tran and Cavanagh, 1984). One explanation of this may be that dormant, imbibed seeds maintain a regular turnover of cellular constituents in order to repair cytological damage (Osborne, 1977). Many dry seeds appear resistant to extremes of temperature but although many remain viable, the subsequent seedlings are often adversely affected (Levitt, 1956). The composition of the atmosphere may also affect longevity. Kidd (1914) reported that the life-span of Herea brasiliensis seed increased in the presence of 40-45% CO_2, and lettuce and onion seed in high levels of CO_2 show reduced numbers of chromosomal aberrations during mitosis at germination (Harrison and McLeish, 1954).

3.2.2 Ageing: Loss of viability is not a sudden switch in state, but results from gradual deterioration which is accompanied by slow germination and a decrease in vigour of the resulting seedling. The causes of such deterioration are (1) generic, (2) physiological/cytological ie membrane damage, lack of vital cell constituents or inability to tolerate detrimental chromosome changes, and a propensity to permanent damage after temperature extremes or anaerobiosis, (3) pathological, ie field fungi invade seeds developing in the field causing ovule destruction (eg Alternaria fusarium and Helminthsporia) or storage-fungi (Xerophytic) mainly Aspergillus species (4) mechanical damage ie faulty harvesting/extraction/handling.

A number of physiological and cytological changes indicate declining viability in seeds. Activities of the dehydrogenase and peroxidase enzymes alter in response to storage conditions and an increase in free fatty acids formation, especially in oily seeds, accompanies loss of viability. Decrease in viability in cereals is paralleled by citric acid content (Barton, 1961). Non-viable seed embryos of rye and maize have a disrupted appearance about their mitochondria which leads to lysis upon imbibition. ATP content falls markedly as does the activity of some respiratory enzymes eg cytochrome oxidase. Chromosome breakage during anaphase of the first mitotic division of the root tips is typical of the chromosomal abberrations which occur, and although the total amount of DNA in dry non-viable embryos is equivalent to that in viable ones, the "spool" value decreases indicating DNA fragmentation.

Roberts (1987) reported critical moisture contents of 28-30% in non-oily seed and 15-18% in oily-types, above which, some repair of DNA lesions incurred during ageing was possible.

Electron micrographs of the axial tissue of dry, non-viable embryos reveals membrane damage which becomes further disrupted upon imbibition eg (i) breaks in plasmalemma and contraction from cell wall, (ii) endoplasmic reticulum bereft of polysomes (iii) monoribosomes in cytoplasm (iv) disintregration of plastids. An accelerated ageing study of sunflower seed deterioration revealed that in dry seeds stored at high relative humidity (95%), a gradual decrease in total phospholipid content was concomitant with a decline in germination potential. More precisely this was due to the loss of phosphatidylcholine and phosphatidlethanolamine (Halder, et-al., 1983). It has been suggested that membrane disruption is through the action of free-radicals when their production in ageing tissue becomes favoured over removal by the superoxide dismutase/catalase system (Villiers and Edgcombe, 1978). However there is conflicting literature as to whether lipidoxidation is associated with ageing. For example, Harman and Matlick (1976) claimed that dienoic and trienoic fatty acid components of the membrane were lost in deteriorating seeds, while Priestley and Leopold (1979) reported no evidence of a change in the unsaturated fatty acids during accelerated ageing of soybean seeds. Priestley, et al (1980) also failed to detect any change in the level of organic free-radicle.

3.3 Imbibition

Water uptake is a vital initial process in germination; it acts as a medium for transportation of reserves and nutrient substances and participates directly in many biochemical processes. However germination is not strictly a function of water uptake as long as a certain minimum amount is absorbed.

3.3.1 Uptake forces: Most species of seed are air dry at shed, the water content being less than 15% of the fresh weight and depending on the nature of the reserves (ie higher if starch rich rather than oil) and the relative humidity of the atmosphere at storage. Exposure of air dry seeds to free-water with a water potential of zero, creates a steep gradient leading to imbibition. An initial rapid period of uptake is followed by a slower linear phase which gradually tails off.

The low water potential of dry seeds is principally due to matric forces. Imbibition is a physical process, occurring regardless of the viability of the seed, and the extent is determined by the permeability of the seed coat, availability of water and composition of the seed. The chief component in the seed responsible for water uptake is proteins. The extent of swelling depends on the pH of the solution and since proteins are zwitterions, is at a minimum at the isoelectric point. With respect to other colloids, dependance on pH is related to their dissociation constants.

The seed size is also relevant to imbibition eg relatively more moisture is needed to swell small seeds of beet (Beta vulgaris) than the larger types. This confers an advantage of large seeds in being able to initiate formation of de novo products and take up mineral nutrients faster and hence enter the autotrophic state earlier. Heterogeneity of seed size within a lot is one of the factors which causes unequal water uptake and hence non-uniform germination eg in clover (Trifolium) alfalfa (Medicago sativa) and other leguminous plants. Chemical and anatomical changes in the seed coat can also render a certain percentage of seeds impenetrable to water and oxygen, which will slow or suppress germination (Orcharov, 1969).

3.3.2 External factors: Environmental factors which can influence imbibition extent and rate include oxygen, which if present during the drying of Pisum elatius seeds, renders them impermeable to water (Marbach and Mayer, 1974). Ambient temperature, although not affecting imbibition through a chemical change (Q_{10} values of imbibition in Xanthium and pea seeds = 1.5-1.8 (Shull, 1920)) has a complex interaction with the viscosity and kinetic energy of water. Temperature also effects the seeds colloidal structure such that decreased temperatures give increased volume (although this is, in practice, a very minor effect). Blacklow (1972) formulated an equation to relate imbibition and temperature in Zea mays:

$$\frac{dW}{dt} = K(f(t) - W) + b$$

K = permeability of seeds to water during exponential phase of uptake

f = water capacity of seed
W = initial water content of seed

b = linear phase of water uptake
t = time

Seeds of tropical/subtropical origin are susceptible to damage if they imbibe cold water eg cotton, lima bean and maize suffer chilling injury if exposed to water at 5°C for the first few minutes of imbibition. Once imbibed at 31°C, however, transfer to 5°C has no significant effect (Simon, 1978).

Time of imbibition prior to germination can effect the quality of the subsequent seedling. In the case of broad beans (Vicia faba) pre-soaking for 72 hours allows more rapid germination and a faster growth rate. However pea, sunflower and common beans respond with a lowered rate of germination and less vigorous plants. This is observed even under sterile conditions and so is not due to micro-organism activity. Anaerobiosis may be the general cause as in Phaseolus vulgaris where flooding of the cavity between the cotyledons results in ethanol self-poisoning (Crawford, 1977). Tolerance of water-logged conditions appears correlated with reduced production of ethanol, as seen in rice (Oryza sativa).

3.4. Dormancy

3.4.1 Introduction: Dormancy is conventionally considered as the state in which a viable seed will not germinate even in the presence of adequate conditions. Many species require a period of after-ripening prior to germination and this may occur in dry-storage. Others require some form of light or temperature stimulus while in the imbibed state. This can be a complication in vigour tests which frequently take germination percentage and/or rate as an indication of field potential. Seedling and developmental abnormalities occur if the embryo's dormancy is not completely broken. Dormancy is also largely responsible for heterogeneity of germination (considered a low vigour trait) as degree of dormancy can vary within a batch and this is exaggerated if the conditions are not optimal (Perry, 1981).

The state of dormancy has an important role in enabling seeds to survive adversity eg heavy frosts, dryness, excessive moisture. Also impermeability of seeds eg cotton (*Gossypium hirsutum*) (Rolston, 1978) helps seed to maintain quality under adverse (ie wet) harvest conditions, and high humidity during storage.

3.4.2 After-ripening: The temperature dependent after-ripening required by some seed in dry-storage, is related to the presence of volatile fatty acids (C_6- C_{10}) which can act as inhibitors of germination. Berrie, *et al* (1978) reported that loss of these fatty acids by evaporation or degradation is an essential process in the after-ripening of *Avena* species. C_7, C_8 and C_9 fatty acids were especially effective in inhibiting germination when added to non-dormant cultivated oat seed, and were found to be present in the developing seed. However this data has not been confirmed by other workers, perhaps due to the wide varietal differences in dormancy characteristics displayed by wild oat species.

3.4.3 Dormancy: Three levels of true dormancy exist (Roberts, 1972):

(i) Innate or primary - this prevents germination during development and maturation on the parent plant and sometimes for a further period.

(ii) Secondary - if innate dormancy is lost, germination may still not occur if conditions are unfavourable. This state continues even when the seed is returned to an optimal environment. Environmental factors which can induce this condition include temperature, absence of light or oxygen, presence of volatile inhibitors.

(iii) Enforced (imposed) - this is mediated purely by external conditions and the seeds germinate immediately upon removal of the environmental constraint.

A common physical cause of primary dormancy is a hard seed coat. This prevents germination by conferring impermeability to water and gases, and mechanically constraining the embryo. Impermeability to water is most often found in the Leguminosae which have a wax-like layer. Whether the general reason for impermeability is impregnation of cell walls with hydrophobic substances or due to a process of shrinkage causing closer packing of the cell in the seed coat is not known. Water uptake in the Leguminosae prior to germination is via the strophiolar cleft which can be reversibly opened. Other hard coated species may lack such a cleft and rely upon abrasion, either mechanical (eg cracking by alternating temperatures) or microbial (eg digestion by an animal gut) to induce permeability of the coat.

Frequently coats can be impermeable to gases (ie CO_2 and O_2) although not necessarily to water as well. For example, Xanthium fruits yield two seeds which differ in their oxygen requirement for germination. This difference was not found between the excised embryos and therefore was attributed as a property of the seed envelope. Temperature can have a significant effect on the permeability of the seed coat; eg apple seeds imbibing at 20°C display a rapid fall in permeability to O_2 whereas at 4°C, o_2 is readily permeated. The permeability of lucerne to O_2 is subject to influence by saponins from the soil which are released by decomposition of plant material (Marchaim et al, 1972).

The mechanical restraint of the seed coat can also prevent germination if the thrust developed by the embryo on imbibition is insufficient to rupture the testa. Mechanical weakening of the seed coat can bring about lifting of dormancy and there is some suggestion that enzymes from the radicle may chemically dissolve it.

Secondary dormancy may be invoked if all the appropriate germination conditions except one are provided eg illumination inhibits seed of Nigella, and temperatures too far either side of the optimum lead to secondary dormancy in lettuce. In Eragrostis, dormancy is reinduced by cycling temperatures during imbibition and high CO_2 tensions have a similar effect in Brassica alba. It is generally assumed that the underlying mechanisms of secondary dormancy are the same as those of dormancy in general, the new requirements for germination indicating that metabolic changes occurred in the seed whilst exposed to the conditions that imposed the secondary state.

3.4.4 Dormancy breakage: Dormancy is controlled by gene repression/derepression and similarly to other physiological phase changes, is under the control of a balance of hormonal promotor-inhibition levels. Certain species require low temperature treatment while in the imbibed state (stratification) to break dormancy before transfer to the germination temperature, eg in cherry seeds at 5°C the embryonic axis increases in cell number, dry weight and total length and there is increased O_2 uptake in the embryonic axis and leaf primordia (Mayer and Polakoff-Mayber, 1978). Although it is possible to force seeds which require stratification to germinate by removal of the seed coat, the resulting seedlings are dwarfed and deformed until subjected to cold treatment.

Whether the effects of this stratification are due to gene-derepression (ie mRNA synthesis) or an increase in protein synthesis is subject to controversy and similarly for other methods of dormancy breaking. In lettuce (Lactuca sativa) delivery of the required light stimulus does not bring about an increase in rate of RNA synthesis until 12 hours later, by which time germination has started (Frankland et al, 1971). The difficulty lies in interpreting the metabolism associated with the break of dormancy separately from that of germination and growth. The assay techniques used may not be sensitive enough to detect small, discrete changes in RNA patterns as implied by Tuan and Bonner's hypothesis (1964) that dormancy is the direct result of repression of part of the genome.

3.4.5 Growth hormones: Growth regulators are generally considered to play a key role in control of the events during dormancy breakage. However the nature of the experimental evidence casts doubt on whether they play as vital a physiological role (Trewavas, 1981). Experiments with exogenously applied growth regulators (GRs) show a log-linear relationship between concentration and response but, in contrast, endogenous levels change very little. This may imply that a change in sensitivity of the tissue to internal hormones is occurring instead.

Abscissic acid: The best evidence for a direct role in dormancy is shown by the data for Abscissic Acid (ABA). Maturing seeds eg wheat (*Triticum aestivum*) and even species which do not show true physiological dormancy in the seed shed eg pea (*Pisum sativum*) accumulate ABA. The majority of ABA in hazel seed is found in the pericarp and testa. Removal of the seed coat at harvest allows germination even in the absence of a stratification period. That this is not a physical effect is shown by the maintenance of inhibition upon incubation of the isolated embryos and seed coats in a growth medium. Chilling of the seed renders it almost insensitive to ABA inhibition suggesting that it is concerned with the onset of dormancy rather than later events. Karssen (1982) demonstrated that ABA is necessary to impose primary dormancy by use of single gene mutants of *Arabidopsis thaliana* which had severely reduced levels of ABA associated with a lack of dormancy.

Gibberellins: Application of exogenous gibberellic acid (GA) can induce the germination of previously dormant seed, substituting for chilling or light stimuli. However measured increases of endogenous GA were far below those required to be added externally, until transfer to the germination temperature after chilling. Also addition of CCC (an inhibitor of GA biosynthesis) could inhibit germination even if added after stratification (Bradbeer, 1968). Therefore chilling appears to relieve some unknown block to GA biosynthesis, but this synthesis occurs upon transfer back to the warmth, indicating that GA is involved with the growth and metabilism of the germinating seed and not the metabolism of dormancy breakage. This conclusion is further supported by the observed lag phase and slower germination of the unchilled seed stimulated by exogenous GA, compared to cold-treated seeds.

Cytokinins: Exogenously added cytokinins (CK) have been observed to allow GA to induce germination of lettuce seed inhibited by exogenous ABA where GA alone fails. This perhaps indicates a permissive role for cytokinis. However the data concerning endogenous changes is less clear. The measured increases in CK do not correspond to a uniform ability to germinate and the rise in CK only occurs subsequent to a decrease in ABA levels.

Ethylene: GLC assays provide a rapid and accurate measurement of ethylene levels and have shown that in non-dormant varieties of peanut (Arachis hypogea) evolution of ethylene is associated with onset of radicle growth (Ross, 1984). Dormant cultivars of the same species showed low levels which were less than those required for induction of germination. Therefore it may be that the inability to produce sufficient ethylene is the block to germination here. Schonbeck and Egley (1981) working on red root pigweed demonstrated that high levels of temperature and light led to increased sensitivity to ethylene as did water stress despite its adverse effect on germination. Perhaps stress conditions cause a decrease in endogenous production which allows lower exogenous levels to bring about a response. The interaction of ethylene with light and high temperature appeared to have maximum effect 12 hours after imbibition started. By 48 hours, all three factors together gave 75% germination, whereas delivered singly, were ineffective. This led the authors to suggest a dual role of ethylene in breaking primary dormancy early after imbibition and then, if germination was prevented by unfavourable conditions, secondary dormancy was induced which could only be alleviated once ethylene sensitivity had redeveloped.

Auxins: The effects of exogenous auxin on germination are in dispute.

Conflicting results of inhibition or stimulation are obtained depending on concentration used and seed type. The most general result is absence of response to physiological level. However endogenous levels have been reported to rise during germination. Indole-3-acetic acid (IAA) concentrations fell after a period at 5°C (in maple seeds) and then rose, in contrast to control samples held at 17°C in which the concentration remained low. But since excised embryos returned to 20°C were already able to germinate, even when IAA levels were at a minimum, it is most likely that IAA is associated with the germination process rather than having a significant role in overcoming dormancy.

3.4.6 Light: The role of light triggering dormancy-break has ecological implications regarding position of the seed with respect to the surface (ie light or dark?), photosynthetic competition (ie quality of spectrum altered by leaf canopy) and the season (ie photoperiod lengths). Sensitivity to light increases with time of imbibition, reaching a maximum point in approximately one hour. The stimulatory effect of light is retained if delivered while the seed is imbibed, even upon subsequent drying-back.

Stimulation is sensitive to the wavelength and can be reversed by the delivery of a far-red pulse (730nm) following a red (670nm) light stimulation. These wavelengths correspond to the absorption peaks of the two forms of the pigment phytochrome which is normally in a state of equilibrium between either type.

$$P_r \underset{(730\text{nm})}{\overset{(670\text{nm})}{\rightleftharpoons}} P_{FR}$$

The change in the position of the equilibrium is dependent on the intensity and duration of the light pulse, and in the dark, P_{FR} reverts gradually to P_R. Whether or not germination is stimulated depends on the amount of P_{FR} expressed as a percentage of total P, and the critical proportion varies with the species, eg in *Nigella*, the required P_{FR} level is 1-3% whereas in tomato seed, it must exceed 22%. In addition the P_R/P_{FR} ratio is influenced by the redox state of the tissue.

Blue light is also capable of transforming P_R to P_{FR} but is less efficient. Comparison of absorption spectrum has led to the proposal of a second pigment, (possibly a flavoprotein) responsible for the response of seeds to blue-light. But there is very little evidence as yet to support this. In some species, it can substitute for red-light eg lettuce and Kalanchoe, but other seeds where germination is promoted or inhibited by light eg Juncus maritmis, GA is not effective. That red-light and GA are not the same is also indicated by their pattern of interaction together and with temperature and far-red light; the latter's influence cannot always be reversed by addition of GA (Ikuma and Thimann, 1969).

Gibberellin can also influence the photoperiodic response of some species, eg Begonia shows a true long day requirement but the critical daylength can be reduced by exogenous application of GA. This is probably also related to the phytochrome system. Thus it can be seen that alleviating dormancy to allow normal germination is a complex process involving the interaction of physiological processes and external factors in, as yet, a little understood fashion. In addition, the critical conditions vary widely between the species and the germination response to a given environment is not uniform even within a single seed lot.

3.5. Seed metabolism

3.5.1 Introduction: The biochemical and physiological characteristics of qualitatively different seed have pronounced effects on the metabolism of seedlings. A high rate of transformation of reserves in large germinating seeds and of assimulation of nutrients from the external medium provides favourable conditions for the faster germination of seeds and growth of the seedlings. The extent of the root system is particularly important to allow uptake of water and mineral nutrients which promote the synthetic processes in the roots and aerial parts. For example, levels of ascorbic acid, which is generally absent in dormant seed, rise sharply upon germination. This is due to rapid biosynthesis of vitamin C, vital to the germinating seed because of the role of the ascorbic acid-glutathion system in oxygen absortion (Orcharov, 1969). The favourable effect of this on the vigour of the seedling can be seen by comparing the performance of maize (Zea mays) seed which have different rates of vitamin C synthesis depending on which part of the cob they originate from; those from the middle section have a higher rate than seeds from the top (Kizilova and Orcharov, 1965).

3.5.2 Respiration: Most of the enzymes responsible for metabolic events before drying are fairly resistant to dessication and become reactivated upon imbibition. This includes those enzymes responsible for respiration. Rate of respiration is negligible in dry seed but rises rapidly as imbibition occurs. This is the combined result of enhanced respiration in individual cells and the increasing number of cells wetted as the water front penetrates the seed. The rise in overall respiration is triphasic, the initial increase reaching a plateau before a second rising phase as cell division starts in the tissues of the root-shoot axis.

The observations that the environmental treatments which break dormancy bring about a concommitent increase in respiration led to the proposal that dormancy was imposed by a block in energy metabolism which was broken by increased availability of phosphate acceptors due to hydrolysis of stored forms. However, several chemical inhibitors of respiration (eg cyanide) can also promote germination and the paradox is still unexplained. One possibility is that an alternative cycle is involved which bypasses the terminal cytochrome oxidase of the mitochondrial electron transport chain (Ross, 1984). However the effectiveness of other inhibitors acting at different target sites led Roberts (1973) to suggest the involvement of the pentose phosphate pathways (PPP). He proposed that in dormant seed the cyanide-insensitive oxidase of the PPP was in competition with conventional electron transport. Inhibition of the conventional system or oxidation of NADPH would then stimulate the PPP, the intermediates of which could be responsible for dormancy breakage

Morohashin and Shimokoryame (1975) also believed that initial breakdown of respiratory substrates was via a different pathway than later and from experiments with labelled glucose suggested that mainly fermentation occurred early on in germination before mitochondrial activity rose sufficently for the Kreb's cycle to operate.

3.5.3. Protein synthesis: In the developing seed, large amounts of protein synthesis occur which gradually decreases towards maturity to practically zero in the dry seed. On germination, the synthesis restarts but the protein profile (as seen by 2-D separation techniques - O'Farrell, 1945) is changed such that some novel types are produced eg α-amylase. There is also supression of the developmental proteins. This synthesis of specific germination proteins is stimulated by imbibition (Dasgupta and Bewly, 1982) and the very prompt start upon water uptake (less than one hour in wheat) indicates that most of the biochemical and structural components are already present in the dry seed. Further increases after imbibition has halted may be due to a rising proportion of polysomes, or an increase in the ATP and GTP levels.

mRNA: In the developing seed, the decline in protein synthesis is associated with disassembly of the polysomes to monosomes as individual ribosomes reach the end of a transcript. Cell free systems have shown that the ribosomes are capable of translation and the availability of amino-acids is not the limiting factor. Therefore the synthesis of protein is permeated by a lack of mRNA (Simon, 1984). However mRNA preparations can be isolated from dry seeds of pea, bean, rape and rye embryos which are capable of acting as templates in a wheat germ system to give high molecular weight proteins (Caers et al, 1979). Also seeds which are allowed to imbibe in the presence of α-amanitin (an inhibitor of RNA synthesis) are still capable of protein synthesis. This apparent paradox may be resolved by the explanation that in the mature seed, mRNA transcripts are present but unavailable to the ribosome due to their subcellular location, eg in the mucleus or as ribonucleotide particles.

Pulse-labelling experiments show the half-life of mRNA, in general, to be 1-2 hours at imbibition. Degradation at this point is therefore faster than synthesis. Smith and Bray (1982) concluded that this initial rapid loss of mRNA is probably the degradation of much of the stored message which may, therefore, have little role once imbibition occurs.

DNA: Cell division ends quite early in the development of peas and bean seeds and the embryo enters a phase of cell expansion. In wheat embryos, it has been demonstrated that synthesis of the DNA polymerase enzyme is a pre-requisite for the resumption of DNA synthesis (Mory *et al*, 1975). Radio-labelled thymidine is incorporated in wheat and rye embryos about five hours after imbibition, but their lag phase can vary widely.

3.5.4 Mobilisation of reserves:

Starch: Rapid mobilisation of nutrient reserves is an important factor in determining seedling establishment ensuring early attainment of the autotrophic state. Starch is the most common carbohydrate accumulated and its mobilisation has been best studied in the cereals. During germination and seedling establishment, hydrolytic events result in starch being rapidly mobilised. Degradation of cell wall deposits such as mannans make a significant contribution to the carbohydrates. The major starch-hydrolysing enzyme, α-amylase is synthesised and secreted by the barley aleurone layer in response to GA_3 (Yomo and Varner, 1971) and this action is specifically antagonised by ABA (Jacobsen, 1973).

Experiments using radio-labelled cDNA to measure specific mRNA levels in the embryo suggest that GA_3 probably acts at the transcriptional level rather than by activating precursor molecules. However, despite the fact that measured levels of free GA in germinating grain appear adequate to bring about this secretory response in the aleurone, doubts regarding the relationship have been expressed. For example Trewavas (1982) considers the apparent absence of a lag between the appearance of GA and the first detectable levels of α-amylase to point to the two events being concommitant and independent. A large amount of variation in response to GA has been noted between seed batches within varieties and from year to year. The differences may be in sensitivity or amounts of endogenous GA which could be the result of the different environmental factors prevailing during seed development and maturation, eg the GA response of wheat aleurone appears dependent on the extent of dehydration (Armstrong et-al., 1982), and temperature influences the induction of aleurone sensitivity to GA_3 in wheat and barley (Nicholls, 1982). The duration and conditions of storage also can affect the GA response.

Lipids: Not all events in the aleurone are controlled by GA and this includes lipid mobilisation. Storage lipids are degraded after a relatively short lag following germination initiation. The lag depends on the species and growth condition of the seedlings. Conversion of lipid to carbohydrates (gluconeogensis) is the main pathway in all oil seeds. The major product, sucrose, is directed along the embryo axis to promote seedling growth. Unlimited seeds are primed for lipid degradation by possession of glyoxysomes which are synthesised during seed maturation and persist through dessication and germination. However the glyoxysomal enzymes are synthesised de novo and added post-translationally during the period of lipid degradation.

The control of lipid mobilisation has not been well studied. Lipid mobilisation and seedling growth appear to be synthronised processes suggesting that the embryo has a direct influence of reserve mobilisation. However the effect of excision of the embryonic axis is not consistent, eg mobilisation is inhibited in excised cucumber and peanut cotyledons but not in castor-bean endosperm. Axial control could take the form of a hormonal stimulus or a source-sink relationship between the storage organ and axis during early seedling development.

Oxygen availability appears to exert some control over enzyme activity (Leegood and Rees, 1978). A decrease in oxygen tension was shown to be paralleled by a drop in incorporation of [2-^{14}C] acetate into sugar in excised marrow cotyeldon. In cucumber, an intact testa substantially inhibits oxygen uptake by cotyledons. Normally the testa is removed soon after germination by a peg if tissue on the lower surface of the hypocotyl which acts as a lever. Upon its removal, rapid hydrolysis of lipid reserves starts (Ashford and Gubler, 1984).

4. Predicting field performance of seeds by vigour testing

4.1 Introduction: The need for vigour prediction

At present, few commercial seed companies test their stocks for vigour levels as there is a tendancy for results from such tests to be unreproducible and unreliable with regard to predicting field emergence. The costs are also prohibitive. Despite this there is demand for a quick and cheap test, that can be standardised throughout the trade, to assess the relative quality of seed lots with respect to expected field performance.

The importance of seed quality to emergence and yield realisation is illustrated by the study by Perry and Harrison (1979) in which barley seed was artificially deteriorated by storage at supra-optimal levels of moisture and temperature. Sowing of this seed in the early spring exposed it to the adverse conditions of a low soil temperature and excessive moisture. A significant interaction was shown between varying levels of deterioration and seed bed condition. The more severely deteriorated seed emerged proportionally less well in comparison to control samples which had not been artificially aged.

Avoidance of low vigour lots in agriculture is important as partial emergence failure will lead to loss of yield if the resulting stand falls below optimum population levels. Also if initial plant growth rate is slow as is typical of low vigour crops, this may reduce the yield in crops which are planted at low density and/or harvested early. However crops at densities high enough to give interplant competition and allowed to reach full maturity over a longer growing period (eg maize, soybean, wheat) will be unlikely to show consistent yield response to seed vigour (Tekrony, 1987).

Prediction of the rate of initial growth may also be important in terms of yield by coinciding desirable environmental conditions with specific stages of crop maturity. For example, high levels of irradiation are optimally delivered at the stage of heartening in lettuce (Wurr et al, 1987) and similarly, early drought conditions have been shown to decrease the rate of conversion of intercepted light to crop dry matter in sugar beet. This is due to a slow initial growth rate leading to delay of leaf-canopy expansion which results in inefficient light interception during the months of maximum radiation (Brown et at, 1984)

4.2 Fungal pathogens

The common observation that fungicidal seed dressing has a more significant effect on the lower quality seed lots led to some suggestions that low vigour is synonymous with susceptibility to pathogens, Pythium ultimum in particular. Reasons for this could be that aged seed has a greater tendency to leak metabolites and thus encourages fungal growth, or mechanical damage leading to cracks in the seed coat exposes the embryo to infection.

If the case was as simple as this, then field emergence could be easily predicted by assessing resistance to soil pathogens. However it might also be expected that the field emergence of old seed with a fungicidal treatment would be as good as new seed with or without fungicide. However experience shows that although emergence can be markedly improved, seedlings from wheat seed, 3-7 years old still gave smaller seedlings which were more subject to post-emergence failure than those from newer seed (Hering et al, 1987). Pathogen attacks appear to be just one of the many adverse environmental factors by which low vigour seed is affected more extremely than high vigour lots.

4.3 Aims of vigour tests

Laboratory testing of a given seed sample aims to grade vigour on a relative basis to potential performance and thereby assess the comparative risks involved between seed lots. No absolute score can be given as it is highly dependent on the prevailing seed bed conditons which in the field, can be predicted to a very limited extent only.

Ideally a laboratory vigour test should:

(i) give reproducible results

(ii) indicate the proportion of seeds which are capable of germinating to give normal seedlings

(iii) indicate the field emergence potential

(iv) be rapid and inexpensive to perform on large numbers

The types of test currently available can be considered in two basic catagories:

1. Indices of vigour: physical
biochemical
physiological

2. Expression of vigour: germination *in vitro*
exhaustion tests
seedling evaluation
performance in stress
seedling emergence in soil

(Heydecker, 1969).

4.4 Physical tests

4.4.1 Size: Physical tests are based on measurement of seed size as an indication of the reserves available for early growth. X-ray analysis can also be used to assess the physical integrity of the seed.

Bhattacharyza and Dutta (1981) reported an evaluation of jute seed based on specific gravity in which increasing values from 1.0 to over 1.3 were accompanied by a linear increase in laboratory and field germination percentage and also fibre yields. Specific values of less than 1.0 corresponded to non-viable seed. Seed weight of cowpea (Vigna sineusus) correlated with hypocotyl and shoot length and vigour index. Significant differences were observed in these parameters between different grades of seed size (Paul and Ramaswany, 1981). Other species in which the larger seed types correspond to superior vigour include ground nut (Arachis hypogaea) (Godoy and Cunha, 1981) and carrots (Daucus carota) (Perry and Hegarty, 1971; Bedford and MacKay, 1973). Smith et al (1973) concluded that the weight of lettuce seed gave a better assessment of seedling vigour (as measured by the slant board-test) than did size (in terms of width and thickness). The most vigorous seedlings tended to come from the heaviest seed in agreement with the findings of Scaife and Jones (1970). Seed lots were readily separated into various classes using an air-column separation which included the parameters of weight, density and some aerodynamic properties about varying shapes in its fractionation.

Not all species show a simple relationship in seed size paralleling vigour. Aguiar (1981) reported that seeds of soybean (Glycine max) which were smaller or larger than average, displayed reduced germination vigour. Also, in maize seed a number of vigour tests showed that the large round types were the poorest in quality and the small flat seed had faster water uptake rates during the initial stages of germination. However field emergence trials revealed no significant differences in seed performances through the different sizes (Sheih and McDonald, 1981).

However physical dimensions of a seed give no indication as to its physiological condition with respect to ageing or fungal damage and so are only useful in comparing seeds taken from the same batch.

4.5. Biochemical

4.5.1 TTC: More perceptive as regards the general viability of the seed are tests based on biochemical processes. These include various staining techniques which are sensitive to specific enzyme activities, eg Glutamic Acid Decarboxylase (GADA) which indicates the general physiological activity (Heydecker, 1969). Acid fuchsin and Triphenyltetrazolium chloride (TTC) staining are topographical methods, the former assessing the intactness of all membranes and the latter revealing dehydrogenase activity which is a measure of the respiration potential (Heydecker, 1968). TTC is reduced by the terminal oxidase system in living tissue to give a red-water insoluable formazan compound. Complete staining of embryos excised from their endosperm is not required. Diagrams of the staining patterns which distinguish between viable and non-viable embryos have been published (ISTA, 1976) but only so far for a limited number of species, ie cereals and cotton.

The staining pattern of tissue other than the embryo, eg scutellum, aleurone layer and storage tissue may also be important. The aleurone has a key physiological role in germination metabolism concerned with the enzymes responsible for starch hydrolysis. Also necrotic areas will facilitate the entry of pathogens into the endosperm and hence colonisation of the embryo. Vigorous seed are characterised by 100-75% staining of the total aleurone surface. However if the results are to show good correlation with vigour under suboptimal conditions, informed and experienced interpretation is necessary, for example, necrosis near the embryo and scutellum is proportionately more damaging than towards the apex. In commercial seed, root injury is common but often insufficient to prevent production of a normal seedling in laboratory germination tests. Seed with extensive injury may still show good germination values but will be unsuitable for long storage. TTC staining patterns will reveal this susceptibility to deterioration (Flood and MacKay, 1972).

4.6 Physiological

The physiological state of the seed may be assessed by (i) measuring the amount of leachates released into the surrounding medium, (ii) forecasting the seedling growth rate from the respiration rate and respiratory quotient.

4.6.1 Electroconductivity

The basis of testing the degree of leaching during imbibition is that non-germinating seeds do not retain their cell contents as effectively as seeds capable of germination and this can be quantified by measuring the electroconductivity of the soak-water (Matthews and Rogerson, 1976). Leakage from dry seeds is fastest at the start of imbibition and halts after approximately one day. The leachates lost into the medium from intact seeds include amino-acids, organic-acids, sugars, phenolics, phosphate and potassium ions and proteins; the rate is different for each. These solutes are probably of cytoplasmic origin but the reason for their release is unclear.

Perry and Harrison (1970) hypothesised that the steep water potential gradient disrupts the organisation of the cell membrane so dispersing the cytoplasmic contents. However this does not explain why there is such a continued and high loss in embryos, so a second hypothesis was forwarded by Simon and Raja-Harum (1972) that the membranes were not ruptured, but gradually reorganised. The phospholipids and protein of membranes normally adopt the bilayer configuration due to their amphipathic nature which dictates a central hydrophobic domain coated by a polar region which faces the aqueous. Therefore water has a stabilising role so that each cell recovering from the dessicated state will be leaky for a while until the membranes have fully renatured. This leakage will continue while the water front is advancing.

There is little association between the amount of solutes leached and extractable amounts from dry embryos therefore the differences in seed lots does not lie in solute content, but ability to retain them in water. The testa is a barrier to the loss of electrolytes for all seed lots and differences in apparent effectiveness may influence differential leaching. But the majority of difference is due to variation in the embryo condition and not the seed coats. Pea lots with the most dead tissue on the abaxial surface of the cotyledon gave the highest leaching (Matthews and Rogerson, 1976). But leaching variation is a property of the living tissue. Reasons for these differences could be differential sensitivity to the damaging effect of rapid imbibition or ability to retain the solutes by the cytoplasm.

The differences in leaching are observed before the plasma-membrane and tonoplast are considered to have been rendered effectively semi-permeable (Simon, 1974).

Lima bean leakage at imbibition has proved to be temperature sensitive such that a two-fold increase occurs upon transfer from 25°C to 5°C. In soybean it has been shown that cold water only enhances leakage if added at the start of imbibition. Initial exposure to warm water and subsequent chilling does not alter the rate (Simon, 1984). This means standardisation of imbibition temperature needs to be established for comparison of test results.

Experiments assessing the relationship between tests and actual germination usually compare the performance of artificially aged seed with untreated samples. But, in practise, vigour tests need to be able to distinguish between untreated samples which are typical of the material in the seed trade. In this respect, electrolyte analysis has been most effective in species with large living cotyledons. In peas, there is a highly significant correlation with field emergence and the electroconductivity of the soak water is used routinely as a test for vining peas in the UK seed trade (Matthews and Bradnock, 1967). In species such as wheat only a small proportion of the seed is living and therefore, even if a significant proportion of this is dead and readily leached tissue, the conductivity of the soak-water may be increased by only a small amount.

Electroconductivity meters can be set to grade seeds either side of a predetermined limit, ie pass or fail. However this assumes a set level to be the same for all seed lots of any one species. Reasons for differential leaching between batches could be due to size variation or state of the testa, leading to different rates of water uptake (Powell and Matthews, 1979).

4.6.2 Respiration rate

Respiration tests of seed are a good indication of the potential rate of seedling growth, especially the respiratory quotient ie

$$\frac{(\text{Vol } CO_2 \text{ produced})}{(\text{Vol } O_2 \text{ consumed})}$$

However they may need calibration within each cultivar and must eliminate any microbial contribution to respiration (Woodstock, 1967).

In common with the electroconductivity test, bulk populations of seed are considered without any information regarding individual seed performance. Therefore they do not distinguish between lots with many good and a few dead seeds, or seed lots which are uniformly mediocre. This could be resolved by measurement of single seeds, but needs an efficient method of processing large numbers to be statistically informative. A move towards the rapid determination of the conductivity of individual seeds is being made which allows the distribution of levels of leaching within a sample to be assessed.

4.7 Germination test in vitro

Tests based on actual expression of vigour in terms of seed growth response are often simpler with respect to equipment but expenisve in man hours and tend to be more subjective in their assessments.

Laboratory germination tests *in vitro* frequently measure percentage germination as the parameter of vigour, but may include assessment of factors such as time from imbibition to germination and uniformity of the response. Uniformity of results within and between seed testing stations is essential and this is best achieved by using optimal conditions for germination. Such conditions will, however, vary with the species and deviations from this will result in variation of the germination percentage (Perry, 1981). Production of a visible radicle as a physiological criteria of germination is unsatisfactory as the subsequent seedling may be abnormal. Therefore the International Rules for Seed Testing (ISTA, 1976) state the seeds must be grown to the point where it is possible to distinguish between normal and abnormal seedlings.

Because this vigour test measures the proportion of seeds which germinate under optimal conditions in contrast to field conditions which are not, the proportion of seeds producing viable seedlings in the soil environment will be lower than the germination capacity. The significance of the correlation of the test results with field emergence will also decrease the more sub/supra-optimal the seed bed environment becomes. The significance of the correlation also depends on the particular species. In general onion varieties show good correlation of results with a tendency for a lower number of seed emergence to correspond to samples with low germination. The actual numbers are significantly less but are proportional to laboratory germination levels (Bedford and MacKay, 1973). The situation is similar for spring wheat (*Triticum aestivum*) (Bedford *et al.*, 1971). In contrast, regression of field emergence on laboratory germination in carrot seeds is non-significant. However, this situation can be improved by fungicidal seed dressing (Perry and Hegarty, 1971).

4.8. Exhaustion tests: slant-board test

The period which elapses before exhaustion of seedlings grown in the dark and the degree of seedling growth is a measure of the seed reserves which can be drawn on until the autotrophic state is attained. The slant-board test has been best studied for evaluation of lettuce seed lots but should be applicable to other small seeded species as well. Vigour is determined by the root length of seedlings grown on a slant board in the dark. Root length is significantly rank-correlated with transplant dry weight (Wurr and Fellows, 1984). Percentage germination was also significantly correlated with root length. Similarly Smith *et al* (1973) found that the slant test was a useful predictor of emergence rate and seedling growth in lettuce, but in contrast to Wurr and Fellows (1984) also reported a significant correlation with head size at maturity

In lettuce crops, both transplanting and direct drilling methods are used. High germination values are therefore favourable but the seedlings used in transplanting may differ in vigour and hence affect the subsequent crop performance. Therefore the concept of vigour needs to be extended beyond percentage emergence to include seedling evaluation.

4.9. Seedling evaluation

Seedling evaluation as an indication of vigour is made on seedlings grown in the light after emergence from a favourable medium. Characters such as seedling sturdiness and strength were originally the definition of vigour as made by Nobbe in 1876. This method needs calibration for each cultivar by a pre-determined set of criteria which need to be conformed to internationally.

Such morphological manifestations of vigour can be measured in several ways eg root/shoot length, fresh weight of seedlings, axis dry weight. Perry (1969) defined vigorous pea seedlings as those with a strong plumule, well developed stem and dark green in colour, whereas low vigour types, are characterised by a spindly plumule, short stem, incomplete emergence from the testa, chlorotic colour with few or no roots. Vigour assessed by these criteria was better correlated with field performance than to germination test and the slope of the regression line was closer to unity than from in vitro germination, indicating that there was superior distinction between the samples.

Perry (1977) also measured the plumule growth of barley seedlings (Hordeum vulgare) as a parameter of their vigour and found that the results were a better indication of field emergence than germination tests when soil conditions were unfavourable (ie early sowings) but were of little advantage in good growing conditions. Grain yield was also better predicted by plumule quality. However such assessments are subjective and therefore difficult to standardise.

Rate of seedling growth indicates the level of activity and co-ordination of the metabilism of the germinating seed and so is frequently regarded as an expression of seed vigour. Seedling length after a specific period is the product of time from germination and the subsequent growth rate. This is easier to measure than rate which requires several consecutive measurements to establish the relationship with time. Linear measurements are difficult for species such as those of the Leguminosae due to the seedling morphology and low vigour seedlings may have long, spindly shoots. Species which have a single straight plumule eg cereals, or single roots eg lettuce and sugarbeet are the most suitable. However genotypes may have inherently different growth rates which are not related to emergence potential and therefore comparisons can only be made within a genotype. Insecticide/fungicide seed treatments may adversely affect germination and growth on paper. Dormancy will also be a problem if incompletely broken as it decreases the growth potential in a laboratory test but would not necessarily diminish field performance. The action of growth regulators is not sufficiently well understood to permit their use to ensure dormancy alleviation. Rate of germination is also infuenced by initial moisture content which meant that the seed samples to be compared must be equilibrated to the same level.

Although accurate measurements of seedling growth are fairly easily made, and the equipment required is simple and inexpensive, the procedure is time-consuming and sensitive to environmental variation. Therefore the conditions of the vigour test must be carefully controlled to maintain a medium of constant composition and free of phytotoxicity. Moisture content is especially influential and anaerobiosis must be avoided as it increases variability of results among replicates. The seed should be sown at a uniform depth and light must be controlled to prevent etiolation.

Seedling growth can be an inappropriate index of vigour in some cases for example when comparing varieties bred for specific characters eg cold tolerance or disease resistance. Also growth rate is a poor and even misleading parameter when comparing normal and dwarf varieties (Woodstock, 1969).

4.10 Performance in stress conditions

The standard tests described so far have involved evaluating seed performance within a highly controlled environment set to those conditions optimal for germination. Such favourable conditions are untypical of the field and therefore in an attempt to better relate test and field conditions and hence improve the correlation of seed performance in the two, various stress factors have been introduced into the tests. These stresses can be delivered to the seed.

(i) prior to germination

(ii) during germination/seedling growth.

4.10.1 Accelerated ageing

Accelerated ageing of seed through high relative humidity and for temperature treatment was originally used to assess the storage potential of a seed lot but has been developed also as a test for vigour. This is used routinely for soybean in the South East USA. Field trials to date have been limited but germination ability after artificial ageing does appear strongly related to field emergence under adverse conditions (Baskin, 1981).

A refined technique is the controlled deterioration test (Matthew and Powell, 1981) in which there is more precise control of seed moisture content and temperature. Here the seeds gain moisture during the initial period of ageing at elevated temperature and different rates of moisture gain between lots of the same species appears related to variation in extent of ageing within the same time period. Hence, in the controlled method, the initial moisture level is regulated to the same point for all lots prior to the deterioration treatment. Subsequent germination of the treated seed is judged as positive upon radicle emergence.

Matthews (1980) reported this to be a highly repeatable test which was superior to standard germination tests with respect to predicting field emergence and gave a comparable correlation coefficient to the results from leachate electroconductivity.

Controlled deterioration may therefore have potential for use with small-seeded crops such as vegetables and ornamentals.

4.10.2 Germination in stress conditions

Soil conditions in the north of the UK (especially early in the growing season) being adverse to germination give large variations in emergence between different pea seed stocks which are not predicted by the official germination tests (Perry, 1969b). This variation is not entirely due to microbiol activities as it is not eliminated by fungicidal treatments. As soil conditions improve, the difference in performance between lots decreases. This is because emergence of poor samples tends to increase to a greater extent than that of the good samples. Therefore the expression of vigour is dependent on the adversity of the environment which includes edaphic factors (eg excessive soil moisture, too little moisture, low temperature and poor texture) as well as the biotic contributions of pathogenic micro-organisms.

Water stress

Osmotic control of the water potential can be readily achieved in the laboratory by germination of the seed in mannitol or polyethylene glycol solutions. Evaluation of germination and survival over a range of osmotic potentials has been observed to give better distinction between seed lots than does germination in water (Heydecker, 1969).

Where degrees of wetness (rather than dryness as simulated by addition of osmotica) are adversely influential, aeration and microbial activity are the limiting factors.

Cold stress

The major environmental constraint to germination of maize sown in the early spring, is the cold, and therefore germination tests at suboptimal temperatures are more likely to be relevant to the field situation. In the cold test, seeds are sown in soil which often contains known pathogens of maize and placed in cold conditions. Subsequent transfer to a higher temperature allows germination to occur. Results are usually scored on percentage germination but the growth rate may also be influenced (Isley, 1952).

Hiltner test: (Mechanical obstruction):

Mechanical impedance to seedling emergence can also be a characteristic of the soil environment which is not represented in the standard germination tests. The Hiltner Test attempts to assess a seed lot's ability to overcome this by substituting sterilised grit. This test was originally devised in 1911 by Hiltner and Ihssen to detect seedborne infection by Fusarium species as infected coleoptiles are shorter and unable to penetrate a 3 cm layer of brick grit without damage. This test is also capable of detecting other defects and so is viable as a general assessment of vigour. Other medias can be used eg Molochite (Perry, 1978) or Terrapere (Eifrig and Buscher, 1975). A number of modifications in the basic technique may be made such as use of a covering layer which is of a different particle size to the base.

Brick grit has the advantage of being a sterile medium with a closely defined particle size and moisture content. Therefore, reproducibility of results is high. However, actual conditions in the field are very different with much heterogeneity of the chemical, physical and microbiological qualities. Therefore relationship of results ranges from good to poor depending on the particular soil environment for which the prediction is being made (Perry, 1978). Other disadvantages include high costs in terms of time and space, and the level of variation is higher than from the germination test. However the Hiltner test seems to detect samples with low vigour more reliably than the germination test.

The Hiltner test appears to have scope for crops such as cereals (and maize), large legumes, vegetables such as sugar beet and carrots and some small-seeded types eg clover. Comparative tests show that conditions for emergence are relatively more severe for monocotyledous than dicots.

An alternative and somewhat cheaper test is the Fritz penetration method in which the obstacle to emergence is a paper layer (Perry, 1977). Such tests need strict standardisation if results between laboratories are to be comparable.

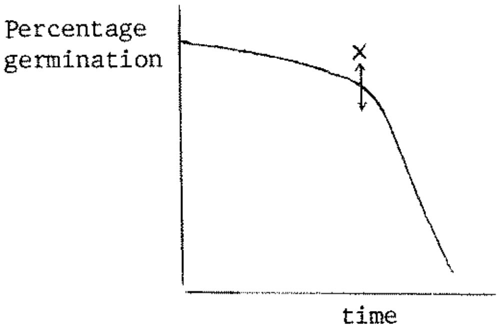
4.11 Seedling emergence in soil

Evaluation of seedling emergence in soil rather than artificial media may overcome the criticism of unrealistic homogeneity, but makes it almost impossible to standardise. Also distinction between the various edaphic and biological factors cannot be made. This makes for low reproducibility of results. Germination and survival in a range of field environments can be assessed, but since the conditions for each location and year are unique, the test conditions may be equally unrepresentative of those to be encountered by the crop. In general, the best approach in such tests is to avoid soil types with extreme properties (Heydecker, 1969) and extrapolation of genotype - environment interactions should only be made with extreme caution.

4.12. Tests of field results

Perry and Hegarty (1971) stated that, providing the field conditions were favourable and a shallow sowing technique was used, then adjustment of the seed rate of onion according to the percentage germination of in vitro tests would be satisfactory. However Perry (1978) concluded in a review that there had, in general, been little success in establishing a link between vigour tests and mean field emergence of vegetable seed lots. Comparisons of various vigour testing methods for accuracy of prediction have presented similarly varying conclusions, depending on the crop species and the particular year of field trials. Polizotto (1981) using soybean seed, ranked the test methods with respect to correlation to crop performance as: warm germination > accelerated ageing > TTC > cold test. Suryatmana et al (1981) reported that, whilst in favourable years, suboptimum conditions were best related to by a combination of standard germination and accelerated ageing, the electroconductivity test was the best single estimate under stress conditions.

Matthews (1981) suggested that the discrepancy between germination and emergence was due to the pattern of seed deterioration. Percentage germinability of a seed lot shows a gradual decline with age before a rapid fall



Any seed samples taken from the shallow gradient section will show little difference in germination, but a sample from the area around x will show significantly less capability when placed under environmental stress.

4.13 Conclusion

Although field performance is related to vigour, seed vigour is an innate quality of the seed and must therefore be measured under controlled conditions in a laboratory (Woodstock, 1969). This means that actual field performance in terms of percentage of emergence cannot be predicted as this is a function of a complex combination of factors over a long period of time. If the seed bed environment is particularly adverse, few seedlings will emerge regardless of their vigour and if conditions approach optimal, emergence may correlate with germination and the vigour tests will show no advantage. It is usually the case that the situation lies between these two extremes and there can be a difference in the field performance of seed lots with similar viabilities. The aim of vigour tests should be to identify those lots with the maximum levels of tolerance or adaptivity.

5. Future improvements of vigour testing

5.1 Test modifications:

An improvement of the single-factor stress tests for testing the vigour of wheat and maize seeds has recently been published as the Complex Stressing Vigour Test (CSVT) (Barla-Szabo and Dolinka, 1988). The seeds were stressed for the first four days by soaking at 20-25°C for 48 hours followed by 2-5°C for a further period. Positioned germination and subsequent seedling evaluation gave results which the authors considered to reflect the general physiological quality of the seed more reliably than the cold test, accelerated ageing or electroconductivity test, giving highly significant correlations with field emergence data.

There have also been suggestions of non-destructive viability tests of single seeds (Matthews, 1984). One approach could be to test and sort seeds that have been hydrated and follow this by dehydration. Experiments have been made in which viable and non-viable seeds of peas, soybean and sprouts have been identified by comparing the electrolyte leakage from single seeds. The problem of defining a demarcation point in the presence of some seeds which are germinable but contain dead tissue still remains however. More simply, Lodge Pole (Pinus contorta) seeds can be separated by comparing the rates of water loss in the drying phase following hydration. This has also shown promise for some vegetable seeds. However the hydration of seed is not desirable in a non-destructive test as this stimulates metabolic activity.

The suggestion that there is a link between seed and membrane deterioration and the autoxidiation of unsaturated fatty acids (Harman and Matlick, 1976) could provide the basis of a novel test of measuring the levels of organic free-radicals in the seed through the electron paramagnetic resonance spectra of individual seeds.

5.2. Statistical methods

Interpretation of vigour test results requires appropriate statistical analysis methods. Favourable and unfavourable soil conditions are generally assessed by the magnitude of the overall mean of seed lots sown in trials without understanding the actual limiting factors. Regression of seed performance on the environment as measured by this biological standard should therefore give low regression coefficients for high vigour lots and high regression coefficients for low vigour lots. This means that before a vigour test can be employed commercially, the emergence behaviour of seed needs to be investigated over a wide range of lots in several environments to establish if a systematic pattern exists (Perry, 1978).

A problem with the application of statistics to comparing germination data is the lack of a good model to quantitatively describe the pattern of germination with respect to time. Polynomial regressions and the normal distribution are not satisfactory, so Bonner and Dell (1976) proposed the three parameter Weibull function (as used in materials reliability testing). This allows calcuation of the cumulative proportion of germination in a seed population.

where: $F(x) = F(t)$

(x = proportion of germinated seed
(t = number of days.

The derivation of F(x) is f(x) which is the probability density function and describes the change at any moment in time in the cumulative proportion germinated. That is, f(x) describes the normal-shaped curve of change while F(x) is the function for the S-shaped cumulative frequency curve.

Such a function however is only concerned with seeds capable of normal germination and does not take into account those which are non-viable.

The Weibull function is readily computerised and through the analysis of germination patterns the relationship of germination in the laboratory and seedling emergence in the field may become clearer.

5.3. Test automation

At present, the labour intensive nature of vigour tests is a major limitation to their general application in the seed trade. Automation of the tests is most readily achieved at the measuring and data handling stage (rather than the setting up of the seed in a germination medium). Keys (1982) described how the electroconductivity test could be combined with CASAS (Computerised Automated Seed Analysis System) to give continuous monitoring of leachates under the control of a micro-computer. The data is therefore fed directly into the computer which performs the analysis and compares it with a pre-set standard. This system could successfully distinguish between deteriorated and untreated samples of peanut (*Arachis hypogaea*).

The CASAS can also be modified to allow automated seedling length measurement. The micro-computer is interfaced with hand-operated calipers which transfer the length measurement directly to the computer thereby cutting the analyst time by 50%. The vast majority of the time saved here is at the imput of data stage; the time for actual measurement of length being reduced only by about a fifth (Keys, *et al.,* 1984).

Number of man-hours could therefore be significantly reduced still further by incorporating machine-vision. Such image analysis techniques have already been shown to be successful in measuring carrot embryos in an automated uniformity test (Keefe and Draper, 1986).

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