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Development of ability to germinate and of longevity in air-dry storage in wheat seed crops subjected to rain shelter or simulated supplementary rainfall
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Corresponding Author:	Richard Harold Ellis, PhD University of Reading Reading, Berkshire UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Reading
Corresponding Author's Secondary Institution:	
First Author:	Richard Harold Ellis, PhD
First Author Secondary Information:	
Order of Authors:	Richard Harold Ellis, PhD
	Gajender Yadav, BSc PhD
Order of Authors Secondary Information:	
Abstract:	Climate change will alter rainfall patterns. The effect of rainfall during seed development and maturation on wheat (Triticum aestivum L.) seed quality (ability to germinate normally; air-dry longevity in hermetic storage at 40oC with c. 15% moisture content) was investigated in field experiments (2011, 2012) by providing rain shelter or simulating additional rainfall. High ability to germinate was detected from mid seed filling until after harvest maturity. Subsequent longevity was more sensitive to stage of development. It increased progressively reaching maximum values during maturation drying at 53-56 DAA, 5-11 (2011) or 8-14 (2012) days beyond mass maturity; maximal values were maintained thereafter in 2011; longevity declined with further delay to harvest in 2012. Post-anthesis protection from rain had no major effect: in later harvests longevity was slightly greater than the control in each year, but in 2011 wetting treatments were also superior to the control. Wetting ears at all stages of development reduced longevity immediately, but considerable recovery in subsequent longevity occurred when seeds re-dried in planta for several days. The greatest damage to longevity from ear wetting occurred with treatments at about 56 DAA, with poorest recovery at 70 DAA (i.e. around harvest maturity) in absolute terms but 56-70 DAA relative to gross damage. Hence, seed quality in highly-dormant wheat varieties is resilient to rain. Net damage is greatest from rain late in maturation. The results also show that seed quality improvement in planta comprises both improvement and deterioration, the former being the greater.

1 **Development of ability to germinate and of longevity in air-dry storage in wheat seed**

2 **crops subjected to rain shelter or simulated supplementary rainfall**

3

4 Running title. Rain, shelter and wheat seed quality development

5

6 **Gajender Yadav[†] and Richard H. Ellis*** School of Agriculture, Policy and Development,

7 University of Reading, Earley Gate, PO Box 237, Reading RG6 6AR, UK

8 [†]Present address: Division of Soil and Crop Management, ICAR-Central Soil Salinity

9 Research Institute, Zarifa Farm, Kachhwa Road, Karnal-132001, India

10 *Correspondence.

11 Address: School of Agriculture, Policy and Development, University of Reading, Earley

12 Gate, PO Box 237, Reading RG6 6AR, UK

13 Telephone: 0118 378 8488

14 Email: r.h.ellis@reading.ac.uk

15 **Keywords: development, germination, longevity, rain, *Triticum aestivum* L., viability,**

16 **wheat**

21

1   Running title. Rain, shelter and wheat seed quality development

2   **Abstract**

3   Climate change will alter rainfall patterns. The effect of rainfall during seed development and

4   maturation on wheat (*Triticum aestivum* L.) seed quality (ability to germinate normally; air-

5   dry longevity in hermetic storage at 40°C with c. 15% moisture content) was investigated in

6   field experiments (2011, 2012) by providing rain shelter or simulating additional rainfall.

7   High ability to germinate was detected from mid seed filling until after harvest maturity.

8   Subsequent longevity was more sensitive to stage of development. It increased progressively

9   reaching maximum values during maturation drying at 53-56 DAA, 5-11 (2011) or 8-14

10   (2012) days beyond mass maturity; maximal values were maintained thereafter in 2011;

11   longevity declined with further delay to harvest in 2012. Post-anthesis protection from rain

12   had no major effect: in later harvests longevity was slightly greater than the control in each

13   year, but in 2011 wetting treatments were also superior to the control. Wetting ears at all

14   stages of development reduced longevity immediately, but considerable recovery in

15   subsequent longevity occurred when seeds re-dried *in planta* for several days. The greatest

16   damage to longevity from ear wetting occurred with treatments at about 56 DAA, with

17   poorest recovery at 70 DAA (i.e. around harvest maturity) in absolute terms but 56-70 DAA

18   relative to gross damage. Hence, seed quality in highly-dormant wheat varieties is resilient to

19   rain. Net damage is greatest from rain late in maturation. The results also show that seed

20   quality improvement *in planta* comprises both improvement and deterioration, the former

21   being the greater.

1   **Introduction**

2   Climate change will increase mean global temperature, but will also affect future rainfall

3   patterns with more intense and more frequent extreme precipitation events

4   (Intergovernmental Panel on Climate Change, 2014). Indeed, during the 20th century

5   precipitation increased by some 10–40% across northern Europe (Klein Tank *et al*., 2002).

6   Seed quality is influenced by environment during seed development and maturation (Gusta *et*

7   *al*., 2004; Hampton *et al*., 2013). Rainfall and temperature are the main environmental factors

8   affecting crop yield and quality of wheat (*Triticum aestivum* L.) in the periods preceding

9   harvest (Smith and Gooding, 1999; Landau *et al*., 2000; Yadav and Ellis, 2016).

10

11   Warmer temperatures in the UK improve wheat seed quality development (Sanhewe

12   *et al*., 1996), whereas rainfall incident upon developing seed crops in cool, wet summers has

13   long been reported to be deleterious to UK wheat seed quality (MacKay, 1972). At the

14   extreme, heavy or prolonged rainfall to wheat varieties with low dormancy results in visible

15   sprouting (Flintham, 2000) - and so poor seed quality.

16

17   Simulated rainfall to developing wheat seed crops in the UK reduced the subsequent

18   air-dry longevity of seeds harvested soon afterwards (Ellis and Yadav, 2016). That study

19   showed that damage to longevity could be detected from a single rainfall event insufficient to

20   promote sprouting, but also that this immediate damage was reversed *in planta* when harvest

21   was delayed and seeds re-dried.

22

23   Post-harvest wetting of mature seed can benefit seed survival (Villiers and

24   Edgecumbe, 1975). Seed hardening (a wetting and drying cycle) has long been applied by

25   farmers in advance of sowing, but the effects of such wetting treatments (e.g. priming) have

1   been reported to both reduce (e.g. Argerich *et al.*, 1989; Tarquis and Bradford, 1992) and

2   improve longevity (e.g. Georghiou *et al.*, 1987; Probert *et al.*, 1991). Priming post-harvest

3   has also been shown to improve subsequent longevity when applied to immature seed but to

4   reduce longevity in seeds closer to maturity (Demir and Ellis, 1992a). Hence the effect of

5   rainfall on longevity may vary with stage of seed development.

6

7   We report here the effect of either rain shelter or additional simulated rainfall on seed

8   quality, assessed by ability to germinate normally with or without desiccation and by

9   longevity in air-dry storage, on seed crops of wheat in two years. The null hypotheses of (a)

10   no effect of rainfall on subsequent seed quality assessed by both ability to germinate normally

11   and by subsequent air-dry seed storage longevity, where the latter is the more sensitive to

12   discriminate amongst high–quality seed lots in seed development studies (Pieta-Filho and

13   Ellis, 1991), and (b) no interaction with developmental stage were tested. Accordingly,

14   treatments were applied at different stages of, and for different durations during, seed

15   development and maturation. Wheat seed quality improves throughout seed development and

16   maturation reaching maximum values at or close to harvest maturity (Ellis and Pieta Filho,

17   1992) and so the response to treatments was assessed against those developmental changes

18   within control crops.

19

20   **Materials and methods**

21   Crops of wheat (*Triticum aestivum* L.) cv. Tybalt were grown from March to August 2011

22   and similarly in 2012 in the field at the University of Reading, Crop Research Unit (CRU),

23   Sonning (51°30' N, 00 °54' W), in split-plot designs. The main plots (each 7.5 m x 2.5 m)

24   were the open, sheltered (protection from rainfall), or wetted (additional simulated rainfall)

1   treatments allocated at random within each block, with serial seed harvest dates as subplots.

2   Full details of agronomy, weather, rain shelter construction, dynamics of seed filling and

3   desiccation, and resultant crop quality are provided by Yadav and Ellis (2016). Rain shelter

4   was provided by polythene covers (2m x 5m, 1.5m tall) - twice the height of the crop and

5   open at the sides to aid air circulation and reduce the possibility of temperature build-up

6   above the crop canopy. Simulated rainfall treatments were provided to an area of 1.5m x 4m

7   marked by bamboo canes at the centre of those plots. The developing ears were wetted with

8   (tap) water equivalent to 2.5 cm rainfall on each occasion in two halves with a 30 minutes

9   gap, as described by Ellis and Yadav (2016).

10

11   The 2011 investigation comprised 22 main plots (11 treatments in each of two

12   blocks). One treatment was untreated and always open to rainfall (C1O; open control) and

13   one covered by full rain shelter from 50% anthesis until the last harvest (C2S; sheltered

14   control). Four treatments were provided with rain shelter for 14 day periods at 0-14 days after

15   50% anthesis (DAA) (S1), 14-28 DAA (S2), 28-42 DAA (S3), or 42-56 DAA (S4). A further

16   five treatments were provided with simulated rain: four were ear wetting treatments at 7 DAA

17   (W1), 21 DAA (W2), 35 DAA (W3), or 49 DAA (W4); the fifth was wetted four times, i.e. at

18   7, 21, 35 and 49 DAA (W5; wetting control). Samples from treatments subjected to ear

19   wetting that day were drawn 30 min after treatments ended.

20

21   Samples of seeds from each treatment were harvested serially by cutting 100 ears

22   from about 0.5 m^2 with scissors at about 1-2 cm below the spikes on up to nine occasions

23   (depending upon when a treatment began) during seed development and maturation, from 14

24   DAA on 23 June and then 25, 32, 39, 46, 53, 60, 67 and finally 74 DAA. The first two

1 harvests (14 and 25 DAA) comprised six treatments, those on 32 and 39 DAA nine, and

2 thereafter all 11 treatments from each block.

3

4   In 2012, there were 20 main plots (ten treatments in each of two blocks). One

5 treatment was always open to rainfall (C1O; open control) and one covered by full rain

6 shelter from 50% anthesis until the last harvest (C2S; sheltered control). The three temporary

7 rain shelter treatments were applied later in development than in 2011 and also differed in

8 duration: S1 (42-70 DAA), S2 (42-56 DAA) and S3 (56-70 DAA). The five ear wetting

9 treatments were also applied later than in 2011: at 42 DAA (W1), 49 DAA (W2), 56 DAA

10 (W3), 63 DAA (W4), or 70 DAA (W5). Samples of seeds from each treatment were

11 harvested serially on up to ten occasions during seed development and maturation: the first

12 four harvests (14, 21, 28 and 35 DAA) comprised two treatments, those on 42, 49, 56, 63 and

13 70 DAA 5, 6, 8, 9 and 10 treatments, respectively, and an additional sample was drawn

14 immediately after each wetting treatment from each block. In the case of W5 only, the last

15 wetting treatment, a sample was also taken at 77 DAA (7 days after wetting). In 2012,

16 samples were also taken from wetting treatments immediately before each wetting as well as

17 afterwards.

18

19   Samples were harvested between 7 and 9 a.m. and seeds threshed from ears by hand.

20 Throughout the study, seeds from the same treatment but from different blocks were analysed

21 separately. With the exception of a sample drawn to determine ability to germinate of freshly-

22 harvested seed, samples were dried to 10-14% moisture content (wet basis) in a drying

23 cabinet maintained at 15-17°C with 12-15% relative humidity. Samples were then drawn to

24 determine seed moisture content and ability to germinate after drying. The remainder of each

1   sample was sealed in a laminated-aluminium-foil bag (Retort laminate, Moore and Buckle

2   Ltd, St Helens, UK) and stored at -20ºC.

3

4   Ability to germinate was tested for two replicates of 50 seeds each between moist

5   rolled paper towels (Kimberley Clark Professional 6803 HOSTESS, Natural, 24 x 35 cm,

6   Greenham Sales, UK) in an incubator at 10°C. Tests were monitored for germination at 7 day

7   intervals up to 28 days. Seedlings were evaluated as normal, abnormal or dead seeds

8   according to the ISTA rules (International Seed Testing Association, 2011). Seeds that had

9   not germinated after 28 days were pricked in order to break any possible dormancy and tests

10   continued until all seeds had either germinated or were no longer fresh (i.e. dead).

11

12   Seed moisture content was determined using the two-stage or the single-stage high-

13   constant-temperature-oven method (International Seed Testing Association, 2011), depending

14   upon expected moisture content; two 100 seed replicates were used in place of two 4-5 g

15   samples due to limited seed supply.

16

17   Seed storage longevity was determined in a constant hermetic environment of 40ºC

18   with c. 15% moisture content. Seed packets were withdrawn from storage at -20º C about

19   four months after harvest. They were first exposed to laboratory temperature within the

20   sealed packets for 24 hours to avoid moisture condensing on seed surfaces. Seed moisture

21   content was then estimated indirectly using a non-destructive equilibrium relative humidity

22   (erh) water activity meter (65% relative humidity at 20°C being the common target for all the

23   seed samples). In order to adjust seed moisture content to 15% (±0.5%), samples were

24   weighed and either placed in a muslin bag to dry at 15°C, 15% relative humidity, or

1   humidified at 20°C above deionised water, depending upon estimated initial moisture

2   content. This adjustment was controlled by weighing repeatedly and determining erh until the

3   desired weight/erh was reached. To enable moisture to equilibrate within and amongst

4   samples, the separate muslin bags were placed together for 15 days in a plastic container at 2-

5   4°C.

6

7   The moisture content of each seed sample was then determined using the high-

8   constant-temperature-oven method (ISTA, 2011). Estimates ranged between extremes of 14.1

9   and 15.7% in 2011 (mean 15.0%) and 14.2 and 15.6% in 2012 (mean 14.7%). Ten

10 subsamples of 100 seeds from each sample were sealed in separate laminated-aluminium-foil

11 packets and stored in an incubator maintained at 40°C. One sample from each treatment was

12 withdrawn from storage after different periods (0 - 49 days) of experimental storage and

13 tested for ability to germinate. Seed survival curves in hermetic storage at 40°C with 15%

14 moisture content were fitted by probit analysis for each sample in accordance with the seed

15 viability equation (Ellis and Roberts, 1980):

16

17      $$v = K_i - p/\sigma \qquad (1)$$

18 where v is probit percentage viability after p days in storage in a constant environment, K_i is a

19 constant specific to seed lot (equivalent to initial probit viability), and σ is the standard

20 deviation of the frequency distribution of seed deaths in time (days) using Genstat (13th

21 Edition, VSN International Ltd., UK). The product of K_i and σ is the period for viability to

22 decline to 50% (p_{50}). The ability of a seed to produce a normal seedling was the criterion of

23 survival. Genstat was also applied to compare seed survival curves.

24

1 **Results**

2 Fifty per cent anthesis occurred on 8 June 2011 (84 days after sowing) or 18 June 2012 (95

3 days after sowing), when the first rain shelters were installed (e.g. C2S). Sheltered plots

4 provided mean temperatures 0.8 to 0.9 ℃ warmer than control plots in both years, with

5 90.2% direct light and 84.6 % photosynthetically-active radiation. Both years provided post-

6 anthesis environments wetter and cooler than long-term site means, with 2011 cooler and

7 wetter than 2012 on average.

8

9 The treatments affected seed filling duration in 2011, only, and towards harvest

10 maturity in both years (Table 1). In 2011, seed filling durations were reduced by rain shelter

11 throughout (C2S) or late within this period (S3). Durations from anthesis to (almost) harvest

12 maturity were reduced by shelter throughout (C2S in 2011 and 2012) or during late

13 maturation drying (S1, S3 in 2012) but only increased by multiple simulated rainfall events

14 (2011, W5).

15

16 *Ability to germinate*

17

18 No pre-harvest sprouting was detected in any treatment. Ability to germinate normally

19 improved greatly amongst early harvests, when it also benefited from post-harvest drying

20 (Figs 1, 2). From 32-74 DAA in 2011, all treatments provided 100% normal germination for

21 dried seed samples (Fig. 1c, d). Ability to germinate without post-harvest drying was slightly

22 lower during this period but also more variable; a marked decline ($P<0.001$) occurred from

23 39-53 DAA with subsequent reversal from 53-67 DAA in 2011 (Figs 1a, b) in contrast to

24 100% normal germination throughout for dried seeds (Figs 1c, d). Ability to germinate

1 normally was also greater with post-harvest drying in 2012 from 35-70 DAA (Fig. 2), but in

2 this case slightly more variation was apparent amongst treatments with lower values during

3 S3 shelter (56-70 DAA; Fig. 2c) and immediately after ear wetting in W2 (49 DAA) and W3

4 (56 DAA) (Fig. 2d).

5

6 *Longevity*

7 The seed survival curves were sigmoidal, conformed to negative cumulative normal

8 distributions, and described well by Equation (1). In each year, seed survival curve

9 comparisons showed significant differences in K_i (P <0.001) and σ (P <0.001), and provided

10 a clear main effect of harvest date on longevity (P <0.001). This main effect provided a

11 somewhat erratic pattern of considerable improvement in longevity until 53 DAA in 2011

12 (Fig. 3a) or 56 DAA in 2012 (Fig. 4a), but with a plateau between 32 and 46 DAA in 2011 or

13 35 and 49 DAA in 2012. The plateau in 2012 was an artefact from meaning treatment results

14 with the control showing continuous improvement (Fig. 4), whereas the plateau was detected

15 in the control in 2011 (Fig. 3). A further difference amongst years occurred after maximum

16 longevity was first attained: longevity declined after 56 DAA in 2012, but was stable between

17 53 and 74 DAA in 2011. The trends were identical between blocks with both giving similar

18 values in 2012 (Fig. 4) and Block 1 initially greater than 2 in 2011 but with closer agreement

19 as seed development and maturation progressed (Fig. 3).

20

21 The treatments had comparatively little effect on the development of longevity in

22 2011, other than that shelter throughout (C2S) may have been in advance of the control

23 (C1O) early on, with close agreement amongst treatments at 53 DAA when maximum

24 longevity was first attained (Figs 3b, c). It is also noteworthy that longevity 21 days later at

1 74 DAA after all five wetting treatments (W1-W5) was not only slightly greater than the

2 control (C1O) but also similar to shelter throughout (C2S).

3

4 Longevity for shelter throughout (C2S) improved in advance of the control (C1O) at

5 28 and 35 DAA in 2012 (Fig. 4b). Moreover, from 56 to 70 DAA longevity was greater from

6 all shelter treatments than the control. Wetting reduced longevity immediately, as shown by

7 W (af) and C1O (Fig. 4c). However, W(af) is not one treatment *per se* but a compilation of

8 all wetting treatments' results immediately after wetting; after a further 7 or more days *in*

9 *planta* all wetting treatments completed by then provided similar longevity at 56 DAA to the

10 control, and only two subsequent wetting treatment observations were below control values

11 (W2 at 63 DAA; W3 at 70 DAA).

12

13 To investigate the effect of the timing of ear wetting more closely, Figure 5 provides a

14 compilation of the 2012 results for longevity immediately before and after ear wetting, and

15 after a further 7 days *in planta* for treatments applied up to 28 days apart late in maturation.

16 Subsequent air-dry longevity was reduced immediately after each of the five wetting

17 treatments, to a similar extent in W2-W5 but to about half this extent in the earliest treatment

18 W1 (42 DAA), but 7 days *in planta* thereafter resulted in improvement to longevity in every

19 treatment. In each case, the difference between the first and second samples (vertical broken

20 line in Fig. 5) provides an estimate of the immediate (gross) damage to seed quality (as

21 assessed by longevity) from simulated rainfall, that between the second and third (solid line

22 in Fig. 5) the reversal of that damage over 7 days. The difference between the first and third

23 samples provides an estimate of net damage, therefore. The magnitude of the initial damage

24 to longevity from simulated rainfall and the subsequent improvement (difference between

second and third samples), in absolute terms and relative to the gross (immediate) damage

from wetting, showed clear patterns over developmental time amongst treatments about a

fulcrum at 56 DAA (when longevity reached maximum values, Fig.4): gross damage showed

a consistent pattern where W1 < W2 < W3 = W4 >W5; for the subsequent improvement in

absolute terms, least in W1 and W5, greatest in W2, and about a quarter of W2 in both W3

and W4; in relative terms, the improvement was similar to initial (gross) damage in W1, more

than twice as great in W2, but just under half of the gross damage in W3 - W5.

Discussion

The general temporal pattern of development in the ability of seeds to germinate during seed

development and maturation was similar in the two years, and largely as expected in wheat

(Ellis and Pieta Filho, 1992). Onset of ability to germinate and of desiccation tolerance was

apparent from the first harvest (14 DAA) early in the seed-filling phase (Figs 1, 2), at which

time seeds were only about 10% filled (Ellis and Yadav, 2016), with full or close to full

ability to germinate achieved 70-80% through the seed-filling phase – and so well before

mass maturity. Post-harvest drying early in seed development promoted ability to germinate.

Such improvements from drying immature seeds are well known (Dasgupta *et al.*, 1982;

Bewley and Black, 1994). The effect was equivalent to several days of further development

in planta, which drying *ex planta* mimics to a certain extent. No decline in ability to

germinate normally of the dried seeds was detected amongst harvests later in development

and maturation in either year, despite sampling until 74 DAA (extremes of 32 days after mass

maturity, or 13 days after seeds dried to 20% moisture content; Table 1).

1   Testing ability to germinate at 10 °C combined with pricking minimized dormancy

2   limiting germination, as expected in temperate cereals (Ellis *et al*., 1987). Nevertheless, the

3   results for freshly-harvested seeds harvested between 39 and 67 DAA in 2011 showed first a

4   decline and then an increase in ability to germinate, whereas seeds first dried showed 100%

5   throughout this period (Fig. 1). This represents late induction and subsequent loss in wheat

6   seed dormancy (Mitchell *et al.*, 1980; Gooding *et al.*, 2012). In sorghum (*Sorghum bicolor*

7   L.), Fenner (1991) noted that plant water status during seed filling affected dormancy. No

8   consistent effect on dormancy induction and loss pattern was detected here, however, with

9   the extreme opposite treatments of shelter (S1, S2) or wetting (W1, W2, W3) during seed

10   filling both providing greater dormancy than the control (Fig. 1).

11

12   Seed longevity can be a more sensitive indicator of differences in seed quality than

13   ability to germinate amongst high quality seed lots (Ellis and Roberts, 1981). This was the

14   case here with substantial differences in seed longevity identified (Figs 3, 4) amongst the

15   high-viability samples (Figs 1, 2) harvested from 25 (2011) or 35 DAA (2012) onwards. Prior

16   to this, both approaches provided a consistent increase in seed quality but whereas ability to

17   germinate then reached maximum values longevity showed continued improvement

18   thereafter, albeit not linear, with further development. Subsequent longevity first reached

19   maximum values during the maturation drying phase at 53-56 DAA (Figs 3, 4), some 5-11

20   (2011) or 8-14 (2012) days after mass maturity and 9-23 (2011) or 1-18 days before seed

21   moisture content declined *in planta* to 20% (Table 1). This confirms earlier studies in barley

22   (*Hordeum vulgare* L.) and wheat in which subsequent longevity continued to improve, and

23   considerably so, beyond the end of the seed-filling phase (Pieta Filho and Ellis, 1991; Ellis

24   and Pieta Filho, 1992; Sanhewe *et al*., 1996; Ellis and Yadav, 2016).

25

1   Longevity remained high, possibly improving a little, from 53 DAA until the last

2   harvest at 74 DAA in 2011 (Fig. 3) but declined, more or less linearly and consistently,

3   between 56 and 70 DAA in 2012 (Fig. 4). A somewhat similar difference between years was

4   reported in a study with barley: longevity declined immediately after improvement ended in

5   1988, whereas in the warmer, drier 1989 longevity was maintained at high values for around

6   14 days, before then declining (Pieta Filho and Ellis, 1991). Decline in longevity after

7   attaining maximum values was detected in wheat in both 2008 and 2009 and was steeper in

8   the wetter and marginally (only 0.1 ºC) cooler 2008 than in 2009 (Ellis and Yadav, 2016).

9   However, the potential assumption that warmer, drier conditions reduce decline in longevity

10   with delay to harvest is not borne out by the current results, because August 2011 was

11   appreciably wetter and cooler than August 2012 (Yadav and Ellis, 2016). Hence, the

12   assumption that seeds deteriorate post peak-longevity more rapidly the wetter the

13   environment is not supported consistently by these inter-annual comparisons.

14

15   A similar lack of clarity for the effect of rainfall is provided by comparison within

16   years between shelter and ear wetting treatments amongst later harvests. In 2011, longevity in

17   the ear wetting treatments (at 7 - 49 DAA) from 54 DAA onwards was similar to or perhaps

18   greater than the control (Fig. 3c), but this was also the case for the rain shelter treatments

19   (Fig. 3b). In 2012, the later harvests (56 DAA onwards) provided consistently greater

20   longevity with shelter than the control (Fig. 4b) and while longevity immediately after

21   wetting (at 42 - 70 DAA, and so applied later than 2011) was reduced, thereafter most

22   wetting treatments provided longevity similar to the control (Fig. 4c). Differences in

23   longevity amongst treatments were slightly greater in 2012 (Fig. 4) than in 2011 (Fig. 3),

24   perhaps because the majority of treatments were applied later, deliberately, during seed

25   development and maturation in 2012. The greater effect of treatments applied later tallies

1   with differences detected amongst previous years (Ellis and Yadav, 2016), but that

2   comparison was confounded with that between field and protected environments.

3

4   Generally, therefore, it would appear that rainfall events result in gross damage to

5   subsequent longevity at all stages of development and maturation, but that the damage is

6   reversed *in planta* in whole or in part thereafter such that there is often little or no net

7   damage. Net damage may be detected from rainfall in the period approaching harvest

8   maturity and thereafter (W3-W5, Fig. 5), however, especially if it is repeated rainfall (Ellis

9   and Yadav, 2016). Moreover, there is a clear trend with respect to developmental stage:

10   simulated rainfall early-mid development is less damaging initially and is more likely to be

11   entirely reversed thereafter *in planta*, than from late maturation drying onwards. Hence, net

12   damage to seed quality from rainfall is more likely in the period approaching harvest maturity

13   and thereafter than earlier. The comparatively limited net damage from ear wetting may help

14   to explain the absence of any consistent effect of rain shelter versus wetting and control

15   treatments amongst the final samples harvested between the years. It also agrees with 2008

16   and 2009 field results in which simulated rainfall early in development delayed the pattern of

17   seed quality improvement but only by a few days, and the more dramatic damage but largely

18   reversible from treatment at close to harvest maturity in a tunnel house in 2010 (Ellis and

19   Yadav, 2016).

20

21   We suggested previously that damage to longevity from rainfall at harvest maturity

22   and subsequent reversal *in planta* might be associated with changes to the glassy state –

23   which may affect air-dry seed survival (Buitink and Leprince, 2004; Walters, 2015), because

24   seeds were at 14-19 % moisture content, the gross damage occurred rapidly, and subsequent

1   recovery was within 24 h (Ellis and Yadav, 2016). Whilst we continue to support that

2   argument for rainfall events close to harvest maturity in very dry seeds, the damage and

3   reversal detected here over a longer period of 7 days and, moreover, earlier in seed

4   development and maturation (W1-W2, Fig. 5) coincides with periods when both

5   oligosaccharides and low molecular weight heat-stable proteins are accumulating, albeit with

6   oligosaccharide accumulation more prevalent during development and heat-stable protein

7   accumulation more during maturation drying (Sinniah *et al*., 1998), and both benefit

8   subsequent air-dry seed survival (e.g., Crowe *et al*., 1984; Galau et al., 1986; Leopold, 1990).

9   A recent network analysis of the co-expression of a considerable number of regulatory genes,

10   associated with seed longevity, during seed development and maturation in *Medicago*

11   *truncatula* L. confirms there is a substantial temporal pattern of expression of different genes,

12   but that gene expression can be detected quite late when moisture content is only c. 20%

13   (Righetti *et al*., 2015). Hence, it is possible that the drivers of both the damage to longevity

14   from rainfall and its subsequent reversal may differ depending upon when during seed

15   development and maturation the rainfall event occurs, which might explain the differences in

16   the magnitude of the gross and net responses at different stages of seed development.

17

18   This research, particularly Fig. 5 here in combination with Fig. 4 of Ellis and Yadav

19   (2016), confirms a long-held suspicion: during the period of seed quality development from

20   the beginning of seed filling until close to harvest maturity, the improvement in seed quality

21   detected is net improvement whereby both improvement and deterioration occur, but the

22   former is (usually) by far the greater. Similarly, once maximum seed quality has been

23   attained, it is net loss in quality that is detected with, again, improvement and deterioration

24   possible, but in this case the latter is (usually) the greater. Hence, the difference between

25   years during late maturation drying of no appreciable change in seed quality between 53 and

1   74 DAA in 2011 (Fig. 3) but consistent decline from 56 to 70 DAA in 2012 (Fig. 4): in the

2   latter deterioration was greater than improvement, whereas in the former they were almost

3   equal, presumably, cancelling each other out. Certain earlier observations in rice (*Oryza*

4   *sativa* L.) and tomato (*Lycopersicon esculentum* Mill.) may also be compatible with this

5   interpretation. Longevity of a *japonica* rice at 32/24 ºC plateaued from mass maturity

6   onwards during the maturation drying phase, whereas longevity continued to improve over

7   this period at 28/20 ºC and in other (*indica*) cultivars in both regimes (Fig. 4a, Ellis *et al.,*

8   1993). We suggest that the former represents no net change, because deterioration and

9   improvement were in balance, whereas in the latter improvement exceeded deterioration.

10   Similarly, tomato seed survival periods were stable over a 40-day period *in planta*, within

11   fleshy fruits, from 55 DAA, 13 d after mass maturity, to 95 DAA (Fig. 3c, Demir and Ellis,

12   1992b) – again representing a possible close match between rates of seed deterioration and

13   improvement.

14

15   Greater tolerance to heat and drought stress are priorities for the genetic improvement

16   of wheat in order to maintain future yield by adapting to climate change (Semenov *et al*.,

17   2014). In the UK at least, warmer summer temperatures from anticipated climate change will

18   benefit wheat seed quality (Sanhewe *et a1*., 1996). Wheat has long been reported to produce

19   poor quality seed in cool, wet UK summers (MacKay, 1972) and similarly poor quality grain

20   for bread-making purposes in wet UK summers (Smith and Gooding, 1999) as a result of

21   germination (visible and so pre-harvest sprouting, or *sensu strictu*). The bread-making quality

22   wheat cultivar selected for this study shows strong dormancy, in order to maintain grain

23   quality in wet UK summers. Clearly, such high dormancy also provides resilience in terms of

24   high seed quality following rainfall events in wet summers. Hence, high seed dormancy is an

25   important character to include in breeding programmes to provide new feed, as well as

1 quality, cultivars adapted to future NW Europe climates, in order to safeguard high quality

2 wheat seed supplies.

3

9

10 **References**

11 **Argerich, C.A., Bradford, K.J. and Tarquis, A.M.** (1989) The effects of priming and aging

12 on resistance to deterioration of tomato seeds. *Journal of Experimental Botany* **40**, 593–598.

13 **Bewley, J.D. and Black, M.** (1994) Seeds: Physiology, Development and Germination (2nd

14 edition). Plenum Press, New York.

15 **Buitink, J. and Leprince, O.** (2004) Glass formation in plant anhydrobiotes: survival in the

16 dry state. *Cryobiology* **48**, 215–228.

17 **Crowe, J.H., Crowe, L.M. and Chapman, D.** (1984) Preservation of membranes in

18 anhydrobiotic organisms: the role of trehalose. *Science* **223,** 701–703.

19 **Dasgupta, J., Bewley, J.D. and Yeung, E.C.** (1982) Desiccation-tolerant and desiccation-

20 intolerant stages during the development and germination of *Phaseolus vulgaris* seeds.

21 *Journal of Experimental Botany* **33**, 1045–1057.

22 **Demir, I. and Ellis, R.H.** (1992a) Development of pepper (Capsicum annuum) seed quality.

23 *Annals of Applied Biology* **121,** 385–399.

24 **Demir, I. and Ellis, R.H.** (1992b) Changes in seed quality during seed development and

25 maturation in tomato. *Seed Science Research* **2**, 81–87.

1 **Ellis, R.H. and Pieta-Filho, C.** (1992) The development of seed quality in spring and winter

2 cultivars of barley and wheat. *Seed Science Research* **2**, 9–15.

3 **Ellis, R.H. and Roberts, E.H.** (1980) Improved equations for the prediction of seed

4 longevity. *Annals of Botany* **45**, 13–30.

5 **Ellis, R.H. and Roberts, E.H.** (1981) The quantification of ageing and survival in orthodox

6 seeds. *Seed Science & Technology* **9**, 373–409.

7 **Ellis, R.H., Hong, T.D. and Jackson, M.T.** (1993) Seed production environment, time of

8 harvest and the potential longevity of seeds of three cultivars of rice (*Oryza sativa* L.). *Annals*

9 *of Botany* **72**, 583–590.

10 **Ellis, R.H., Hong, T.D. and Roberts, E.H.** (1987) Comparison of cumulative germination and

11 rate of germination of dormant and aged barley seed lots at different constant temperatures.

12 *Seed Science and Technology* **15**, 717–728.

13 **Ellis, R. H. and Yadav, G.** (2016) Effect of simulated rainfall during wheat seed

14 development and maturation on subsequent seed longevity is reversible. *Seed Science*

15 *Research* **26**, 67−76.

16 **Fenner, M.** (1991) The effects of the parent environment on seed germinability. *Seed Science*

17 *Research* **1**, 75–84.

18 **Flintham, J.E.** (2000) Different genetic components control coat-imposed and embryo-

19 imposed dormancy in wheat. *Seed Science Research* **10**, 43–50.

20 **Galau, G.A., Hughes, D.W. and Dure, L. III.** (1986) Abscisic acid induction of cloned late

21 embryogenesis-abundant (lea) mRNAs. *Plant Molecular Biology* **7,** 155–170.

22 **Georghiou, K., Thanos, C.A. and Passam, H.C.** (1987) Osmoconditioning as a means of

23 counteracting the ageing of pepper seeds during high-temperature storage. *Annals of Botany*

24 **60**, 279–285.

1   **Gooding, M. J., Uppal, R. K., Addisu, M., Harris, K.D., Uauy, C., Simmonds, J.R. and**

2   **Murdoch, A.J.** (2012) Reduced height alleles (Rht) and Hagberg falling number of wheat.

3   *Journal of Cereal Science* **55**, 305–311.

4   **Gusta, L.V., Johnson, E.N., Nesbitt, N.T. and Kirkland, K.J.** (2004) Effect of seeding date

5   on canola seed quality and seed vigour. *Canadian Journal of Plant Science* **84**, 463–471.

6   **Hampton, J.G., Boelt, B., Rolston, M.P. and Chastain, T.G.** (2013) Effects of elevated

7   CO_2 and temperature on seed quality. *Journal of Agricultural Science* **151**, 154–162.

8   **Intergovernmental Panel on Climate Change.** (2014) *Climate Change 2014: Synthesis*

9   *Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the*

10 *Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A.

11 Meyer (eds.)]. Geneva, The Intergovernmental Panel on Climate Change.

12 **International Seed Testing Association** (2011) *International rules for seed testing. Edition*

13 *2011*. Switzerland, The International Seed Testing Association.

14 **Klein Tank, A.M.G., Wijngaard, J.B., Können, G.P., Böhm, R., Demarée, G., Gocheva,**

15 **A., Mileta, M., Pashiardis, S., Hejkrlik, L., Kern-Hansen, C., Heino, R., Bessemoulin, P.,**

16 **Müller-Westermeier, G., Tzanakou, M., Szalai, S., Pálsdóttir, T., Fitzgerald, D., Rubin,**

17 **S., Capaldo, M., Maugeri, M., Leitass, A., Bukantis, A., Aberfeld, R., Van Engelen, A. F.**

18 **V., Forland, E., Mietus, M., Coelho, F., Mares, C., Razuvaev, V., Nieplova, E., Cegnar,**

19 **T., López, J. A., Dahlström, B., Moberg, A., Kirchhofer, W., Ceylan, A., Pachaljuk, O.,**

20 **Alexander, L.V. and Petrovic, P.** (2002) Daily dataset of 20th-century surface air

21 temperature and precipitation series for the European Climate Assessment. *International*

22 *Journal of Climatology* **22**, 1441–1453.

23 **Landau, S., Mitchell, R.A.C., Barnett, V., Colls, J.J., Craigon, J. and Payne, R.W.**

24 (2000) A parsimonious, multiple-regression model of wheat yield response to environment.

25 *Agricultural and Forest Meteorology* **101**, 151–166.

Leopold, A.C. (1990) Coping with desiccation, pp. 57–86 *in* Alscher, R.G. and Cumming, J.R. (Eds) *Stress responses in plants: adaptation and acclimation mechanisms.* New York, Wiley-Liss Inc.

MacKay, D.B. (1972) The measurement of viability, pp. 172–208 *in* Roberts, E.H. (Ed.) *Viability of seeds.* London: Chapman and Hall.

Mitchell, B., Armstrong, C., Black, M. and Chapman, J. (1980) Physiological aspects of sprouting and spoilage in developing *Triticum aestivum* L. (wheat) grains, pp. 339–356 *in* Hebblethwaite, P.D. (Ed.) *Seed Production.* London: Butterworths.

Pieta-Filho, C. and Ellis, R.H. (1991) The development of seed quality in spring barley in four environments. I. Germination and longevity. *Seed Science Research* **1**, 163–177.

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

Righetti, K., Vu, J.L., Pelletier, S., Vu, B.L., Glaab, E., Lalanne, D., Pasha, A., Patel, R.V., Provart, N.J., Verdier, J., Leprince, O., and Buitink, J. (2015) Inference of longevity-related genes from a robust coexpression network of seed maturation identifies regulators linking seed storability to biotic defense-related pathways. *Plant Cell* **27**, 2692–2708.

Sanhewe, A.J., Ellis, R.H., Hong, T.D., Wheeler, T.R., Batts, G.R., Hadley, P., and Morison J. (1996) The effect of temperature and CO_2 on seed quality development in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **47**, 631–637.

Semenov, M.A., Stratonovitch, P., Alghabari, F. and Gooding, M.J. (2014) Adapting wheat in Europe for climate change. *Journal of Cereal Science* 59, 245–256.

Sinniah, U.R., Ellis, R.H. and John P. (1998) Irrigation and seed quality development in rapid-cycling Brassica: soluble carbohydrates and heat-stable proteins. *Annals of Botany* **82**, 647–655.

1 **Smith, G.P. and Gooding, M.J.** (1999) Models of wheat grain quality considering climate,

2 cultivar and nitrogen effects. *Agricultural and Forest Meteorology* **94**, 159–170.

3 **Tarquis, A.M. and Bradford, K.J.** (1992) Prehydration and priming treatments that advance

4 germination also increase the rate of deterioration of lettuce seeds. *Journal of Experimental*

5 *Botany* **43,** 307–317.

6 **Villiers, T.A. and Edgecumbe, D.J.** (1975) On the cause of seed deterioration in dry

7 storage. *Seed Science and Technology* **3**, 61–74.

8 **Walters, C.** (2015) Orthodoxy, recalcitrance and in-between: describing variation in seed

9 storage characteristics using threshold responses to water loss. *Planta* **242**, 397–406.

10 **Yadav, G. and Ellis, R. H.** (2016) Effects of rain shelter or simulated rain during grain

11 filling and maturation on subsequent wheat grain quality in the UK. *Journal of Agricultural*

12 *Science* (in press)

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Table 1. Effect of rain shelter or ear wetting on durations to mass maturity and 20% moisture content (i.e. approaching harvest maturity) in wheat cv. Tybalt in 2011 and 2012. From Yadav and Ellis (2016).

Treatment	Duration to mass maturity (DAA)	Duration to 20% moisture content (DAA)
2011		
Control (C1O)	46.3	70.0
C2S (Shelter 0-56 DAA)	41.8	62.0
S1 (Shelter 0-14 DAA)	46.1	72.5
S2 (Shelter 14-28 DAA)	46.7	72.0
S3 (Shelter 28-42 DAA)	43.6	71.0
S4 (Shelter 42-56 DAA)	46.3	69.0
W1 (Wetting 7 DAA)	45.8	72.5
W2 (Wetting 21 DAA)	48.3	71.5
W3 (Wetting 35 DAA)	48.3	70.5
W4 (Wetting 49 DAA)	46.6	71.5
W5(Wetting 7-49 DAA)	46.8	76.0
$LSD_{0.05}$	2.17	4.61
P	*0.002*	*0.007*
2012		
Control (C1O)	42.8	74.0
C2S (Shelter 0-70 DAA)	45.6	56.5
S1 (Shelter 42-70 DAA)	42.8	58.0
S2 (Shelter 42-56 DAA)	48.5	74.0

S3 (Shelter 56-70 DAA)	45.3	59.0
W1 (Wetting 42 DAA)	41.8	74.0
W2 (Wetting 49 DAA)	42.3	74.0
W3 (Wetting 56 DAA)	44.6	74.0
W4 (Wetting 63 DAA)	42.3	74.0
W5 (Wetting 70 DAA)	44.6	74.0
W (30 mins after wetting)	47.1	74.0
$LSD_{0.05}$	4.92	1.49
P	*0.155*	*0.001*

Captions to Figures

Figure 1. Ability of fresh (a, b) or dried (c, d) seeds of wheat cv. Tybalt to germinate

normally when tested at 10°C after harvesting at different times during seed development and

maturation when grown (a, c) under rain shelter (C2S, S1, S2, S3, S4) or (b, d) with ear

wetting (W1, W2, W3, W4, W5) compared with a control (C1O) in 2011.

Figure 2. Ability of fresh (a, b) or dried (c, d) seeds of wheat cv. Tybalt to germinate

normally when tested at 10°C after harvesting at different times during seed development and

maturation when grown (a, c) under rain shelter (C2S, S1, S2, S3) or (b, d) with ear wetting

(W1, W2, W3, W4, W5, Waf) compared with a control (C1O) in 2012. Note that Waf

represents results of samples harvested immediately after ear wetting.

Figure 3. Improvement in subsequent air-dry seed storage longevity (p_{50}, days, provided by

probit analysis), in hermetic storage at 40°C with c. 15% moisture content, during seed

development and maturation in wheat cv. Tybalt in 2011 comparing amongst blocks (a; mean

of all treatments), rain shelter (b; mean of both blocks), or ear wetting treatments (c; mean of

both blocks)**.** Standard errors of estimates shown (but largely similar to or smaller than

symbols). Control treatment results (C10) are shown in both b and c. Further details as Figure

1.

Figure 4. Improvement, and later decline, in subsequent air-dry seed storage longevity (p_{50},

days, provided by probit analysis), in hermetic storage at 40°C with c. 15% moisture content,

during seed development and maturation in wheat cv. Tybalt in 2012 comparing amongst

blocks (a; mean of all treatments), rain shelter (b; mean of both blocks), or ear wetting

treatments (c; mean of both blocks)**.** Standard errors of estimates shown (but largely similar

to or smaller than symbols). Control treatment results (C10) are shown in both b and c.

Further details as Figure 2.

Figure 5. Immediate damage from ear wetting to subsequent seed storage longevity (p_{50}) of

wheat cv. Tybalt (— — —) and subsequent improvement with 7 days' natural re-drying *in*

planta (——) from treatments at different times during seed development and maturation: W1

(■), W2 (□), W3 (▲), W4 (△),W5 (◆). Data repeated from Figure 4.

Figure 5