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Characterizing critical phases of germination in winterfat and malting barley with isothermal calorimetry

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Abstract

The heat production of seeds during germination comes from metabolism as well as hydration. Previous studies either lack continuous measurements, or are based on samples composed of more than one seed, thus failing to characterize differences among the critical phases of germination. This study examines the potential of isothermal calorimetry to characterize water uptake and metabolism in single seeds. Seeds of malting barley (*Hordeum vulgare* L.) and winterfat [*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit], two species with contrasting seed size, structure, composition and selection history, were used to determine patterns of heat production rate by isothermal calorimetry during water uptake and germination. Embryos of malting barley contributed less than 4% of total seed weight, and metabolic heat production during Phase I of germination was negligible compared to that due to hydration. Embryos accounted for 74% of seed mass for winterfat, and the majority of heat produced in Phase I was due to metabolic heat release. The total heat production rate in Phase I decreased rapidly in malting barley due to slowing of hydration reactions, but increased gradually in winterfat due to an increasing metabolic rate. The heat production rate at the end of Phase II was about twice as high in malting barley as in winterfat. This indicates a higher metabolic activity for malting barley than for winterfat seeds during germination, which may have also contributed to the rapid increase in the heat production rate of malting barley seedlings during Phase III, compared to the gradual increase in heat production rate of winterfat. The comparison between excised embryos and intact seeds indicates that the covering tissues delay radicle emergence in malting barley, but not in winterfat, due to differences in seed structure between the two species.

Keywords: calorimetry, germination phases, *Hordeum vulgare*, *Krascheninnikovia lanata*, metabolic heat production, water uptake

Introduction

The seed germination process can be characterized by a tri-phasic water uptake curve (Bewley and Black, 1994). Changes in physiological and biochemical activities during seed germination have been associated with these phases. The repair of cell membranes and DNA, and protein synthesis using stored mRNAs, are thought to occur during water uptake in Phase I. Activation of reserves and metabolic activities, i.e. mitochondria and protein synthesis using new mRNAs, commences in Phase II, and growth resumes as a result of cell elongation and division in Phase III (Bewley and Black, 1994; Buchanan *et al.*, 2000). The application of image analysis to water uptake during imbibition and germination provides a continuous and non-destructive measure of changes in seed water status (McCormac and Keefe, 1990; Dell'Aquila *et al.*, 2000), but does not provide direct evidence on metabolic changes.

Viable and non-viable seeds cannot be distinguished based on water uptake in Phase I, because it is a physical process driven by the water potential gradient between the seed and its surroundings, even though respiratory metabolism can commence within minutes of water uptake in viable seeds (Bewley and Black, 1994). Similarly, both dormant and non-dormant seeds are metabolically active in Phase II, but only when the non-dormant seed enters Phase III can the two states be separated. Uneven distribution of ATP was found between radicle and cotyledons of non-dormant wild-type tomato (*Lycopersicon esculentum*

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Mill.) seeds, while ATP distribution is more uniform in dormant seeds (Spoelstra *et al.*, 2002). The biochemical processes required for cell growth and wall expansion in the embryonic axis at the end of Phase II remain to be elucidated (Buchanan *et al.*, 2000). Plant metabolism can be studied with calorimetry, which measures metabolic heat production rate (R_q), as well as heat from all other processes (Thygerson *et al.*, 2002). The application of calorimetry to seed germination may provide bioenergetic information for understanding physiological and biochemical processes and readiness for germination.

Heat production rate is positively correlated with metabolism (Criddle *et al.*, 1997) and is a good indicator of seed vigour of germinating radish (*Raphanus sativa* L.) seeds (Yamaguchi *et al.*, 1990). Increases in heat production rate of seeds during germination and after radicle emergence have been reported for melon (*Cucumis melo* L.) seeds (Edelstein *et al.*, 2001); however, changes in heat production rate during the phase transition in an individual seed, especially at the time of radicle emergence, were not measured due to the non-continuous method in their study. Hu *et al.* (2001) established the relationship between heat production rate and water uptake during seed germination of *Robinia pseudoacacia* Linn. with calorimetry. The results showed a continuous increase in heat production rate with increasing water content. Only one sample with 20 seeds was tested, and the point of radicle emergence could not be detected because of variation in germination times. Sigstad and Garcia (2001) and Sigstad and Schabes (2000) generated thermal power–time curves of quinoa (*Chenopodium quinoa* Willd.) seeds using five seeds per sample, but the time of germination was not directly visible in these curves. These previous studies on heat production rate of seeds during germination either lack continuous measurements or are based on samples composed of several seeds. Thus, they fail to characterize differences in heat production rate among the critical phases of germination.

Variability in seed physiological traits, especially the time required for germination, exists among individual seeds of a population (Garcia-Huidobro *et al.*, 1982; Ellis *et al.*, 1986). Modelling of seed germination is based on the concept of subpopulations (i.e. Bradford, 1995, 2002). Therefore, studies of heat production rate of seeds among subpopulations, and of correlations between heat production rate and other physiological traits of seeds, such as seed water uptake, may provide more information about physiological processes during seed germination. Such results would be useful in understanding the metabolic processes leading to seedling establishment, and to relate subpopulations to variations in metabolic rates within a seed population.

We hypothesize that changes in the heat production rate of an individual seed during germination may be correlated with the tri-phasic water uptake curve; seeds with different structures (endospermic versus reduced-endospermic) may have different heat production patterns during germination. To test the potential of isothermal calorimetry to provide answers to these questions, seeds of two species, winterfat (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit; from the *Chenopodiaceae* family) and malting barley (*Hordeum vulgare* L. cv. CDC Kendall), were studied. Seeds of these species were chosen because they have very different sizes, structures, compositions and selective histories, thus representing extremes of the spectrum of seeds physiology. Winterfat is a small, dicot shrub native to the Great Plains of North America, and the endosperm of a mature seed is reduced. Malting barley with a starchy endosperm was used for comparison because it is the most tested cereal grain for germination, especially the biochemical processes during imbibition (Simpson, 1990). The objectives of this study were to: (1) test our calorimetric method for measuring the heat production rate of individual seeds during germination, particularly at the initial water uptake phase and at the point of radicle emergence; (2) characterize and correlate heat production rate with germination phases, such as imbibition and radicle emergence; and (3) compare the characteristics of heat production rates between winterfat and malting barley seeds.

Materials and methods

Seed sources and characteristics

Malting barley seeds were obtained from the Crop Development Centre, Department of Plant Sciences, University of Saskatchewan. Seeds were harvested in August 2002 and stored at room temperature. Winterfat seeds (diaspores) were purchased from Wind River Seed (Manderson, Wyoming, USA). Diaspores were stored in a warehouse for approximately 7 months after harvesting in October 2001. They were air-dried at room temperature for at least a week after purchase, and then cleaned by rubbing, fanning and passing serial sieves and blowers. Cleaned seeds were sealed in plastic bags and stored at −18°C until use. In order to calculate the embryo to whole seed ratio, embryos were excised from seeds after 2–4 h of imbibition in distilled water. The dry weights of seed and embryo, and seed water content, were determined after oven drying at 100°C for 24 h. All weighing was done using a microbalance (Cahn C33, CAHN Instrument, Inc., Cerritos, California, USA) with a precision of ±0.001 mg. The seed water contents (WC)

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of winterfat and malting barley were within a narrow range between 5.5 and 6.5% WC (dry weight basis), and there was no significant difference between the two species ($P > 0.05$) (see Table 1).

Seed water uptake during imbibition

Individual air-dried seeds of both species were weighed and placed on top of two layers of Whatman No. 1 filter papers in Petri dishes with distilled water. Fifteen seeds of each species were used, and the experiment was conducted in darkness (except when the seeds were retrieved for weighing) at controlled room temperature (22.5 ± 1.0°C), which was monitored with a data logger (21X, Campbell Scientific (Canada) Corp., Edmonton, Alberta, Canada). Seeds were individually retrieved and quickly blotted using tissue paper, and weighed at hourly intervals until radicle emergence. Seed water uptake was then calculated based on a seed air-dry weight (ADW) basis.

The time course of individual seed water uptake was best described with a linear logarithmic function determined using both P value and R^2:

$$Y = a + b\ln X \quad (1)$$

where a and b are constants, X is the imbibition time (h), and Y is the seed water content (% ADW).

Measurements of heat production rate by calorimetry

Heat production rates were measured with three Multi-Cell Differential Scanning Calorimeters (MC-DSC; Model 4100, Calorimetry Sciences Corporation, American Fork, Utah, USA) operated as isothermal calorimeters. An individual seed was placed on top of a paper disc (0.6 cm in diameter, Whatman No. 1) in each of the three sample ampoules of the calorimeter, with 20 or 80 µl of distilled water for winterfat or malting barley, respectively. Ampoules were then sealed, weighed and placed inside the calorimeter. The fourth ampoule was left empty as a blank reference. Temperatures of water baths and calorimeters were set to 22.5°C, which was close to the ambient temperature, to maximize the equipment stability and the precision of measurement. Data were collected at 7-second intervals. Data in the first 50 min were discarded to allow for thermal equilibration. Baseline data were obtained with empty ampoules both before and after each run. Sealed ampoules were weighed again after each run, and data from ampoules with a weight loss greater than 0.3 mg (from water evaporation from the ampoule) were discarded, based on results from preliminary tests.

Measurements of heat production rates of viable and non-viable seeds during imbibition

Approximately 50 winterfat seeds and 25 malting barley seeds were weighed (ADW) individually. Half of the seeds were sealed individually in tin capsules (Leco Instrument Ltd., Mississauga, Ontario, Canada) and heated at 100°C for 24 h to produce dead seeds. Heated seeds were cooled at room temperature within the sealed capsules for 24 h, allowing re-absorption of moisture. The death of heat-treated seeds was confirmed by the absence of germination under favourable conditions.

Prior to calorimetric measurements, seeds were placed in marked plastic cassettes and soaked in 1% sodium hypochlorite for 10 min for surface sterilization, and then rinsed several times with distilled water. Heat production rates of viable and dead seeds were measured continuously for 8 h during the initial stage of imbibition. The value of the heat production rate for each seed was baseline-corrected and expressed as µW (mg ADW)$^{-1}$ of the whole seed. Heat production rates were compared between dead seeds (heat of water uptake) and live seeds to separate the heat production rate of metabolism from that of hydration. Because of the small seed mass and low heat production rate of individual winterfat seeds during imbibition, the above procedures were also used to measure heat production rate for samples containing 10 seeds/sample for comparison.

Table 1. Characteristics of malting barley and winterfat seeds used in the study. Values are means ± SE (n)

Species	Water content (%, DW)	Seed weight (mg, DW)	Embryo weight (mg, DW)
Winterfat	6.39 ± 0.08 (21) a*	2.25 ± 0.18 (33) b	1.66 ± 0.15 (33) a
Malting barley	5.89 ± 0.05 (21) a	45.56 ± 1.24 (21) a	1.76 ± 0.07 (21) a

DW, oven dry weight.
* Means with the same letter within a column are not significantly different at $P \leq 0.05$.

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Measurement of heat production rate during radicle emergence

Approximately 100 winterfat seeds and 50 malting barley seeds were weighed (ADW) individually, surface sterilized and rinsed, as previously described. Sterilized seeds were placed in Petri dishes on top of a moistened filter paper (Whatman No. 1) and imbibed at 22.5 ± 0.5°C in an incubator in darkness for a total of 8 h, including pretreatment time. The same procedures for the measurement of heat production rate as previously described were followed, and the heat production rate was measured for 16 h. The value of the heat production rate for each seed was baseline-corrected and expressed as $\mu W\,mg^{-1}$ embryo weight (ADW). The selection of 16 h for the study of radicle emergence was based on results from preliminary tests, which indicated that most seeds started germination after 8 h of imbibition at 22.5°C, and that at least 50% of the seeds germinated between 8 and 24 h. When sudden changes occurred in the curves of heat production rate versus time during the preliminary tests, data collection was halted and ampoules opened 30 min after the sudden change, to determine the germination phase of seeds.

Measurement of heat production rate of excised embryos

Approximately 50 winterfat seeds and 50 malting barley seeds were surface sterilized and rinsed as described previously. This experiment was conducted approximately 1.5 years after the DSC experiments on intact seeds, and seeds of malting barley were from a different harvest year. Sterilized seeds were placed in Petri dishes on top of a moistened filter paper (Whatman No. 1) and imbibed at 22.5 ± 0.5°C in an incubator in darkness for approximately 3 h. Embryos were excised from seeds, and the heat production rates of individual embryos were measured for 30–40 h at 22.5°C as described previously. The incubation and excision procedures were conducted under sterilized conditions. The lengths of embryos were estimated before and after the calorimetric measurement, and embryos with an increase in length of 3 mm were considered 'germinated'. Oven dry weight (DW) of individual embryos was determined after the test, as described previously.

Data analysis

Regression analysis (SAS Institute, 1995) was used to determine the relationship between seed mass and final seed water content before radicle emergence, as well as water uptake time courses. The differences between the two species were compared using a one-way ANOVA.

Results

Morphological attributes of winterfat and malting barley seeds

Malting barley seeds were more than 20-fold heavier than winterfat seeds (Fig. 1, Table 1). However, embryo weights were not significantly different between the two species ($P > 0.05$). The endosperm and seed coat of malting barley accounted for over 96% of the seed mass, compared to 26% in winterfat seeds. The embryo of malting barley seed is partially surrounded by the endosperm. In contrast, the endosperm of winterfat seed is surrounded by the embryo, which is enclosed in a membranous seed coat.

Individual seed water uptake phases during imbibition

Seed water content (% ADW) at 22.5°C increased rapidly in the first 5 h of imbibition, followed by a gradual increase until radicle emergence for both winterfat and malting barley (Fig. 2). However, there was no clear separation of Phase II from Phase I. For winterfat, the slopes of fitted individual seed water uptake curves were between 25 and 43, except for the two seeds that germinated within 10 h of imbibition. The slopes for individual malting barley water uptake curves were between 13 and 17, indicating a slower and less variable water uptake among barley compared with winterfat. Final water content immediately before radicle emergence was between 100 and 160% ADW for winterfat seeds, and between 50 and 70% for malting barley seeds; the difference between the two species was significant ($P < 0.05$). Seed water content before radicle emergence decreased linearly with increasing seed mass in winterfat ($P < 0.05$). A reverse trend, although statistically non-significant ($P = 0.097$), was found in malting barley.

Heat production rate during seed imbibition

The heat production rates of viable, air-dried seeds without the addition of water were $0\,\mu W\,mg^{-1}$ within the limit of the resolution of the measurement, indicating that metabolic activities of unimbibed seeds were negligible (Table 2). Heat production was detected during imbibition of both viable and dead seeds of both species, indicating that heat was produced during physical hydration (Fig. 3). For barley seeds, the heat production rate decreased with

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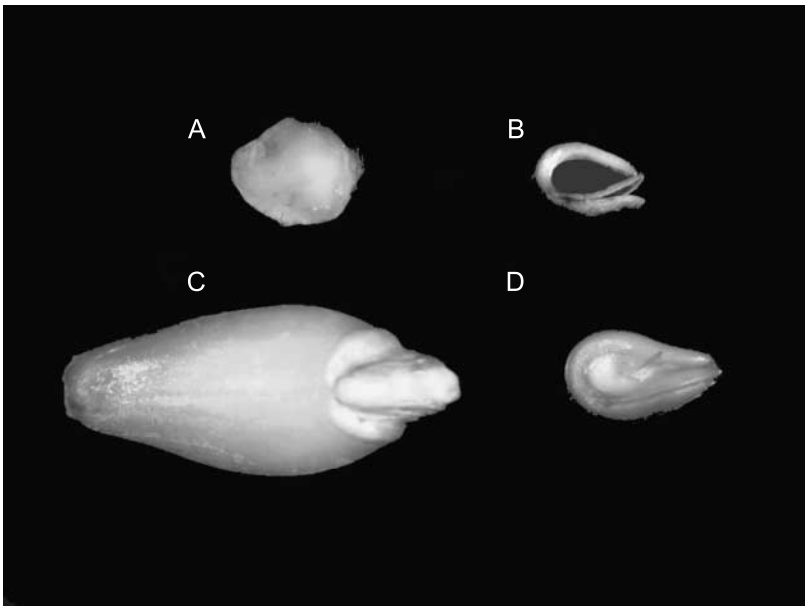

Figure 1. Seed structure of winterfat and malting barley. (A) Embryo of malting barley; (B) embryo of winterfat; (C) whole seed of malting barley; and (D) whole seed of winterfat.

increasing duration of imbibition in both dead and viable seeds, and the patterns were similar between dead and viable seeds. The decrease in heat production rate slowed and the heat production rate became nearly constant after 6 h of imbibition. Heat production rates measured for individual seeds of winterfat exhibited a large variation among seeds, with some values in the negative range, probably due to errors in measuring the small heat production rate. Measurements using 10 seeds per sample provided more reliable results, as indicated by the reduced range of variation and similar patterns among samples. Dead seeds of winterfat had a different heat production pattern from that of viable seeds, showing a much lower and relatively constant heat production rate during imbibition. The pattern of heat production rate in viable winterfat seeds was different from that of viable barley seeds. A gradual increase in heat production rate was observed in viable winterfat seeds, which reached its maximum at 4 h of imbibition and then decreased slightly.

Heat production rate during radicle emergence

Radicle emergence of intact seeds in both species was marked by an increase in heat production rate and associated with a continuous increase in heat production rate afterward (Fig. 4). Radicle emergence of winterfat occurred after approximately 9.5–16.5 h of imbibition, while that of malting barley occurred after 10.5–21.5 h. The heat production rate immediately before radicle emergence was higher in malting barley than in winterfat (Table 2). The increase in heat production rate after radicle emergence was gradual for winterfat but abrupt for malting barley. For ungerminated seeds of both species, heat production rate exhibited little change during the same time period.

Heat production rate of excised embryos

The heat production rate of excised embryos (Fig. 5) was within the range of that measured in intact seeds, based on embryo weight (Fig. 4), confirming that the heat production at Phases II and III was mainly from metabolism. The exact point of time for radicle emergence during heat production measurements cannot be directly validated because of the gradual increase in embryo length and the lack of seed coat to mark radicle emergence. For most winterfat embryos, there was an increasing trend in the heat production rate between 8 and 24 h after imbibition, the time period during which most winterfat seeds germinated at 22.5°C, based on preliminary experiments. However, large variation existed among individual embryos, with some having no change, while others exhibited a decline near the end of the measurement. Similar to patterns observed at radicle emergence in intact seeds (Fig. 4), the increase in heat production rate seen in malting barley embryos was more dramatic than in winterfat. The time for radicle emergence of isolated barley embryos was less than

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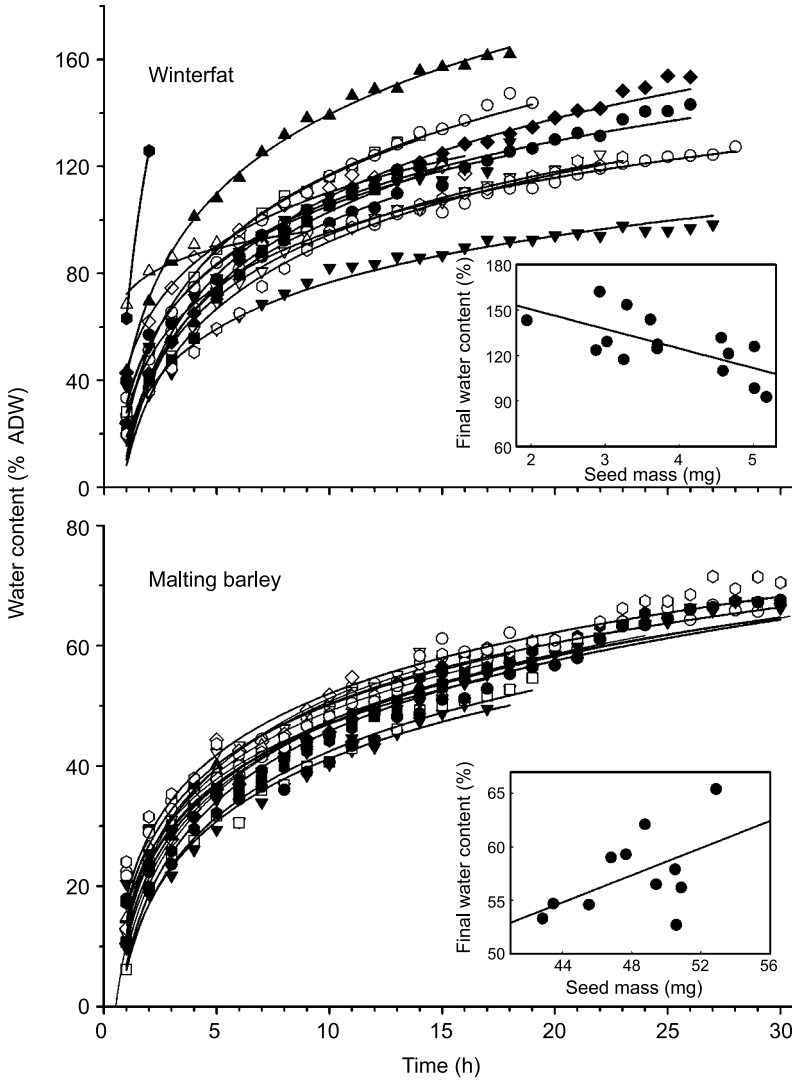

Figure 2. Seed water content of winterfat and malting barley during imbibition, based on hourly weighing of individual seeds. The last data point for each seed represents seed water content within 1 h of radicle emergence. The fitted lines of water uptake curves were based on a linear logarithmic function [equation (1)]. The figure inserts show the relationship between seed mass and final seed water content.

8 h after imbibition, which was faster than that for intact seeds. A decrease in heat production rate similar to that in intact seeds was found during Phase III in malting barley embryos.

Discussion

Using a single seed to study germination is important because samples containing a group of seeds would not reveal germination phases or the extent of biological variation between individuals (Dell'Aquila *et al.*, 2000). The heat production rate with more than one seed per sample represents the combined effect of seeds at different physiological stages, which cannot then characterize changes in heat production rate between phases of germination. However, for small-seeded species, such as winterfat, the heat production rate of a single seed before radicle emergence can be less than 5 μW seed^{-1} at 22.5 °C, and therefore difficult to measure. In this study, calorimeter performance was optimized by using the same temperatures for the calorimeter measurement and the water bath, and by maintaining a constant temperature in the surroundings. A relatively high temperature (22.5°C) was selected to study the

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Table 2. Variations in heat production rate of winterfat and malting barley seeds among germination phases at 22.5°C in darkness. Values are means ± SE (*n*). Heat production rate was based on a per whole seed mass for dry seeds and a per embryo mass for others

	Heat production rate [μW (mg ADW)$^{-1}$]		Relative change in heat production rate (% h^{-1})	
Seed type/Germination stage	Winterfat	Malting barley	Winterfat	Malting barley
Dry seed	0.03 ± 0.01 (10) a*	−0.01 ± 0.00 (18) a	–	–
Before radicle emergence (dead)**	–	–	−20.6 ± 4.5 (10) a	−17.2 ± 0.5 (15) a
Before radicle emergence (viable)**	–	–	19.9 ± 1.5 (10) a	−17.8 ± 0.3 (15) b
At radicle emergence	4.06 ± 0.32 (23) b	8.98 ± 0.44 (12) a	–	–
After radicle emergence***	–	–	29.4 ± 2.9 (23) b	58.4 ± 5.5 (12) a

* Means with the same letter within a row and parameter are not significantly different between species at $P \leq 0.05$.
** 1.5–4 h imbibition for winterfat and 1.5–6 h for malting barley. Relative change in rate was calculated using $(R_{q2} - R_{q1})/R_{q1}/T \times 100$, where T was the time duration in hours, R_{q2} was the heat production rate at the end of the period and R_{q1} was the initial heat production rate of the period.
*** 0–5 h after radicle emergence. Relative change in rate was calculated using the same equation as in **.

germination process of the two species because the heat production rate of seeds during germination increases with temperature (Sigstad and Prado, 1999) within the suboptimal temperature range. This temperature is within the optimal temperature range for winterfat seed germination (Wang *et al.*, 2004), and close to the 25°C optimal temperature for barley seeds (Briggs, 1992).

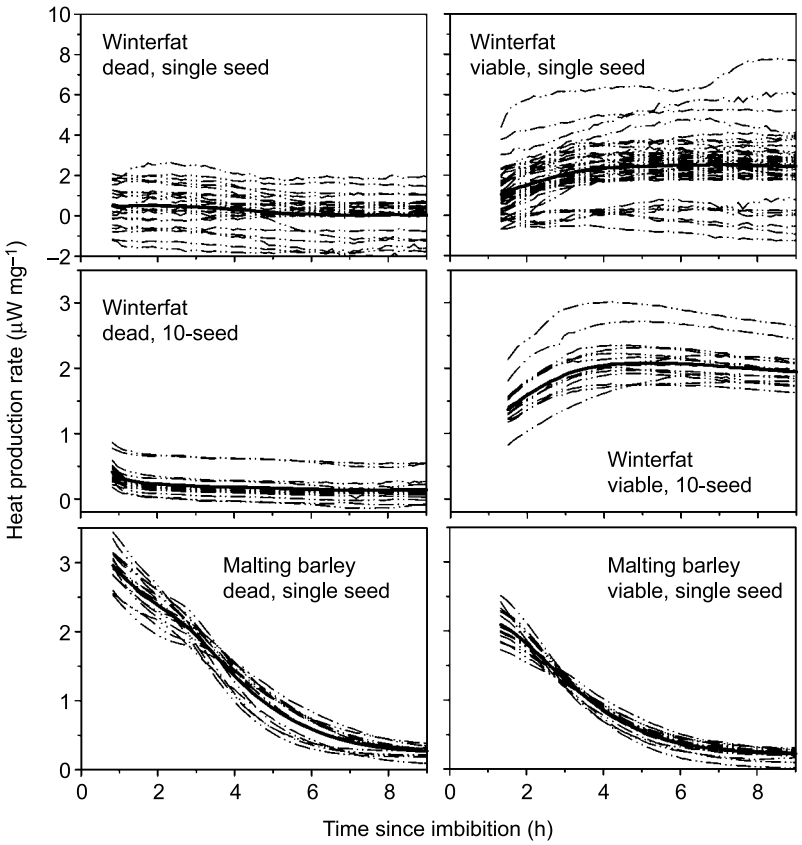

Figure 3. Heat production rate of dead (heat-killed at 100°C for 24 h) and viable seeds of winterfat and malting barley during the early stage of imbibition at 22.5°C, as expressed per unit whole seed mass (ADW). Data for malting barley were based on single-seed samples, while those for winterfat were based on both single-seed samples and 10-seed samples. Solid lines were the averages of all samples.

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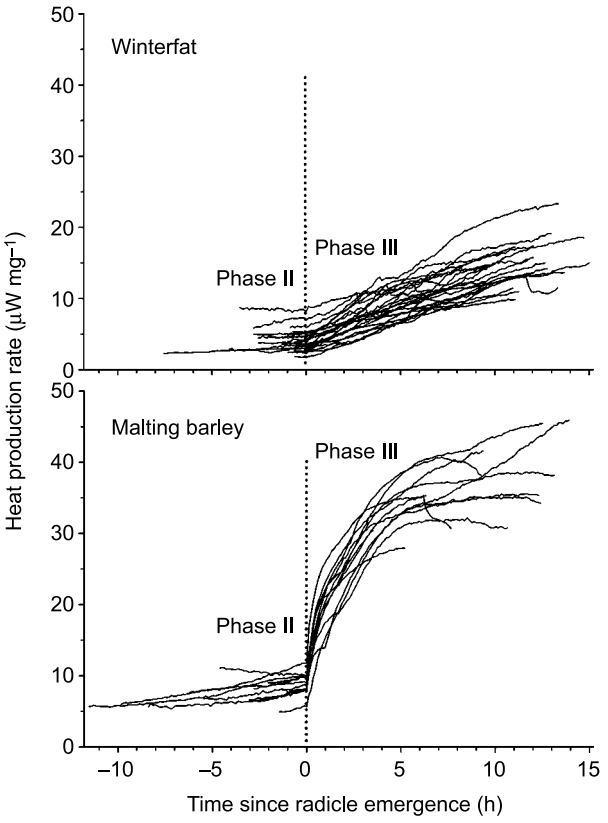

Figure 4. Heat production rate of winterfat and malting barley seeds during radicle emergence at 22.5°C, as expressed per embryo mass (ADW). Seeds were imbibed at 22.5°C in a growth chamber for 8 h before being transferred into calorimeters. The time of radicle emergence (the end of Phase II) was defined as 0 h.

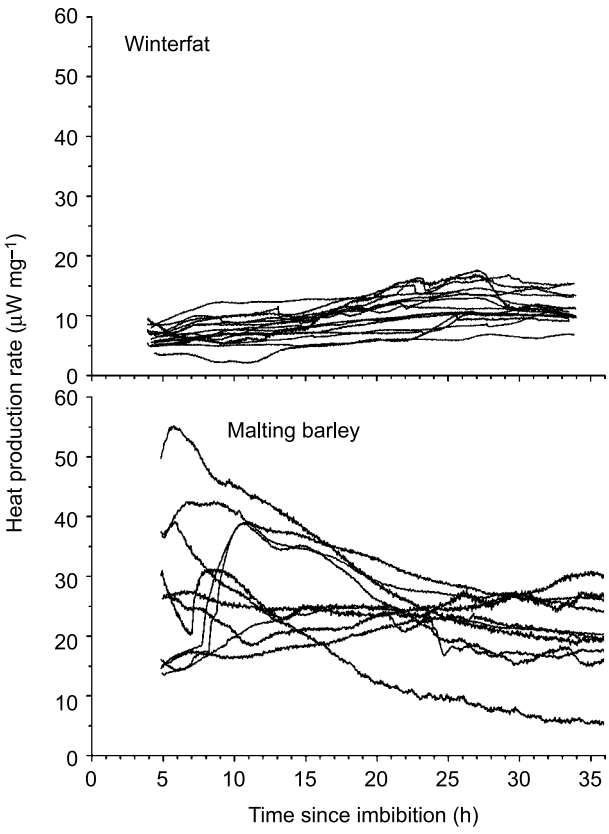

Figure 5. Heat production rate of single, excised embryos of winterfat and malting barley at 22.5°C, as expressed per embryo mass (DW).

For the comparison between dead and viable seeds during the early stages of imbibition, total heat production rate per seed of winterfat is near zero and variations due to baseline reproducibility of the calorimeter were encountered. Decreasing the calorimeter detection limit by use of a different type of calorimeter is possible (Hansen, 1996), but only at the expense of longer equilibration times and loss of the convenience offered by the particular design of the MC-DSC 4100 calorimeter (Criddle and Hansen, 1999). Therefore, the use of several seeds per sample may be unavoidable for some species with small seed mass or low heat production rates.

Water uptake curves of both species exhibited a rapid initial increase in seed water content followed by a gradual increase, as described by the logarithmic model. However, the 'lag phase' typical of Phase II was not apparent, and ungerminated seeds continued to increase in water content. The lack of a lag phase in winterfat seeds was also reported in a previous study when seeds of three collections were imbibed at four temperatures (Bai *et al.*, 1999). The embryo and the seed have similar water uptake patterns in winterfat (Wang, 2005). Barley is also a typical species without a lag phase (Bewley and Black, 1978). There is no simple explanation for the lack of a lag phase in the seed water-uptake curve, but it is possible that O_2 and respiration are not restricted in such seeds during imbibition, and this species has rapid metabolism, enabling it to germinate rapidly. For both winterfat and malting barley, embryos hydrate before endosperm, as evidenced by nuclear magnetic resonance (NMR) studies (Gruwel *et al.*, 2002; Wang, 2005). Viable seeds could not be easily separated from non-viable seeds based on water uptake alone, even though embryos of non-viable barley seeds hydrate faster than those of viable seeds (Gruwel *et al.*, 2002). Changes in heat production rate of viable winterfat seeds during the early stage of imbibition clearly distinguished the phase of rapidly increasing metabolism and the phase of stable metabolism. Changes in heat production rate of malting barley seeds distinguished the phase of rapid hydration from a phase of stable hydration. Neither process is correlated with

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changes in seed water content. Therefore, the traditional tri-phase characterization of the germination process needs to include parameters other than seed water content for species such as winterfat and malting barley, which do not exhibit a distinct Phase 2 lag in water uptake.

The pattern of seed metabolic activities during seed germination can be correlated with O_2 consumption (Bewley and Black, 1994), heat evolution and CO_2 production (Sigstad and Schabes, 2000; Edelstein *et al*., 2001). The heat evolution rate due to metabolism is interpretable as a measurement of overall metabolic rate, and has advantages over biomass measurement, O_2 uptake and CO_2 release rate for the determination of metabolic rate (Criddle *et al*., 1988). Sigstad and Prado (1999) obtained the heat production rate due to metabolism by subtracting heat production rate due to imbibition [using potassium cyanide (KCN)-treated seeds] from total heat production rate. An alternative method was used in the present study, in which the heat production rates of viable and dead seeds were compared, and an interaction between seed structure and heat production rate was found.

The heat production rate of both dead and viable malting barley seeds decreased rapidly for the first 6 h of imbibition, and the patterns were similar. This suggests that, due to a high starch content, heat production associated with hydration during the early stages of imbibition accounts for nearly all of the heat production rate. The pattern of heat production rate for winterfat seeds was different between dead and viable seeds. A relatively constant, low heat production rate was found in dead seeds, while an increasing heat production rate for the first 4 h of imbibition was found in viable seeds. Heat production due to hydration is minimal during imbibition in winterfat because of its high embryo:seed ratio (0.74) and small portion of endosperm. The total heat production rate is mainly due to embryo metabolism. Winterfat seeds have more soluble and lower molecular weight carbohydrate reserves than barley seeds (Wang, 2005). The greater heat production rate from hydration of malting barley endosperm is consistent with hydration of polymer molecules, which varies with molecular structure (Bogracheva *et al*., 2002). Differences in seed structure and composition between winterfat and malting barley seeds thus explain not only the different patterns in heat production rate during seed imbibition between the two species, but also the similar patterns between dead and viable barley seeds.

The rate of heat production per mg embryo at the end of Phase II, before radicle emergence, for malting barley was almost twice as high as for winterfat in intact seeds. The higher heat production rate does not exclusively represent a higher metabolic activity in malting barley than in winterfat, because the continuous hydration of the endosperm of malting barley seeds at this stage also releases heat. After radicle emergence in Phase III, the relative rate of change of heat production of malting barley was also twice that of winterfat. This was confirmed by the measurements of excised embryos, indicating that malting barley seedlings are more metabolically active than winterfat seedlings. The pattern of heat production rate was similar between intact winterfat seeds and excised embryos, indicating that embryos were not oxygen-limited prior to emergence from the seeds. The structure of winterfat seeds, with an embryo surrounding the endosperm, probably permits faster gas exchange between the embryo and the environment than in malting barley seeds. The fact that excised malting barley embryos took less time to emerge than in intact seeds indicates that the covering tissues delay radicle emergence in intact seeds, possibly due to oxygen limitation to embryos within intact barley seeds.

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References

Bai, Y., Booth, D.T. and Romo, J.T. (1999) Imbibition temperature affects winterfat (*Eurotia lanata* (Pursh) Moq.) seed hydration and cold-hardiness response. *Journal of Range Management* **52**, 271–274.

Bewley, J.D. and Black, M. (1978) *Physiology and biochemistry of seeds in relation to germination. Volume I. Development, germination and growth*. Berlin, Springer-Verlag.

Bewley, J.D. and Black, M. (1994) *Seeds: Physiology of development and germination* (2nd edition). London, Plenum Press.

Bradford, K.J. (1995) Water relations in seed germination. pp. 351–396 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Bradford, K.J. (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50**, 248–260.

Briggs, D.E. (1992) Barley germination: Biochemical changes and hormonal control. pp. 369–412 *in* Shewry, P.R. (Ed.)

https://doi.org/10.1079/SSR2005213 Published online by Cambridge University Press

Barley: Genetics, biochemistry, molecular biology and biotechnology. Wallingford, CAB International.

Bogracheva, T.Y., Wang, Y.L., Wang, T.L. and Hedley, C.L. (2002) Structural studies of starches with different water contents. *Biopolymers* **64**, 268–281.

Buchanan, B.B., Gruissem, W. and Jones, R.L. (2000) *Biochemistry and molecular biology of plants*. Rockville, Maryland, American Society of Plant Biologists.

Criddle, R.S. and Hansen, L.D. (1999) Calorimetric methods for analysis of plant metabolism. pp. 711–763 *in* Kemp, R.B. (Ed.) *From macromolecules to man (Handbook of thermal analysis and calorimetry, Vol. 4)*. Amsterdan, Elsevier.

Criddle, R.S., Breidenbach, R.W., Lewis, E.A., Eatough, D.J. and Hansen, L.D. (1988) Effects of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings measured by calorimetry. *Plant, Cell and Environment* **11**, 695–701.

Criddle, R.C., Smith, B.N. and Hansen, L.D. (1997) A respiration based description of plant growth rate responses to temperature. *Planta* **201**, 441–445.

Dell'Aquila, A., van Eck, J.W. and van der Heijden, G.W.A.M. (2000) The application of image analysis in monitoring the imbibition process of white cabbage (*Brassica oleracea* L.) seeds. *Seed Science Research* **10**, 163–169.

Edelstein, M., Bradford, K.J. and Burger, D.W. (2001) Metabolic heat and CO_2 production rates during germination of melon (*Cucumis melo* L.) seeds measured by microcalorimetry. *Seed Science Research* **11**, 265–272.

Ellis, R.H., Covell, S., Roberts, E.H. and Summerfield, R.J. (1986) The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *Journal of Experimental Botany* **37**, 1503–1515.

Garcia-Huidobro, J., Monteith, J.L. and Squire, G.R. (1982) Time, temperature and germination of pearl millet (*Pennisetum typhoides*, S & H). 1. Constant temperature. *Journal of Experimental Botany* **33**, 288–296.

Gruwel, M.L.H., Yin, X.S., Edney, M.J., Schroeder, S.W., MacGregor, A.W. and Abrams, S. (2002) Barley viability during storage: use of magnetic resonance as a potential tool to study viability loss. *Journal of Agricultural and Food Chemistry* **50**, 667–676.

Hansen, L.D. (1996) Instrument selection for calorimetric drug stability studies. *Pharmaceutical Technology* **20**, 64–74.

Hu, Y.C., Zhou, P.J., Wang, C.X. and Qu, S.S. (2001) Thermogenetic curves and thermokinetics of seed germination of *Robinia pseudoacacia*. *Journal of Forestry Research* **12**, 176–178.

McCormac, A.C. and Keefe, P.D. (1990) Cauliflower (*Brassica oleracea* L.) seed vigour: Imbibition effects. *Journal of Experimental Botany* **41**, 893–899.

SAS Institute Inc. (1995) *SAS system for Windows V8*. Cary, North Carolina, SAS Institute Inc..

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

Sigstad, E.E. and Prado, F.E. (1999) A microcalorimetric study of *Chenopodium quinoa* Willd. seed germination. *Thermochimica Acta* **326**, 159–164.

Sigstad, E.E. and Schabes, F.I. (2000) Isothermal microcalorimetry allows detection of 'aquaporines' in quinoa seeds. *Thermochimica Acta* **349**, 95–101.

Simpson, G.M. (1990) *Dormancy in grasses*. Cambridge, Cambridge University Press.

Spoelstra, P., Joosen, R.V.L., Van de Plas, L.H.W. and Hilhorst, H.W.M. (2002) The distribution of ATP within tomato (*Lycopersicon esculentum* Mill.) embryos correlates with germination whereas total ATP concentration does not. *Seed Science Research* **12**, 231–238.

Thygerson, T., Harris, J.M., Smith, B.N., Hansen, L.D., Pendelton, R.L. and Booth, D.T. (2002) Metabolic response to temperature for six populations of winterfat (*Eurotia lanata*). *Thermochimica Acta* **394**, 211–217.

Wang, R. (2005) Modeling seed germination and seedling emergence in winterfat (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit): Physiological mechanisms and ecological relevance. PhD Thesis, University of Saskatchewan, Saskatoon, Canada.

Wang, R., Bai, Y. and Tanino, K. (2004) Effect of seed size and sub-zero imbibition temperature on the thermal time model of winterfat (*Eurotia lanata* (Pursh) Moq.). *Environmental and Experimental Botany* **51**, 183–197.

Yamaguchi, T., Tsukamoto, Y. and Takahashi, K. (1990) Calorimetry as an analytical tool for germination tests of plant seeds. *Tokai Journal of Experimental and Clinical Medicine* **15**, 381–386.

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