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Isothermal micro-calorimetry: a tool to predict seed longevity?

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Abstract

This paper describes the exploratory use of isothermal micro-calorimetry (IMC) to measure directly the heat flow produced as seeds age. Heat flow was recorded in primed and non-primed (control) seeds of *Ranunculus sceleratus* L., aged in a micro-calorimeter at 35°C at three different seed water contents [*c.* 0.12, 0.075 and 0.045 g H_2O (g dw)$^{-1}$]. The rate of heat flow and total heat generated (an indicator of extent of reaction) were generally greater in control seeds, which aged at a faster rate, than in primed seeds. Total heat generated over a given period also increased with increasing water content. The power–time curves did not indicate first- or second-order rate kinetics, consistent with the probability that seed ageing is complex and involves a number of reactions. Even after the capacity to germinate had ceased, there was a residual power signal. As a method, IMC gave consistent results using independent samples at different times. Therefore, short-term experiments at relatively high water contents and/or temperatures may have the potential to predict the relative longevity of seed-lots, at least within a species.

Keywords: isothermal micro-calorimetry, priming, *Ranunculus sceleratus*, seed longevity

Introduction

Seeds from different species show considerable differences in longevity in dry storage (Priestley, 1986; Walters *et al.*, 2005). Indeed, in order to enable the efficient management of seed bank accessions, 'a major goal of seed ageing research is to identify "good keepers" and "bad keepers" before deterioration begins and to provide predictive tools to tell us the rate at which deterioration will proceed' (Walters, 1998). Ideally, such a diagnostic tool would be non-destructive and would avoid the utilization of valuable germplasm.

Isothermal micro-calorimetry (IMC) – the measurement of very small amounts of heat produced or absorbed by a sample that is surrounded by a heat sink maintained at a constant temperature – is an innovative method of thermal analysis that has already been considered by the food and pharmaceutical industries as a tool for predicting the rate and degree of ageing of products. By examining the kinetics of heat production, there is the potential to determine the reaction(s) involved in such deterioration and/or extent of reaction (Beezer *et al.*, 1999). IMC might be used similarly to increase our understanding of the kinetics and possible mechanism(s) of ageing in seeds. The storage of food products is particularly analogous to the storage of seeds because both seeds and many foods have heterogeneous compositions.

IMC has been used to assess seed quality by measuring heat flow during the early stages of germination (Prat, 1952; Mourik and Bakri, 1991; Yamaguchi *et al.*, 1996). Furthermore, Sigstad and García (2001) used the relative heat output during germination of seeds equilibrated to different water contents to predict the optimum water content for storage of quinoa (*Chenopodium quinoa* Willd.) seeds. However, IMC appears not to have been used to measure directly the heat flow produced by seeds during ageing. IMC may be a non-destructive tool for potentially predicting seed longevity or how much ageing has already occurred, at the population level, or perhaps, at an individual seed level, depending on seed size and/or sensitivity of the calorimeter. Furthermore, it may be a technique that will help to ascertain the mechanisms of seed ageing.

The typically sigmoid shape of survival curves for a population of seeds (% germination versus time) stored in a constant environment has been well described, and various models have been used to explain this shape (e.g. Ellis and Roberts, 1980; Walters, 1998). The rate of viability loss is dependent largely on the water content of the seeds and the

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temperature at which they are stored; however, it is generally recognized that, for a given storage environment, inter-species variation in seed longevity is greater than intra-species variation (e.g. Roberts, 1972; Priestley, 1986).

Intra-species differences in longevity may arise due to differences in seed maturity (Hay *et al.*, 1997), growth conditions (Sinniah *et al.*, 1998) or cultivar (Kraak and Vos, 1987). Longevity can also change following a controlled hydration (priming) treatment. For example, reduced resistance to ageing has been observed in seeds of lettuce (Tarquis and Bradford, 1992), leek and carrot (Dearman *et al.*, 1987), and pepper (Lanteri *et al.*, 1997) following priming treatments. In contrast, in *Ranunculus sceleratus*, priming resulted in a four- to fivefold increase in longevity in seeds subsequently stored at 35°C and 9.2% water content (fresh weight basis) (Probert *et al.*, 1991). This ability to manipulate longevity may be used to provide experimental material to study the fundamental basis of both intra- and inter-species differences in seed longevity and/or find methods to predict rates of viability loss.

This paper describes the use of IMC to measure the heat produced by primed and non-primed seeds of *R. sceleratus* during ageing at 35°C over a range of water contents. Specifically, the following hypotheses were tested: (1) Does IMC detect heat produced by seeds as they age? (2) Does heat flow depend on the storage conditions of the seeds, increasing with increases in water content? (3) Is heat flow greater in non-primed *R. sceleratus* seeds (which age at a faster rate) compared with primed seeds?

Materials and methods

Seed production

Plants of *R. sceleratus* were raised under glass at the Royal Botanical Gardens Kew, Wakehurst Place, UK, in 2000. Seeds were harvested on 31 July (shaking the plants over sheets of paper), dried to equilibrium in a dry-room maintained at 15% relative humidity (RH) and 15°C, sealed in air-tight containers, and stored at −20°C.

Experiment 1: Seed priming

In March 2002, the seeds were removed from storage and allowed to warm to room temperature before taking six samples, each weighing 1 g (dry-room equilibrated weight). Three of these samples were primed for 7 d at 15°C in the dark on two layers of Whatman No. 1 filter paper wetted with a −1.5 MPa solution of polyethylene glycol (PEG) 6000 (Fisher Scientific, UK). Following priming, each sample was rinsed in distilled water and surface-dried by placing the seeds in a sintered glass funnel attached to a vacuum pump. The primed seeds were then re-dried to equilibrium in the dry-room. An unweighed sample of seeds was also primed in this way.

Experiment 1: Seed equilibration

A 1 g sample of each of the primed and non-primed (control) seeds was equilibrated at 5°C in the dark over each of three non-saturated solutions of lithium chloride: 230 g l^{-1}, 410 g l^{-1} and 580 g l^{-1} giving *c.* 70%, 41% and 23% RH, respectively. Additional primed and control seeds were also equilibrated under these conditions for initial determination of seed water content and viability (see below).

Experiment 1: Seed ageing in the thermal activity monitor (TAM)

This first experiment was carried out using a thermal activity monitor (TAM™; Thermometric AB, Sweden) on loan to the Seed Conservation Department (SCD) from Thermometric Ltd, UK. Each calorimeter unit of the TAM had a sample and a reference vessel surrounded by a heat sink that was maintained at a constant temperature. The TAM used in this study had two calorimeter units, and the temperature was set at 35°C. Heat produced or absorbed by a sample resulted in a temperature difference between the sample and the heat sink that caused heat flow; this heat flow was detected by highly sensitive thermopiles. The heat flow detected by the reference thermopiles was subtracted from the heat flow detected for the sample and was expressed in units of power (J s^{-1}).

After 2 weeks of equilibration, each 1 g sample of seeds was sealed inside a 3 ml glass TAM ampoule, crimping an aluminium lid with a rubber lining over the ampoule opening; the seeds occupied most of the space inside the ampoule. These, along with two empty sealed 3 ml ampoules, were placed at 35°C to allow for temperature equilibration. The following day, the ampoule containing the unprimed control seeds equilibrated over 230 g l^{-1} LiCl was slowly lowered into one of the TAM calorimeter units in synchronization with one of the empty reference ampoules. They were held for 30 min in the suspended position before complete lowering to allow for final temperature equilibration. The ampoule containing the primed seeds equilibrated over 230 g l^{-1} LiCl was similarly lowered with the second reference ampoule into the other calorimeter unit. Sample loading was carried out as slowly and smoothly as possible to minimize heat

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created by friction. Heat flow was recorded every 60 s for the duration of all the experiments.

The samples in the TAM were changed every 3–4 d, cycling between seeds equilibrated over different LiCl solutions. When the ampoules were not in the TAM, they were placed in an incubator at 35°C to maintain a constant rate of ageing.

Samples of the additional seeds, which were equilibrated alongside the 1 g samples, were used for gravimetric determination of seed water content (three replicates of *c.* 50 seeds each, dried in a ventilated oven at 103°C for 17 h; expressed as a proportion of dry weight) and for initial assessment of seed quality. This was determined by sowing four replicates of 50 seeds each on 1% (w/v) distilled water agar at 25/10°C (8 h/16 h), with light provided during the warm phase, and determining both total germination and the mean time to germinate (MTG).

At the end of the TAM storage experiment (39 d), all samples were removed (either from the TAM or from the 35°C incubator) and ampoules unsealed. The total number of seeds in each ampoule was estimated by weight, and sub-samples were taken for determination of water content and seed quality as before. The experiment conducted with the higher water content samples was repeated as above, except the experiment duration was 20 d.

Experiment 2

A similar experiment, commencing in December 2004, was carried out using a TAM at the University of London, School of Pharmacy (ULSOP). At the SCD, eight seed samples of 1 g were taken from the same bulk collection as before. Four of these samples were primed, rinsed and dried as in Experiment 1. After equilibration in the dry-room, three of the primed samples and three of the non-primed (control) samples were sealed in Wheaton vials for transport to ULSOP.

The remaining primed and control samples were each divided into three 'extra' sub-samples, two of which were similarly sealed in Wheaton vials; the third was used for initial (before ageing in the TAM) determination of seed water content and quality (total germination only), as before, of 'dry' seeds.

At ULSOP, one each of the 'dry' primed and control samples was transferred to TAM ampoules and placed in the TAM at 35°C for ageing. Empty ampoules were used as references. Before lowering, ampoules were suspended within the calorimeter unit to allow for temperature equilibration. At the same time, one of each of the primed and control 1 g samples, and of the primed and control 'extra' samples, were placed over a 230 g l^{-1} LiCl solution at 5°C for equilibration; the remaining samples were stored at 5°C (within the sealed vials in which they were transported).

After *c.* 14 d, the 'dry' samples were removed from the TAM and, still sealed in the ampoules, sent overnight to the SCD for determination of final (after ageing) water content and viability, and to estimate the number of seeds in each ampoule. At the same time, the 1 g primed and control samples equilibrated over the 230 g l^{-1} LiCl solution were sealed inside TAM ampoules, brought to room temperature and then placed in the TAM for ageing at 35°C, suspending the samples to allow for temperature equilibration as before. The 'extra' seeds equilibrated over the 230 g l^{-1} solution were also sent to the SCD for pre-ageing determination of water content and viability. The remaining 1 g and 'extra' primed and control samples of seeds were equilibrated over 410 g l^{-1} LiCl at 5°C.

After 14 d, the samples were again exchanged, and aged samples and equilibrated 'extras' were sent to the SCD for determination of water content, viability, and seed number (aged samples only) as before. Finally, after the last 14 d ageing period, the 410 g l^{-1}-equilibrated samples were removed from the TAM and returned to the SCD where water content, viability and seed number were determined as before.

Results

Experiment 1

Seeds equilibrated over the different LiCl solutions had significantly different water contents ($P < 0.05$), ranging from *c.* 0.045 g (g dw)$^{-1}$ for seeds equilibrated over the 580 g l^{-1} solution, to *c.* 0.12 g (g dw)$^{-1}$ for seeds equilibrated over the 230 g l^{-1} solution (Table 1). The differences between primed and control seeds were not significant ($P > 0.05$), and there was only one case where the water content appeared to differ significantly ($P < 0.05$) at the end of the storage period, compared with the initial water content (control seeds equilibrated over the 230 g l^{-1} solution). The water contents of the two sets (primed and control) of seeds equilibrated over the 230 g l^{-1} solution did not differ significantly ($P > 0.05$) between the two runs.

The degree of ageing that had occurred in the cycled samples by the end of the storage period (39 d) depended on the water content; both primed and control seeds stored at the highest water content were completely non-viable after the 39 d (Table 1). Indeed, the repeat experiment showed that all the seeds had died by 20 d under these storage conditions. For seeds stored at the two lower water contents, the rate of ageing differed between the primed and control seeds, with control seeds having lower viability and/or higher MTG by the end of the storage period,

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Table 1. Water content and quality of primed and unprimed seeds of *Ranunculus sceleratus* L. before and after ageing at 35°C, after pre-equilibration at 5°C over various LiCl solutions (Experiment 1)

		Initial			Final*			
LiCl ($g\,l^{-1}$)	Treatment	Water content [$g\,(g\,dw)^{-1}$] mean ± SE	Germination (%)	Mean time to germinate (d)	Water content [$g\,(g\,dw)^{-1}$] mean ± SE	Germination (%)	Mean time to germinate (d)	Number of seeds in ampoule
230	Primed	0.120 ± 0.0097	99.5	7.86	0.112 ± 0.0025	0	–	4114
230	Control	0.097 ± 0.0073	99.5	10.78	0.125 ± 0.0004	0	–	4077
410	Primed	0.082 ± 0.0031	99	8.15	0.072 ± 0.0009	98	9.58	4059
410	Control	0.075 ± 0.0052	98.5	10.82	0.070 ± 0.0002	56	12.13	4269
580	Primed	0.039 ± 0.0019	98	8.28	0.045 ± 0.0018	99	8.64	4144
580	Control	0.046 ± 0.0031	94	10.51	0.052 ± 0.0003	95	13.51	4197
230	Primed	0.107 ± 0.0008	98	8.15	0.109 ± 0.0139	0	–	
230	Control	0.127 ± 0.0005	97	11.34	0.119 ± 0.0035	0	–	

*Seeds aged for 39 d (first six rows) or 20 d (last two rows; 'repeat').

compared with primed seeds. For example, for primed seeds stored at *c.* $0.08\,g\,(g\,dw)^{-1}$, although there was no loss in viability, MTG increased from 8.2 to 9.6 d; in contrast, for control seeds stored at the same water content, viability fell from 98.5 to 56%, and MTG increased from 10.8 to 12.1 d. The only sample that did not appear to age significantly during the course of the experiment was the primed seeds stored at *c.* $0.045\,g\,H_2O\,(g\,dw)^{-1}$.

All samples generated heat flow (Fig. 1). In the main experiment (panels A, C and D), the samples were cycled every 3 d, and there are interruptions in the plots. There was also the loss of some data, corresponding to the samples stored at the lowest water content, due to computer failure. Nonetheless, it is clear that the heat produced by the seeds was related to their water content, with seeds at the highest water content causing the greatest heat flow. There were also differences between primed and control seeds, with priming of seeds consistently resulting in less heat flow, compared with control seeds, at least initially (Fig. 1A). In the case of seeds stored at the highest water content, the heat produced by primed seeds surpassed that produced by control seeds after *c.* 13 d storage. This is clearer in the repeated 20-d experiment with seeds stored at the same water content (Fig. 1B). For this run, the seeds remained in the TAM continuously. The power–time curves from both the primed and control seeds in this second run were identical to those observed in the first run (over the periods when data were recorded; compare Figs 1A and B).

In general, there appeared to be a smooth decline in heat flow over the duration of each experiment; however, analysis of the curves does not indicate first- or second-order kinetics (results not shown). Furthermore, there was a large and sudden drop in the heat flow for control seeds after *c.* 6 d storage at $0.12\,g\,H_2O\,(g\,dw)^{-1}$ (Fig. 1B). Again, because the traces are identical over the periods when data were being recorded, this same decline presumably would have been observed in the first run had the data been collected throughout. Although heat flow declined, the seeds were still producing some heat, even after 34 d at the highest water content, when all the seeds had presumably died (Fig. 1A).

Experiment 2

In the second experiment, the water contents of the different samples were comparable with those for Experiment 1 and, with the possible exception of the LiCl $230\,g\,l^{-1}$ sample (initial water content was not replicated), did not change during ageing (Table 2). The viability losses during ageing in the TAM were not expected to be the same as the losses in Experiment 1, since the seeds were aged for different

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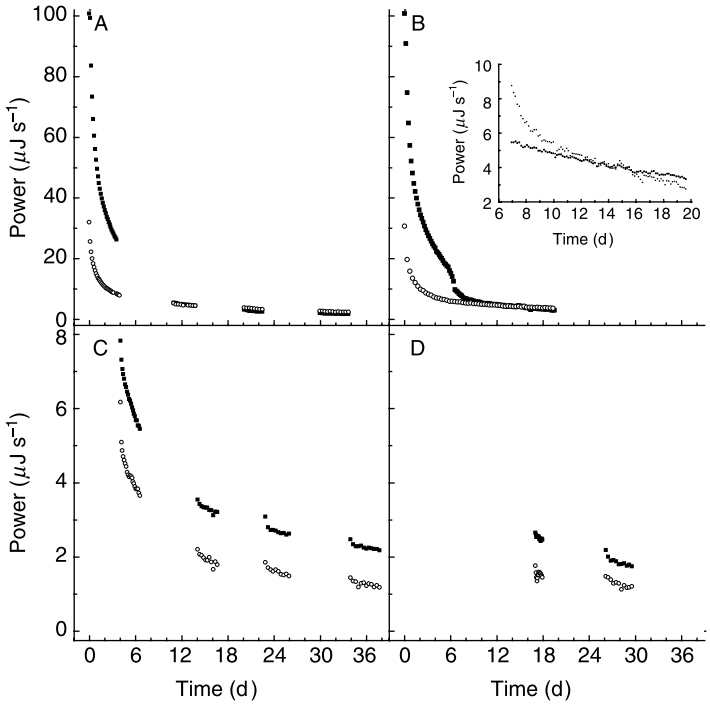

Figure 1. Heat flow curves measured on samples of approximately 4100 primed (○) or control (■) seeds of *Ranunculus sceleratus* aged at 35°C. Seeds pre-equilibrated at 5°C over LiCl: (A, B) 230 g l^{-1}; (C) 410 g l^{-1} or (D) 580 g l^{-1} (Experiment 1). The insert in panel B expands the data between 7 and 20 d. For clarity, only a small proportion of the recorded data is shown.

lengths of time. Nonetheless, after 14 d both primed and control seeds at the highest water content had completely lost viability (Table 2). There was some loss in viability for seeds at the intermediate water content and, while some ageing was expected to have occurred in the dry seeds, there was no loss of viability.

As in Experiment 1, heat flow was detected for both primed and control seeds at all three water contents (individual power–time curves not shown). The rates of heat flow were very similar to those observed in the first experiment, and the same sharp reduction in heat flow was observed in the control seeds at the highest water content, after the same period of ageing. Since it was not necessary to cycle the samples in this experiment, it was possible to calculate the total amount of heat produced by the ageing seeds by integration of the power–time curves over a certain period of time; this was done for the first 6 d (before the change in kinetics observed in the high water content control seeds, Fig. 1B). This confirmed that there were considerable differences between control and primed seeds, with control seeds (which aged at a faster rate) generally having greater total

Table 2. Water content and quality of primed and unprimed seeds of *Ranunculus sceleratus* L., before and after ageing for 14 d at 35°C. Seeds were taken directly from the dry-room or pre-equilibrated at 5°C over, 230 or 410 g l^{-1} LiCl

		Initial		Final		
Equilibration environment (g l^{-1} LiCl)	Treatment	Water content [g (g dw)$^{-1}$] mean ± SE*	Germination (%)	Water content [g (g dw)$^{-1}$] mean ± SE*	Germination (%)	Number of seeds in ampoule
230	Primed	0.145	96.5	0.117 ± 0.0005	0	4163
230	Control	0.123	95.5	0.117 ± 0.0035	0	4203
410	Primed	0.070 ± 0.0004	98	0.069 ± 0.0009	93.5	4077
410	Control	0.072 ± 0.0002	95	0.072 ± 0.0003	93	4112
Dry-room	Primed	0.048 ± 0.0039	96	0.046 ± 0.0014	96	4046
Dry-room	Control	0.047 ± 0.0008	92	0.047 ± 0.0006	93.5	4063

* Mean and standard error of five replicates, except where SE is not given (no replicates were available).

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heat generated than primed seeds (Fig. 2). The total heat generated also depended on the water content of the seeds, with significant ($P < 0.05$) linear relationships between total heat and water content.

Discussion

Priming is often used as an invigoration treatment to increase the speed and uniformity of seed germination. However, there is often a negative effect on the subsequent longevity of the seeds in dry storage. In contrast, Probert *et al.* (1991) reported that there was a four- to fivefold increase in longevity of *R. sceleratus* seeds following priming. In the experiments on *R. sceleratus* seeds described here, priming also improved the rate of germination and reduced the rate of seed ageing. This improvement in longevity was seen as a smaller loss in viability and/or a smaller reduction in the MTG following ageing (Table 1). As expected, the amount of ageing incurred depended on the water content of the seeds during storage, with a greater decline in viability and/or increase in MTG as the water content increased (Table 1).

The heat generated over the course of these ageing experiments was consistent with the differences in extent of ageing observed. The rate and total amount of heat generated by unprimed control seeds (which aged at a faster rate) were generally higher than those for primed seeds at each water content (at least initially; the heat flow of primed seeds at the highest water content surpassed that of control seeds after 13 d; Fig. 1B, inset). For the second experiment, the total heat flow over the first 6 d was calculated by integrating the area under the power–time curves; total heat produced by control seeds at 0.12 g H_2O (g dw)$^{-1}$ was *c.* 16 J compared with 7 J by primed seeds (Fig. 2). Heat flow also increased with increases in water content. For example, for control seeds, total heat produced over 6 d was 1.5 J at 0.045 g H_2O (g dw)$^{-1}$ and increased to 16 J at 0.12 g H_2O (g dw)$^{-1}$ (Fig. 2).

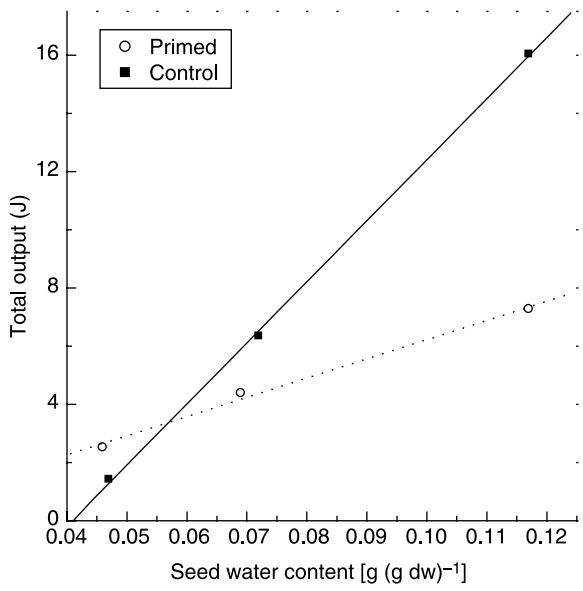

Figure 2. The effect of seed water content on the total heat produced over the first 6 d by primed or control seeds of *Ranunculus sceleratus* aged at 35°C (Experiment 2).

During the storage of seeds, a number of reactions may cause, or at least contribute to, declining vigour and the subsequent loss of viability (for reviews, see Priestley, 1986; Walters, 1998). The generally sigmoid curves of viability loss may be indicative of 'co-operativity' of different reactions (Bernal-Lugo and Leopold, 1998; Walters, 1998). In addition, the type and rate at which different ageing reactions occur may vary depending on the conditions of storage. For example, Walters (1998) presented a model of the rates of chemical degradation of different seed components (protein, phospholipids and carbohydrate) at different water contents.

While we hypothesize that the heat flow detected was entirely due to detrimental ageing reactions, there may have been non-detrimental reactions taking place and contributing to the heat flow detected. Furthermore, different reactions will result in different rates of heat flow (or may even be endothermic). This, along with the fact that more molecules will be in their 'unaged' state at the start of the experiment, may account for the shape of the power–time curves, which generally showed an initial phase of rapidly declining heat flow (e.g. over the first 36 h for seeds aged at the highest water content); initial ageing reactions may be both more numerous and/or more exothermic than subsequent reactions. Clearly, for both primed and control seeds, reactions were still occurring and resulting in an overall exothermic heat flow after seeds had lost the ability to germinate. Failure to germinate may only reflect the fact that a critical number of (specific) cells, e.g. in the radicle of a seed, have died, and other reactions will still be taking place elsewhere. Indeed, reactions may still occur even after a cell is, in essence, deemed non-viable.

The power–time curves generally take the same form, with the exception of control seeds at the highest water content, for which there is a sudden decline in the heat flow after 6 d, followed by a return to a process with similar kinetics (Fig. 1B). This was observed repeatedly. In a reaction system, a sudden decrease in heat flow could be caused by a number of different factors, the most obvious being a sudden exhaustion of a particular substrate necessary for a chemical reaction(s) in the ageing pathway (loss of the active site of an enzyme may also account for this observation). For example, within a sealed system

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where a sample is respiring, the gas around the sample may become exhausted of oxygen, resulting in a sudden decrease in heat flow. In this study, the seeds were not at a high enough water content for respiration to occur, and although some ageing reactions involve oxygen, there is likely to have been a sufficient volume of air around the seeds such that it did not become a limiting factor. A decline in heat flow may not occur until an equivalent point in the total ageing process (i.e. after a longer period of time) for primed seeds or for seeds at lower water contents, or it may be prolonged and therefore less likely to be detected.

The power–time curves also showed that the heat flow of primed seeds surpasses that of control seeds after *c.* 15 d for seeds at the highest water content, and suggest that the control seeds reached zero heat flow before the primed seeds (Fig. 1B, inset). The greater heat flow produced by the primed seeds during the latter part of the experiment was consistent with a slower rate of ageing and more reactants still being available. However, it is not clear whether the total heat produced by the primed and control samples would equalize, if the experiments were run longer.

The total heat produced over a set period indicated the extent of reactions occurring over that time frame; clearly, the higher the water content, the more activity that was present (Fig. 2). This in not surprising; the effects of water content on the rate of seed ageing are well known. However, the primed seeds exhibited a significantly lower change in extent of reaction as a function of water content, compared with that observed for unprimed control seeds. This is consistent with a suggested decrease in weak water-binding sites, resulting in a reduced effect of water content on rate of ageing as a consequence of priming in this species (Sun *et al.*, 1997). Further experimentation may help to explain the mechanism(s) at the molecular level by which priming enhances (or otherwise alters) seed longevity.

Finally, the results showed the repeatability of IMC measurements made using different TAM machines on independent, identical samples of seeds at different times and in different laboratories; individual power–time curves can be superimposed almost exactly (compare Figs 1A and 1B; data from second experiment not shown). This adds to the potential of IMC as a reliable diagnostic tool for seed science applications.

Conclusions

IMC detected heat flow in seeds that we attribute to ageing reactions. Differences in heat flow reflected differences in longevity in response to a priming treatment, and as a consequence of the storage environment (water content). We have further demonstrated, to a limited extent, the repeatability of this method in the analysis of seed ageing kinetics. By deconvoluting power–time curves, it may be possible to gain a much greater understanding of the ageing reactions in seeds, although considerable further experimentation would be required, working on whole seeds and purified samples of different seed components.

Heat was detected even after the seeds had lost viability; this may limit the use of the IMC method if, as a consequence, it could not distinguish between a very slow rate of viability loss and a collection of seeds with low initial viability. If IMC is to be diagnostic of the amount of ageing that has occurred, it may be necessary to determine initial viability and heat flow during ageing at two or three water contents.

Ageing experiments, in particular experiments that are intended to be more comparable with seeds stored under seed-bank conditions, are necessarily lengthy, and so determining kinetics using IMC at a wide range of water contents and temperatures may be impractical. However, it may help to discriminate 'good-keepers' from 'bad-keepers' at the seed-lot and/or species level; such a screening could be carried out over much shorter time periods than those used in this preliminary study.

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References

Beezer, A.E., Gaisford, S., Hills, A.K., Willson, R.J. and Mitchell, J.C. (1999) Pharmaceutical microcalorimetry: applications to long-term stability studies. *International Journal of Pharmaceutics* **179**, 159–165.

Bernal-Lugo, I. and Leopold, A.C. (1998) The dynamics of seed mortality. *Journal of Experimental Botany* **49**, 1455–1461.

Dearman, J., Brocklehurst, P.A. and Drew, R.L.K. (1987) Effects of osmotic priming and aging on the germination and emergence of carrot and leek seed. *Annals of Applied Biology* **111**, 717–722.

Ellis, R.H. and Roberts, E.H. (1980) Improved equations for the prediction of seed longevity. *Annals of Botany* **45**, 13–30.

Hay, F.R., Probert, R.J. and Smith, R.D. (1997) The effect of maturity on the moisture relations of seed longevity in foxglove (*Digitialis purpurea* L.). *Seed Science Research* **7**, 341–349.

https://doi.org/10.1079/SSR2006238 Published online by Cambridge University Press

Kraak, H.L. and Vos, J. (1987) Seed viability constants for lettuce. *Annals of Botany* **59**, 343–349.

Lanteri, S., Belletti, P., Marzach, C., Nada, E., Quagliotti, L. and Bino, R.J. (1997) Priming-induced nuclear replication activity in pepper (*Capsicum annuum* L.) seeds: effect on germination and storability. pp. 451–460 *in* Ellis, R.H.; Black, M.; Murdoch, A.J.; Hong, T.D. (Eds) *Basic and applied aspects of seed biology.* Dordrecht, Kluwer Academic.

Mourik, J. and Bakri, A. (1991) Application of microcalorimetry to plant technology: germination and initial growth. *Thermometric Application Note 2201*. Thermometric AB, Sweden.

Prat, H. (1952) Microcalorimetric studies on germinations of cereals. *Canadian Journal of Botany* **30**, 379–394.

Priestley, D.A. (1986) *Seed aging*. Ithaca, New York, Comstock Publishing.

Probert, R.J., Bogh, S.V., Smith, A.J. and Wechsberg, G.E. (1991) The effect of priming on seed longevity in *Ranunculus sceleratus* L. *Seed Science Research* **1**, 243–249.

Roberts, E.H. (1972) Storage environment and the control of viability. pp. 14–58 *in* Roberts, E.H. (Ed.) *Viability of seeds*. London, Chapman & Hall.

Sigstad, E.E. and García, C.I. (2001) A microcalorimetric analysis of quinoa seeds with different initial water content during germination at 25°C. *Thermochimica Acta* **366**, 149–155.

Sinniah, U.R., Ellis, R.H. and John, P. (1998) Irrigation and seed quality development in rapid-cycling *Brassica*: seed germination and longevity. *Annals of Botany* **82**, 309–314.

Sun, W.Q., Koh, D.C.Y. and Ong, C.-M. (1997) Correlation of modified water sorption properties with the decline of storage stability of osmotically-primed seeds of *Vigna radiata* (L.) Wilczek. *Seed Science Research* **7**, 391–397.

Tarquis, A.M. and Bradford, K.J. (1992) Prehydration and priming treatments that advance germination also increase the rate of deterioration of lettuce seed. *Journal of Experimental Botany* **43**, 307–317.

Walters, C. (1998) Understanding the mechanisms and kinetics of seed aging. *Seed Science Research* **8**, 223–244.

Walters, C., Wheeler, L.M. and Grotenhuis, J.M. (2005) Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* **15**, 1–20.

Yamaguchi, T., Wakizuka, T. and Takahashi, K. (1996) Application of calorimetry to investigate viability of crops seeds. *Netsu Sokutei* **23**, 2–4.

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