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Seed heteromorphy influences seed longevity in *Aegilops*

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Abstract

The genus *Aegilops* belongs to the secondary gene pool of wheat and has great importance for wheat cultivar improvement. As a genus with only annual species, regeneration from seeds in *Aegilops* is crucial. In several species in *Aegilops*, spikes produce different seed morphs, both in size and germination patterns. However, little is known about the ecology of seed germination, nor about the seed longevity in this genus. Here we investigated the germination phenology of *Ae. neglecta* under laboratory and field conditions and assessed longevity of different seed morphs of five additional *Aegilops* species using controlled ageing tests. Large seeds were short-lived and germinated faster than small seeds in most of the species. Field experiments with *Ae. neglecta* showed that large seeds of the dimorphic pair germinated 3 months after dispersal in contrast to 14 months for smaller seeds. Differences in longevity were detected not only in dimorphic seed pairs, but also among seeds from different positions on the spike. Our results indicate that different longevities in seed morphs of *Aegilops* may reflect a different soil seed bank persistence, with smaller seeds able to maintain a higher viability after dispersal than larger ones, thereby spreading seedling emergence over two years. Differences of seed germination and longevities between seed morphs in *Aegilops* may have important implications for *ex situ* seed conservation and reinforce the hypothesis of a bet-hedging strategy in the germination ecology of this genus.

Introduction

Seed heteromorphy is defined as the production of different types of seeds by a single plant and is a common strategy to cope with unpredictable climatic conditions (Bhatt and Santo, 2016). Seed heteromorphy influences seed dispersal ability, germination, seed dormancy and persistence in the soil (Duràn and Retamal, 1989; Puga-Hermida *et al.*, 2003; El-Keblawy and Bhatt, 2015). Seed dimorphism is considered the main type of seed heteromorphy and it is expressed mostly in annual plants (Venable, 1985).

The *Aegilops* genus includes 23 species of annual grasses, occurring in southern Europe, North Africa, the Near East and western and central Asia. *Aegilops* species occur in various dry, thermophile and low nutrient, often disturbed habitats (e.g. pastures, roadsides, field margins, wastelands; van Slageren, 1994). Some *Aegilops* L. species (Triticeae Dumort.) produce caryopses (hereafter referred to as seeds) of different size and shape and different testa colours. Seed dimorphism was also detected and shown to be linked to a complex dispersal strategy (Dyer, 2004; Marañon, 1989; Matilla *et al.*, 2005). In *Aegilops geniculata* Roth, *Ae. kotschyi* Boiss., *Ae. neglecta* Req. ex Bertol. and *Ae. triuncialis* L. dimorphic pairs of seeds are found in the lower fertile spikelets, with one seed larger and lighter coloured than the other one (Datta *et al.*, 1970; Wurzburger *et al.*, 1976; Dyer, 2004; Marañon, 1989). In *Ae. geniculata*, only the larger seeds of the dimorphic pair within the spikelets readily germinate, whereas the germination of the smaller seeds is inhibited probably due to the presence of an inhibitor in the glumes (Datta *et al.*, 1970). A similar behaviour was detected in *Ae. cylindrica* Host and *Ae. triuncialis* (Fandrich and Mallory-Smith, 2005; Dyer, 2017).

Seed heteromorphism in *Aegilops* is supposed to reflect a bet-hedging strategy to cope with variable environments with unpredictable resource availability (Dyer, 2004). Germination delay for some of the seeds increases the chances that at least some offspring survive, reducing also the competition among sibling seedlings (Nave *et al.*, 2016). This strategy could be particularly functional in *Aegilops*, considering its very limited dispersal capacity (Dyer, 2004; van Slageren, 1994; Volis, 2016). It has been hypothesized that in *Aegilops* the largest seeds of the dimorphic pair germinate after the first rainfall at the end of summer, or beginning of autumn, whereas the smallest seeds germinate later (Datta *et al.*, 1970; Marañon, 1989), probably during the following spring (Onnis *et al.*, 1995). Consequently, smaller seeds should be able to maintain a high viability for lingering after dispersal than larger ones. Interestingly, the smaller

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seeds of *Ae. triuncialis* remained viable longer during storage under laboratory conditions compared with larger seeds (Dyer, 2017) and similar patterns were found in dimorphic seeds of *Dasypyrum villosum* (L.) P. (De Pace *et al.*, 2011), suggesting that different germination patterns across seed morphs could reflect different seed longevities, i.e. with small seeds being inherently longer-lived than large seeds, since they have to persist longer in the soil before germination. Despite this, little is known about differences of longevity across seed morphs in *Aegilops*.

The longevity of seeds is an important plant trait, allowing not only seed persistence in the soil but also long-term *ex situ* plant conservation in germplasm banks (Walters *et al.*, 2005; Probert *et al.*, 2009). In orthodox seeds, longevity is mainly determined by seed moisture content and temperature during storage. By experimentally increasing these two factors, it is possible to accelerate ageing processes in the laboratory, thereby estimating seed longevity from survival curves (Ellis and Roberts, 1980) and predicting with caution seed persistence in the field (Long *et al.*, 2008). Understanding seed longevity differences among species (see e.g. Walters *et al.*, 2005; Probert *et al.*, 2009; Mondoni *et al.*; 2011; Bernareggi *et al.*, 2015; Davies *et al.*, 2016) is crucial for the management of *ex situ* seed conservation facilities, as it allows to plan re-collection or define regeneration intervals (Walters, 2003).

Ex situ seed conservation is particularly important for crop wild relatives (CWR) whose collections should be representative of the largest possible genetic variability, in order to safeguard it and provide useful traits for crop improvement (Warschefsky *et al.*, 2014). *Aegilops* forms part of the secondary gene pool of wheat, playing a key role in wheat evolution and domestication (van Slageren, 1994) and being extensively used in breeding programmes (Maxted *et al.*, 2008; Kilian *et al.*, 2011). Furthermore, some *Aegilops* species are endangered (e.g. *Ae. uniaristata* Vis., *Ae. biuncialis* Vis. and *Ae. tauschii* Coss.; Bilz *et al.*, 2011; Rossi *et al.*, 2013), underlining the need for an effective *ex situ* conservation of this genus. Therefore, a better understanding of the ecology of these wheat wild relatives is essential for their exploitation in breeding programmes and for their conservation (McCouch *et al.*, 2013; Dempewolf *et al.*, 2014).

The objective of this study was to characterize germination and longevity of heteromorphic seeds in different *Aegilops* species, integrating field and laboratory investigations. In particular, we aimed to: (1) estimate the longevity of different seed morphs in six *Aegilops* species using controlled ageing tests (CAT), and (2) investigate germination phenology in seed morphs of *Ae. neglecta* under laboratory and field conditions.

Materials and methods

Study material

Dispersal units of all *Aegilops* species were collected in 2015 at the time of natural dispersal (May–June) in nature, except those of *Ae. biuncialis* that were collected from a first generation population cultivated in Cyprus (Table 1). After collection, seeds were extracted from the dispersal units. For the *Aegilops* species in which the whole spike is the dispersal unit and therefore spikelets do not disarticulate at maturity (i.e. *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrina* (Hack.) Maire and Weiller and *Ae. uniaristata*), the basal fertile spikelet was separated from the upper ones and the dimorphic seed pairs extracted from it. After seed cleaning, seeds were kept at 15% relative humidity (RH) and 15°C (ISTA, 2018) until use. Germination phenology of *Ae. neglecta* both in the laboratory and in the field were studied using fresh seeds, re-collected from the same population in July 2016 and immediately sown. Seed mass was determined by weighing 20 individual seeds, kept at 15% RH, randomly sampled from each seed lot, using an analytical balance (Kern ALJ 220-4 nm).

Ageing experiments and longevity assessment

Seed longevity was estimated using a modified protocol for accelerated ageing tests (Newton *et al.*, 2009). Ageing experiments started in April 2016 and lasted for 6 months. Prior to storage, seeds were first rehydrated for 14 days in open Petri dishes over a non-saturated solution of LiCl in a sealed 300 × 300 × 130 mm sealed electrical enclosure box (Ensto UK Ltd, Southampton, UK) at 47% RH and 20°C. Thereafter, seed equilibrium relative humidity (eRH) was verified with an AW-DI0 water activity probe used in conjunction with a HygroPalm 3 display unit (Rotronic Instruments UK Ltd, Crawley, UK). Once the seed lot was judged to have reached eRH, the initial germination was assessed using triplicates of 20 seeds using the protocol described in the next section. Next, seed lots were stored in sealed box over a non-saturated LiCl solution at 60% RH at 45 ± 2°C in the dark placed in a compact incubator (Binder FD53, Binder GmbH, Tuttlingen, Germany). The RH generated by the LiCl solution in the box was monitored with a data logger inside the enclosure box (Tinytag View 2 Temperature/Relative Humidity Logger, Gemini Data Logger, Chichester, UK). When necessary, the bulk solution was adjusted by adding distilled water, stirring and allowing the solution to equilibrate (Hay *et al.*, 2008; Newton *et al.*, 2009). At three to four intervals during storage, tri-replicates of 20 seeds were removed to assess viability using germination. Different seed lots were exposed to different periods of storage according to their lifespan so that seeds could experience a complete loss of viability.

Seed germination in the laboratory

Triplicates of 20 seeds each per lot were sown on 1% agar in 50 mm diameter Petri dishes that were placed, randomly interspersed, in an alternating temperature (20/10°C) and 12 h daily photoperiod (photosynthetically active radiation 40–50 µmol m^{-2} s^{-1} ; LMS 250A, LMS Ltd, Sevenoaks, UK; Guzzon *et al.*, 2015). Germination was scored twice a day when the emerging radicle had reached approximately 2 mm. At the completion of each germination test (4 weeks after sowing), ungerminated seeds were cut to confirm whether they were empty. Final germination percentages were adjusted based the number of filled seeds.

Seed germination in the field

A triplicate of 70 spikes from *Aegilops neglecta* was sown in July 2016 at the original collection site (Table 1). One plot (50 × 60 cm) per each replicate was prepared by removing the vegetation and flattening the soil. The spikes were inserted in the soil surface to simulate natural dispersal (Perrino *et al.*, 2014). Seedling emergence from each spikelet was recorded every 2 weeks for the first 3 months and thereafter, once a month, for the following 11 months until the end of September 2017. Only the emergence of the dimorphic seed pairs of the basal fertile spikelet was recorded. One year after sowing, 20 spikelets per

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Table 1. Geographical locations of the sampled populations and type of the six *Aegilops* species

Species	Country	Latitude	Longitude	Section	DU	Biological status	Seed lots
Ae. cylindrica	Italy	N 44° 57′ 25.02″	E 9° 11′ 53.88″	*Cylindropyrum*	Spikelet	Wild	a/b/s
Ae. biuncialis	Cyprus	N 34° 55′ 55.96″	E 32° 21′ 01.8″	*Aegilops*	Spike	Cultivated	1/2/3
Ae. geniculata	Italy	N 44° 57′ 25.02″	E 9° 11′ 53.88″	*Aegilops*	Spike	Wild	1a/1b
Ae. peregrina	Cyprus	N 34° 43′ 53.69″	E 33° 21′ 01.8″	*Aegilops*	Spike	Wild	1a/1b/2a
Ae. neglecta	Italy	N 44° 48′ 26.6″	E 8° 54′ 16.7″	*Aegilops*	Spike	Wild	1a/1b/2a
Ae. uniaristata	Italy	N 40° 37′ 0.73″	E 16° 58′ 9.08″	*Comopyrum*	Spike	Wild	1a/1b

The type of dispersal unit (DU) is indicated. The seed lots extracted in each species by the DU are also provided.

replicate were retrieved and the viability of ungerminated seeds was assessed using a germination test in the laboratory as described above. Data loggers recording temperature and RH were placed at the soil surface (WatchDog data logger, Spectrum Technologies, Inc.) and buried 2 cm deep in the soil (Supplementary Figure 1).

Statistical analysis

Non-parametric Kruskal–Wallis tests were applied to evaluate if the difference in seed masses was significant among different species and if the mass of the different seed lots varied significantly within species.

To estimate p_{50}, as measure of seed longevity, probit analysis of the seed viability data was carried out using GenStat for Windows, version 9 (VSN International Ltd, Oxford, UK) by fitting the seed viability equation:

$$v = K_i - p/\sigma,$$

where v is the viability in normal equivalent deviates (NED) at time p (days); K_i is the initial viability or the intercept on the y-axis (NED), and σ is the standard deviation of the normal distribution of seed deaths in time. Previous studies have highlighted that in some *Aegilops* species the dimorphic seed pairs extracted from the spikelet show differences in the germination timing, with larger seeds germinating faster than the smaller ones (Datta *et al*., 1970; Marañon 1989). To test this observation, differences in germination timing (mean time to germinate, MTG) were recorded of fresh seeds (i.e. not exposed to CAT). The mean-time to germination MTG was calculated as:

$$\mathrm{MTG} = \Sigma(\mathrm{nT})/N,$$

where n is the number of seeds that germinated within consecutive intervals of time, T is the time between the beginning of the test and the end of a particular interval of measurement, and N is the total number of seeds that germinated. The MTG was calculated using the hour of sowing as initial time.

ANOVA was used to test the significance of the differences, in terms of p_{50} and MGT detected among the different seed lots and species. Bonferroni *post-hoc* tests were used to pairwise compare the differences in p_{50} and MTG between seed lots of the species with three seed lots. Data were checked for normality and homogeneity of variance to meet the ANOVA assumption. To this end, MGT data were log-transformed. The F-test was used to determine whether there was a significant increase in residual deviance by constraining seed survival curves for reduced seed number and standard longevity test data to a single slope (σ^{-1}) and intercept (K_i). Seed germination was modelled using a binomial probability distribution and a logit link function by means of a generalized linear model (GLM). The Kruskal–Wallis tests, GLMs and ANOVAs were performed using SPSS for Windows, version 21.0.

Results

Seed heteromorphy

Based on the morphology of the spike and the presence/absence of seed dimorphism within-spikelet, the six species tested in the current experiment were divided into three groups (Fig. 1), and the seed morphs identified were considered as different seed lots during the germination experiment:

(1) In *Ae. geniculata*, *Ae. peregrina*, *Ae. neglecta* and *Ae. uniaristata*, a clear seed dimorphism was observed in the seed pair of the basal fertile spikelet. The larger and brighter coloured (here after referred as 1a) and the smaller and darker (hereafter referred to as 1b) seeds of the seed pairs, were considered as different seed lots during the ageing experiment (Table 1, Fig. 1). In *Ae. peregrina* and *Ae. neglecta*, an additional seed lot was considered (hereafter referred as 2a), consisting of one seed of the second fertile spikelet (only the larger and brighter in case of a dimorphic pair; Table 1, Fig. 1).

(2) *Ae. biuncialis* shows a similar spike morphology to the aforementioned species, although in our accession no clear size and colour dimorphism could be noticed between seeds from the same spikelet. Therefore, three seed lots were considered for *Ae. biuncialis* including seeds from the first (basal) fertile spikelet, seeds from the second fertile spikelet and seeds from the third fertile spikelet (hereafter referred as 1, 2 and 3; Table 1, Fig. 1).

(3) In *Ae. cylindrica* the spike disarticulates at maturity into several (6–8) spikelets, therefore the spikelet serves as the dispersal unit (van Slageren, 1994). Three seed lots were considered for this latter species, including the larger (a) and smaller (b) seed of the dimorphic pair inside the spikelet and the solitary seed (s) when there was only one seed inside the spikelet (Table 1, Fig. 1). The dimorphic pair in *Ae. cylindrica* showed only size dimorphism but no colour differences.

Larger seeds from the dimorphic pairs are defined as 'primary seeds' (seed lots 1a, a) while the smaller ones are 'secondary seeds' (seed lots 1b, b). Example pictures and X-ray scans of the spikes and pictures of the seed morphs are provided in Fig. 2.

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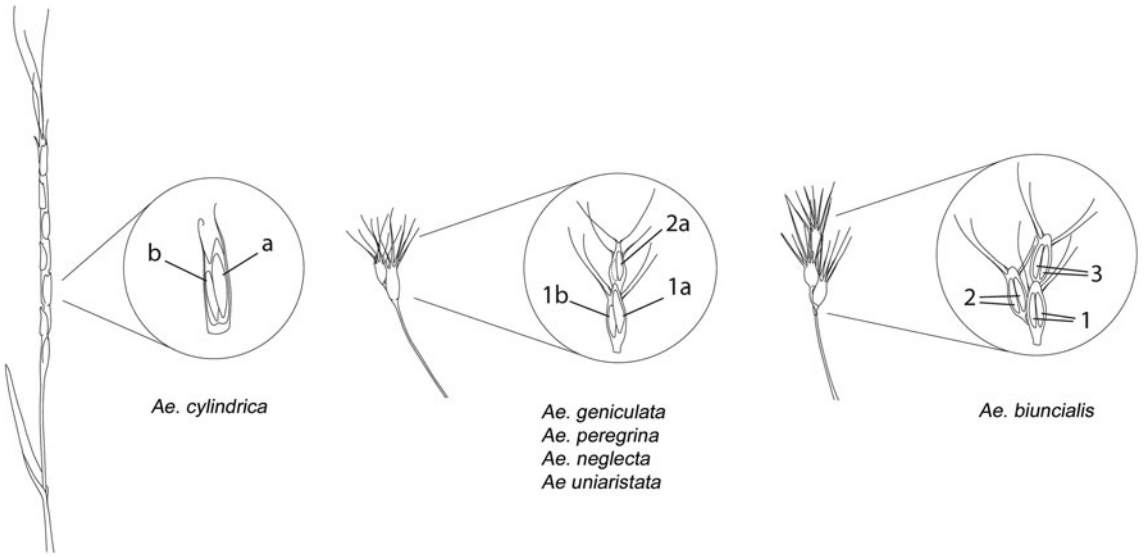


Fig. 1. Schemes of the spikes and internal spikelet morphologies (in the insets) encountered in the tested *Aegilops* species. On the left, a spike and a spikelet of *Ae. cylindrica* with the larger (a) and smaller (b) seeds. In the middle, the spike and spikelet morphology of *Ae. geniculata*, *Ae. uniaristata*, *Ae. peregrina* and *Ae. neglecta*. First fertile spikelet had two dimorphic seeds: one larger (1a) and one smaller (1b). In *Ae. peregrina* and *Ae. neglecta* another seed lot was considered (2a) consisting of solitary seeds of the second fertile spikelet or larger seeds in case of the presence of a dimorphic pair also in the second spikelet (smaller seed in dashed line). On the right, spike morphology of *Ae. biuncialis*, a species that does not show within-spikelet seed dimorphism. Three seed lots were considered, seeds of the basal fertile spikelet (1), seed of the second fertile spikelet (2), and seeds of the third fertile spikelet (3).

Seed mass

Seed mass varied significantly both among species (Kruskal–Wallis, $P < 0.001$) and within all the species among seed lots ($P < 0.001$). Hence in all the species showing dimorphic seed pairs (e.g. *Ae. cylindrica*, *Ae. geniculata*, *Ae. neglecta*, *Ae peregrina* and Ae. *uniaristata*; Fig. 1) primary seeds (a, 1a) were significantly heavier than secondary (b, 1b; Table 2; $P < 0.05$). In *Ae. neglecta*, seed lot 2a was lighter than both 1a and 1b ($P < 0.001$), while in *Ae. peregrina* 2a was lighter than 1a ($P = 0.020$) and not significantly different from 1b (Table 2; $P = 0.159$). The seed lot s of *Ae. cylindrica* was significantly lighter than a ($P < 0.001$) but not different in seed mass from b ($P = 0.779$). Finally in *Ae. biuncialis*, the only tested species not showing within spikelet dimorphism (Fig. 1), seeds 1 were lighter than 2 and 3 ($P < 0.001$) while seed lots 2 and 3 did not different significantly between them (Table 2; $P = 0.799$).

Seed longevity

The estimated longevity of seeds (p_{50}) ranged from 22.74 ± 2.803 days (*Ae. biuncialis* 2) to 85.72 ± 2.203 days (*Ae. neglecta* 1b; Table 2). Species and seed lot had a significant effect on the p_{50} (d.f. = 5; $F = 11.413$; $P < 0.001$ and d.f. = 8; $F = 11.078$; $P < 0.001$). Seed longevity was not statistically different among seed lots in *Ae. peregrina* ($P = 0.284$), as well as between seed lot 3 and both 1 ($P = 0.084$) and 2 ($P = 0.186$) in *Ae. biuncialis*. In *Ae. geniculata*, *Ae. neglecta* and *Ae. uniaristata*, the secondary seeds (1b) of the second fertile spikelets were always longer lived than the primary ones (1a) ($P < 0.05$), although to a different extent across species. In particular, estimated secondary seeds (1b) longevity was 1.4- to 1.9-fold greater than primary ones (1a) for *Ae. geniculata*, *Ae. neglecta* and *Ae. uniaristata*. Similarly, in *Ae. cylindrica* the estimated longevity of the secondary seed lot (b) was significantly ($P < 0.001$) 1.5-fold greater than the primary seed lot (a). At the same time, the estimated seed longevity of the solitary seeds (s) was intermediate between that of seed lot a ($P = 0.026$) and b ($P = 0.001$). Similarly, in *Ae. neglecta* p_{50} of seed lot 2a was intermediate between that of 1a and 1b ($P < 0.001$) (Fig. 3, Table 2).

Within each species, the slopes (σ^{-1}) of the survival curves across seed lots were not significantly different ($P > 0.05$). On the other hand, differences in the intercept (K_i) of the survival curves were significant ($P < 0.05$) in all the pairwise comparison, except in *Ae neglecta* 1a and 2a ($F_{1,3}$=3.655), *Ae biuncialis* 1 and 3 ($F_{1,3}$=5.988), *Ae biuncialis* 2 and 3 ($F_{1,3}$=2.422) and in all pairwise comparisons between seed lots in *Ae. peregrina* (1a and 1b, $F_{1,3}$=3.693; 1a and 2a, $F_{1,3}$=0.948; 1b and 2a, $F_{1,3}$=1.927).

Seed germination in the laboratory

Under laboratory conditions, seed morphs of all species showed high germination percentage (on average >90%), without significant differences across both, species (d.f. = 5, Wald chi-square = 5.342, $P = 0.376$) and seed morphs (d.f. = 8, Wald chi-square = 12.603, $P = 0.126$). Mean time to germinate (MTG) was significantly different across species and seed lots (d.f. = 5; $F = 2.457$; $P < 0.05$ and d.f. = 8; $F = 7.330$; $P < 0.001$).). In *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrina* and *Ae. uniaristata*, the MGT was significantly greater (1.6, 2.3, 1.7 and 1.5-fold respectively) in the secondary seeds (1b) of the first spikelet then in the primary ones (1a) ($P < 0.05$). In *Ae. peregrina* the MTG of seed lot 2a was similar to both those showed by seed lots 1a ($P = 0.196$) and 1b ($P = 0.266$), while in *Ae. neglecta* seed lot 2a germinated significantly faster than seed lot 1b ($P < 0.05$), but similarly to seed lot 1a ($P = 0.195$). Finally, no significant differences were observed in the MTG within seed lots in *Ae. cylindrica* (a,b,s) and *Ae. biuncialis* (1,2,3) ($P > 0.05$) (Table 2). Supplementary Figure 2 outlines germination progress curves among the different seed lots.

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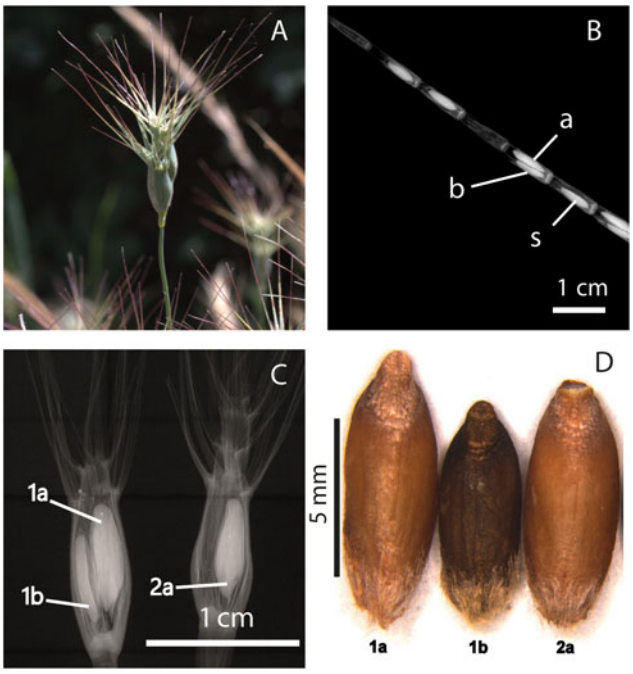


Fig. 2. (A) Spike of *Ae. geniculata* in the field. (B) X-ray scan of a spike of *Ae. cylindrica*. Different spikelets can be observed enclosing either one seed (seed lot s) or a dimorphic seed pair with one seed (seed lot a) larger than the other (seed lot b). (C) X-ray scan of a basal fertile spikelet of *Ae. geniculata* (on the left); the bigger (1a) and the smaller seed (1b) of the dimorphic seed pair can be observed. Moreover, the 2a seed of the second fertile spikelet (on the right) can also be observed. 2a seed lot was not considered for *Ae. geniculata* in the experiment, but was tested in *Ae. neglecta* and *Ae. peregrina*, species with a similar spike morphology to *Ae. geniculata*. The X-ray scanning was performed with a Faxitron X-ray scanner at the Millennium Seed Bank of the Royal Botanic Gardens, Kew. (D) Seeds extracted from the same spike and belonging to seed lots 1a, 1b and 2a of *Ae. neglecta*. The picture was taken using a microscope (Zeiss Stemi SV 11) with camera (Zeiss AxioCam). All the pictures were taken by F. Guzzon and edited by M. Canella (University of Padua).

Seed germination in the field

Ten weeks after sowing (and few days after the first rains; end of September 2016) 41.43 ± 14.8% of the primary seeds of the first basal fertile spikelets (seed lot 1a) of *Ae. neglecta* had emerged (Fig. 4). Two weeks later, 90 ± 8.5% of the primary seeds emerged. Moreover, 1 year after sowing (July 2017) the secondary seeds of the first basal fertile spikelets (seed lot 1b) were all still ungerminated. However, by that time 100% of a subsample of them, retrieved from the soil and extracted from the spikes, could germinate in 3 days under laboratory conditions. Finally, germination of seeds left in the field within the spikes was complete (100%) in September 2017, 14 months after sowing and 1 year after the emergence of primary seeds (Fig. 4).

Discussion

In this study we estimated the longevity of heteromorphic seeds in six different species of *Aegilops*. Our aim was to clarify longevity across species and seed morphs of the same dispersal units, thereby providing novel insights into the germination patterns of *Aegilops*. Results from controlled ageing tests (CAT) were implemented with a field germination experiment on *Ae. neglecta* to better understand emergence phenology and its relation to seed lifespan.

All species studied here, except *Ae. biuncialis*, showed within-spikelet seed dimorphism, with primary seeds being always significantly heavier than secondary seeds. At the same time, seeds from different spikelets also differed in their mass. The seed heteromorphy detected in the study accessions is consistent with the findings of Datta *et al.* (1970), Marañon (1989) and Dyer (2017) in *Ae. geniculata*, *Ae. neglecta* and *Ae. triuncialis*, suggesting that a significant difference in the mass of the different seed morphs produced by the same spike and especially the presence of dimorphic seed pairs is a widespread characteristic in the genus *Aegilops*.

Dimorphic seeds studied here reflected differences in both germination and longevity. In particular, the germination of seeds in the laboratory was high (on average >90%) and quick (i.e. with MGT ranging from 44 to 112 hours; Table 2) indicating the absence of dormancy mechanisms when seeds are extracted from their dispersal units. At the same time, the secondary seeds (1b) of the dimorphic pair showed a delayed germination compared with the primary seeds (1a), i.e. in *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrina* and *Ae. uniaristata*, confirming previous observations (Datta *et al.*, 1970; Marañon, 1989). However, in *Ae. cylindrica* the MTG was similar between the dimorphic seed pair, underlining that different germination patterns may exist across species in *Aegilops*. A delayed germination in the smaller seeds of several *Aegilops* has also been observed in natural conditions (i.e. with seeds enclosed in the dispersal unit) and explained by the influences of both maternal and sibling factors, through the presence of an inhibitor in the glumes, internal levels of gibberellins or competition for essential metabolites among sibling seeds (Lavie *et al.*, 1974; Wurzburger and Leshem, 1967; Wurzburger and Leshem, 1969; Wurzburger and Koller, 1973; Dyer, 2004). However, the implications of such constrains on the emergence phenology in nature were not well understood. Here we have shown that differences in MTG between dimorphic pairs of *Ae. neglecta* were exacerbated under field experiments, with primary seeds (1a) emerging soon after dispersal (i.e. 3 months later, in autumn, after the first rains) and secondary seeds (1b) germinating 14 months after dispersal (Fig. 1), indicating the possibility of dormancy mechanisms in the secondary seeds imposed by the spikelet (Datta *et al.*, 1970). The dormancy of the secondary seed may be released when the primary, larger seed is not present any more in the dispersal unit and the effects of the water-soluble inhibitor have been weakened by several wet and dry periods (Dyer, 2004). Nevertheless, further studies are needed to clarify the dormancy of *Aegilops* secondary seeds when enclosed in the dispersal units, since other explanations of this phenology (e.g. different thermal requirement for the germination of the different seed morphs) cannot be completely ruled out. The delay in the germination of the secondary seeds in the field is an interesting and confirmatory observation, outlining that the shift in the germination timing between seed morphs in nature spreads the seedling emergence over two consecutive years.

Supporting a previous observation that CAT holds a great potential to predict seed persistence in soil seed banks (Long *et al.*, 2008), our results show that seed longevity of the different seed morphs reflected their soil seed bank persistence in *Ae. neglecta*. In this species, primary seeds (1a) were significantly shorter lived than secondary seeds (1b) from the same spikelets and, consistently, the former germinated much sooner after dispersal than the latter (see above). Consequently, it appears that the higher resilience to ageing of the secondary seeds detected in *Ae. neglecta* may be needed to maintain high viability after

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Table 2. Values (mean ± standard error) of seed mass, parameters obtained through fitting of the basic seed viability equation (p_{50}, K_i, σ^{-1}) and the initial MTG (before the accelerated ageing treatment) for each seed lot

Species	Seed lot	Mass (mg)	p_{50} (days)	K_i (NEDs)	σ^{-1} (days^{-1})	MTG (h)	Time points (days)
Ae. biuncialis	1	6.56 ± 0.12	36 ± 2.4	1.882 ± 0.272	0.052 ± 0.006	58 ± 3	0, 17, 44, 75
	2	11.87 ± 0.20	22.7 ± 2.8	1.230 ± 0.277	0.054 ± 0.009	61 ± 3	0, 17, 44, 75
	3	11.51 ± 0.14	28.2 ± 2.4	1.620 ± 0.283	0.057 ± 0.008	58 ± 1	0, 17, 44, 75
Ae. cylindrica	a	17.91 ± 0.15	41.7 ± 2.4	2.136 ± 0.280	0.051 ± 0.006	74 ± 2	0, 17, 44, 75
	b	10.67 ± 0.06	79.8 ± 2.4	4.229 ± 0.513	0.053 ± 0.006	64 ± 2	0, 17, 44, 75, 102
	s	11.88 ± 0.24	54.1 ± 2.2	3.639 ± 0.450	0.067 ± 0.008	61 ± 4	0, 17, 44, 75
Ae. geniculata	1a	14.76 ± 0.16	32.7 ± 1.1	3.968 ± 0.475	0.121 ± 0.014	47 ± 1	0, 17, 30, 44, 75
	1b	7.54 ± 0.11	62.3 ± 1.5	9.04 ± 1.08	0.145 ± 0.017	75 ± 5	0, 30, 51, 75
Ae. peregrina	1a	11.86 ± 0.17	46.8 ± 3.3	2.210 ± 0.277	0.047 ± 0.006	62 ± 1	0, 17, 30, 75
	1b	4.6 ± 0.08	60.2 ± 4.4	1.951 ± 0.334	0.032 ± 0.005	105 ± 8	0, 30, 75, 102
	2a	5.8 ± 0.30	50.2 ± 2.9	2.567 ± 0.394	0.051 ± 0.007	81 ± 3	0, 30,75, 102
Ae. neglecta	1a	30.12 ± 0.18	51 ± 2.6	3.458 ± 0.364	0.068 ± 0.007	48 ± 0.4	0, 17, 30, 75
	1b	13.33 ± 0.12	85.7 ± 2.2	5.063 ± 0.595	0.059 ± 0.007	112 ± 7	0, 30, 51, 75, 102
	2a	9.94 ± 0.26	59.26 ± 2.	4.094 ± 0.609	0.069 ± 0.01	65 ± 4	0, 17, 51, 75
Ae. uniaristata	1a	14.72 ± 0.14	36.4 ± 1.3	4.847 ± 0.578	0.133 ± 0.016	44 ± 0.1	0, 17, 30, 51, 75
	1b	6.16 ± 0.12	51.7 ± 1.6	4.888 ± 0.652	0.094 ± 0.013	68 ± 1	0, 30, 51, 75

The experimental time points at which seed viability was tested are also indicated.

dispersal to fulfil germination phenology. Additionally, differences in p_{50} across seed morphs could be linked to differences in their initial seed viabilities (K_i) and not to differences in the deterioration rate (σ^{-1}) (Fig. 3). This strategy allows secondary seeds to retain full viability during the initial stages of ageing for a longer time compared with primary ones, both under CAT and in the field. Indeed, results from field experiments in *Ae. neglecta* showed that longer-lived secondary seeds retained the full viability for over 1 year after sowing, with 100% of them emerging 14 months later. The same dimorphism in seed longevity was shown in all other species with within-spikelet dimorphism (except *Ae. peregrina*), indicating that a similar germination phenology should be expected also in these taxa. The delayed germination and high resilience to ageing showed by secondary seeds reinforce the hypothesis of a bet-hedging strategy in the germination ecology of *Aegilops* (Dyer, 2004). Supporting this view, seed heteromorphy has been associated with a bet-hedging germination strategy in several plant genera (Silvertown, 1984; Imbert, 2002).

A major question is whether the mechanisms of seed ageing under CAT resemble those of the cool dry conditions of seed banks (i.e. 15% RH, –20°C), rather than those occurring in the wild (Long *et al.*, 2008). Despite the fact that we could not investigate the biochemical process of ageing and the exact seed water content, the air humidity the spikes of *Ae. neglecta* experienced at the soil surface after dispersal was (on average) similar to that used in CAT (60% RH) for most of the time (i.e. 50–70% for 10 months, December to September), although temperatures were much lower (i.e. <15°C for 6 months, then between 20 and 27°C) (Supplementary Figure 1). In this regard, longevity of seeds at RH <70% is increased at lower temperatures, explaining the high survival 1 year after sowing of the secondary seeds. Temperature enhances the rate of deteriorative reactions occurring in seeds. In particular, above a critical temperature (e.g. estimated in approximately 40°C in *Vigna radiate*; Murthy *et al.*, 2003) the solid-like glassy state may soften into the rubbery or even liquid state (i.e. glass transition temperature), enhancing molecular mobility, thereby the deteriorative reactions proceed rapidly (Walters, 1998; Buitink and Leprince, 2008). Consequently, our results indicate that differences in the deterioration process that seeds experience in the wild and under CAT might exist mostly due to temperature differences.

Despite our data reinforcing the hypothesis that in the dimorphic seed pairs of *Aegilops*, the primary and shorter-lived seeds germinate soon after the dispersal, while the secondary and longer-lived seeds create a more persistent soil seed bank (Datta *et al.*, 1970; Marañon, 1989; Onnis *et al.*, 1995), the similar longevity found among seed lots in *Ae. peregrina* indicates that the regeneration strategy may follow different patterns in this species. Additionally, in seed lot 2a (consisting of seeds of the second fertile spikelet; Fig. 1) of *Ae. neglecta* the p_{50} was intermediate to that of primary and secondary seed lots (1a and 1b). Similarly, in *Ae. cylindrica*, seed lots from single-seeded spikelet (s) had an intermediate p_{50} comparable to that of the primary (a) and the secondary (b) seeds from the double-seeded spikelet. Moreover, in *Ae. biuncialis*, even if seed dimorphism was not detected, seeds from different spikelets on the same spikes significantly differed in their p_{50}. Therefore, further studies are needed to understand whether longevity differences across these latter seed morphs may correlate with their germination phenology (and/or vice versa), as shown here for dimorphic pairs of *Ae. neglecta*. Supporting this view, previous studies highlighted some germination differences across a range of seed morphs from different spikelets in *Aegilops* (Datta *et al.*, 1970; Fandrich and Mallory-Smith, 2006; Orsenigo *et al.*, 2017).

Seed longevity guarantees the long-term *ex situ* conservation of plant genetic resources (PGR) within gene banks (Walters *et al.*, 2005). The longevity ranking of seed lots based on data from

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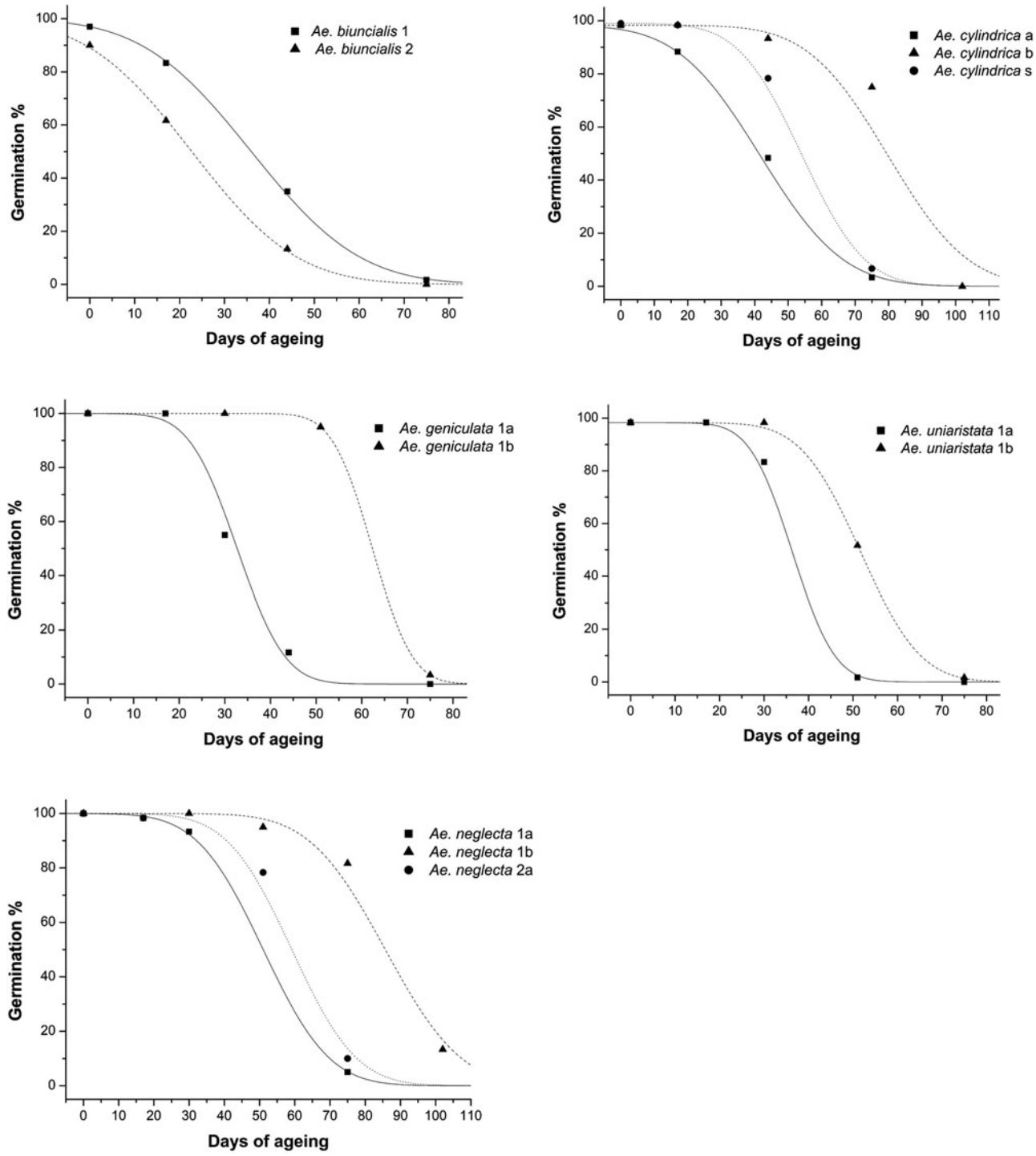


Fig. 3. Survival curves fitted by probit analysis of indicated *Aegilops* species. In *Ae. biuncialis* the curve of the seed lot 3 is not presented as the p_{50} values of this seed lot was not different from seed lots 1 and 2 of the same species (see text). Survival curves of *Ae. peregrina* are not presented as the values of p_{50} for the three seed lots tested for this species (1a, 1b, 2a) were not significantly different (see text).

controlled ageing tests is expected to be similar to that for seed lots in long-term storage, i.e. seed lots that are identified as losing viability quickly in the controlled ageing test will also lose viability quickly during long-term conservation storage (Probert *et al.*, 2009; Hay and Whitehouse, 2017). The significant differences in seed longevity among heteromorphic seeds in most of the *Aegilops* species tested here, suggest that different seed morphs are also likely to have different longevities under *ex situ* seed bank storage, with secondary seeds being longer lived than primary seeds. The fundamental aim of *ex situ* seed collections of CWR is to capture all the possible adaptive traits in the seed samples (Warschefsky *et al.*, 2014). Several differences have been detected at the biochemical, genetic and morphological levels, as well as in abiotic stress resistance among different seed morphs in species of Poaceae (Datta, 1970; De Gara *et al.*, 1991; Cremonini *et al.*, 1994; Frediani *et al.*, 1994; Orsenigo *et al.*,

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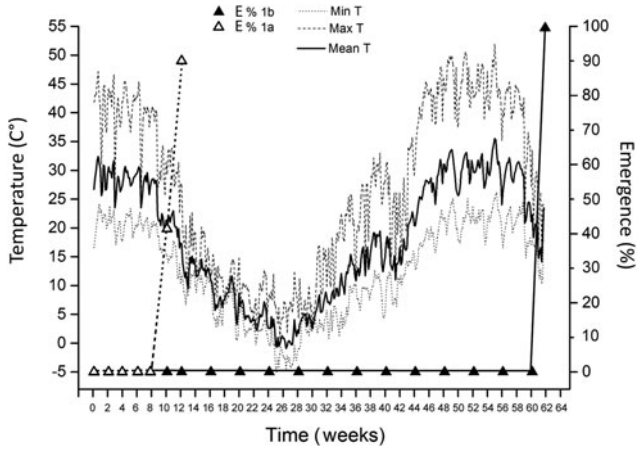


Fig. 4. Percentages of the emergence from primary (1a; white triangles) and secondary (1b; black triangles) seeds of *Ae. neglecta* in the field. Also shown is the mean daily soil temperature (continuous black line), mean minimum soil temperature (dotted line) and mean maximum soil temperature (dashed line).

2017). It follows that different seed morphs of *Aegilops* should be held separately in storage and their viability, regeneration or recollection intervals (Hay and Probert, 2013) should follow different timings, in order to avoid important loss of genetic resources.

Finally, seed longevity in the *Aegilops* species tested here was, on average, longer compared with that of other grasses. For example, across 722 seed collections of Poaceae exposed to CAT, Probert *et al.* (2009) found an average p_{50} value of 26.7 days. Similarly, Mondoni *et al.* (2011) could not find any grasses with a p_{50} higher than 38 days. However, in our study overall p_{50} in *Aegilops* was on average higher (i.e. about 50 days), with some seed lots showing a p_{50} of about 80 days (*Ae. cylindrica* b, *Ae. neglecta* 1b). Thus, the seed lots in our study can be described as having a medium longevity in air-dry storage, based on a logarithmic scale to categorize species according to their relative longevity (Probert *et al.*, 2009; i.e. $p_{50} \leq 1$ day indicates 'very short' seed longevity; p_{50} >1 to ≤10, 'short'; p_{50} >10 to ≤100, 'medium'; p_{50} >100 to ≤1000, 'long'; p_{50} >1000, 'very long'). Such higher seed longevity of *Aegilops* compared with that shown by other Poaceae may be due to specific adaptations of seeds of this genus to warm and dry environments, where seeds need to persist, in a dry state, for long periods between bouts of rainfall sufficient to allow successful establishment (Probert *et al.*, 2009). Supporting this view, seeds produced by species growing in Mediterranean habitats, as the ones in which *Aegilops* thrives, are known to be among the longest lived found worldwide (Merrit *et al.*, 2014).

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S096025851800034X

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