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Contrasting seed moisture sorption behaviour between two species and the implication for seed longevity

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Research Paper

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Abstract

Understanding seed moisture desorption and adsorption isotherms is important for seed quality maintenance and better predicting seed storage lifespan. Freshly harvested oilseed rape and barley seeds were dried and then rehydrated twice. Seed equilibrium relative humidity (eRH) and moisture content (MC) were determined at different humidity levels so that two cycles of desorption and adsorption could be constructed. In addition, seeds were dried to 30% RH and then rehydrated to 50% RH for five cycles to determine whether they shift to the adsorption isotherm. Monolayer MC was determined using the Gugenheim-Anderson-de Boer model. Storage experiments were conducted for seeds equilibrated at 30, 40, 50, 60 and 70% RH for two cycles of desorption and adsorption at 45°C. Isotherm curves' shapes were similar for oilseed rape and barley, although spanning a greater MC range in barley. The hysteresis effect was observed for oilseed rape and barley seeds when dried over silica gel at <10% RH. However, this effect was only observed for barley seeds when dried to 30% RH, but not for oilseed rape seeds. Longevity was greater for adsorbing seeds than desorbing seeds at a given eRH, however, there was no significant difference in σ (the standard deviation of the normal distribution of seed deaths over time)–MC log–log relationship. The relationship shifted for seeds on the second cycle. In conclusion, if seed lots are stored at a specific RH, reaching equilibrium by desorption or adsorption can strongly influence their longevity. Also, when seeds of different species are dried to low RH, they will respond differently to a subsequent increase in RH, which could profoundly affect their longevity.

Introduction

Seed longevity is a complex, quantitative quality trait, defined as the period during which seeds retain their ability to germinate. Seed viability and vigour during storage are two critical quality factors for commercial seed lots and for seeds stored in genebanks (Hay, 2022; Hey et al., 2022b; Reed et al., 2022). The vigour of a seed lot relates to its ageing tolerance, dormancy, viability and rapid germination and seedling establishment, especially under suboptimal growing conditions (Reed et al., 2022). The handling of seeds and postharvest practices have a significant impact on seed quality; and even though different lots are handled similarly and have initial high germination, they will naturally differ in vigour (Hay, 2022). Moisture content is an important factor that determines the rate at which seeds age and decline in viability and vigour, along with the temperature and gaseous composition of the storage environment (Sinha and Wallace, 1977; Roberts and Ellis, 1989; Walters et al., 2005; Probert et al., 2009; Merritt et al., 2014; Whitehouse et al., 2015; De Vitis et al., 2020; Buitink and Leprince, 2022; Hay, 2022). Generally, seed viability and vigour are maintained longer when the seeds have a low moisture content and are at low temperature in an oxygen-limited environment. Hence, seeds are typically dried to a very low moisture content for long-term storage, to equilibrium with 10–25% RH at 5–20°C according to the Genebank Standards (FAO, 2014). However, commercial seed lots are not dried to such low moisture levels, nor are they always hermetically packaged before being sold to consumers. Short-term seed storage is usually in commercial seed warehouses conditioned to 30–35% RH and 15–20°C. Seeds stored under such conditions can lose viability relatively quickly, over some months (Yahaya et al., 2022). It is therefore important to determine seed moisture content and consider differences between species for better seed warehouse management.

Seed moisture is a dynamic property of the seeds which fluctuates in direct relation to the surrounding air, i.e. relative humidity (RH) and temperature (Elias et al., 2018). A 'moisture sorption isotherm' is defined as the relationship between moisture content (MC) and corresponding equilibrium relative humidity (eRH) or water activity (a_w; where eRH $\cong a_w \times 100$), at a constant temperature (Basu et al., 2006; Hay et al., 2022a). An adsorption isotherm is obtained by allowing dry material to take up moisture (hydration/humidification). A desorption isotherm is obtained by making wet material lose moisture (dehydration/drying).

Moisture sorption isotherms are essential to have a better understanding of the water sorption mechanisms, thermodynamic functions and interactions of the absorbed/desorbed water (Mujumdar and Devahastin, 2000). The isotherm shape and position vary between seeds of different species and depend on the temperature (Hay et al., 2022a). Most orthodox seeds show typical type II isotherm characteristics, being 'reverse sigmoidal' or 'S-shaped' (Brunauer et al., 1940; Vertucci, 1989). The type II isotherm curve is divided into three regions with predominantly strongly bound water in region 1, loosely bound water that can act as a solvent in region 2 and weakly bound water in region 3 (Walters et al., 2002, 2005; Okos et al., 2019; Hay et al., 2022a). Isotherms can be determined by equilibrating seeds in an environment with a specific relative humidity (for example by using saturated and unsaturated salt solutions) or by adjusting the moisture content (adjusting based on the change in weight by drying or humidification) and measuring their eRH and then determining their moisture content (Hay et al., 2008, 2022a). Numerous equations have been developed to model the relationship between seed water content and relative humidity (for a review of equations used in the literature, see Hay et al., 2022a; supplementary Table S1; Menkov, 2000; Mallek-Ayadi et al., 2020). The Gugenheim-Anderson-de Boer (GAB) model is a theoretical model which includes a parameter described as the 'monolayer moisture content' (M_m). M_m is the point where ionic-bound water cannot participate in chemical reactions (Caballero-Cerón et al., 2015). Above M_m, more water becomes available to act as a solvent. Therefore, the rate of reactions increases, resulting in a loss of quality and acceleration of ageing (Bell and Labuza, 2000). Below M_m, the rates of chemical reactions and, therefore, the rates of quality loss in foods, are negligible (Bell and Labuza, 2000). The M_m typically occurs between 0.2 and 0.4 on the a_w scale in foods (Rahman and Labuza, 2007).

Sorption hysteresis refers to the difference in the moisture content of material between adsorption and desorption isotherms at the same RH (Wolf et al., 1972; Yang et al., 1997); more water is held within desorbing seeds than within adsorbing seeds between upper and lower closure points where the isotherms come together again (Ellis et al., 1989; Bell and Labuza, 2000; Silva et al., 2018). Hence, most of the hysteresis effect occurs in region II of the isotherm (Roberts and Ellis, 1989). The magnitude of hysteresis varies between seeds of different species, for example being higher for starchy seeds compared with oily seeds (Hay et al., 2022a). The magnitude is also affected by temperature, history and physicochemical properties of the seeds, as well as the equilibration method (Yang et al., 1997; Hay et al., 2022a). This effect has not yet been systematically characterized for seeds across diverse species, or for seeds with different compositions. Also, even if seeds are equilibrated with the same method and temperature, repeated cycles of desorption and adsorption may not give the same isotherms as the initial cycle (Li et al., 2011; Hay et al., 2022a). Furthermore, seed storage experiments (SSEs) tend to be conducted for seeds equilibrated in region II. This region can be described by the viability equations, in terms of either storage moisture content (longevity–MC relationship) or seed relative humidity (longevity–eRH relationship) (Ellis and Roberts, 1980a; Roberts and Ellis, 1989). Taking into account these two relationships may help ensure the validity of comparisons between seeds from different species (Hay and Timple, 2016).

This work aimed to:

(1) determine cycles of desorption and adsorption isotherms for freshly harvested seeds of oilseed rape, as a seed with high oil content, and barley, with low oil content, to determine the extent of hysteresis in terms of both magnitude and RH range over which it occurs;
(2) determine whether seeds shift to the adsorption isotherm when dehydrated to 30% RH before subsequently rehydrating to 50% RH, and test the hypothesis that the shift is related to M_m;
(3) compare the longevity–moisture content relationship for these two species, depending on the sorption cycle.

Material and methods

Materials (seeds)

Winter oilseed rape (*Brassica napus* var. DK-Exlibris) and spring barley (*Hordeum vulgare* var. KWS Irina) seeds, produced on the experimental fields at the Dept. Agroecology, Aarhus University-Flakkebjerg, were harvested on 24 July 2021 and 16 August 2021, respectively. After threshing and cleaning, they were transferred to the laboratory (the same day that seeds were harvested) and a sample was taken for initial moisture content (MC) and water activity (a_w; expressed as equilibrium relative humidity, eRH where eRH $\cong a_w \times 100$) determination. Water activity was determined at 20°C using a Rotronic AwTherm (C.K. Environment A/S, Værløse, Denmark). The moisture contents were determined gravimetrically, using the 'low constant temperature' oven method (ISTA, 2021), drying samples of 2–5 g seeds at 103°C for 17 ± 1 h. Upon removal from the oven, the seeds, within the covered crucibles, were allowed to cool for about 1 h in a glass desiccator with silica gel at room temperature before re-weighing. Seed moisture contents are expressed on a fresh weight basis (f.wt.).

Isotherm determination

The seeds of each species (oilseed rape and barley) were divided into four groups for two cycles of isotherm determination: (i) desorption-1; (ii) adsorption-1; (iii) desorption-2 and (iv) adsorption-2. The seeds for the initial desorption were further subdivided and placed over silica gel and non-saturated LiCl solutions (20, 30, 40, 50, 60, 70 or 80% RH) inside 300 mm × 300 mm × 132 mm Ensto Cubo electrical enclosure boxes (Ensto Industry, Finland) with non-transparent lids at 20°C. The lithium chloride solutions were prepared according to Hay et al. (2008) a week before the start of the experiments and were kept at 20°C. After at least 1–3 weeks, the a_w and MC of Group 1 'desorption-1' was measured for seeds equilibrated at each RH. To make sure that seeds had equilibrated to a specific RH, a sample was taken and its a_w was measured using the Rotronic (before measuring MC). Freshly regenerated silica gel was used to dry seeds below 10% RH (because seeds could dry down to just 11% RH using LiCl solution). Silica gel was dried in the oven at 103°C for 24 h prior to starting and the a_w of silica gel was checked regularly.

The seeds of the other three groups (i.e. adsorption-1, desorption-2 and adsorption-2) were placed over silica gel for 3 weeks. Group 2 seeds for adsorption cycle-1 were then placed in the LiCl boxes as above and the a_w and MC measured after at least 1–3 weeks. Groups 3 and 4 seeds for desorption-2 and adsorption-2, respectively, were transferred to a CTS Climatic Test Cabinet (Series C; Citrotek ApS, Hillerød, Denmark) at 20°C, 95% RH for rehydration. After at least 1–3 weeks, a_w and MC rehydration were measured on seeds from groups 3 and 4, after which group 3 seeds were transferred to LiCl boxes for desorption-2 and the same measurements continued for this group. Seeds from group 4 were transferred to silica for 3 weeks, after which samples were taken for a_{w} and MC determination. Next, seeds were subdivided and placed in the separate RH electrical enclosure boxes as described above, to allow moisture uptake to determine the adsorption-2 isotherm. After at least 1–3 weeks of equilibration, a_{w} and MC were measured again. The isotherm experiment is summarized below:

i. Group 1 (desorption-1): equilibrated at 80, 70, 60, 50, 30 and 20% RH.
ii. Group 2 (adsorption-1): equilibrated over silica → 20, 30, 40, 50, 60, 70 and 80% RH.
iii. Group 3 (desorption-2): equilibrated over silica → 95% RH → 80, 70, 60, 50, 30 and 20% RH.
iv. Group 4 (adsorption-2): equilibrated over silica → 95% RH → silica → 20, 30, 40, 50, 60, 70 and 80% RH.

Partial moisture desorption and adsorption cycling between 30 and 50% RH

Separate samples of freshly harvested oilseed rape and barley seeds were dried to 30% RH and then rehydrated to 50% RH over LiCl salt solutions for five cycles. The seeds were equilibrated at the respective RH for at least 1–3 weeks. After equilibration, seed moisture content (MC) and water activity (a_{w}) were determined at 20°C using the Rotronic AwTherm.

Storage procedure

After the seeds were equilibrated at 30, 40, 50, 60 and 70% RH for each separate isotherm (i.e. desorption-1, adsorption-1, desorption-2 and adsorption-2), samples were subdivided and sealed inside 80 mm × 110 mm (width × length) laminated aluminium foil packets (eight packets for each seed lot × relative humidity × species) and placed at an incubator at 45°C. Each aluminium foil packet contained >90 seeds needed for germination testing. The packets of seeds were taken out from storage at different time intervals depending on species and sorption cycle. A further six aluminium foil packets containing seeds for each relative humidity were similarly prepared and stored at 45°C to check the MC of seeds on the first (three packets) and final day of storage (three packets). Seeds equilibrated at 80% RH were not considered in the storage experiment as they may be beyond the applicable limits of the viability equations (Hay et al., 2022a,b).

Seed germination

For oilseed rape, three samples of 30 seeds were sown with one bottom filter paper wetted with 7500 µl 0.2% KNO_3 solution in 90-mm diameter Petri dishes. For barley, three samples of 30 seeds were sown with one bottom and one top filter paper wetted with 7500 and 4750 µl 0.2% KNO_3 solution, respectively, in 90-mm diameter Petri dishes. The covered dishes were placed at 20°C with a 12-h light and 12-h dark period per day. Water was used for moistening as needed (ISTA, 2021). Seeds were recorded as germinated upon confirmation of radicle emergence (RE) by at least 2 mm based on ISTA (2021). Non-germinated seeds were tested for a further 3–7 d and then evaluated to see if they were dead, soft or mouldy. The germination percentage was calculated by dividing the number of seeds showing RE by the total number of seeds sown.

Statistical analysis and model fitting

The moisture desorption-1, adsorption-1, desorption-2 and adsorption-2 isotherms were fitted using the GAB model (equation 1) using non-linear curve fitting in OriginPro 2018 (Bell and Labuza, 2000):

$$m = \frac{M_{\mathrm{m}} C k a_{\mathrm{w}}}{(1 - k a_{\mathrm{w}})(1 - k a_{\mathrm{w}} + C k a_{\mathrm{w}})} \tag{1}$$

where m is moisture content (% dry weight), M_{m} is the monolayer moisture content (% dry weight). C and k are constants. C is associated with the water sorption heat of the first layer (monolayer) and k involves the water sorption heat of multilayer.

Seed survival data, i.e. the ability to germinate upon removal from the experimental storage regime (v), were fitted by probit analysis using Genstat for Windows, 21st Edition (VSN International Ltd., UK), thereby fitting the seed viability equation (equation 2):

$$v = K_i - \frac{p}{\sigma} \tag{2}$$

where σ is the standard deviation of the frequency distribution of seed deaths in time (days), K_i is the estimated probit viability of the seed lot at the start of storage and P is the storage period.

Estimates of the time (days) for viability to fall to 50% (p_{50}) were also estimated. Approximate F-tests were used to determine whether the survival curves could be constrained to a common estimate of K_i within each species × sorption cycle. The FITNONLINEAR directive was then used to model the effect of change in moisture content, m, on the standard deviation of seed deaths over time, constraining K_i within each species × sorption cycle, thereby fitting equation (3):

$$v = K_i - \frac{p}{10^{K - C_{\mathrm{W}} \log m}} \tag{3}$$

This one-step approach (Hay et al., 2003, 2014; Crawford et al., 2012) takes into account all the data in estimating the constants K and C_{W}. Including the data for all the sorption cycles, F-tests were used to see whether K and/or C_{W} could be constrained among sorption cycles for each species. The universal values for the temperature constants, C_{H} and C_{W} (0.0329 and 0.000478, respectively), were then used to determine K_{E}, as equation (4):

$$K_{\mathrm{E}} = K + C_{\mathrm{H}} t + C_{\mathrm{Q}} t^2 \tag{4}$$

where t is temperature in °C, thereby solving all the parameters of the full viability equation (equation 4; Ellis and Roberts, 1980a; Ellis, 2022):

$$v = K_i - \frac{p}{10^{K_{\mathrm{E}} - C_{\mathrm{W}} \log m - C_{\mathrm{H}} t - C_{\mathrm{Q}} t^2}} \tag{5}$$

Results

Moisture sorption isotherms

The isotherm curves showed the typical type II sigmoidal shape and were similar for oilseed rape and barley, although spanning

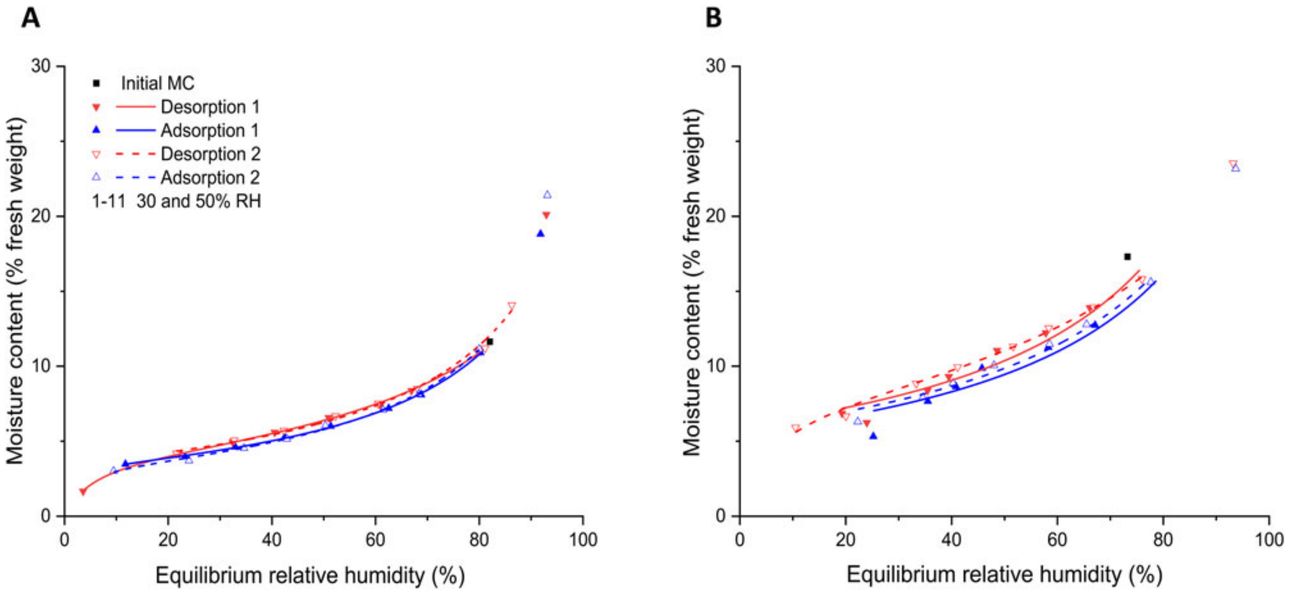

Figure 1. Moisture desorption (red) and adsorption (blue) isotherms of seeds of winter oilseed rape (*Brassica napus* var. DK-Exlibris) (A) and spring barley (*Hordeum vulgare* var. KWS Irina) (B) at 20°C. Solid lines represent isotherm cycle-1, and dashed lines represent isotherm cycle-2. The isotherms were fitted using the GAB equation (equation 1).

a greater MC range in barley compared with oilseed rape (Fig. 1). For example, for barley seeds at between 33 and 67% eRH, moisture content ranged from 8.4 to 13.9% f.wt., while for oilseed rape seeds, the moisture content ranged from 5.0 to 8.4% f.wt. over the same RH range (see supplementary Table S2). The moisture content of the seeds was systematically higher during the desorption process (red symbols) than the adsorption process (blue symbols) between 20 and 80% eRH for both species, due to a hysteresis effect (Fig. 1A, B). The maximum difference in the MC of desorbing and adsorbing seeds was 0.57% f.wt. for oilseed rape seeds and 1.19% f.wt. for barley seeds. Isotherms were fitted using the GAB model (equation 1), which accounted for 93–99.9% of the variance, for each cycle of desorption and adsorption for both oilseed rape and barley (Table 1). According to the GAB modelling, oilseed rape seeds had a M_m value of 4.11% f.wt. for the first desorption cycle and 3.34% f.wt. for the first adsorption cycle. The point where the hysteresis effect started to appear was at 3.7% moisture content and 17% eRH (Figs 1A and 2A). The M_m value decreased for the second desorption cycle compared to the first desorption cycle, to 3.60% f.wt., but was similar for the two adsorption cycles. For barley seeds, the M_m values were higher than for oilseed rape: 6.02% f.wt. for the first desorption cycle and 5.58% f.wt. for the first adsorption cycle (Table 1). The point where the hysteresis became apparent was at 6.7% moisture content or 16% eRH (Figs 1B and 2B) for barley seeds. In contrast with oilseed rape, the M_m of barley seeds increased for the second desorption and adsorption cycles (Table 1).

Partial moisture adsorption and desorption cycling between 30 and 50% RH

The effect of sequential cycles of drying to 30% RH and then rehydrating to 50% RH differed between the oilseed rape seeds and the barley seeds (Fig. 2A, B, numbers 1–11). For oilseed rape, no hysteresis was observed: the moisture content of seeds dried to 30% RH and then rehydrated to 50% RH (multiple cycles) was not significantly different ($P > 0.05$) to that of seeds dried to 50% RH (Figs 2A and 4A). In contrast, for barley seeds, the hysteresis effect was observed (Figs 2B and 4B): upon

Table 1. Parameters (estimates and standard error, SE) of the GAB equation (equation 1) for oilseed rape and barley seeds for two cycles of desorption and adsorption (data and fitted isotherms shown in Fig. 1)

Species	Isotherm cycle	C (estimate and SE)	K (estimate and SE)	M_m (estimate and SE;% f.wt.)	eRH (%) at M_m	R^2
Oilseed rape	Desorption-1	21.191 (2.273)	0.823 (0.010)	4.11 (0.08)	22	0.999
	Adsorption-1	113.674 (49.120)	0.895 (0.006)	3.34 (0.05)	13	0.999
	Desorption-2	227.387 (1421.894)	0.887 (0.019)	3.60 (0.23)	14	0.994
	Adsorption-2	43.875 (14.492)	0.910 (0.011)	3.32 (0.09)	13	0.998
Barley	Desorption-1	2.347×10^{45} (1.692×10^{45})	0.892 (0.049)	6.02 (0.38)	34	0.957
	Adsorption-1	-2.259×10^{43} (3.402×10^{45})	0.868 (0.060)	5.58 (0.54)	11	0.930
	Desorption-2	22.385 (5.434)	0.763 (0.034)	7.61 (0.40)	23	0.994
	Adsorption-2	-1.807×10^{45} (8.171×10^{44})	0.866 (0.038)	5.84 (0.33)	12	0.979

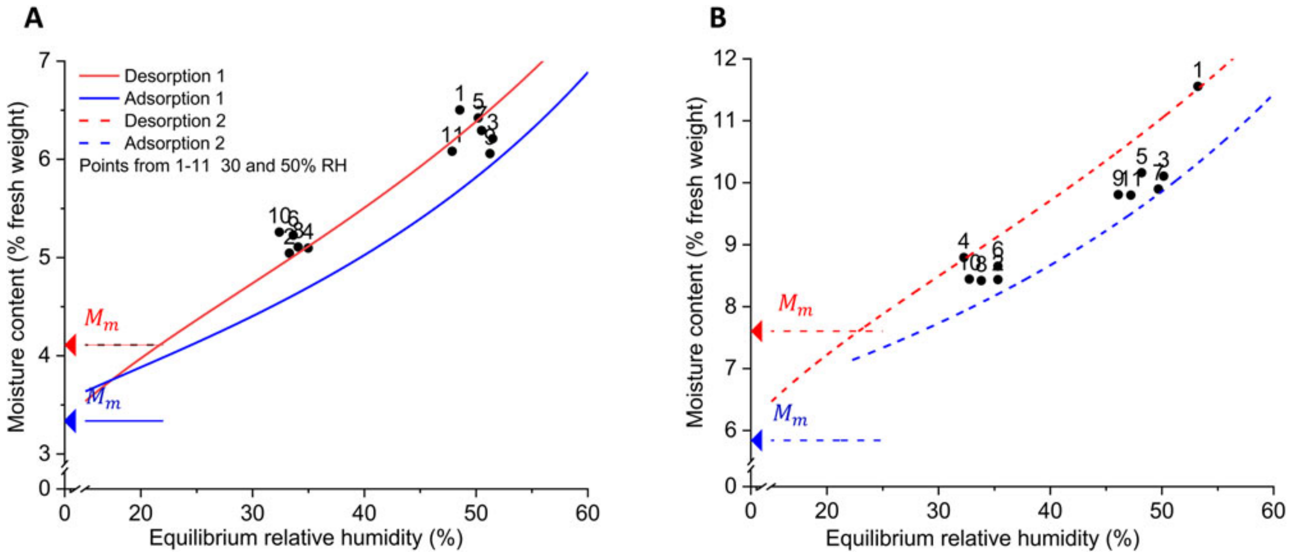

Figure 2. Parts of the moisture desorption (red) and adsorption (blue) isotherms at 20°C of seeds of winter oilseed rape (*Brassica napus* var. DK-Exlibris) (A) and spring barley (*Hordeum vulgare* var. KWS Irina) (B), as shown in Fig. 1. A and B show the results of cycling seeds between 30 and 50% RH (numbered in order from 1 to 11), plotted with isotherm cycle-1 (desorption and adsorption-1) for oilseed rape and with isotherm cycle-2 (desorption and adsorption-2) for barley seeds, respectively. The GAB model gave the best fit to the experimental desorption and adsorption-2 data points ($R^2 = 0.99$) compared with desorption and adsorption-1 ($R^2 = 0.95$) for barley seeds (Table 1). Monolayer values (M_m) are shown in A corresponding to the M_m of desorption-1 (red arrow) and adsorption-1 (blue arrow); and B corresponding to the M_m of desorption-2 (red dashed arrow) and adsorption-2 (blue dashed arrow). Numbers in A and B indicate the sequential cycles of sorption and desorption.

rehydrating to 30% RH-dried seeds at 50% RH, the moisture content values were significantly different from those predicted by the fitted desorption isotherm (using the measured eRH values; $P < 0.001$), but were not signficantly different from those predicted by the adsorption isotherm ($P > 0.05$).

Seed longevity

Since the moisture content of desorption and adsorbing seeds differs, there may be differences in the longevity of desorption and adsorbing seeds equilibrated in the same environment. To determine the effect of adsorption or desorption on longevity, desorption and adsorbing seeds (two cycles) were sealed inside aluminium foil pouches and stored at 45°C. *T*-tests confirmed that the moisture content did not differ significantly ($P > 0.05$) between the first and last sampling times for these storage experiments, for most of the seed lots equilibrated in the same environment (see supplementary Fig. S1).

As expected, the survival curves from the storage experiments followed a negative cumulative normal distribution (see supplementary Figs S2 and S3). Fitting equation (2) to these data, it was possible to accept a common estimate for K_i within each sorption series (desorption-1, adsorption-1, desorption-2 and adsorption-2) for both oilseed rape and barley, without a significant increase in residual deviance (*F*-statistic probability < 0.05; see supplementary Table S2).

For both species, the longevity (p_{50}, the time for viability to fall to 50%) of seeds that were adsorbing moisture was greater than that of those that were equilibrated at the same RH but which were desorbing moisture (see supplementary Table S2). For example, the longevity (p_{50}) of oilseed rape seeds equilibrated at 30% RH (cycle-1) was 196.7 and 132.7 days for adsorbing and desorbing seeds, respectively (see supplementary Table S2). Similarly, adsorbing barley seeds had greater longevity compared to desorbing seeds, having p_{50} values of 110.9 and 50.5 days, respectively, for seeds equilibrated at 30% RH (cycle-1). A similar trend of greater longevity for adsorbing seeds was seen for cycle-2 compared to cycle-1. For example, at 30% RH in cycle-2, the p_{50} values for adsorbing and desorbing seeds were 170.5 and 123.9 days for oilseed rape, and 100.0 and 47.8 days for barley seeds, respectively (see supplementary Table S2).

Fitting equation (3) to the survival curves for each species, it was possible to constrain estimates of K and C_W to common values within each moisture sorption cycle (desorption and adsorption-1 or desorption and adsorption-2) for each species (see supplementary Table S3). However, it was not possible to constrain estimates of K or C_W to common estimates for the two moisture sorption cycles without a significant increase in residual deviance. In other words, the relationship between σ and moisture content differed between seeds on the first or second sorption cycle (Fig. 3). Further, the changes in the parameters differed between the two species; both K and C_W increased for oilseed rape seeds in the second sorption cycle, while for barley seeds, they decreased (Table 2). Estimating K_E based on the universal values for C_H and C_Q according to equation (4), the estimates for C_W and K_E were not too dissimilar from published values, with the exception of the estimate of K_E for barley seeds, which was estimated to be lower in our experiments, compared with the reported estimate in the literature.

Discussion

Seed moisture sorption isotherms, describing the relationship between seed moisture content and RH at a constant temperature, can be used to infer the types and relative rates of different reactions occurring in seeds (Yang et al., 1997). Indeed, in food science, water activity and RH are considered more informative than moisture content in assessing the relative stability of food products (Bell and Labuza, 2000; Hay et al., 2022a). This simplistic framework is, however, complicated by a phenomenon

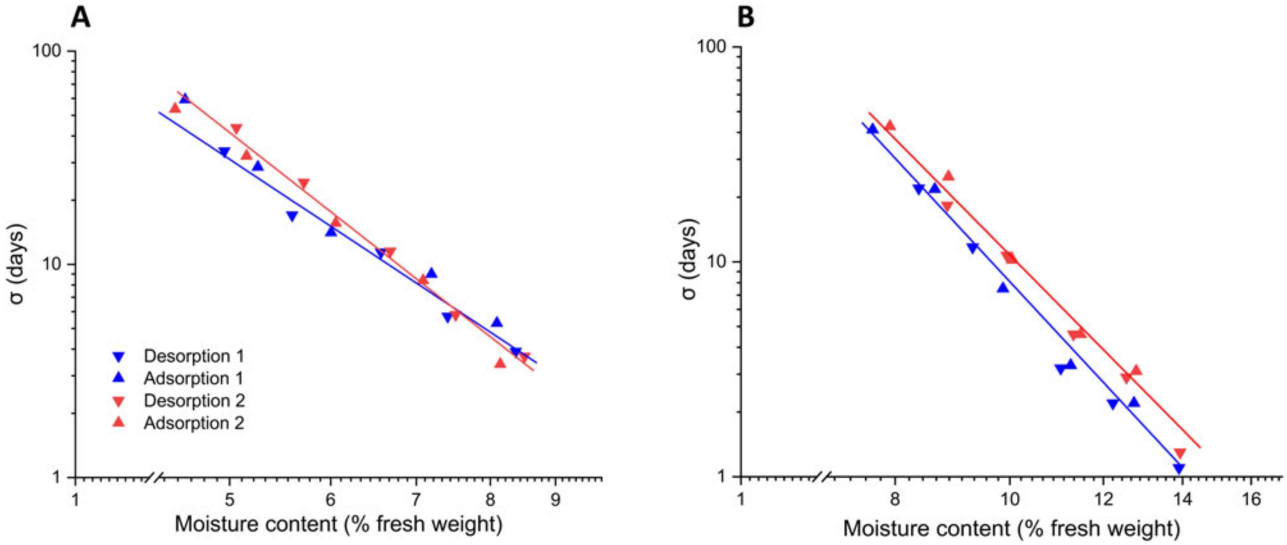

Figure 3. The relationship between σ (the standard deviation of the normal distribution of seed deaths in time determined by fitting equation 2; supplementary Table S2) and storage moisture content for (A) oilseed rape and (B) barley seeds stored at 45°C. The fitted lines show the results of one stage fitting of equation (3) for cycle-1 (blue) and cycle-2 (red) of desorption and adsorption, constrained to a common slope and intercept within each cycle and to independent slope and intercept between the two cycles (see supplementary Table S3). Both axes are logarithmic scales.

known as hysteresis, whereby the moisture content differs at a specific RH depending whether the material is losing or gaining water. In the current study, the shapes of the isotherm curves were similar for oilseed rape and barley, although spanning a greater MC range in barley compared with oilseed rape (Fig. 1). This is consistent with barley seeds having a lower oil content than oilseed rape seeds. An important observation is that seeds of both species showed a hysteresis effect, with the isotherms shifted to lower moisture contents during adsorption compared with desorption at the same RH when dried over silica gel below 10% RH. It is reported that repeated cycles of desorption and adsorption may shift the isotherms away from the first desorption and adsorption isotherms (Li et al., 2011; Okos et al., 2019). However, there are only a few studies where samples have been exposed to repeated cycles of desorption and adsorption. The shift in the isotherms on the second cycle of desorption and adsorption was much greater for barley seeds compared to oilseed rape (Fig. 1), although both species shifted to higher moisture contents compared to cycle-1. In the case of oilseed rape, the shift on the second cycle varied, diverging away from the first isotherms at low RH and converging towards the first isotherms at higher RH.

The M_{m} value, above which the rate of quality loss in food samples begins to increase (Mujumdar and Devahastin, 2000), was determined as part of fitting the GAB equation to the isotherm data. It was higher for barley seeds compared with oilseed rape seeds (Fig. 2 and Table 1). Rahman and Labuza (2007) reported that the M_{m} typically occurs between 20 and 40 on the RH scale in foods, however, we observed that M_{m} occurred between 13 and 22 for oilseed rape seeds and 11 and 34 for barley seeds. According to Rahman and Labuza (2007), materials must be dried below M_{m} before they will cross over to the adsorption isotherm upon rehydration. For oilseed rape, drying seeds to 30% RH (5% MC) implied they were not below the M_{m}, and hence, as expected, the seeds did not shift to the adsorption isotherm upon transfer to 50% RH (Figs 2A and 4A). In contrast, barley seeds partially switched to the adsorption isotherm upon transfer to 50% RH after drying to just 30% RH (8.5% MC) close to the M_{m} value (7.6% MC; Figs 2B and 4B). The results for barley are in agreement with Hay and Timple (2016), who

Table 2. Estimates of the species constants of the seed viability equation (equation 3) for oilseed rape and barley seeds (with ±SE) and estimates from the current study and published estimates

Species	Sorption cycles	K	C_{W}	C_{H}	C_{Q}	K_{E}	Reference
Oilseed rape	Desorption and adsorption-1	4.271 (0.108)	3.974 (0.120)	0.0329	0.000478	6.719 (0.108)	Current research
	Desorption and adsorption-2	4.899 (0.099)	4.693 (0.111)	0.0329	0.000478	7.347 (0.099)	
Barley	Desorption and adsorption-1	6.829 (0.151)	5.921 (0.143)	0.0329	0.000478	9.277 (0.151)	
	Desorption and adsorption-2	6.597 (0.128)	5.565 (0.117)	0.0329	0.000478	9.045 (0.128)	
Oilseed rape	Desorption and adsorption (hybrid[a])	4.54 (0.13)	3.56 (0.11)	0.0329	0.000478	6.989	Ellis et al. (1989)
Barley	Desorption and adsorption (hybrid)	–	5.896 (0.248)	–	–	9.983	Ellis and Roberts (1980b)

A one-step approach has been used to fit the viability model for seeds aged at a storage temperature (45°C) and a range of seed moisture levels. The constant values for C_{H} and C_{Q} were used for both seed species. Common K_i was used from the accepted model within each isotherm, desorption-1, adsorption-1, desorption-2 and adsorption-2 from the first step of the two-step analysis.

[a] Hybrid isotherm: Seed MC adjusted from its initial value, dried below the initial value (desorption) and rehydrated from the initial value (adsorption).

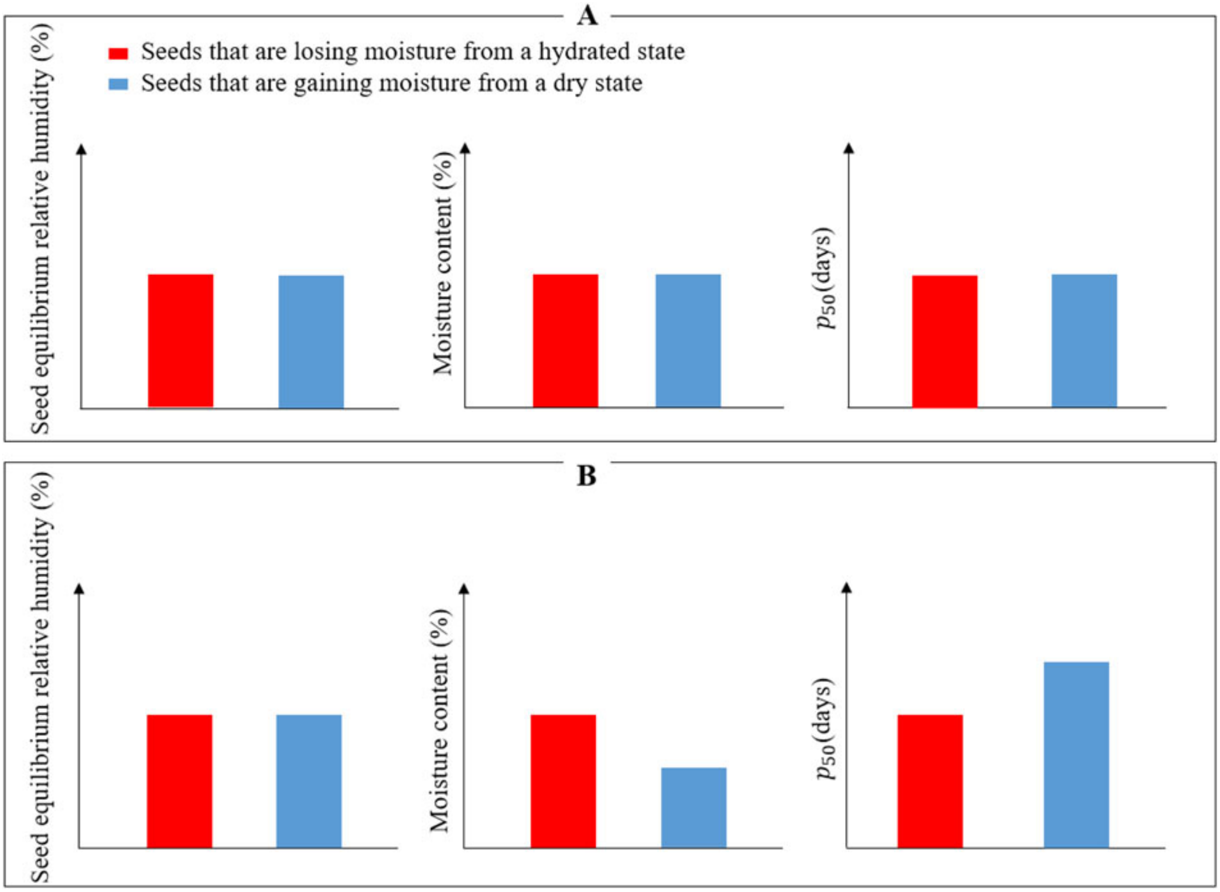

Figure 4. Schematic showing how seeds of different species respond when they are exposed to a higher relative humidity (50% RH) after drying to a relatively low RH (30% in the case of the experiments in this study). In A, the seeds that are adsorbing water have the same eRH and moisture content as desorbing seeds, and hence their longevity is also the same, and not increased by having shifted to the adsorption isotherm (oilseed rape in this study). In contrast, in B, the seeds that are adsorbing water have the same equilibrium relative humidity (eRH) as seeds that are desorbing water, but, because they switch to the adsorption isotherm after drying, a lower moisture content. Consequently, the longevity of adsorbing seeds is greater than that of desorbing seeds (barley in this study).

found that rice seeds switched to the adsorption isotherm upon rehydration, even after drying to just 45% eRH (10% MC). Thus, seeds of different species appear to behave differently in response to fluctuations in the RH of the air to which they are exposed. Seeds of some species (barley, rice) do not need to be dried to low moisture levels for the seeds to switch to the adsorption isotherm, whereas seeds of others (oilseed rape) will remain on the desorption isotherm. These latter seeds will therefore 'immediately' respond to an increase in RH and overall, moisture content will remain on the higher, desorption isotherm relation. Consequently, such seeds are at greater risk of higher rates of ageing. Based on these results, we can conclude that for seeds, the monolayer value does not appear to inform hysteresis behaviour for all species. It should be noted that M_m is a theoretical parameter; however, the accuracy and hence utility of the parameter may be dependent on the quality and range of the data. Indeed, we found significant variation in the estimates of M_m within each species (depending on the isotherm), in some cases, greater than the maximum difference between the desorption and adsorption isotherms (Fig. 2). Clearly, more research is needed to better understand variability in M_m and whether or not it is helpful to inform seed behaviour.

In the mid-region of the isotherm, the effect of change in moisture content on seed longevity can be described by the viability equation (Roberts and Ellis, 1989; Hay et al., 2022a,b). However, this equation does not take into account hysteresis; nor was it considered when Roberts and Ellis (1989) converted the equation to describe longevity on the basis of RH rather than moisture content. Within this mid-range of RH values, greater seed longevity was observed when seeds were adsorbing moisture compared to desorbing moisture (see supplementary Figs S2 and S3 and Table S2). This can be attributed to the lower moisture content of adsorbing seeds compared with desorbing seeds (Fig. 1). Despite these apparent differences in seeds equilibrated at the same RH, the actual relationship between seed longevity (σ) and MC did not differ depending on whether seeds were desorbing or adsorbing moisture (Fig. 3). As a result, the viability constants K and C_W did not differ between desorbing and adsorbing seeds within each cycle (Fig. 3 and Table 2). Similarly, in the second cycle, for both species, viability constants K and C_W did not vary depending on whether the seeds were desorption or adsorbing water and could be constrained to common values, although a bit different from the estimates from cycle-1 (Fig. 3 and Table 2).

Based on the literature, if using the Dickie et al. (1990) 'universal' values for C_H and C_Q, K_E was estimated as 9.275 and 6.719 for barley and oilseed rape seeds, respectively (Table 2), which are not dissimilar to the previous reported values of 9.983 and 6.989 (Ellis and Roberts, 1980a; Ellis et al., 1989). It should be mentioned that viability constants reported by Ellis and Roberts (1980b) and Ellis et al. (1989) for barley and oilseed rape, respectively, were calculated from seeds that were both desorption and adsorbing water, depending on the storage moisture content. Overall, the longevity–MC relationship does not vary depending on whether the seeds are adsorbing or desorbing, which is consistent with other studies (Bello et al., 2011; Bradford et al., 2015; Hay et al., 2016). Furthermore, we can conclude that if the σ–MC relationship is the same for desorption and adsorbing seeds, the same cannot be said for the σ–RH relationship. This is consistent with the results of Hay and Timple (2016) for rice seeds.

To minimize ageing rates during storage, seeds are typically dried to mid- to low moisture contents, and hence, since harvest moisture content is usually higher, seeds are on the desorption isotherm curve (Cromarty et al., 1982; Hay and Smith, 2003; Hay and Probert, 2013). The results of our cycling experiments suggest that seeds of different species will respond differently to subsequent increase in RH. This could have profoundly different impacts in terms of longevity. For some species, such as barley, if the seeds are dried and allowed to take up moisture again, the seeds shift to the adsorption isotherm relation; thus, although the RH is higher, the moisture content is not as high as if the seeds were desorbing to that same higher RH. Consequently, seed longevity will be greater for these adsorbing seeds compared with the desorbing seeds (Fig. 4B). Indeed, this could be a deliberate strategy, to enhance the longevity of seeds for some species, by initially 'overdrying' the seeds but then allowing them to equilibrate to a higher RH for storage, as proposed by Bradford et al. (2015). In contrast, for seeds that stay on the desorption isotherm upon rehydration unless they are dried somewhere below 30% RH (here, we dried seeds over silica to determine the adsorption isotherm), the moisture content will be at the higher, desorption isotherm level and longevity will thus remain relatively short (Fig. 4A). As expressed elsewhere (Hay et al., pers. comm.), how long the hysteresis effect is apparent is not known, but given the time scales used here, would likely be valid for the time periods over which seeds are typically in storage or transit, or even while still on the mother plant, where they could be exposed to fluctuating ambient humidity (Hay and Smith, 2003). The impact of such fluctuations in terms of preharvest ageing may vary between different species, because of this variable hysteresis response phenomenon. Conversely, this may be partly why seeds of different species show different impacts of delayed harvesting (Ellis, 2019).

Another implication of our study relates to research on seeds. Seeds used in experiments have usually been dried, perhaps to about 30% RH in the case of commercial seed lots or lower in the case of conservation-related seed research. When studies of relative longevity are made, seeds are equilibrated to higher moisture contents to enable the collection of data within an acceptable period (i.e. months or a few years, rather than many years or decades in genebank storage) (Probert et al., 2009; Hay et al., 2019, 2022a,b). Hence, for some species, seeds rehydrated to 60% RH will have a lower moisture content compared to seeds that are dried to 60% RH, due to hysteresis. This results in a difference in longevity at a given RH between seeds that lose water from a hydrated state and seeds that gain water from a dry state. Bradford et al. (2015) reported that seeds of lettuce, carrot, radish, pepper and sweet corn lost viability 20–50% more rapidly during storage when on the desorption isotherm than when on the adsorption isotherm, when stored at the same RH. In contrast, seeds of other species, if not dried sufficiently, may not shift to the adsorption isotherm. This further confounds our attempts to characterize relative seed longevity across diverse taxa.

Conclusions

The relationship between seed moisture content and eRH varied between the two species, depending on whether the seeds were desorption or adsorbing moisture and depending on the moisture history of the seeds, i.e. whether or not they had previously been dried to low MC, thereby affecting the longevity of the seed lots. The significance of the moisture history on the behaviour of seeds was emphasized by experiments in which seeds were cycled multiple times between 30 and 50% RH. Indeed, this is the first report that we are aware of, where contrasting responses to such cycling are apparent between seeds of different species. The response will differentially impact the relative rates of ageing of seeds of different species, with seeds that stay on the desorption isotherm showing a greater increase in moisture content in response to rehydration and hence faster relative rates of ageing. The moisture history of seeds should be taken into account when considering the impact of variation in longevity depending on the ambient relative humidity to which seeds are exposed.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0960258523000156.

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