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# **A review of the principles and use of the Q2 Seed Analyser**

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## Summary

The Q2 seed analyser is said to measure oxygen consumption of single seed and is promoted as a means of assessing seed germination and vigour. These claims have been reviewed using evidence provided by those developing and marketing the Q2, and published scientific papers. The Q2 measures oxygen consumption, with different patterns of consumption seen for dead compared to germinating seeds, and for aged and primed seeds. There is however little convincing evidence to support the claims that Q2 assessments predict germination and vigour. In addition there is little work reported that applies the Q2 to commercial seed lots of any species. A number of factors that may influence assessments of oxygen consumption are identified.

## Introduction

ISTA has for over a century developed and validated methods for evaluating seed quality, which are included in the International Rules for Seed Testing. These methods have largely been developed within the technical committees and their supporting laboratories. Any equipment required to complete these tests is typically generic and test methods define only the specifications of the equipment and not a specific make.

The use of metabolic, biophysical and molecular measurements of seed performance have, however, led to the development of equipment that may/may not have potential for use in seed quality testing. Such equipment typically originates in the commercial environment; an example is the Q2 seed analyser. This review was requested by Astec Global the producers of the Q2 (personal communication from B van Duijn, Chair of the ATC to Chair of SSAG). The review is based on articles and data provided by those who produce and market the Q2 (Astec Global), who have been involved in its development and marketing (Johan van Asbrouck, Bert van Duijn) and who complete research on behalf of the Q2 users group (Kent Bradford). In addition, information available online from companies selling the Q2 (Astec Global; Centor Group including Centor Thai, Centor Oceania / India, Rhino Research, Aginnovation; Fytogoras) has been used.

## Claims for use of the Q2 seed analyser

The claim made for Q2 by Astec Global (Frederick Schreurs, Global Director New Business, Astec Global, personal communication, 15/10/15) is '*The technical principle or claim behind the Q2 is the oxygen consumption of a single seed in a closed compartment. This could be used as a performance evaluation (validation?) method or technique*'. A specific use of Q2 in seed testing was not stated. A similar view was expressed by Bert van Duijn (Fytogoras, personal communication, 15/09/15): '*I would look for claims such as: Measurement of oxygen consumption of (germinating) single seeds in (closed) containers*'.

In contrast promotional material for the Q2 makes many very specific claims for the use of the Q2 in seed quality testing:

The Astec Global website (web ref 1) claims that the Q2 is '*a fast germination tool*' with '*germination results in 24-48 hours*', that it gives '*a holistic picture of vigor*', and that the information provided gives support for checking levels of pathogens, breeding programmes and predicting feasibility of priming methods.

The general brochure for the Centor Group (web ref 2) states that the '*Q2 data is more robust and defining than traditional germination tests*' and states that it can identify dead, dormant and actively

germinating seeds, although specific details of seedling abnormalities are not provided. Centor Thai (web ref 3) and Centor Oceania/ Centor India (web ref 4) claim that the Q2 system is a '*practical germination alternative*' (Centor Oceania), that can be used for '*Fast prediction of germination*' (Centor Thai). The same brochures also claim that the Q2 provides '*a holistic view of vigor*' including germination speed, stress tolerance, healthiness and yield potential (Centor Group). In addition data from the Q2 '*correlates respiration rate to the germination and vigor characteristics of the seed, provides a great overview of your seed lots vigor*' and '*results predict how seed lots will perform under water stress*' (Centor Thai); and assess '*quality and vigor*' (Centor Oceania /Centor India).

In the USA, a founding member of the Centor Group, Aginnovation (web ref 5), markets the Q2 under the heading VIM Technology with the claim that the target for the Q2 is '*Seed germination and vigor tests in general*' stating that the Q2 can be applied for '*seed quality and vigor analysis*', and germination analysis under water and germination stress, that it provides '*quantifiable vigor data*' and is the '*most sensitive seed vigor indicator*'. Similarly, Rhino Research, also a member of the Centor Group claims in its brochure (web ref 6) that the measurement from the Q2 is '*a holistic view on vigor*' including stress tolerance, imbibition and water stress.

Finally, Fytgoras (web ref 7) states that the Q2 has many applications including as a '*device to determine the viability of seeds*', providing a '*complete overview of vigor*' and allowing a check of '*bacterial and fungal infections of seeds*'.

**The aim of this review** is to evaluate the claims made above that the Q2:

1. Measures the oxygen consumption of single seeds in a closed compartment
2. Identifies dead, dormant and germinating seeds
3. Is more robust and defining than traditional germination tests
4. Provides a complete view of vigour.

The review will first consider some basic background information provided on oxygen measurements in relation germination. It will then review the links between respiration, germination and vigour, with particular reference to work using non-invasive methods to assess oxygen use by the seed. The evidence relating respiratory assessments to germination and vigour will be considered with particular emphasis on the Q2. The factors affecting use of the Q2 will be identified, followed by the conclusion reached from this review.

### **Respiration and germination**

Germination is an energy requiring process that depends on the respiration of the seed and changes in respiration during germination are well documented, most commonly as changes in oxygen uptake (for example, Bewley and Black, 1994; Black *et al.*, 2006). The consumption of oxygen follows four phases. In Phase I there is a sharp increase in oxygen uptake which increases linearly with hydration, followed by a lag phase in oxygen uptake in Phase II. This phase is also associated with an increase in the Respiratory Quotient (RQ: i.e. carbon dioxide evolution: oxygen uptake). A further rapid increase in oxygen uptake occurs in Phase III, onset of which is often coincident with radicle emergence. Finally in Phase IV a decline in oxygen uptake is observed in storage tissues as they senesce when storage reserves are depleted. Characteristics of individual species such as rate of imbibition, permeability of the seed coat and metabolic rates influence the length of each of these phases.

In some species limited availability of oxygen to the embryo due to seed coat coverings is a major factor contributing to seed dormancy. For example in early work on *Betula* the seed coat

inhibited oxygen diffusion and the level of dormancy decreased when seed coat was scratched (Black, 1956) and in more recent work on barley Bradford *et al.* (2008) showed that dormancy was regulated by combination of physical and physiological factors. Thus the oxygen requirement for dormant intact seed was 400 times greater than for excised embryos. In addition, although growth regulators (GRs: ABA and GA<sub>3</sub>) that influence dormancy had little effect on oxygen requirements for germination, the requirement for GRs varied dependent on oxygen availability. In contrast to the need for oxygen uptake and respiration to achieve germination, the inhibition of respiration by azide and cyanide released dormancy and enhanced germination in *Avena fatua* (Tilsner, 1987).

The concentration of oxygen required for germination is not necessarily the same as that in air. Siegel and Rosen (1962) examined the germination of 20 species in air and in lower concentrations of oxygen. A number of species achieved high germination in 2 and 5% oxygen, while the concentration of oxygen required to reach the same germination as in air never exceeded 10%. Recently Tian (2003) demonstrated that sesame seeds that germinated in low (5%) oxygen had higher oxygen uptake than pea seeds germinated in the same conditions. In addition the seedlings produced showed improved subsequent growth even one month later. In non-dormant barley, the timing of oxygen deficiency had an effect during germination in the malting process (Wilhelmson *et al.*, 2006). Low oxygen early in the process had no effect on germination, but when low oxygen was applied later, germination was delayed.

#### **Links between respiration, germination and vigour:**

The process of respiration has been associated with the ability to germinate and seed vigour, although there has been relatively little work that clearly illustrates this in commercial seed lots. Early work on lima bean (Woodstock and Pollock, 1965) and garden pea (Carver and Matthews, 1975) seeds, reported that the respiration rates in the early stages of germination were positively correlated with seedling development. Differences in oxygen uptake during early imbibition of soyabean axes were subsequently correlated with incidence of deterioration following different simulated pre-harvest deterioration conditions (Amable and Obendorf, 1986). However seeds with 0% germination following different pre-harvest treatments had very different levels of oxygen uptake, from very low levels to levels similar to those found in highly germinating seeds. Respiration, assessed by carbon dioxide release, was also a good indicator of germination metabolism (germination = 2mm radicle) in sorghum (Patané *et al.*, 2006), although in extreme conditions, where respiration continued, the seed population did not germinate rapidly or completely. However, there was a good correlation between CO<sub>2</sub> release at 2 hours and rate of germination (time to 50% germination).

There has been a keen interest in recent years in the development of non-invasive methods for measuring respiration (Chaturvedui *et al.*, 2013). These include optical probes, micro-probes / micro-sensors (Rolletscheck *et al.*, 2009; Sanchez *et al.*, 2008; Sew *et al.*, 2013), near infra-red, hyperspectral imaging, F nuclear magnetic resonance imaging. A range of emerging technologies include EPR oximetry, self-referencing flux sensors and nanosensors (Chaturvedi *et al.*, 2013). Most of these have been used in animal systems, specifically in medical research, although some have been used in some plant investigations.

Three non-invasive approaches to assessing respiration have been used as a means of predicting germination and / or vigour: a micro-optrode technique (MOT) (Xin *et al.*, 2013), a non-invasive micro-test technique (NIMT) (Li *et al.*, 2014) and the Q2 seed analyser (Astec Global, web ref 1). The MOT is a fast non-invasive technique and was used to measure oxygen concentration and flux at the cell surface of between 20 and 30 single seeds of soyabean, wheat and rape after 3 hours

imbibition, with the aim of developing a rapid means of quantifying viability for seed bank maintenance (Xin *et al.*, 2013). Oxygen flux, measured every 10 seconds for at least 5 minutes, was more useful than oxygen concentration and there was a highly significant relationship ( $r^2 \geq 0.954$ ,  $p \leq 0.004$ ) between oxygen influx and the germination of five samples of naturally aged seeds from each of the three species, the germination (% normal seedlings) of which ranged from zero to above 90%. They (Xin *et al.*, 2013) commented that the system used is open, in contrast to Q2 which measures oxygen concentration in a closed chamber in which '*continued attenuation of the oxygen concentration in seeds, causes hypoxia stress on seeds, and significantly reduces the rate of germination*'.

The non-invasive micro-test technique (NIMT) assessed oxygen and hydrogen peroxide flux in relation to germination and vigour in five samples of *Caragana korshinskii* produced after artificial ageing (Li *et al.*, 2014). Oxygen flux, measured after 3 hours imbibition of 10 seeds, decreased as germination (radicle emergence after 5 days for 3 x 50 seeds) declined from 90 to 0%, with a particularly marked decrease as germination fell below 80%. In this work, the hydrogen peroxide flux was more highly predictive of germination ( $r^2 = 0.94$ ) than oxygen flux ( $r^2 = 0.906$ ). The opposite was true for predicting vigour (germination index x seedling dry weight), with the oxygen flux being more predictive of vigour ( $r^2 = 0.974$ ) than  $H_2O_2$  ( $r^2 = 0.64$ ). The authors noted that a disadvantage of this approach was the need to remove the seed coat in order to achieve accurate information.

A number of presentations and papers have described the principles behind and potential use of the Q2 seed analyser. This review will now outline these principles and discuss the evidence for the application of data produced.

#### *Q2: principles and assessments:*

The Q2 assesses oxygen consumption during the process of germination by measuring the depletion of oxygen in a closed system. Thus, even though the process is non-invasive, the seeds are hydrated during the oxygen measurement, as also seen in MOT (Xin *et al.*, 2013) and NIMT (Li *et al.*, 2014).

To measure the oxygen consumption, 100 individual seeds are each sealed in a cell of a micro-titer plate that contains agar or other substrates to provide moisture for imbibition. The covering of the cell has a membrane with a dot in the inner side containing a metal organic dye that changes its fluorescence properties in proportion to the oxygen concentration. As the seed respire, it depletes the oxygen in the sealed well, which increases the fluorescence of the dye. This change is detected by a light source that shines blue light on the dye dot and a sensor that measures the fluorescence intensity. A robotic arm sequentially moves the light source and sensor over each well, measuring the oxygen concentration inside the well at desired time intervals and developing oxygen consumption time courses for individual seeds. The measurements can be repeated as often as desired (generally at 1- to 2-hour intervals) to obtain time courses of respiratory activity (oxygen depletion in the sealed wells). The time course data from each well are collected in a database that is accessible to the Q2 analysis software or can be output to a spreadsheet file.

The data from Q2 oxygen consumption time courses (Figure 1) are computed to give what are referred to as ASTEC values:

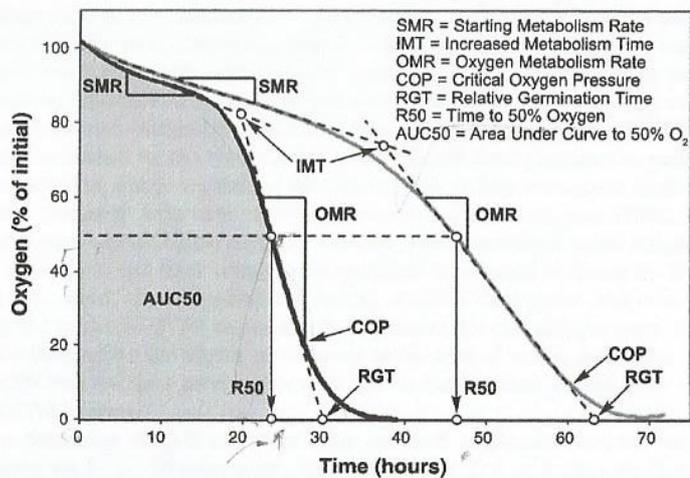


Figure 1. Oxygen consumption curves illustrating the ASTEC values (SMR, IMT, OMR, COP and RGT) and two values used by Bradford *et al* (2013) (R50, AUC50). Examples of two seeds are presented, one germinating rapidly (left hand curve) and one germinating slowly (right hand curve) Figure taken from Bradford *et al.*, (2013).

The ASTEC values calculated from the oxygen consumption curve (Figure 1) are:

**IMT:** Increased metabolism time. This is the time for the seed to begin increasing metabolism It has been said to depend on the water permeability of the pericarp, structure and composition of seed coatings, absorption of water, timing of physiological processes (B3)

**OMR:** Oxygen metabolism (% O<sub>2</sub> consumed/ hour).

**COP:** Critical oxygen pressure (% O<sub>2</sub>). This is where metabolism decreases due to lack of oxygen. It is expressed as an oxygen percentage and is said to give an idea of how well seeds will perform under oxygen stress.

**RGT:** Relative germination time. This value is extrapolated from the curve and is dependent on IMT and OMR.

**HOM:** Homogeneity value. The standard deviation of MGT, said to be indicative of field emergence (B3).

Some seeds e.g. onion do not show a typical s-shaped curve for oxygen consumption, so it is problematic to calculate ASTEC values. Two further indices have therefore been developed (Bradford *et al.*, 2013) that do not depend on the shape of the oxygen consumption curve, namely:

**R50:** the time required to reduce the initial oxygen level to 50%

AUC50: the area under the curve from time zero to 50%

Many presentations have outlined these principles of the Q2 and how the ASTEC values are derived (for example Astec 1 and Halmer, 2010). These have included comments that correlations exist between ASTEC values and seed quality differences that result from ageing, and with factors that have impact on seed quality (Astec 1), but no data was provided to support this. Examples of the O<sub>2</sub> depletion curves for control, aged and primed seeds from an un-named species have illustrated greater variability and lower oxygen consumption in aged seeds and improved uniformity and higher consumption in primed seeds (Astec 1). Specific evidence from pepper and tomato (Astec 2) and in sugar beet (Halmer, 2010) showed that improved uniformity following priming of single seed lots of each species was associated with more uniform and more rapid oxygen consumption.

Work presented in a poster on use of Q2 in sugar beet (van Asbrouck *et al.*, 2007) suggested that the Q2 can distinguish between dead, dormant and germinating seeds and that ASTEC values could allow interpretation of Q2 data. However, oxygen consumption curves were presented for only two lots, one of high quality and one including seeds with either very slow oxygen depletion, described as dormant seeds, or slower depletion, described as lower vigour seeds. Examples of the ASTEC values for five beet seed lots revealed one seed lot to be clearly higher quality than the remaining four, but the rankings of these four lots were not consistent. The poster suggested throughout that the ASTEC values and O<sub>2</sub> depletion curves provided information about field performance of the lots, but no evidence was provided in support of this.

A number of papers have reported relationships between ASTEC values and germination characteristics, with varying degrees of success. Significant relationships were reported between OMR and COP and both the standard germination (SG) and rate of germination of ten lots of supersweet sweetcorn from one cultivar (Zhao *et al.*, 2009). The correlation coefficients ranged from 0.487 to 0.582, and hence the maximum  $r^2$  was only 0.338 (between OMR and SG) i.e. even in the best scenario, the ASTEC values would have predicted SG correctly only 33% of the time. Similarly, in work on malting barley Nielsen *et al.* (2015) found that IMT was correlated with radicle emergence at 24 ( $r = 0.59$ ), 48 ( $r = 0.80$ ) and 72 hours ( $r = 0.73$ ), with similar, but lower correlations between RGT and radicle emergence. This means that the maximum coefficient of determination ( $r^2 = 0.64$ ) at 48 hours would predict radicle emergence 64% of the time. They commented (Nielsen *et al.*, 2015) that the Q2 would not provide a replacement for assessment of germination in the standard germination test, although it does give information on the germination pattern of seed. They concluded that the approach was repeatable, but did not show any repeat experimental runs in the presentation, and they emphasised that further development of the protocol was necessary.

The work of Bopper and Kruse (2010) showed greater oxygen consumption in germinating (normal and abnormal seedlings) than non-germinating seeds of three species (oilseed rape, onion, clover) but not in wheat. Estimates of germination from oxygen consumption data were not possible for onion, clover and wheat, although there was a clear relationship between germination and oxygen consumption for four lots of oilseed rape. The good correlations between the germination percentages of the seed lots from all four species in an ISTA test and the germinations of seeds seed dried after removal from the Q2, supported the claim that the Q2 approach is non-destructive. However, in each species, there were instances where germination after hydration in the Q2 was higher or lower than in the standard test.

Q2 data has also been obtained for five rice seed lots with standard germination values ranging from 70 to 90%, as quoted from a client, and from 64 to 89% as obtained by Astec Global (Astec 3). Histograms and diagrams were presented in support of the Q2 approach. Calculation of the correlations between the ASTEC values (SMR, IMT, OMR, COP, RGT) and both of the sets of the germination data provided in this paper reveal a significant relationship with germination in only one of the ten correlations (SG from Astec Global with COP,  $r = -0.878$ ,  $p \leq 0.05$ ).

Q2 indices have also been examined in relation to laboratory assessments of seed vigour. However there is little evidence to show that the ASTEC values relate to practical expressions of vigour.

Comparisons of four commercial tomato seed lots from Thailand and one EU lot were said to ‘show good correlations between some of the obtained Q2 quality parameters and the germination percentage as well as some different vigour tests (early score and the mean germination time)’ (van Asbrouck and Taridno, 2009). This paper does display straight line relationships for several ASTEC values with aspects of germination: OMR with both early count of root emergence and MGT (N.B. definition of MGT in this case was when 50% of the germinating seeds had germinated); RGT with an early germination count; and ‘strong Q2 stress’ (seeds showing clear sigmoid pattern of O<sub>2</sub> depletion) with mean germination. However, no correlations are quoted in the paper for these figures, and when correlation coefficients are calculated from the data used in the figures (given in Table 1 of this paper) there are no significant relationships. In addition, markedly different early counts are shown following an SG test for an EU seed lot (90% germination) and one of the Thai seed lots (30% germination), despite similar RGT values (Figure 5, van Asbrouck and Taridno, 2009). The data in Table 1 also allows calculation of the correlations between standard germination (%) and the ASTEC values OMR, RGT and ‘Q2 strong’; none of these relationships are significant.

Q2 indices have been examined in relation to seed vigour, expressed as field emergence potential in two Chinese fir species (Chinese fir, *Cunninghamia lanceolata* and Masson pine, *Pinus massoniana*; Zhao and Zhong, 2012) and in rice (Zhao *et al.*, 2013). The ASTEC values that related to field emergence differed in the two fir species: RGT for Chinese fir and OMR for Masson pine. However, calculation of the coefficients of determination ( $r^2$ ) for these indices with field emergence (%) from the correlation coefficients provided for field trials (mean of two years) gives  $r^2$  values of only 0.415 (RGT, Chinese fir) and 0.294 (OMR, Masson pine) which do not suggest good prediction of vigour as expressed in field emergence.

Different indices also correlated best with emergence in the two sub-species of rice: RGR was found to be most closely correlated with emergence in *indica*, while OMR gave the best correlation for *japonica*. In this work, the best differentiation of differences in final emergence in the field was the rate of germination (production of a 1mm radicle) and Zhao *et al.* (2013) concluded that ‘oxygen sensing technology is not able to predict reliably the field emergence of conventional rice seeds because of the relatively low correlation coefficients.’

Relationships between ASTEC values and assessments of germination and vigour in vegetable crops have been examined in work by Bradford and colleagues (Bradford *et al.* 2012, 2013) using seed lots of cabbage, Chinese cabbage, turnip and cauliflower. Significant coefficients of determination ( $r^2$ ) were reported between IMT, OMR, RGT and HOM and vigour as expressed by the early germination count in an ISTA standard germination test, the results being combined for seed lots of all species, (Bradford *et al.*, 2012). However the  $r^2$  values did not exceed 0.2967, indicating poor

prediction of early germination by the ASTEC values; germination would be predicted less than 30% of the time. This paper also introduced the calculation of two new values determined from the Q2 data, namely the QT50, or time to 50% Oxygen and the AUC50, the area under the depletion curve to QT50 (see figure 1).

Data from the same seed lots (except that two cabbage lots were excluded) showed no correlation between the ASTEC values and either final germination or soil emergence (Bradford *et al.*, 2013). In fact the only measurement made in the Q2 analyser that significantly correlated ( $p \leq 0.05$ ) with aspects of germination (first count in a standard test, final germination in the Q2) was not a respiration index but the rate of radicle emergence in the wells of the Q2, which was expressed as the time to 50% germination in the Q2 ( $Q_{T50}$ ). Even so, the  $r^2$  values of 0.157 (first count) and 0.413 (final germination in Q2) indicated a low level of predictability. The  $Q_{T50}$  was more closely related to R50 (time to reduce the oxygen level to 50%;  $r^2 = 0.560$ ) and AUC50 (area under the curve to 50% oxygen depletion,  $r^2 = 0.396$ ) (Bradford *et al.*, 2013).

In these four species tested (Bradford *et al.*, 2013), the authors used the time to 50% radicle emergence in the vials ( $Q_{T50}$ ) as a measure of vigour under the conditions of the Q2 test. However, only one species, Chinese cabbage (six lots) gave a significant correlation of  $Q_{T50}$  with soil emergence, i.e. vigour ( $r^2 = 0.726$ ). The significant (IMT, SMR and COP) and highly significant (RGT) relationships of ASTEC values with  $Q_{T50}$  have led to the suggestion (Bradford, personal communication) that the Q2 would provide an accurate and repeatable assessment of rate of radicle emergence. However, highly repeatable and reproducible assessments of RE are currently obtained in the RE vigour test for maize, oilseed rape (ISTA, 2016a) and radish (ISTA, 2016b), which can be achieved both manually and automatically using computer-aided machine vision. In addition, a substantial number of publications show that RE provides convincing predictions of field emergence and standard germination after storage (Powell and Matthews, 2012)

### **Factors affecting performance of the Q2 and the accuracy its assessments.**

*Protocol used:* The importance of the protocol to be used, when applying the Q2 to assess oxygen consumption, has been consistently emphasised (Asbrouck and Taridno, 2009; van Asbrouck *et al.*, 2007; Nielsen *et al.*, 2015). Important aspects include precision in water availability and substrate application (Asbrouck and Taridno, 2009) and well size in relation to seed size and temperature (van Asbrouck *et al.*, 2007). The importance of the test conditions was emphasised by Halmer (2010), who noted that the respiration curves can vary with species, seed lot, seed physiological state (dormancy, germination, infection), embryo size, seed size : cell ratio, imbibition liquid.

*Seed size:* Seed size is certainly a factor that should be taken into account of when assessing respiration, particularly in a closed system, although published protocols for the Q2 do not take account of this. Corbineau *et al.* (1995) highlighted the importance of seed size to sensitivity to oxygen deprivation, such as may occur when seeds respire in a closed system as in the Q2. They (Corbineau *et al.*, 1995) reported that larger seeds are significantly more sensitive to oxygen deprivation and in addition, that cultivars show differing sensitivity to low oxygen tension. Pons *et al.* (1986) also noted differences between species in response to reduced, although not decreasing, oxygen and Kleinwachter *et al.* (2012) found that germination is inhibited by oxygen deficiency and high CO<sub>2</sub>.

*Variability in seed/embryo size:* This may influence accuracy of the assessments, since the Q2 takes no account of its influence on oxygen uptake. Most commonly respiration measurements are expressed per seed weight to account for variability within and between lots / cultivars. Bradford *et al.*, (2013) have also commented that the embryo size in relation to any other respiring tissue in the seed may influence the sensitivity with which differences can be detected by respiration measurements.

*Species:* ASTEC values that relate to germination characteristics apparently differ between species (Zhao and Zhong, 2012; Bradford *et al.*, 2013) and even sub-species (Zhao *et al.*, 2013). This led Bradford *et al.*, (2013) to comment that the optimal ASTEC values for assessing vigour may therefore depend on the respiratory characteristics of the species. However, some species may not even show the typical s-shape pattern of oxygen depletion, as seen in onion where the oxygen uptake pattern was more linear with no clear distinctions between the slow initial phase and the second rapid oxygen consumption phase (Bradford *et al.*, 2013). This made it difficult to obtain reliable ASTEC values for onion that could be related to germination characteristics. Bradford *et al.* (2013) commented that ‘*it is often the case that individual seeds in a lot exhibit different types of oxygen consumption patterns, this can be problematic for determining ASTEC values for comparisons across all seeds, which are based on various components of the typical oxygen consumption time courses*’

*Accuracy:* A number of the papers on use of the Q2 emphasise the accuracy of the assessments, since they are obtained from 100 single seeds. However in most cases the ASTEC values calculated from oxygen consumption curves appear to be the mean values from one replicate of 100 seeds. This compares to an ISTA standard germination test where the germination percentage is quoted as the mean of four replicates of 100 seeds. Furthermore, there does not appear to be any evidence to illustrate that repeated tests on the same commercial seed lots give the same final results.

*Timing:* Much is made of the timing of a test of seed quality using the Q2, and that it is a quick test to complete. This will however depend on the rate of germination of a species and therefore be very variable. Times for the test completion of 10 to 72 hours are quoted, but some of the timings in published papers indicate the completion of the oxygen consumption curves for up to 5 days in slower germinating species.

These factors all point to the need for careful development of protocols that are adapted for different species and different seed sizes. Currently purchasers of the Q2 need to develop the protocols for new species since they are only available for a limited number of species.

## **Conclusions**

1. The Q2 measures oxygen consumption in a closed system from which oxygen consumption curves can be derived. The repeatability of these measurements using the same seed lots in more than one experimental run has not been demonstrated
2. Dead, dormant and germinating seeds show different patterns in their oxygen consumption curves
3. Populations of non-aged, aged and non-aged but primed seeds may show different oxygen consumption characteristics, but there is limited experimental evidence to either illustrate this in samples from commercial seed lots or link the oxygen consumption to seed lot performance.

4. There is little evidence that the Q2 data is ‘*more robust and defining than traditional germination tests*’ (Centor group, web ref 2).
  - a. Experimental work has largely been done on artificially aged seeds and seeds with widely different germination levels (e.g. 0 and 90%). There is little work using commercial seed lots. Where commercial seed lots have been used in the work reviewed there are few significant relationships between Q2 data and seed lot germination and no convincing predictions of germination
  - b. There is no published experimental work within one laboratory to illustrate that repeat samples from the same seed lot give comparable results.
  - c. There are species and test variables that may influence both the use of the Q2 and interpretation of the results that have not been investigated.
5. The Q2 has not been shown to give a complete view of vigour. Little experimental work examines the relationship of ASTEC values with expressions of vigour (field emergence or storage potential). No significant relationships with these expressions of vigour have been shown.
6. Research using different techniques does not provide clear evidence that oxygen consumption relates to seed quality and this has yet to be proven in commercial seed lot.
7. Even though techniques are non-invasive, seeds have been hydrated during oxygen assessments for 3 hours (MOT, NIMT) or much longer (Q2). There has been insufficient research so far to determine if these techniques could also be considered non-destructive i.e. the seeds can be dried back and used for sowing purposes.

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