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REGULAR PAPER

Conditional dormancy of *Stipa lagascae* (Poaceae) bulk-harvested on seed increase plots in South Tunisia: a reassessment and a surprise

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Background and aims – With the perspective to reseed degraded drylands, grass seeds are often stocked for several years. This common practice overlooks conditional dormancy and the necessity to preserve it. This paper reports on the germination ecology of *Stipa lagascae* Roem. & Schult., which is a circum-Mediterranean winter-growing bunch grass of high grazing value. However, the published record on its germination ecology is scarce and inconsistent.
Methods – This record was reassessed through a series of germination trials in combination with dormancy breaking treatments on seeds that were mainly harvested on a seed increase plot in South-Tunisia.
Key results –The surprise finding was that *Stipa lagascae* exhibits a particular kind of conditional dormancy for many months after harvest. Whereas dormant seeds barely germinate at 10°C in classical Petri dishes or on germination tables, they germinate massively (but not fully) when allowed full contact with a water-saturated substrate at 7–10°C in boxes. Dehulling provokes fast germination of near 100% of the seeds, thus showing that the substrate effect at low temperatures breaks most but not all dormancy in a particular seed lot. This remaining or residual dormancy is not conditional, as it can only be broken through dehulling. There are thus two distinct germination windows: a very broad one for non-dormant seed and a narrow one for conditionally dormant seed.
Conclusions – A pattern is suggested whereby each seed lot evolves through a continuum from full over conditional to non-dormancy and finally mortality. However, only the state of conditional dormancy times germination optimally with regard to the start of the winter growing season in South-Tunisia. Its ecological significance should be interpreted in combination with its trypanocarpy. Reseeding for restoration purposes and to render grazing value to depleted drylands should thus use conditionally dormant seed.

Keywords – *Stipa lagascae* Roem. & Schult.; conditional dormancy; Type I nondeep physiological dormancy; dry after-ripening; ecological restoration; Mediterranean steppe.

INTRODUCTION

Many dryland reseeding programmes struggle to keep a reliable stock of seeds. Reliable seed will time its germination in the field as a function of the "right" combination of temperature and moisture. One overlooked mechanism to do so is conditional dormancy. A seed lot is said to be in a status of conditional dormancy when seeds are in between full dormancy (no germination under any set of environmental conditions) and loss of dormancy (germination over a wide range of environmental conditions). Under this status of conditional dormancy most seeds germinate only within a narrow range of environmental conditions, to be specified

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through extensive testing. Conditional dormancy is thus a transitory state of each seed that is evolving from full dormancy to no dormancy, during which germination happens over an ever-widening range of environmental conditions (Baskin & Baskin 2004). Conditional dormancy enables a seed to germinate only in very particular circumstances that give the seedling the best chance of survival during the following establishment year (Thompson & Ooi 2010; Baskin & Baskin 2014). Loss of conditional dormancy of a seed lot means it has lost its capacity to time germination appropriately. However, the exact conditions under which conditional dormant seed germinates are rarely taken into account to design routine germination capacity tests, which instead test viability of non-dormant seed. As a result, many dryland reseeding operations use non-dormant seed that has lost its capacity to sense the "right" combination of temperature and rainfall.

Germination ecology of *Stipa lagascae*

This paper reports on the conditional dormancy of *Stipa lagascae* Roem. & Schult., a winter growing C_3-grass of circum-Mediterranean drylands (Freitag 1985). It is the ecological complement of the summer growing C_4-grass *Cenchrus ciliaris* L. (Visser et al. 2008). In North Africa, the presence of a limited number of highly palatable bunch grass species gives permanent grazing value to shrublands dominated by grazing-resistant dwarf shrubs of the Asteraceae (Floret & Pontanier 1982; Jauffret & Visser 2003), but extensive areas have lost these perennial grasses. *Cenchrus ciliaris* has all but disappeared (M'Seddi et al. 2002) and *S. lagascae* is regarded by some as the last not yet extinct palatable perennial grass species of what used to be extensive grasslands bordering the Sahara desert. In South Tunisia, efforts were made to multiply seed of this species. The work underlying this paper was a seed increase programme of a selection of individual genotypes of high grazing value coming from a starter collection that united a high number of clonal lines from many South-Tunisian accessions (Visser & Reheul 2001, 2002).

In the Mediterranean area, seed dispersal of *Stipa lagascae* occurs with the onset of the summer drought in May. The dispersal unit is the caryopsis with the actual seed firmly enclosed by the lemma and palea; hereafter we call this unit a seed. Seeds are exceptionally heavy and long. With a 1000 seed mass of 6.590 g (Neffati 1994), seeds have a far greater mass than seeds of any other grass of the North African steppe. *Stipagrostis ciliata* (Desf.) De Winter comes second with an average 1000 seed mass of 2.533 g (Neffati 1994). Seed length varies between 12 and 17 mm but stays relatively stable within one genotype since 82% of the total variation is due to differences between seed lineages (Visser 2001). The lemma and palea are covered with hairs arranged in distinct rows and pointing toward a very sharp hilum. The lemma carries a long awn that needs to be removed during seed cleaning to allow for seed handling and storage. However, as with all *Stipa* species, in nature the awn is hygroscopically active and plays an important role in active seed burial. This mechanism is called trypanocarpy (Ghermandi 1995). The two distinct parts of the awn combine with the high seed mass, the sharp basis and the hairs to actively bury the seed through a "shaft and drill" effect (Peart 1979, 1981, 1984; Ghermandi 1995; Gasque & García-Fayos 2003; Schöning et al. 2004). Trypanocarpy protects *Stipa* seeds not only from the worst of the summer heat but also from predation (Schöning et al. 2004). It is therefore reasonable to assume that naturally dispersed seeds spend the dry and hot summer months buried in the soil. Moreover, the depth of burial corresponds with the optimal seeding depth of 5 cm as determined in sand containers (Neffati et al. 1992; Neffati 1994; Ouled Belgacem et al. 2006).

Neffati (1994) studied bulk-harvested seeds from wild populations in South-Tunisia and published on temperature profiles and germination as a function of storage conditions (Neffati et al. 1992; Neffati et al. 1996). Neffati (1994) put the temperature optimum for germination at 20°C, as determined on Petri dishes in seed germination chambers, yet the maximum germination percentage did not exceed 75%. Neffati et al. (1996) however stated 15°C as the temperature optimum, and this figure was taken over by Baskin & Baskin (2014) who classified *S. lagascae* seeds as showing "type I nondeep physiological dormancy" (see also Baskin & Baskin 2004). This published temperature optimum is thus for non-dormant seed. Still according to Neffati (1994), at dispersal in May, seeds exhibit a primary dormancy that is progressively lost over the summer period (June–August). Seeds simply stored in the laboratory and incubated on Petri dishes at the optimum of 20°C one, two and three months after harvest (in June, July and August respectively) germinated to 10%, 20% and 75% respectively. Under these storage conditions, germination capacity remains around 60% for up to 20 months after harvest. After that, germination capacity decreases rapidly (to 20% 45 months after harvest), because dormancy loss is followed by loss of viability. Airtight storage improves and maintains germination percentages for a longer time. Dry storage in hermetic containers at low temperatures (5°C) slows down dry after-ripening since germination capacity peaks after 30 months of cold storage only.

Inconsistent reports requiring a reassessment

Up to here, the germination as ruled by dry after-ripening of *S. lagascae* seeds seems rather straightforward and similar to many grass seed dormancy stories (Simpson 1990; Baskin & Baskin 1998). However, there are reports on the germination behaviour of *S. lagascae* (studied with other *Stipa* species) that do not corroborate Neffati's findings. Kigel (1995) classified *S. lagascae*, *S. bromoides* (L.) Dörfl. and *S. capensis* Thunb. among the species whose germination capacities are significantly higher at 10°C than at 25°C. More recently, Hamasha & Hensen (2009) compared the germination behaviour (but not dormancy) of different Jordanian accessions of four *Stipa* species, including *S. lagascae.* When Neffati's data are compared with Kigel (1995) and Hamasha & Hensen (2009), germination of *S. lagascae* shows widely different temperature optima (low, intermediate and high temperatures) and only Neffati (1994) has considered dormancy, without mentioning possible conditional dormancy dynamics. The reported optima also contrast with the repeated observation of spontaneous germination of *S. lagascae* seeds in the field from October onward as soon as the first heavy au-

Table 1 – Chronology of germination trials: timing, conditions, seed specifications and resulting figures (figs 1–5 in this paper).

	Trial				
	1	**2**	**3**	**4**	
Period	October 1997–January 1998	January 1998	February 1998	December 1998	August and December 1999
Treatment	temperature profile of fresh seed compared to old seed	dehulling compared to four different controls followed by germination	stratification for 30–60 days followed by germination at 25°C	harvest, harvest site, seed age	harvest, harvest site, seed age, germination environment
Number of seeds	70 (5 × 14)	20	70 (5 × 14)	15 (L96, L98) or 5 × 14 (CHEN97, CHEN98)	15 (L99) or 5 × 14 (CHEN97, CHEN98)
Number of replicates	4	2	4	4	4
Temperature (°C)	10 to 30 with leaps of 5	alternating 25/10	3.5 or 7 during stratification, then 25	25	10 and 25
Conditions	table	table	cold room or refrigerator followed by table	table	cold room and table
Seed covers	bells	bells	boxes during stratification, then bells	bells	boxes and bells
Origin	CHEN97 and wild	CHEN97	CHEN97	1. CHEN98 2. L98 from Chenchou 3. CHEN97 4. L96 from rainfed collection	1. L99 from rainfed collection 2. CHEN98 3. CHEN97
Seed age at trial (months)	5 to 8	8	9	1. 7 2. 7 3. 19 4. 31	1. 3 and 7 2. 15 and 19 3. 27 and 31
Resulting figure	1	2	3	5	4

tumn rains arrive and when temperatures drop sharply, well below 15–25°C.

This paper reassesses germination and dormancy of *S. lagascae* seed. In particular, it reports on three trials that were run on the same bulk-harvested seed lot of *S. lagascae* with one single but diversified genotypic make-up. A fourth trial combines bulk-harvested and manually harvested seed lots to check findings on the first three trials. These four trials were selected among an extensive series of trials that were run to find a way to break unusually strong dormancy of recently harvested seeds of *S. lagascae* (Delfosse 1998; Visser 2001). Each of these trials was selected because it adds additional light on the issue.

MATERIAL AND METHODS

General methods

Trials were run with germination tables or cold rooms and seeds were incubated in two different setups. The first setup follows the specificity of germination tables under plastic germination bells (10 cm diameter). This setup is the standard in seed certification laboratories and emulates germination on Petri dishes whereby seed is incubated on top of moist filter paper. The second setup allows for a more complete contact between the seed and a moist substrate. We used plastic germination boxes (3 × 16 × 11 cm) with a transparent lid. A filter paper covers the bottom. An accordeon-like pre-folded filter paper comes on top and fills the box. The seeds are placed in the folds (3 cm high) and the folds are compressed so that both sides of each fold are in contact with the seeds. Each time the seeds are checked for germination, tap water is added with the pipette until saturation. These boxes can be laid out on a germination table or stacked in a germination chamber, cold room or refrigerator.

For the setup under germination bells, a row of five bells, each covering 14 seeds, corresponded to one replicate of 70 seeds (exceptions in Trial 2 and 4, see table 1). For the setup with boxes, one box corresponded to one replicate.

Within each germination environment, the replicates were organised in fully randomised blocks. Table 1 compares the details of each trial. On the germination tables, the light regime was the day-and night cycle of each day of the year. Following Neffati (1994), no additional light sources were used. At the end of each trial, any intact remaining seed was tested for viability with the tetrazolium test as described by Moore (1985).

Trial succession

The trials were conducted at the University of Ghent in Belgium, between October 1997 and December 1999 (table 1). The chronology follows a logic where the results of the previous test were used to design the next one, within the limits of seed and germination table availability and taking into account that dormancy was being lost through dry after-ripening as seeds aged. Trials 1 to 3 were carried out with seeds from one bulk harvest (May 1997) of an irrigated seed increase plot of 47 genetically different seed lineages in Chenchou, near Gabes in South Tunisia (described in Visser & Reheul 2002). The seeds to establish this seed increase plot came from 47 selected clonal lines within a rainfed starter collection (described in Visser & Reheul 2001) The ripe inflorescences were bulk-harvested with sickles and then allowed to dry outside. This straw was threshed and manually winnowed in the month that followed the harvest. Seeds were then stored at ambient summer conditions of the laboratory in Tunisia (no air conditioning). We call this seed lot CHEN97.

For Trial 4 we used two types of seed: seed bulk-harvested within the same irrigated seed increase plot in 1997 and 1998 (called CHEN97 and CHEN98) and manually harvested seed of the same 15 clonal lines either in the irrigated seed increase plot (L98) or in the rainfed starter collection (L96 and L99).

TRIAL 1: temperature profile of bulk seed October 1997, January 1998 –The temperature optimum was checked via a comparison between old (harvest 1994 on a wild population, with many genotypes bulked from one accession, stored at ambient laboratory conditions in Tunisia) and recently harvested (harvest 1997 CHEN97, 5–8 months old) seeds on two germination tables under bells. The old seeds came from a stock of straw that was unbeaten, which means that the awns had been kept intact until preparation for the trial. We tested the range from 10 to 30°C with lapses of 5°C.

TRIAL 2: seed dehulling, January 1998 – In many grass seeds, dormancy is related to the hull (lemma and palea). Seed dehulling means taking away lemma and palea. This is only possible after soaking. Since the seed lot of CHEN97 (8 months old) already contained seed with damaged or partially detached seed coats, seed dehulling needed to be compared with different control treatments. All treatments were replicated twice with twenty seeds each:

- seeds with intact seed coat, not soaked (control);
- seeds with intact seed coat, soaked for 12 hours in tap water;
- seeds with intact seed coat, soaked for 12 hours and dehulled;
- seeds with damaged seed coat, not soaked;
- seeds with damaged seed coat, soaked for 12 hours in tap water.

The soaking happened simultaneously. One and the same person dehulled the seeds. Since dehulling of even a small amount of seeds requires several hours of work, the soaked and already dehulled seeds were kept moist on Petri dishes. After dehulling, all the seeds of all treatments were incubated on a germination table under bells. This time, the temperature regime was alternating: 8 hours at 25°C (day) followed by 16 hours at 10°C (night).

TRIAL 3: wet cold, February 1998 – Since *Stipa lagascae* seeds naturally germinate in South-Tunisian autumn conditions from October onward, when the rains arrive and temperatures drop sharply, the question was whether wet cold conditions could trigger germination.

Seeds of CHEN97 (9 months old) were incubated in germination boxes at 7°C in a refrigerator (dark when closed) and at 3.5°C in a cold room (dark when closed). These temperatures were combined with two substrates: filter paper and water-saturated sand. The regime of 7°C was maintained for 30 and 60 days but the regime of 3.5°C was maintained for 30 days only. This makes for six different treatments:

- 60 days, folded filter paper, 7°C;
- 60 days, sand, 7°C;
- 30 days, folded filter paper, 7°C;
- 30 days, sand, 7°C;
- 30 days, folded filter paper, 3.5°C;
- 30 days, sand, 3.5°C.

All treatments ended the same day. That same day, all remaining non-germinated seeds were incubated on a germination table at 25°C under bells.

TRIAL 4: final check, December 1998 and August and December 1999 – To consolidate previous findings, new seed was sourced in May 1998 and 1999 and compared with older references from 1996 and 1997. In December 1998 (at the age of 7 months for L98) L98 was put to germinate together with L96, CHEN97 and CHEN98 at 25°C on one germination table. In August and in December 1999 (at the age of 3 and 7 months for L99), L99 was put to germinate together with CHEN97 and CHEN98 simultaneously in wet cold (10°C in boxes in a cold room) and at 25°C on a germination table.

Data analysis

Germination rates are characterised by both the proportion of seeds that germinates and the speed of germination within the time frame of one particular trial. For each trial and each treatment, and as a basis for the deciding the setup of the next trial, we calculated two parameters: (1) overall germination percentage (number of germinated seeds/total number of seeds, averaged over replicates); (2) mean germination time, expressed as the average number of days needed for a particular seed to germinate, averaged over replicates (Scott et al. 1984).

We focused on germination percentages and used germination times when appropriate to further distinguish treatments. Strictly speaking, these parameters do not obey the assumptions of the normal distribution and arcsine transformation used to be the standard advice (Sokal & Rohlf 1981; Scott et al. 1984; Baskin & Baskin 2014). Arcsine transformed data of Trial 2 and 3 were subjected to one-way ANOVA. Arcsine transformed data of Trial 1 were subjected to two-way ANOVA accounting for interaction between temperature and seed lot.

For Trial 4, overall germination percentage of the August and December 1999 trials were tested for treatment (wet

cold versus 25°C under bells, seed age and the interaction between treatment and seed age. A Mixed Model was used in order to account for harvest and trial-date random effects, as recommended by Fang & Loughin (2012) for binomial data. The model was fitted with logit transformed final germination percentage as the response variable, treatment, seed age (in months) and the interaction between treatment and seed age as the fixed effects, and harvest and trial date as random effects.

Pairs of treatments (in case of a significant one-way ANOVA) or of treatment combinations (in case of a significant two-way ANOVA) were compared using the Tukey Honestly Significant Difference (HSD) test. For graphical output, either Box-plots were chosen over error bar plots, to be more transparent about the variation of within-group errors (Trial 1 and 3) or cumulative germination percentages are shown with error bars per observation date (Trials 2 and 4).

All analysis was carried out with R (R Core Team 2014).

RESULTS

The comparison between the temperature profiles of recently harvested seed versus old seed under bells (fig. 1) suggests a significant optimum at 25°C (as indicated by the Tukey HSD-test following a two-way ANOVA of germination percentage as a function of temperature and seed age) and shows a very highly significant ($p < 0.001$) difference in germination percentage between old and recently harvested seeds at 20°C, 25°C and 30°C, but not ($p > 0.05$) at 10°C and 15°C. The common temperature optimum was decided to be 25°C since only at this temperature the difference in germination time between both seed lots was significant ($p < 0.05$). At this optimum indeed, 88% of the old seeds germinated after 4.5 days on average, whereas only 48% of the recently harvested seeds germinated after 8.0 days on average. Temperatures < 20°C were clearly inhibitory for both old and recently harvested seed.

During Trial 2, dehulled seeds germinated in great numbers on day three and four, and up to 98% in six days on average (fig. 2). All other treatments required more time (10–12 days on average) to finally reach 48–75% germination at day 25. However, because of the low number of seeds per replicate and lack of replicates, the ANOVA on the final germination percentage did not yield a significant F-value ($p > 0.05$). Therefore, an ANOVA on germination percentage at day four was also carried out. A significant F-value was obtained and the Tukey HSD test showed that dehulling significantly ($p < 0.01$) increased early germination (in less than four days) compared to the different controls.

During Trial 3, seeds germinated to 61–78% already during the first two weeks of the stratification phase at 7°C. However, no seeds germinated at 3.5°C (fig. 3). The seeds that had not germinated during stratification were then incubated at 25°C under bells. Few of the non-germinated seeds stratified at 7°C subsequently germinated at 25°C. Conversely, the seeds stratified at 3.5°C germinated to only 24–33%. Tetrazolium tests showed the remaining seeds were mostly

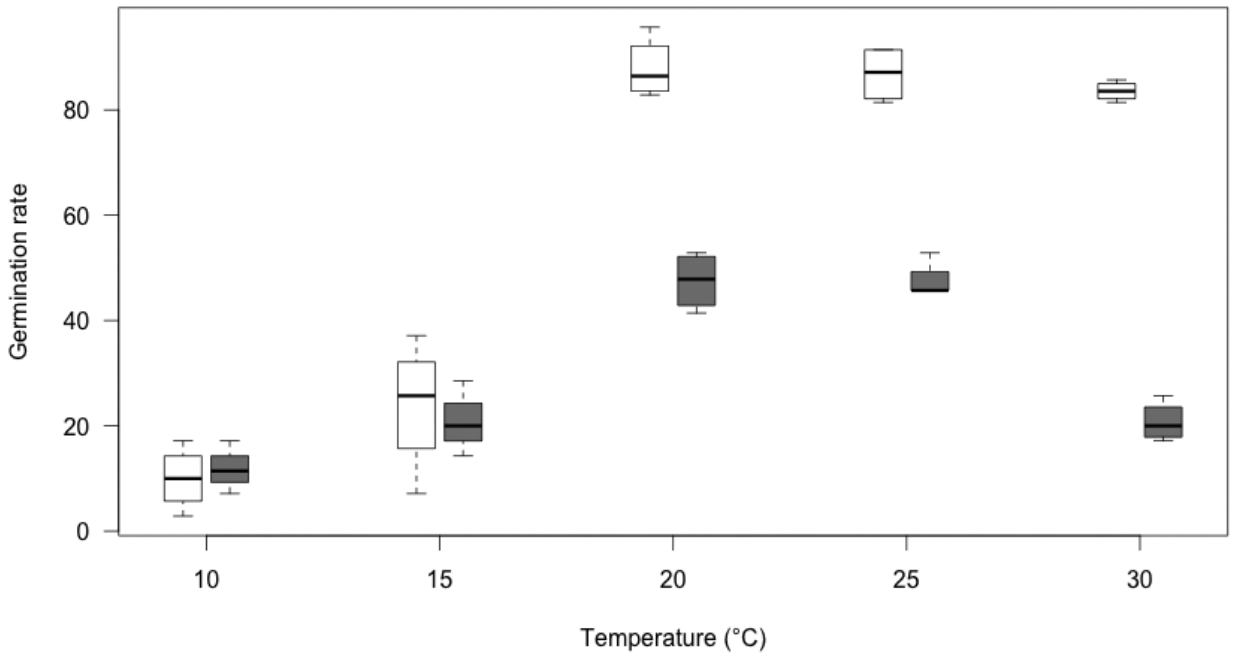

Figure 1 – Trial 1, October 1997–January 1998. Box-plots of final germination rates of 4-years old seeds (white boxes) and 4–6-months old seeds (CHEN97, grey boxes) of *Stipa lagascae* at a range of temperatures under bells.

still viable. Germination percentage of Trial 3 (24–33%) is much less than the 63% obtained by the control of Trial 1.

Trial 4 confirms the stark difference in behaviour of fresh seed put to germinate at the temperature optimum of non-dormant seed (25°C under bells) and in wet cold as emulated by boxes at 10°C (close to the 7°C boxes of Trial 3). The 1999 harvest showed much lower final germination percentages at 25°C under bells (10% and 16% for the August and December trials respectively), than in wet cold (94% and 93%). On the other hand, these differences seemed to fade for older seeds: as compared to young seeds, final germination percentages reached higher levels under bells at 25°C (58–65% for seeds from the 1997 harvest), but lower levels in the boxes at 10°C (59–71%, 1997 harvest).

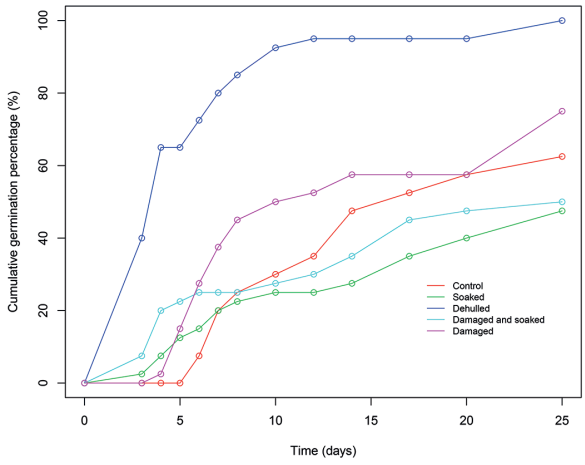

Figure 2 – Trial 2, January 1998. Cumulative germination rates of *Stipa lagascae* seeds (CHEN97, 8-months old) after dehulling compared with several controls (final germination percentage, average germination time): soaked (48%, 12 days); damaged (75%, 11 days), dehulled (98%, 6 days); damaged and soaked (50% and 10 days); control (63% and 12 days). Temperature regime of 25/10°C under bells.

Trial 4 also suggests the shift in conditional dormancy over time, for fresh seed between August and December on the one hand (results not shown), and between different harvests on the other (fig. 4). Significance testing confirms these observed patterns, with seed age having a highly significant effect on seed germination ($p = 0.02091$), and treatment as well as the interaction between seed age and treatment having a very highly significant effect on final germination percentages ($p < 2 \times 10^{-16}$ for both predictors).

The December 1998 testing at 25°C under bells made only non-dormant seed germinate. The non-germinated seeds could be classified as dormant or dead with a tetrazolium test. Figure 5 thus suggests how the proportions of germinating, dormant and dead seed evolve over time. In young seed, conditional dormancy prevents germination at a temperature optimum of 25°C. As seed ages, this conditional dormancy disappears as the proportion of dead seed increases.

DISCUSSION

Because of the inconsistencies between different authors and between the literature and practical observations, the starting point of this paper was to reassess dormancy and germination requirements of *Stipa lagascae* with the perspective of seed increase, storage and use for steppe reseeding and restoration purposes.

Trial 1 points to a common temperature optimum of 25°C under bells but different germination percentages be-

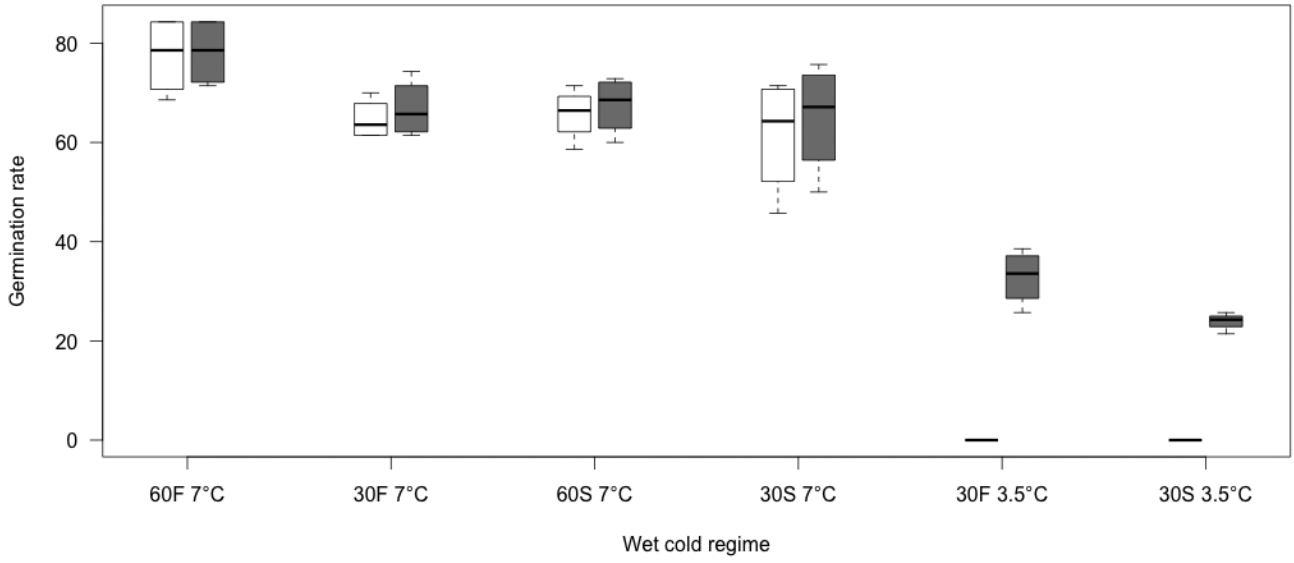

Figure 3 – Trial 3, February 1998. Box plots of final germination rates of *Stipa lagascae* seeds (CHEN97, 9-months old) after six variations of stratification (in white) and subsequently at 25°C under bells (in grey). 30 and 60: number of days, F and S: in filter paper (F) or in sand (S). The number of seeds that germinated at 25°C under bells after the actual stratification were added to those germinated during stratification.

tween 6–8-month old seed and 4-year old seed. Even though three months (the length of the summer) were supposed to be enough for dry after-ripening (Neffati 1994), this comparison shows that only half of the young seed lot germinated at its demonstrated temperature optimum. It can be concluded that the other half of the younger seed lot was still dormant, but how dormant?

Trial 2 shows that dehulling is highly effective in removing all dormancy. With just two replicates of 20 seeds each, dehulling showed that seed dormancy of *S. lagascae* is mainly due to the hull. Since simple soaking does not improve germination (Trial 2), dormancy is probably not related to a leachable chemical component in the hull, unlike what Gasque & García-Fayos (2003) suggested for *S. tenacissima*.

Trial 3 reveals the true conditionality of dormancy of *S. lagascae* seeds. Intermediate between the extremes of Trial 1 (low germination capacity) and 2 (almost 100% capacity), Trial 3 shows a surprise substrate effect that makes most but not all seeds germinate at 7°C. Yet at 3.5°C it induces secondary dormancy. The contrast between 10°C under bells from Trial 1 and 7°C in boxes from Trial 3 was indeed striking. Arguably, this was related to the way the seeds were incubated at low temperatures (in full contact with a moist substrate or not). Yet the confounding factors needed to be checked: the difference between germination in boxes and under bells at 7, 10 and 25°C corresponded also with differences in light regimes (quasi-continuous dark in refrigerator at 7°C versus natural photoperiods under the bells at 10°C and 25°C in Trial 1). These confounding factors were checked by Delfosse (1998) and Visser (2001). The conclusion was there is only a substrate effect within a narrow window from 7 to 10°C and none at 25°C, while the light regime does not make any difference. A supplementary observation in this sense was that seeds incubated under bells at 10°C in Trial 1 hardly imbibed. By contrast, seeds incubated in boxes at 7°C (Trial 3) showed a characteristic progressive swelling followed by the emergence of the radicle.

Trial 4 confirms that this striking difference in germination capacity of young seed is not due to an experimental artefact but due to conditional dormancy: 25°C under bells allows the germination of non-dormant seeds, while 10°C in boxes allows the germination of both non-dormant and conditionally dormant seeds. After the first year, conditional dormancy gives way to non-dormancy, as shown by the coming together of both cumulative germination curves going from L99 over CHEN98 to CHEN97. Of CHEN97, seed germi-

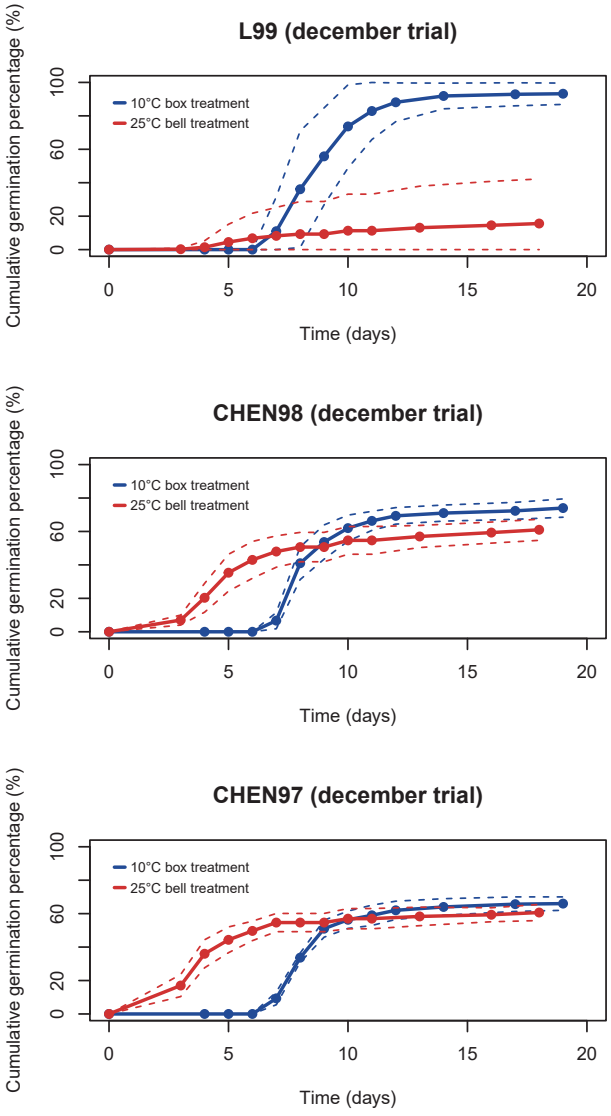

Figure 4 – Trial 4, December 1999. Mean cumulative germination rates of *Stipa lagascae* seed lots in wet cold (10°C in boxes) and at 25°C under bells: fresh seeds (L99, 7-months old) compared with older references CHEN98 and CHEN97. Dashed lines represent standard errors.

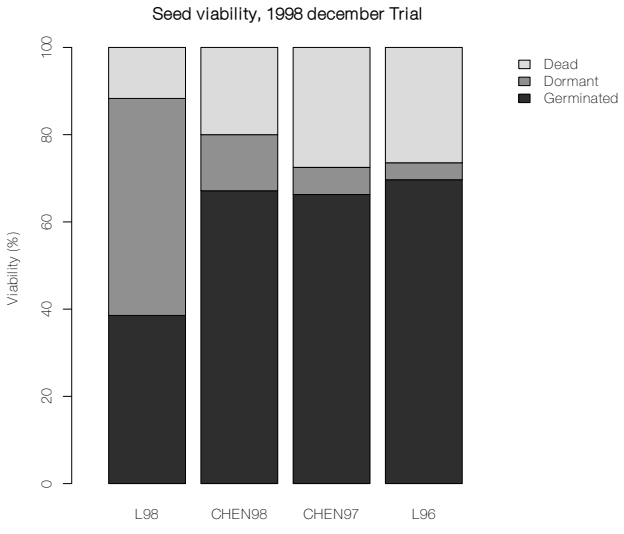

Figure 5 – Trial 4, December 1998. Proportions of dead, dormant and germinated seeds of *Stipa lagascae* as revealed by tetrazolium tests after germination at 25°C under bells: fresh seeds (L98 and CHEN98, 7-months old), compared with older references CHEN97 and L96.

nates in similar proportions under both conditions, meaning that there is no conditional dormancy left.

Trial 4 confirms the shifting nature of this conditional dormancy with age. During the first months after harvest, most but not all primary dormancy gives way to conditional dormancy, and what remains of this primary dormancy is called residual dormancy. Tetrazolium tests (fig. 5) show that most remaining seed after germination in wet cold is viable yet dormant in case the seed is less than one year old (L98, CHEN98) but increasingly dead in case the seed is older (CHEN97 and L96). Interestingly also, L98 shows more dormancy than CHEN98, which suggests that manually harvesting selects the ripest and most dormant seeds of an inflorescence.

The following paragraphs discuss the meaning of these findings and how they fit within what was already known on the germination ecology of *S. lagascae*.

Substrate effect and induced dormancy

The hitherto unreported yet pronounced substrate effect illustrates well the evolving conditionality of dormancy of *S. lagascae*. This substrate effect breaks dormancy of very recently harvested seeds within a very narrow low-temperature window (7–10°C). However, that window widens (seeds germinating within an ever wider temperature range and accepting ever less moisturizing conditions) as dry after-ripening proceeds; hence the specification of "type I nondeep physiological dormancy" according to the typology of Baskin & Baskin (2014). The concept of a germination window, introduced by Baskin & Baskin (1998) and illustrated by Kigel (1995) for *S. capensis*, a widespread Mediterranean winter annual, applies thus very well to the dormancy behaviour of *S. lagascae*.

The most plausible reason why Neffati (1994) and Kigel (1995) found contrasting temperature optima for *S. lagascae* may lie in an unspecified difference in their experimental setups (were the seeds allowed full contact with moisture or not?). Yet both the low and high temperature optima relate to one ecological reality. Low temperatures, indicative of the arrival of the Mediterranean winter season, can break primary dormancy of *S. lagascae*, but only on the condition that seeds are allowed full contact with a water-saturated substrate, which does not happen in a Petri dish or under bells on germination tables. In natural conditions however, seed dispersal leads to the gradual drilling of the seeds into the soil over summer. Full contact with water then happens as soon as rainfall can saturate the soil down to the depth of the seed burial. Indeed, under an arid Mediterranean climate, many rainfall events never wet the soil down to this depth and are thus deemed unsafe from the *Stipa* seed point of view.

So early autumn rains (September, when the soil is still hot) trigger only the non-dormant seeds to germinate and these early autumn conditions (Floret & Pontanier 1982) match the demonstrated temperature optimum of 20–25°C. The dormant seeds can be triggered to germinate by later autumn rains, when temperatures drop sharply, on the condition to become fully immersed (rains being heavy enough), and this matches the substrate effect at 7–10°C. If however the autumn rains fail (a typical feature of Mediterranean drylands near the Sahara) and winter temperatures drop to near (rarely below) 0°C, part of the seed bank could be induced into secondary dormancy, which is what the results of Trial 3 suggest. It would be interesting to study whether co-existing winter-growing Mediterranean grasses with much smaller but similarly awned seeds (from the genus *Stipa* but also *Stipagrostis* and *Aristida*) also show this conditional dormancy and substrate effect to break it.

Very gradual dormancy breakdown and residual dormancy

Neither Neffati (1994) nor Kigel (1995) reported on the gradualness of dormancy breakdown of stored seeds and on the occurrence of residual dormancy. Even if dry summer heat putatively hastens dormancy breakdown in seeds buried in the soil (Baskin & Baskin 2014), in stored seeds dry after-ripening is clearly not complete once the autumn starts. Dry after-ripening continues for many months after harvest, as Trial 4 showed.

The 75% germination percentage Neffati (1994) found 3 months after harvest contrasts strongly with the 48% maximum of 4 to 8-month old bulk-harvested seeds (Trial 1) and the < 20% of 3 to 8-month old manually harvested seeds of individual lines (Trial 4). An explanation should be sought in the very different growing conditions and maturity levels at harvest. First, CHEN97 came indeed from a half-shaded irrigated seed increase plot, whereas the seeds Neffati (1994) used were harvested in unshaded and rainfed conditions. Simpson (1990) stated that, as a general rule, humid and colder conditions during seed maturation increase the extent of dormancy whereas dry and hot conditions decrease the extent of dormancy. Second, primary dormancy is stronger in the first grass seeds in the ear to ripen (Simpson 1990), typically those that are harvested manually as was shown for the young seed of L98 and L99 in Trial 4.

The difference in germination percentages between dehulled seeds (Trial 2) and the intacts seeds in wet cold (Trial 3) showed that, even if it is possible to induce germination at low temperatures (7–10°C) with a substrate effect that allows a full contact between the seed and a moist substrate, not all seeds germinate in these conditions. A small fraction keeps its primary dormancy for a long time. Only removal of the hull induces germination.

As dry after-ripening proceeds, there is a shift from full dormancy (becoming residual dormancy over time) over conditional dormancy to non-dormancy. Once seeds have become non-dormant, the proportion of dead seeds increases while the proportion of residually dormant seeds decreases.

Implications for seed conservation, testing and use in restoration reseedings

Overall, a dynamic dormancy continuum of *S. lagascae* seeds was revealed that can be interpreted as a multi-featured strategy spreading ecological risk in a dry climate where rainfall is highly unpredictable. These patterns are suspected to occur in other valuable but threatened grasses as well (other *Stipa*, or *Stipagrostis* and *Aristida* species) of the steppes of North Africa. Yet the existence of conditional dormancy

such as the one shown for *S. lagascae*, as well as its loss during conventional seed storage did not get attention so far.

Hu et al. (2014) recommended using one-year old non-dormant seeds for reseeding *Stipa bungeana* Trin. ex Bunge in Northwestern China in order to increase the chances to obtain a homogeneous stand. This thinking mirrors old work on dormancy of *Stipa viridula* Trin. in the USA when the variation of dormancy was studied to breed homogeneous low-dormancy varieties (Rogler 1960; Schaaf & Rogler 1960). However, for the purpose of ecological restoration requiring reseeding in hazardous dryland conditions, seeds of *S. lagascae* are best used when in full conditional dormancy (as shown by young seed), so as to emulate its natural risk spreading behaviour. As things stand today, this conditional dormancy is not routinely tested.

It remains to be seen whether it is possible to stock seeds in such a way, for example by dry cold storage in hermetically sealed containers, that conditional dormancy can be conserved for more than one year. If not, seed stocks would need an annual turnover to avoid using non-dormant seeds in hazardous dryland reseeding operations.

Conclusion

Seeds of *S. lagascae* have a particular "type 1 nondeep physiological dormancy" *sensu* Baskin & Baskin (2014) that is mainly related to the hull. In the early stages of dormancy breakdown, the hull can inhibit germination. In standard germination conditions (on germination tables under bells or on Petri dishes), seeds show a wide temperature optimum of 15–25°C, for non-dormant seeds. By contrast, both non-dormant and conditionally dormant seeds of the seed lot are capable of germinating at low temperatures (7–10°C) if and only if these are combined with high substrate moisture (wet cold). Yet even in this wet cold, a small proportion will still not germinate. Only removal of the hull breaks this residual dormancy. The very narrow temperature optimum of conditionally dormant seed contrasts with the broad temperature optimum of non-dormant seed.

Dormancy of *Stipa lagascae* evolves gradually through dry after-ripening but does not fully disappear, and the difference between a dormant and non-dormant seed lot is not clearcut. Rather, each single seed evolves at its own speed over a dormancy continuum, from primary over conditional to maybe induced (or secondary) dormancy and finally non-dormancy. The ecological relevance of this variation is high. Seeds multiplied for ecological restoration purposes should be stored in conditions that conserve conditional dormancy up to the moment of use in the field. This pattern might be revealed among several dryland grasses of North-Africa and merits a particular attention.

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