

## Seed Vigour: From Concept to Internationally Validated Test Methods

ALISON A POWELL\* AND STAN MATTHEWS

Institute of Biological and Environmental Sciences, University of Aberdeen, AB24 3UU, UK  
\*agr791@abn.ac.uk

The aim of this review is to examine how seed vigour has developed from a concept to an accepted aspect of seed quality that is used commercially throughout the world. We shall consider why seed vigour is important in crop production before reviewing vigour tests. We shall consider how vigour tests were initially developed empirically before being hypothesis-based, giving emphasis to tests that have been validated for use by the International Seed Testing Association (ISTA) and are included in the ISTA Rules for Seed Testing. We shall focus particularly on the ageing/repair hypothesis, its development and application.

### CONCEPT OF SEED VIGOUR

The concept of seed vigour was first recognized in 1876 by Friedrich Nobbe who introduced the term '*Triebkraft*', meaning driving force [1]. This led to numerous debates and developments regarding the definition of vigour and methods of vigour testing which have been reviewed on many occasions [2,3 & 4]. The ISTA Vigour Committee was first established in 1950 as the ISTA Biochemical and Seedling Vigour Committee by ISTA President WJ Franck. The committee has discussed the definition of vigour for many years and has investigated the effects of seed vigour and methods of testing vigour. The committee's work ultimately led to the definition of vigour proposed in 2001 and the acceptance of Seed Vigour Testing as Chapter 15 in the ISTA Rules by the ISTA Ordinary Meeting. This definition states that 'Seed vigour is a sum of those properties that determine the activity and level of performance of seed lots of acceptable

germination in a wide range of environments'. The characteristics of a seed lot associated with vigour are the rate and uniformity of seed germination and seedling growth, the emergence ability of seeds under unfavourable environmental conditions and seed lot performance after storage, particularly the retention of the ability to germinate (normal germination).

The above characteristics are illustrated for several crops in Table 1. Clear differences in field emergence can be seen for commercial seed lots from both field (maize and oilseed rape) and vegetable (radish, cucurbits and pepper) crops. These are particularly evident when seeds have been sown in stressful conditions such as low temperature or where there is mechanical impedance. Seed lots sown in more controlled environmental conditions, as seen during transplant production in modular trays, often do not show marked differences in emergence (Table 1). However, in this case differences in performance are reflected in the rate of emergence (mean emergence time) and variation in seedling size (Table 1).

### SIGNIFICANCE OF SEED VIGOUR TO CROP PRODUCTION

The significance of the impact of the vigour differences illustrated in Table 1 depends on the nature of the crop in question. Where low vigour leads to reduced crop establishment, this can have a direct effect on yield. In many cereals, however, this effect is limited if emergence is only slightly reduced, due to the ability of the plant to tiller and hence produce compensatory growth.

Table 1. Characteristics of commercial seed lots of a range of species, illustrating differences in performance of lots having high normal laboratory germinations

Species (no. of lots)	Range of normal germination(%)	Field (F) or module (M) emergence (%)	Germination after storage (%)	Mean emergence time (days)	Coefficient of variation in plant height
Maize(29)	92-98	48-98(F)			
Oilseed rape <sup>1</sup> (9)	89-99	65-93(F)			
Radish <sup>2</sup> (9)	79-100	50-95(F)	37-92 12 Months Equilibrated at 75% RH25°C		
Watermelon <sup>3</sup> (9)	90-100	12-58(F)			
Watermelon <sup>3</sup> (10)	94-98	35-79(F)			
Watermelon <sup>4</sup> (10)	>98	80-35 (F: low temperature sowing) 82-13 (F: mechanical stress, deep sowing)			
Melon <sup>4</sup> (10)	>98	82-40 (F: low temperature sowing) 82-30 (F: mechanical stress, deep sowing)			
Cucumber <sup>4</sup> (9)	>98	82-35 (F: low temperature) 83-28 (F: mechanical stress, deep sowing)			
Pepper <sup>5</sup> (13)	86-97	71-96 (F: low temperature sowing) 63-93 (F: high temperature sowing)	56-92 8 months, 8.1% MC, 5°C		
<i>Avena sativa</i> <sup>6</sup> (15)	80-93	Sowing 1(F) = 47-78 Sowing 2(F) = 76-92			
<i>Elymus nutans</i> <sup>6</sup> (15)	70-84	Sowing 1(F) = 23-56 Sowing 2(F) = 27-60			
Cabbage <sup>7</sup> (12)	72-100	43-95 (M)			
White cabbage <sup>8</sup> (3)	83-98	90-98 (M)		4.4-6.3	13.6-36.9
Red cabbage <sup>8</sup> (2)	85-97	98-100 (M)		4.6-6.3	22.1-32.5
Calabrese <sup>8</sup> (3)	88-99	91-96 (M)		4.1-4.6	14.7-25.6
Brussels sprouts <sup>8</sup> (4)	81-100	44-100 (M)		4.2-5.0	19.7-27.7
Cauliflower <sup>8</sup> (4)	81-98	83-92 (M)		4.4-6.2	21.5-28.7

<sup>40</sup>Matthews et al. (2012); <sup>41</sup>Mavi et al. (2014); <sup>42</sup>Mavi and Demir (2007); <sup>39</sup>Mavi et al. (2010); <sup>48</sup>Basak et al. (2006); <sup>42</sup>Ly et al. (2016)

Nevertheless, if emergence is markedly reduced, so is yield. In contrast maize does not tiller and is sown to a specific plant stand. Thus whenever seeds fail to emerge and there are gaps in the crop, yield is reduced.

In vegetable crops, the impact of reduced establishment is often on marketable yield, since particular plant densities are required to produce specifically sized crops for the market [5]. In this case the rate and uniformity of emergence are also critical to the production of a product that is uniform in size and stage of maturity [5]. Seeds that show rapid, synchronous germination and emergence typically produce larger and more uniform seedlings [6 & 7]. Thus in the examples in Table 1, the seed lots of cabbage, calabrese and Brussels sprouts that had a higher mean emergence time, that is emerged more slowly, were also those that produced seedlings that were not only smaller, but more variable in size. These differences in size are maintained throughout crop growth and therefore impact on the production of a uniform crop for once-over harvests.

If the plant populations produced by high and low vigour seeds are the same, the impact of vigour on yield of annual crops depends largely on the nature of the harvested material [8]. Thus crops harvested in the vegetative state e.g. lettuce, cabbage, radish, carrot, and crops such as peas and tomatoes, which are harvested during early reproductive growth, have shown a positive yield response to use of high vigour seed. In contrast, in crops harvested at full reproductive maturity, i.e. the grain crops in which only the seeds are harvested for yield, there was no relationship between vigour and yield [8].

### SEED VIGOUR TEST DEVELOPMENT

The approach to seed vigour test development has changed over the years from one that was empirically-based i.e. based on observations, to one that is hypothesis-based. Thus the early development of tests such as the Hiltner [9] and cold tests [10 & 11] were based on observations of seed lot emergence under mechanical and cold, wet conditions respectively, and simulated such conditions in the laboratory. Similar observations

of differences between seed lots in seedling growth led to seedling growth tests.

In contrast the electrical conductivity test was developed on the basis of both observation and hypothesis. Early work investigated the relationship between conductivity and germination [12] and illustrated greater loss of electrolytes (higher conductivity) from samples with low germination. There was however no comment on the differences that were seen between samples with high germination. Later work associated differences in leakage amongst highly germinable seed lots of maize [13] and castor bean [14] and inversely related to emergence in soil.

Observations on pea seeds [15, 16 & 17] had noted that failure of commercial seed lots to emerge was associated with infection of the seeds by the facultative soil-borne parasite *Pythium* spp. Furthermore, high incidence of infection was seen in seed lots that showed high levels of leakage from the seed, assessed by the electrical conductivity of seed leachates. In contrast lower levels of infection were seen when leakage was low [17]. They suggested that leachate conductivity might therefore be a test of emergence potential of pea seed lots. The leachate from the seeds included sugars and amino acids as well as electrolytes [17] and seed lots with high leakage included dead tissue on the cotyledons [18]. This led to the hypothesis [18] that increased predisposition of seed lots to infection, and subsequent failure to emerge, may result from the dead tissue acting as an initial site of infection and from the leakage of solutes into the soil causing an increase in inoculum potential. Bradnock and Matthews, 1970 [19] subsequently illustrated that differences in field emergence of peas were clearly predicted by conductivity. The negative correlation between conductivity measurements of solute leakage and the ability of seed to achieve good field establishment has been shown on many occasions since then [20] and is well recognised as a test for seed vigour. The conductivity test for garden peas was one of the first two tests to be validated by ISTA and included in the ISTA Rules for Seed Testing in 2002.

Possibly the first vigour test to be based on a hypothesis was the accelerated ageing (AA) test developed by [21]. In their work they aimed to predict differences between seed lots in the decline in germination, i.e. their storage potential, in the hot, humid conditions of Mississippi and tropical climates. The test is based on the pattern of ageing in a population of seeds that is described in the seed survival curve (Fig. 1), whereby there is a long slow decline in germination, followed by more rapid, then linear decrease in germination until the ability to germinate is lost. The test is based on the well-known rapid deterioration, or ageing, of seeds having a high moisture content and at high temperature. Their hypothesis [21] was that if seeds were held at a high relative humidity the seed moisture content would increase. This high moisture content, along with a high temperature would simulate the effects of prolonged storage in less extreme, but still unfavourable storage conditions, and results in rapid seed ageing. Thus after the accelerated ageing test, the seed lots that had good storage potential would retain a high germination whereas those lots with poor storage potential would have a low germination. Their work on AA showed that test results reflected the storage potential of a wide range of species, including soybean [22], maize [21] and ten vegetable species [21]. Additional studies have illustrated that the AA test also predicts field emergence in a range of crop species [23]. Following sowing lots under stressful conditions, AA had a higher correlation with field emergence than standard germination in both soybean [24 & 25] and maize [26]. The AA test for soybean was one of the first two tests to be validated by ISTA and included in the ISTA Rules for Seed Testing in 2002.

A further test based on the hypothesis that rapid ageing would reveal differences in seed vigour is the controlled deterioration (CD) test for small-seeded vegetable species. However, this test differs from the AA test in that the seed moisture content is raised to a pre-determined level (usually 20%) before seeds are held at a high temperature. This approach was selected following the observation of widely differing seed moisture contents for onion seed lots after 1 day AA. As a result of these differences in moisture

content the extent of ageing would differ amongst the lots and be reflected in their subsequent germination [27]. Thus, the vigour of seed lots could not be compared. The deterioration of seed lots at the same precisely raised seed moisture content and high temperature has ensured that all seed lots are subjected to the same degree of ageing. This means that seed lots that are placed at different positions on the seed survival curve (Fig. 1a) will move equal distances along the curve during the CD test, resulting in clear differences in germination after CD (Fig. 1b). The germination after CD has been related to field emergence and storage potential of a number of vegetable species (Matthews, 1980) and the test has been validated for *Brassica* spp. and included in the ISTA Rules since 2010. More recently, following work at the French Official Seed Testing Station, alternative procedures for two stages of the CD test have been validated and included in the ISTA Rules (2017) [28].

The vigour test that has been added to the ISTA Rules most recently is the Radicle Emergence (RE) test. This is based on the ageing/repair hypothesis and there is evidence that the test will be applicable to a very wide range of species. The remainder of this review will focus on the ageing/repair hypothesis and the RE test. We shall also reveal how this hypothesis explains the basis of vigour and all other vigour tests.

### SEED AGEING/REPAIR HYPOTHESIS

Mean germination time as a measure of the rate of germination was first suggested by Ellis and Roberts, 1980 [29] and is calculated for a seed lot as:  $\frac{\sum t}{n}$ , where  $n$  is the number of seed newly germinated to radicle emergence at time  $t$  and on day  $\sum t$  is the total of all germinating seeds. This measure of the rate of germination is in fact the average delay for a seed lot from the start of imbibition to the time of radicle emergence, as pointed out by [30]. This delay, sometimes referred to as a lag period [31], was described by [32] in aged rye embryos. She explained this lag period as the time needed for the metabolic repair of DNA damage before germination could proceed. [30] suggested that the longer lag period (higher MGT) seen in aged, low vigour seed lots resulted from the need for

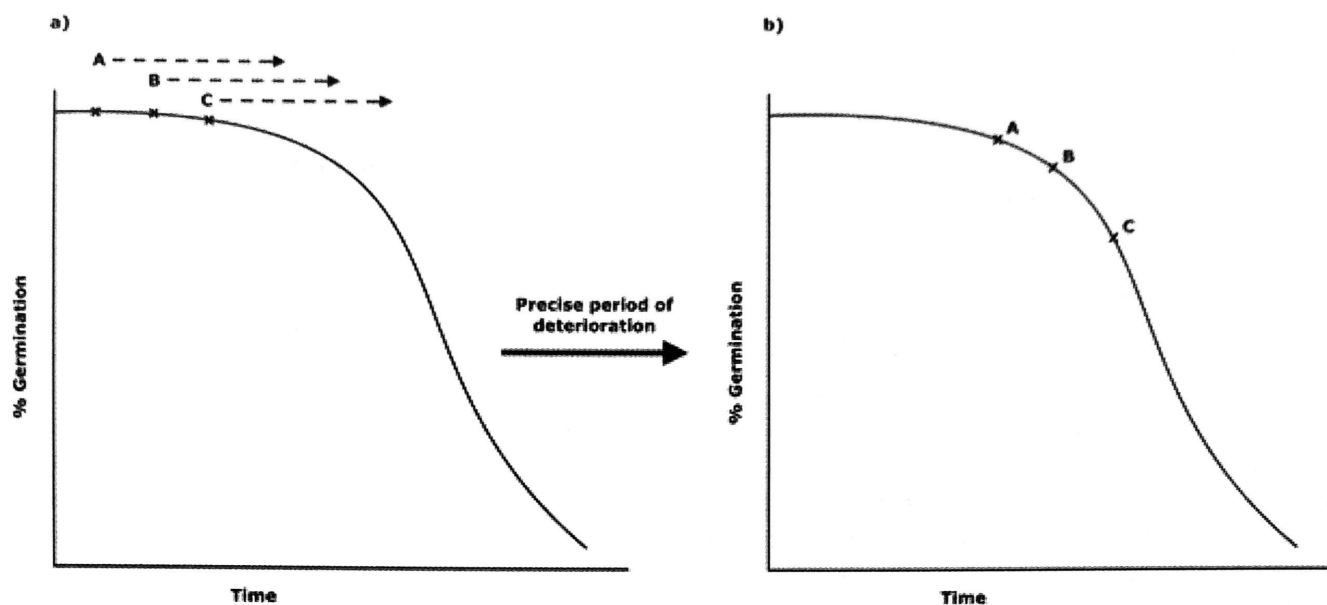


Fig. 1. Seed survival curve, illustrating the position of seed lots on the curve a) before and b) after controlled deterioration

more repair compared to unaged, faster germinating (lower MGT) seed lots. This proposal [30] was supported by the observation that in many species where MGT was related to emergence performance, the slow germinating lots were more aged, as measured by accelerated ageing or controlled deterioration [33]. Furthermore, lower temperatures prolonged the lag period and increased MGT, although the relative rates of germination between lots were maintained. The greater delay to RE at the lower temperature could be explained by slower, therefore less, metabolic repair at a reduced temperature.

Observations on the effect of priming can also be explained on the basis of the hypothesis. Aerated hydration priming of Brassica seed reduced MGT through a shortening of the lag period, which was greater in aged than unaged seeds [34]. In tomato, reduced oxygen levels down to 3% reduced the final germination to less than 10% in unprimed seed and seeds primed in polyethylene glycol for 3 days [35]. In contrast, after 7 days priming germination was more than 80% in 3% oxygen. Our interpretation would be that oxygen was needed for metabolic repair early in the germination process, but not later. Thus

the longer priming treatment enabled sufficient repair for germination to proceed at 3% oxygen. In addition, hydration treatments in air for 5 hours, followed by drying back to the initial seed moisture content, significantly increased the rate of RE in aged seed of maize [30] and radish [36].

#### A NEW VIGOUR TEST BASED ON RADICLE EMERGENCE (RE)

Mean germination time (MGT) is determined from many counts of RE over the germination period of a seed lot. This does not lend itself to application in routine seed testing. Single early counts of RE have however been shown to be highly predictive of MGT, with  $R^2$  mostly above 0.90 for seven species [33]. Work at the French National Seed Testing Station (Wagner, personal communication) has shown this for 17 species, using an automated image analysis system to determine MGT. A high early count of RE indicates a low MGT and high vigour, leading to rapid and high emergence in the field [33].

The rate of germination to the radicle emergence (RE) stage has been found to relate to the final emergence in the field or in modules of seed lots from many species with high and commercially acceptable levels of germination.

This has been reported for maize [37], cotton [33], pepper [38], watermelon, melon and cucumber [39], as well as oilseed rape [40] and radish [41]. More recently the close relationship between RE and field emergence has been reported in China for two fodder crop species, *Avena sativa* and *Elymus nutans* [42].

Following international comparative tests in a number of laboratories, the RE test has been validated by ISTA for several species and is now included in the International Rules for Seed Testing (Table 3; ISTA, 2017). Comparative tests have been completed for a further two species, are planned for three species and work is in progress in the ISTA Vigour Committee to establish the test conditions for another six species (ISTA Vigour Committee, 2016). In addition, guidelines have been published to enable individuals to investigate the development of the test for new species that are not included in the Vigour Committee's Work Programme [31].

All tests of seed vigour, both validated and non-validated (Table 2) can be explained in terms of the ageing/repair hypothesis. In the cold test for maize, counts of normal seedlings made after holding seeds in saturated soil for 4 days followed by 3 days at 25°C were closely related to MGT at 13°C and the age of the seed assessed by accelerated ageing [43]. The low temperature and relatively low oxygen concentrations in the cold test would reduce the metabolic repair in aged seeds and hence the percentage of normal seedlings.

Mean germination time summarises the germination progress curve of a seed lot and therefore the spread of germination over time [40]. The more spread the germination, the more variable is the size of the seedlings, since the time between RE and seedling measurement determines seedling size [44]. Support for this suggestion is found in work on peppers [38], maize [37] and *Brassica napus* (Argentine canola) [45]. Thus differences seen in seedling growth tests (Table 2) would have their origin in timing of RE as result of ageing and repair, and the subsequent time available for growth before seedling assessment.

In the cool test, differences in the number of normal seedlings of a specific size will, like in the cold test, be determined by the extent of repair of any deterioration in seed lots. In addition, differences in seedling growth will result from differences in the timing of RE.

Conductivity measurements from bulks of seeds will be determined by ageing of seeds within the population. Seed leachates are higher for lots with a high proportion of dead seeds, seeds producing normal and abnormal seedlings that are slower to RE, as seen in radish [36]. This work on radish also revealed a lower loss of electrolytes into soak water when seeds had been hydrated for 5 hours then dried back, suggesting the possible repair of membranes during early imbibition. The tetrazolium test reflects the overall effect of ageing by evaluation of the incidence and location of living, deteriorated and dead tissue. Finally, the assessment of vigour by imposing periods of rapid ageing in the accelerated ageing and controlled deterioration tests reflect the whole of the ageing process.

## CONCLUSION

The approach to the development of new vigour tests within ISTA has been to ensure that:

- tests have a sound theoretical background, as described above,
- test results relate to a practical expression of vigour e.g. field or module emergence, storage potential
- tests have been developed using seed material that is commercially available, i.e. differences in quality are not artificially created
- they are repeatable within a laboratory and reproducible between laboratories.

All these points are also contained in the ISTA Method Validation Programme [46], a key feature of test development throughout ISTA

Commercial developments in seed testing may not always follow the approach above. Seed testing laboratories will be aware of the promotion of equipment that aims to speed up

Table 2 : Summary of vigour tests and their explanation on the basis of the ageing/repair hypothesis

Vigour test	Species/modification of method	Validated (in ISTA Rules)	Test length	Result reported	Interpretation on the basis of aging/repair hypothesis
A) ISTA validated tests					
Accelerated Ageing (AA)	<i>Glycine max</i> Soyabean	2001 (2002)	11 days	% Normal seedlings	Ageing and position of seed lot on the survival curve
Conductivity test (EC)	<i>Pisum sativum</i> Garden pea	2001 (2002)	24 hours	Conductivity of seed leachate ( $\mu\text{S cm}^{-1}\text{g}^{-1}$ )	Leakage from dead seeds and dead/damaged tissue following aging and imbibition damage
	<i>Phaseolus vulgaris</i> French/green/garden bean	2009 (2010)	24 hours		
	<i>Glycine max</i> Soyabean	2010 (2011)	24 hours		
	<i>Cicer arietinum</i> Chickpea (Kabuli type)	2013 (2014)	24 hours		
Controlled deterioration (CD)	<i>Brassica</i> spp.  Modified moisture raising method	2009 (2010)  2016 (2017)	10-17 days depending on spp. (3 days+ germination test) 10-17 days depending on spp. (3 days+ germination test)	% total germinated seeds (normal plus abnormal seedlings)  Or Conductivity of seed leachate ( $\mu\text{S cm}^{-1}\text{g}^{-1}$ )	Ageing and position of seed lot on the survival curve
Radicle emergence (RE)	EC as alternative to germination test to assess deterioration <i>Zea mays</i> Maize	2016 (2017) 2011 (2012)	5 days 66 hours: $20\pm 1^\circ\text{C}$ 144 hours: $13\pm 1^\circ\text{C}$	% radicle emergence	Time for repair of aging

Table 2 : Contd ....

	<i>Brassica napus</i>	Oilseed rape	2014	(2015)	30 hours:	20±1°C	
	<i>Raphanus sativus</i>	Radish	2016	(2017)	48 hours:	20±1°C	
Tetrazolium (TZ)	<i>Glycine max</i>	Soyabean	2016	(2017)	24 hours		% vigorous seeds Pattern of living/dead tissue
B) Non-validated tests							
Cold test (CT)	<i>Zea mays</i>	Maize	-		10-13 days		% normal and abnormal seed- lings; % dead seeds Incomplete repair at low temperature (and especially in anaerobic conditions) increases abnormal seedlings
Saturated salt accelerated ageing	Small seeded species		-		48-140 hours ageing plus 7-21 days germination, depending on species		% Normal seedlings Ageing and position of seed lot on the survival curve
Seedling size and uniformity	Various species		-		Length of germi- nation test		Varies e.g. seedling dry wt, length or uniformity Differences in the timing of RE
Cool test (18°C)	Cotton		-		7 days		% based on no. Incomplete repair at lower temperature of normal seed- lings with hypocotyl+ root length>4cm slows RE giving smaller seedlings

and/or replace traditional seed testing methods. One such example is the costly Q2 seed analyser, which measures oxygen uptake of single seeds and claims to predict germination and vigour. The claims for this equipment have been evaluated by the ISTA Seed Science Advisory Group (SSAG). The SSAG found (Powell, 2016) that on the basis of the evidence provided, both by those involved with the Q2 and that available in the public domain, the claims made for use of the Q2 in seed testing to predict germination and vigour of commercial seed lots are not supported. This highlights the need for those involved in seed testing to be aware of the criteria outlined above for the development of new vigour tests, which help to ensure that a new approach is both practical and useful.

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