

Electrical Conductivity Vigour Test: Physiological Basis and Use

By Stan Matthews and Alison Powell, ISTA Seed Vigour Committee Chair
School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland, a.a.powell@abdn.ac.uk

Introduction

The electrical conductivity (EC) test for garden peas (*Pisum sativum*) is one of only two vigour tests included in the ISTA Rules for Seed Testing (ISTA, 2006). Bulk samples of 50 seeds are soaked in deionised water and the EC of the soak water is measured after 24 hours. The test is both quantitative and repeatable. In many reports on peas, the EC readings for lots have been found to relate significantly to field emergence (Matthews and Powell, 1981). High levels of leakage are a characteristic of low vigour lots with acceptably high levels of laboratory germination (e.g. above 80%) but low field emergence, particularly in cold, wet soils.

The earliest example of EC as a vigour test was for castor bean (Thomas, 1960) when performance in a soil test was related to the EC of seed soak water. This observation gave the lead to later work on garden or vining peas, when the frozen food industry in the UK came up against a problem. The programme to spread the harvesting of peas ready for freezing included early season sowings, which exposed weak, so-called low vigour seed lots, that were satisfactory in laboratory germination tests, but failed in the field. The relationship between field emergence and EC turned out to be not only interesting, but useful in practical seed technology (Matthews and Bradnock, 1967). An understanding as to the physiological basis of this relationship came later as we describe in this brief review. We have focussed on the two main explanations for high leakage from low vigour seeds for which there is substantial evidence, namely seed ageing and imbibition damage.

Seed ageing

When seed scientists discuss the causes of the leakage from low vigour seeds, they almost always start with a reference to the

deterioration of membranes, resulting from either artificial or natural ageing. Biochemical analyses and hypotheses about seed deterioration suggest impaired membrane activity is a factor leading to leakage (McDonald, 1999). This can have a role in the early stages of seed ageing (Powell and Matthews, 1977). However, the greatest increases in leakage seen in commercially available seed lots are often associated with an increase in dead tissue on the cotyledons which does not result in the failure to germinate in the laboratory (Matthews and Rogerson, 1976). Similar seeds can be produced by artificial ageing (Powell and Matthews, 1977). In terms of the seed survival curve (Figure 1) these seeds would be placed on the slow initial decline in germination.



Figure 1: Seed survival curve. Seed germination initially declines slowly, followed by a steep fall in germination.

The effect of ageing on cotyledon tissues of peas is illustrated in Figure 2, which depicts staining with tetrazolium chloride (TZ) of cotyledons after ageing and slow imbibition in moist tissues. This vital stain, TZ, is well known to seed technologists, being extensively used in viability testing. The red formazan which is formed by the reduction of TZ by dehydrogenases indicates respiratory activity in living cells (ISTA, 2003).

As ageing progresses from the top row of em-



bryos in Figure 2, the areas of the cotyledons that remain unstained increase, even though the staining of the embryonic axes indicates that the seeds will germinate. It is these dead areas that contribute much of the electrolyte leakage into seed soak water (Matthews and Rogerson, 1976). Legumes with large, normally living, cotyledons are good candidates for the EC vigour test to indicate field emergence, because they still germinate in the laboratory even with considerable areas of dead tissue on their cotyledons, provided that critical areas of the embryo remain living (ISTA, 2003).

Analyses of seed soak water have shown that potassium is a major constituent of the electrolytes leaked out in peas (Matthews and Rogerson, 1976) and in soyabeans (Dias *et al.*, 1996). The conductivity of pea seed soak water was found to be closely correlated with both the sugar and amino acid content, indicating general solute loss from seeds (Mat-



Figure 2: Tetrazolium staining of pea cotyledons reveals an increase in the incidence of dead tissue as the extent of seed ageing increases.

thews and Carver, 1971). This is also a feature of leachates from other species (Schroth and Cook, 1964; Duke and Kakefuda, 1981). The levels of leakage were not associated with the extractable solute content of the embryos (Matthews and Rogerson, 1976).

A further reason why the conductivity test works as a vigour test can be illustrated by looking again at the seed survival curve, and particularly at the slow decline in germination. The further to the right that a seed lot is placed on the initial decline, the lower the germination. A germination of 80% is often taken as an acceptable minimum for the use of a seed lot, based on the laboratory germination of a sample of 400 seeds. If a sample of 50 seeds from the same seed lot is soaked in water, then on average 10 of the 50 seeds would be non-germinable. Work on many species has shown that high levels of leakage are characteristic of seeds incapable of germinating (Steere *et al.*). Thus, as we go down the slow initial decline, the conductivity will increase as the laboratory germination falls, in addition to the reduced ability of germinable seeds to retain cell contents.

Imbibition damage

Imbibition damage is a phenomenon that has been clearly shown to influence vigour in a range of temperate and tropical grain legumes (Powell *et al.*, 1984), although it may also occur in small-seeded dicotyledonous crops (Thornton and Powell, 1992; Draper and Keefe, 1990). Imbibition damage results from the rapid entry of water into the cotyledons during imbibition, leading to cell death and high solute leakage from the seeds (Powell and Matthews, 1978a). When seeds are soaked in water, the almost instantaneous occurrence of damage within 2 minutes of the beginning of imbibition suggests that it results from physical damage, possibly through the disruption and disorganization of cell membranes. Certainly, the extensive loss of cellular material and enzymes from the seeds (Powell and Matthews 1981a; Duke and Kakefuda, 1981) indicates extensive membrane disruption.

Imbibition damage is prevented by slowing down the rate of water uptake and is greater at low temperatures in peas (Powell and Matthews, 1978a), soyabean (Leopold and Musgrave, 1979) and lima bean (Pollock, 1969), possibly because the membrane components are held more rigidly and are therefore more

sensitive to physical damage from the considerable forces generated by the imbibition process. These forces result from the difference in water potential between pure water (0 mega Pascals [MPa]) and that of seed tissues. The water potential of seeds is generated by the slightly hydrated starch and protein molecules and can be lower than -3.0 MPa. This is extremely low, with the result that an air dry seed can initially pull in water against a pressure equivalent to 30 atmospheres and more (Hadas and Russo, 1974).

As the seed hydrates, its water potential increases to reduce the differential between the seed and the outside. Conversely, the drier the seed tissues, the lower the water potential and the greater the differential between the seed and the outside substrate, be it soak water, laboratory germination media or soil. The relevance of imbibition damage to field emergence has been clearly shown for peas, when seeds imbibed in wet field soil were removed and stained with TZ (Powell and Matthews, 1980).

The observation of imbibition damage is most apparent when seed coats have been removed from dry seeds before imbibition. This has highlighted the role of the testa in protecting the cotyledons from the damaging effect of water uptake (Powell and Matthews, 1978a; Tully *et al.*, 1981). In addition, these observations on imbibition damage have brought into question the evidence used by Simon and Raja Harun (1972) to support their hypothesis of changes in membrane conformation during early imbibition of dry embryos. They proposed that the high leakage of solutes in the first few minutes of imbibition that they observed from pea embryos (seed minus testa) resulted from the membranes of the dry seed being in a porous hexagonal state. The subsequent decline in leakage as the embryos imbibed was explained by the formation of the normal bilamellar membrane following hydration, which restricted leakage. However, Powell and Matthews (1978a) suggested that the initial high leakage during imbibition resulted from the death of the outer cells of the cotyledons due to imbibition damage and that the decline in leakage represented a slower loss of solutes from undamaged tissue within the seed. This was in part suggested on the basis of the TZ staining of cotyledons after imbibition damage, where only the outer layers of cotyledon cells failed to stain (Powell and Matthews, 1978a). This

was later confirmed in soyabean by Tully *et al.* (1981). Indeed subsequently, we went on to demonstrate that the pattern of leakage observed by Simon and Raja Harun (1972) occurs from completely dead embryos and from any spherical structure containing ions (Powell and Matthews, 1981a).

In some species, such as peas and soyabean, the presence of an intact testa limits the incidence of imbibition damage (Powell and Matthews, 1979; Oliveira *et al.*, 1984). In these species, the incidence of damage to the testa influences the extent of imbibition damage and the vigour of the seeds. Thus seed lots showing extensive testa damage imbibe rapidly and exhibit a high incidence of imbibition damage (Powell and Matthews, 1979, 1981b). These lots emerge poorly in the field (Powell and Matthews, 1980; Oliveira *et al.*, 1984) and therefore have low vigour. In contrast, seed lots with little testa damage imbibe slowly, show little imbibition damage and have high emergence. This emphasizes the importance of the integrity of the testa in determining the vigour of some grain legume species and hence the need to minimize damage to the testa during harvest and processing.

In other grain legume species however, there is also a genotypic component to the susceptibility of seeds to imbibition damage. Thus cultivars of *Phaseolus vulgaris* (Powell *et al.*, 1986a, 1986b), soyabean (Tully *et al.*, 1981), chickpea (*Cicer ciceris*; Legesse, 1991), long bean (*Vigna sesquipedalis*; Abdullah *et al.*, 1991) and cowpea (*Vigna unguicularis*; Legesse and Powell, 1992), in which the testa is partially or completely unpigmented, imbibe more rapidly and show greater levels of imbibition damage compared with cultivars having pigmented testae. As a result, the vigour of unpigmented cultivars is reduced, leading to poorer field emergence than is found in pigmented cultivars (Powell *et al.*, 1984). The close association between pigmentation and slower rates of imbibition has been demonstrated in studies on seed development (Legesse and Powell, 1996) and on isogenic lines of peas, differing only in the A gene for testa pigmentation (Powell, 1989). In some species, for example *Phaseolus vulgaris* (Powell *et al.*, 1986b) and chickpea (Legesse and Powell, 1996), the close adherence of the pigmented testa to the cotyledons appears to limit the rate of water movement within the seed. In cowpea, however, testa permeability

also has a role (Legesse and Powell, 1996). Identification of the factor associated with pigmentation that leads to reduced rates of water uptake, could lead to the introduction of this characteristic to unpigmented cultivars through breeding programmes, thereby increasing the seed vigour of these cultivars.

Interaction of imbibition damage and ageing

The two major causes of reduced vigour in grain legumes, ageing and imbibition damage also interact, with aged seeds being more susceptible to imbibition damage seen in reduced TZ staining and increased leakage into soak water. When the testae of seeds of peas (Powell, 1985) and cowpeas (Asiedu and Powell, 1998) were scarified before imbibition, leading to more rapid water uptake, the extent of living tissue declined much more markedly in aged than in unaged seeds. The increased susceptibility of aged seeds to imbibition damage could be the result of the weakening of cell membranes by physiological deterioration, with the result that the membranes are more sensitive to physical damage during imbibition (Powell, 1985; Asiedu and Powell, 1998).

Leachates and emergence in soil

The nutrient status of seed leachates has been suggested as a stimulatory factor for fungi close to the germinating seed in, for example, *Phaseolus* beans (Schroth and Cook, 1964) and peas (Perry, 1973). There is also experimental evidence (Matthews, 1971) to suggest an additional explanation, that dead areas on cotyledons, which result from ageing and/or imbibition damage, act as a focal point and a food base for infection by the weakly parasitic soil-borne fungus *Pythium ultimum*

Extending the use of the conductivity test

Conductivity tests have also been applied to detect vigour differences in many other grain legumes and indeed some other species (ISTA, 1995). The methods remain to be developed and standardised for these species which include many grain legumes such as soyabean (Oliveira *et al.*, 1984; Yaklich *et al.*, 1979), longbean (Abdullah *et al.*, 1991) and *Phaseolus* (Abdullah, 1988; Powell *et al.*, 1986a). However, the correlations between conductivity readings and field emergence are encouraging (Table 1).

Prediction of viability by EC

The assessment of the conductivity of single

Table 1 Correlation coefficients (r) of EC of seed soak water and field emergence for 18 seed lots¹ of soyabean (*Glycine max*), 80 lots² of soyabean, 30 lots of *Phaseolus vulgaris*³ and 11 lots of long bean⁴ (*Vigna sesquipedalis*)

Soyabeans			<i>Phaseolus vulgaris</i> ³		Longbean ⁴
Sowing 1 ¹	Sowing 2 ¹	Range for 12 sowings ²	Sowing 1	Sowing 2	
-0.89***	-0.92***	-0.67*** to -0.79***	-0.82***	-0.87***	-0.86***

¹Oliveira *et al.* (1984); ²Yaklich *et al.* (1979); ³Powell *et al.* (1986a); ⁴Abdullah *et al.* (1991); ***p ≤ 0.001

seeds was proposed as an assessment of seed viability (Steere *et al.*, 1981), following the development of an instrument to measure the conductivity of the leachate of 100 single seeds simultaneously. Studies with pea, soyabean, cotton, *Phaseolus* bean, maize and small-seeded crops provided evidence that analysis of the single seed leachate conductivity, could indicate both standard germination and seed vigour. The instrument does not however adjust readings to take account of seed weight. It is therefore recommended that each of the 100 seeds be weighed prior to testing so that the average reading can be recorded per gram individual seed weight (Hepburn *et al.*, 1984) i.e. as $\mu\text{amps cm}^{-1} \text{g}^{-1}$ or $\mu\text{S cm}^{-1} \text{g}^{-1}$, depending on the instrument used.

As a routine measure of germination, the original proposed use of the instrument, the determination of single seed conductivity, has not proved successful because of the lack of a clear and consistent conductivity for each species that separates viable from non-viable seed (Hepburn *et al.*, 1984). Work on cotton (Perl and Feder, 1983) confirmed the difficulty of predicting laboratory germination, but showed that the percentage of seeds below a relatively low conductivity after 18 hours soaking was closely related to both the rate of emergence and final emergence when the same seeds were sown in the field.

Much earlier work on the use of electrical conductivity of seed soak water to predict viability used bulks of seed, not single seeds. Hibbard and Millar (1928) found that in bulk samples of wheat, peas and timothy grass that showed wide ranges of germination, the conductivity of the soak water increased (or in their measurement, the resistance to electricity decreased) as germination decreased. In cotton, Presley (1958) generated different levels of viability by artificial ageing and showed that the conductivity of soak water increased as germination decreased. More recently Mirdad *et al.* (2006) generated samples of cauliflower and cabbage contain-

ing different proportions of germinable seeds by ageing seeds at raised seed moisture content and high temperature and found that the conductivity of soak water using bulks of 50 seeds related well to germination. This applied to a wide range of germination levels, similar to those in early work, with r^2 values of from 0.79*** to 0.96*** (cauliflower and cabbage) and also to germination levels from 90 to 98% (r^2 0.98*** for cauliflower). There is clearly potential for a 24 hour test of germination based on the electrical conductivity of the soak water of bulk samples of seeds.

Future developments

The challenges for the future include extension of the validated routine EC method to other crops beyond peas, starting with *Phaseolus* and soyabeans. Work on these crops is underway within ISTA, and will move on to other grain legumes. This requires a systematic approach on appropriate seed material, taking account of some of the scientific understanding that we already have. An example would be the effect of a low initial seed moisture content. This has been shown to slow down imbibition in peas (Powell and Matthews, 1977) leading to a misleadingly low reading. In contrast low seed moisture content in soyabean can lead to inappropriately high readings (Loeffler *et al.* 1988). This may result from the increased sensitivity to imbibition damage in both soyabeans (Obendorf and Hobbs, 1970) and lima beans (Pollock, 1969) observed in very dry seeds having extremely low water potentials. Hence in routine EC testing the moisture content of peas and soyabeans must be adjusted to within the range 10 – 14% before EC testing.

The measurement of EC as an assessment of viability goes back as far as Hibbard and Millar (1928). We believe their bulk method has a potential that has not yet been realised. To achieve a repeatable measure of viability in 24 hours or less would be valuable in seed production and commercial seed testing. The challenge is by no means insurmountable, needing a methodical scientific approach

and would not require technically elaborate equipment.

The measurement of EC could also have a role alongside ageing-based vigour tests, like the accelerated ageing and controlled deterioration tests, by giving a measure of viability following ageing in 24 hours in place of a germination test of 7 days or longer (Thornton *et al.*, 1990). In our own work EC after rapid ageing predicted the longevity of pea seeds in commercial storage (Powell and Matthews, 1978b).

The main intention in writing this brief review was to explain the basis of the EC test. We also hope that others will be encouraged to take up the challenges of extending the use of conductivity in seed technology.

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