Seed Science Research (2010) **20**, 237–242
doi:10.1017/S0960258510000218
© *Cambridge University Press 2010*

Combinational dormancy in winter annual *Fabaceae*

Jozef A. Van Assche* and Filip E.A. Vandelook
Laboratory of Plant Ecology, Catholic University Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium

(Received 3 March 2010; accepted after revision 29 June 2010; first published online 30 July 2010)

Abstract

Among 14 species of herbaceous *Fabaceae*, all six winter annuals show a marked non-deep physiological dormancy of the embryo in addition to physical dormancy. This physiological dormancy is apparent at 23°C, but not at lower temperatures of 10°C and 5°C and disappears after 3 months of dry storage. These results corroborate the hypothesis that combinational dormancy is a double safety mechanism for delaying germination during summer: physical dormancy postpones germination, and even in early softened seeds germination is prevented by physiological dormancy of the embryo. Softened, swollen seeds of *Medicago arabica* tolerate a subsequent desiccation and remain viable even after five cycles of dehydration and rehydration. The rate of natural softening of *M. arabica* seeds increases exponentially at higher temperatures, with a Q_{10} between 3.4 and 5.1, and obeys the Arrhenius equation. This indicates that a chemical reaction might be involved in breakdown of physical dormancy. Winter annuals with hard seeds show similar properties as winter annuals with permeable seeds: the need for afterripening and requirement of lower temperatures delay germination until autumn. Only one species, *Vicia sativa*, loses physical dormancy during dry storage. Drying during summer might be a supplementary cue for germination in autumn.

Keywords: afterripening, dehydration, legumes, *Medicago arabica*, physical dormancy

Introduction

Nearly all temperate and arctic species of *Fabaceae* produce seeds with a water-impermeable seed coat that causes physical dormancy. This dormancy can be broken artificially by scarification, by fire or by passage through the digestive tract of animals (reviewed by Baskin and Baskin, 1998).

Germination of the seeds of the Mediterranean species *Trifolium subterraneum* is enhanced by a pretreatment at daily fluctuating temperatures between 30°C and 60°C (e.g. Ballard, 1958; Taylor, 1981). However, it is not known how germination is regulated for most species in ecosystems with a temperate climate where fires occur very rarely. In previous work, we showed that seeds of some *Fabaceae* germinated in spring after a defined temperature treatment of chilling followed by relatively low daily alternating temperatures (Van Assche *et al.*, 2003).

Seeds of most *Fabaceae* remain impermeable during dry storage, but Morrison *et al.* (1992) showed that some Australian species lose physical dormancy during dry storage. Dry storage acts as an environmental cue for germination in several *Geraniaceae* (Van Assche and Vandelook, 2006). This phenomenon might be more common, but it is poorly documented. Timing of germination may be regulated by the presence of a dormant embryo, so that germination does not start immediately after softening or scarification. The presence of both systems is called combinational dormancy (Baskin and Baskin, 2004). Ballard (1958) studied in detail embryo dormancy in *T. subterraneum*, and Thomson (1965) showed its presence in other *Trifolium* species. Expression of embryo dormancy is transitory and temperature dependent. Scarified seeds of *T. subterraneum* do not germinate at 30°C, but they do so readily at 15°C (Katznelson and Carpenter, 1972). Swollen seeds of *Trifolium* sp. germinate to a higher percentage at lower temperature (Thomson, 1965).

The combination of a hard seed coat and a dormant embryo is interpreted as a double safety mechanism to prevent early germination after out-of-season rains in arid regions and Mediterranean climates (Kigel, 1995; Norman *et al.*, 1998). However, it is not known whether combinational dormancy occurs frequently in *Fabaceae* growing in a temperate climate. Furthermore, it has not been demonstrated whether seeds that are swollen after summer rains can survive subsequent drought

*Correspondence
Fax: + 3216321968
Email: jozef.vanassche@bio.kuleuven.be

https://doi.org/10.1017/S0960258510000218 Published online by Cambridge University Press

and delay germination until autumn. As far as we know, only one study deals with this problem: Jansen and Ison (1994) showed that seed viability of *Trifolium* sp. decreased during storage after imbibition and dehydration.

The first aim of this study was to determine if combinational dormancy is frequent in herbaceous *Fabaceae* growing in a temperate climate and if it affects germination time. We compared the germination rate of scarified seeds of 14 species and characterized embryo dormancy by a delay in germination of scarified seeds. As embryo dormancy is described as a transitory phenomenon, we compared fresh seeds and seeds stored for 3 months. The second aim was to analyse temperature effects on softening of the seed coat and on growth of the embryo of *Medicago arabica*, the two distinct processes in germination of hard seeds. The third aim was to determine if swollen seeds of *M. arabica* tolerate desiccation. Finally, we tested the hypothesis that drying might be a possible environmental cue for breaking of dormancy in *Fabaceae*.

Materials and methods

Fourteen species of herbaceous *Fabaceae* were used in this study; six species were winter annuals and eight species had another kind of life cycle (Table 1). Most seeds were collected from one population growing along roadsides near Leuven (Belgium). The following species were grown in a garden: *Lathyrus aphaca* and *M. arabica* were collected in Ambleteuse (northern France) and *Lathyrus nissolia* in Halle (Brabant).

Pods were collected as soon as they were ripe. Seeds were separated by drying the pods in laboratory conditions [*c.* 40% relative humidity (RH)], by rubbing on a sieve or breaking them manually. Fresh seeds were used within 1 week after harvest. Other samples were stored for 3 months at room temperature (18–22°C and *c.* 40% RH) or for a longer time as mentioned in the results.

Table 1. A list of the 14 studied species and their life-form: B, biennial; P, perennial; SA, summer annual; WA, winter annual

Lathyrus aphaca L.	WA
Lathyrus nissolia L.	WA
Lathyrus pratensis L.	P
Medicago arabica (L.) Huds.	WA
Medicago lupulina L.	SA
Medicago sativa L.	P
Melilotus albus Med.	B
Trifolium arvense L.	SA
Trifolium dubium Sibth.	WA
Trifolium pratense L.	P
Trifolium repens L.	P
Vicia cracca L.	P
Vicia hirsuta (L.) S.F. Gray	WA
Vicia sativa L. subsp. *segetalis* (Thuill.) Gaudin	WA

Scarification and germination rate at different temperatures

Seeds were scarified by cutting a small part of the seed coat at the concave side of the seed with a surgical knife. Three replicates of 50 seeds each were placed in Petri dishes (diameter 10 cm) on moist filter paper (MN400, Macherey Nagel, Düren, Germany) and incubated at 23°C, 10°C and 5°C. Seeds were exposed to diffuse daylight in the laboratory (PAR between 2.6 and 6.5 $\mu mol\, m^{-2}\, s^{-1}$), but numerous unmentioned experiments showed no difference in germination in light and darkness. Germinated seeds were counted daily until germination was complete, or for a maximal period of 60 d. From the cumulative germination curve, we derived T_{50}, the number of days to attain 50% germination of the total seed population. For *M. arabica*, we determined germination rate, expressed as $1/T_{50}$, at 5°C intervals between 5°C and 35°C. At the optimal temperature, germinated seeds also were counted after 12, 16 and 20 h imbibition.

Softening rate of seeds

For each temperature treatment, three lots of 100 seeds of *M. arabica* were placed in Petri dishes on filter paper (MN 400) moistened with distilled water. Seeds were incubated for 3 years at 5°C, 10°C, 20°C and 30°C. They were exposed to diffuse daylight (see above). Water was added if necessary to keep the filter paper moist. Swollen seeds were counted weekly and removed. Three Petri dishes were placed at 40°C. Here, swollen seeds were counted three times a week, and the experiment was terminated after 20 weeks. From the cumulative curve, we derived T_{50}, the number of weeks to attain 50% swelling, and the rate of softening was expressed as $1/T_{50}$.

Drying of naturally softened seeds

About 2000 fresh seeds of *M. arabica* were placed on moist filter papers at 23°C and 30°C. During 1 month, naturally swollen seeds were selected every 2 d to start the treatment. One series was dried immediately; others were incubated further at 23°C and 30°C for 1, 2, 3 and 4 weeks. Germinated seeds were counted and removed. At the end of the incubation period, non-germinated seeds were dried. Drying was started by placing seeds on dry filter paper in open Petri dishes. After 1 d under laboratory conditions (*c.* 40% RH), the open Petri dishes were placed in a desiccator over silica gel for 1 week at room temperature (*c.* 20°C).

https://doi.org/10.1017/S0960258510000218 Published online by Cambridge University Press

Germination was tested at 10°C on wet filter paper. In each treatment, three replicates were used with at least 50 seeds. The desiccation treatment was repeated up to five times in another series of experiments. After drying, filter papers were wetted and placed at 23°C. Seeds swelled in 24 h and desiccation was repeated as described above.

Effect of dry storage

Germination of intact seeds was tested immediately after harvesting and after 3 months of dry storage, or up to 1 year for *Vicia sativa*. Three replicates of 100 seeds were placed at 23°C on MN400 filter paper. Germinated seeds were counted after 14 d.

Statistical analysis

For winter annuals, the interactive effect of dry storage at room temperature and incubation temperature on T_{50} was analysed statistically for each species separately using a two-way analysis of variance (ANOVA) (SPSS, version 16.0; SPSS Inc., Chicago, Illinois, USA). In case of species with a life cycle other than winter annual, the effect of dry storage on the germination rate at 23°C was analysed for individual species using a *t*-test.

Results

Germination rate of fresh and stored seeds after scarification

There was a large difference in germination rate between the 14 species (Tables 2 and 3). At 23°C, some species germinated very rapidly with a T_{50} of 1 d or even less, while other species remained in a swollen state for a long time, and germination was delayed, with a T_{50} up to 48 d, indicating the presence of a dormant embryo.

A delay in germination was observed in all winter annuals, but was less obvious in species with a different life cycle, so we classified two groups of species (Tables 2 and 3). Storing seeds dry at room temperature for 3 months significantly affected T_{50} for most species. After storage, T_{50} for winter annuals was greatly reduced (except for *L. nissolia*), and some species germinated without delay (*Vicia* spp. and *M. arabica*). In species with a different life cycle, germination was in general more rapid after storage, but the effect of storage on germination rate was smaller than in winter annuals.

Incubation temperature had a significant effect on germination rate, as fresh seeds of winter annuals germinated much faster at 10°C than at 23°C, and even at 5°C germination rate was only slightly reduced compared to 10°C. The germination rate of stored seeds at lower temperatures was similar or slightly higher than that of fresh seeds, resulting in a significant interaction effect between storage and incubation temperature for all species except *L. nissolia* (Table 2).

Rate of softening and germination at different temperatures

We compared the effects of temperature on softening of the seed coat and on radicle protrusion, the two distinct processes of germination of hard seeds. Embryo growth and radicle protrusion of 3-month-old scarified seeds of *M. arabica* occurred very rapidly at 20°C (Fig. 1). After 24 h, 80% of the seeds had germinated, and T_{50} was attained in 17 h. Compared to 20°C, germination rate decreased slightly at 5–15°C, whereas it decreased sharply at 30°C and 35°C. In contrast, softening, measured by counting swollen seeds that were not scarified, is very slow at lower temperatures. At 5°C, only 41.4% swelled after 3 years. Rate of softening increased exponentially at higher temperatures with a Q_{10} between 3.4 and 5.1 (Fig. 1). Incubation at 40°C for 20 weeks led to swelling of 93%

Table 2. Mean germination rate of winter annuals at different temperatures. Seeds were either fresh or stored for 3 months and scarified. Germination rate is expressed as time in days to attain 50% germination. For individual species the interactive effect of storage (S) and incubation temperature (T) was analysed statistically using a two-way ANOVA, where *** indicates $P < 0.001$; **, $P < 0.01$ and NS, $P > 0.05$

	Fresh			Stored					
	23°C	10°C	5°C	23°C	10°C	5°C	S	T	S × T
Lathyrus aphaca	39.3	4.8	9.3	18.7	4.7	8.5	**	***	**
Lathyrus nissolia	48.7	5.5	8.3	49.3	5.8	9.8	NS	***	NS
Medicago arabica	11.1	6.7	8.9	1.5	2.5	4.2	***	***	**
Trifolium dubium	17	6.3	10.7	3.8	2.5	5.5	***	***	***
Vicia hirsuta	48.3	5.7	8.3	2.7	3.5	7.5	***	***	***
Vicia sativa	28.7	4	10.2	1.7	3.5	6.7	***	***	***

https://doi.org/10.1017/S0960258510000218 Published online by Cambridge University Press

Table 3. Mean germination rate of species with a life cycle other than winter annual at 23°C. Seeds were either fresh or stored for 3 months and scarified. Germination rate is expressed as time in days to attain 50% germination. For individual species the effect of storage (S) was analysed statistically using a *t*-test, where *** indicates $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ and NS, $P > 0.05$

	Fresh	Stored	S
Lathyrus pratensis	11.8	5.2	*
Medicago lupulina	5.3	2.3	***
Medicago sativa	1.5	<1	NS
Melilotus albus	1.3	<1	NS
Trifolium arvense	10.7	7.3	*
Trifolium pratense	<1	<1	NS
Trifolium repens	8.2	4	**
Vicia cracca	5.5	2	NS

of seeds. A linear correlation of $\ln(1/T_{50})$ versus 1000/T indicates that the softening rate obeys the Arrhenius equation (Fig. 2).

Survival of Medicago arabica *seeds after drying*

Fresh seeds that swelled naturally were selected and further incubated for up to 4 weeks at 23°C and 30°C. At 23°C, few seeds germinated in the first weeks due to embryo dormancy, whereas in the third and fourth weeks about 25% germinated. The ungerminated swollen seeds were dried and they all germinated within 7 d after rewetting (Table 4). At 30°C, still fewer seeds germinated, due to the combination of embryo dormancy and supra-optimal temperature. Again, all ungerminated swollen seeds survived the desiccation treatment.

This experiment was repeated with 3-month-old seeds. Swollen seeds that were immediately dried survived this treatment. During a longer period of incubation at 23°C, however, swollen seeds germinated and could not survive after dehydration. Up to five hydration re-drying cycles had little effect on seed viability, with germination remaining between 98.5 and 100%.

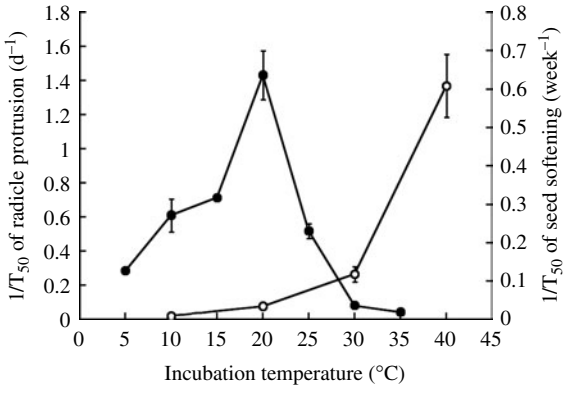


Figure 1. Effect of temperature on the rate of softening (open circles) and on the rate of germination (closed circles) of seeds of *Medicago arabica*. Vertical bars denote SD.

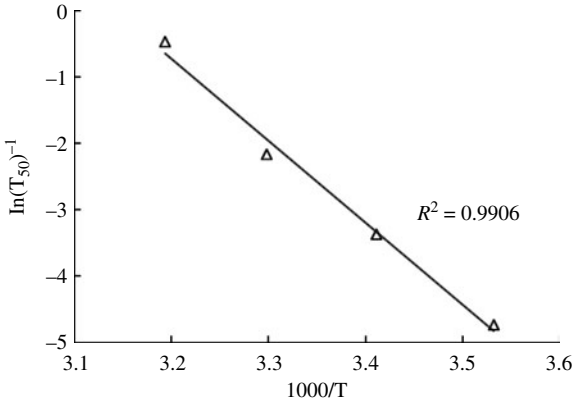


Figure 2. Arrhenius plot of ln of softening rate $[\ln(T_{50})^{-1}]$ of seeds of *Medicago arabica* versus 1000/T. (T_{50} is expressed in weeks).

Effect of dry storage on physical dormancy

Germination at 23°C of all species was tested after dry storage. In only one species, *V. sativa*, seeds became gradually water permeable in 1 year, and then germinated promptly in 14 d (Table 5). All other 13 species were tested after 3 months of dry storage. Germination percentages were lower than 5% and nearly identical to that of fresh seeds, indicating that all seeds remained impermeable (results are not shown).

Table 4. Mean germination percentage (±SD) of swollen seeds of *Medicago arabica* after different pretreatments followed by drying. Germination after drying was determined after 7 d of imbibition

	Germination (%)	
Pretreatment	During pretreatment	At 10°C after drying
0	–	100
1 week 23°C	1.2 ± 1.4	100
2 weeks 23°C	12.4 ± 11.4	100
3 weeks 23°C	23.6 ± 11.4	100
4 weeks 23°C	25.5 ± 8.0	100
1 week 30°C	0.4 ± 1.0	100
2 weeks 30°C	0.8 ± 1.2	100
3 weeks 30°C	4.3 ± 2.0	100
4 weeks 30°C	15.0 ± 2.1	100

https://doi.org/10.1017/S0960258510000218 Published online by Cambridge University Press

Table 5. Germination percentage of seeds of *Vicia sativa* after different periods of dry storage. Germination percentage was determined after 14 d of imbibition

Storage time (months)	Germination percentage
0	0
3	19.0 ± 1.7
6	58.3 ± 1.5
12	87.8 ± 5.0

Discussion

Scarified seeds of nearly all studied species germinated faster after 3 months of dry storage than immediately after harvest. We concluded that the embryos of nearly all species have, to a greater or lesser degree, some form of non-deep physiological dormancy that is lost within 3 months. However, embryo dormancy was much more marked in winter annuals than in species with another life history. The difference between fresh and stored seeds was very striking in some species, such as *V. hirsuta*, *V. sativa* and *M. arabica*. Only one species, *Lathyrus nissolia*, did not change in germination response during 3 months of storage.

Expression of physiological dormancy was evident only at the relatively high temperature of 23°C. Dormancy of fresh seeds was not observed at the lower temperatures of 10°C and 5°C. Also, *L. nissolia* seeds did not change during storage at 23°C, but fresh and stored seeds germinated much faster at lower temperatures. This relative dormancy at higher temperature is typical of winter annuals that have permeable seeds, e.g. *Agrostemma githago* and cereals like wheat and barley. Fresh seeds of these species germinate immediately at temperatures lower than 15°C, while storage is needed to enable germination at higher temperatures (Borris, 1940; Bewley and Black, 1994).

Physiological dormancy of the embryo might be quite common in herbaceous *Fabaceae* and presumably more frequent in Mediterranean than in other climates (Thomson, 1965; Kigel, 1995). Grant Lipp and Ballard (1959) showed that seeds of several legume species germinated very slowly after scarification. Germination was stimulated in an atmosphere enriched in carbon dioxide and increased for some species after dry storage. Combinational dormancy also occurs in other families, such as *Geraniaceae* (Baskin and Baskin, 1974; Van Assche and Vandelook, 2006), *Convolvulaceae* (Meulebrouck *et al.*, 2008), *Anacardiaceae* (Baskin *et al.*, 2000) and in *Cercis canadensis* (*Caesalpiniaceae*) (Geneve, 1991). Profumo and Gastaldo (1977) showed that the endosperm imposes dormancy in *Cercis siliquastrum*.

Natural softening of seeds of *Fabaceae* species presumably occurs by opening of the lens (Baskin, 2003), but the exact mechanism and its regulation remain unknown. As for most *Fabaceae*, softening of hard seeds of *M. arabica* is a very slow process at relatively low temperatures, ensuring that seeds can survive in the soil for many years. The rate of softening increases exponentially at higher temperatures, with a Q_{10} between 3.4 and 5.1. A Q_{10} of 2–3 or higher is typical for chemical reactions, whereas a Q_{10} of 1.0 or slightly above indicates a physical process (reviewed by Berry and Raison, 1981). Furthermore there is a mathematical relation between logarithm of rate and 1/T, identical to the Arrhenius equation that is used generally to describe kinetics of chemical reactions. It is possible that softening of the seed coat is the consequence of an unknown chemical reaction, a chemical breakdown of the seed coat or a reaction in the lens. This supposed reaction might occur in hydrated parts of the seed coat. We found that the softening rates of *Geranium pratense* and *G. robertianum* also obeyed the Arrhenius equation (unpublished experiments).

Winter annuals of *Fabaceae* show many ecological adaptations that are commonly found in winter annuals with permeable seeds (Grime *et al.*, 1981). Germination during summer is prevented by a double safety mechanism: an impermeable seed coat and physiological dormancy that is expressed at higher summer temperatures. Embryo dormancy disappears during dry storage (afterripening), and a fraction of the seed population that has become permeable germinates at the optimal lower autumn temperatures. The remaining impermeable seeds survive in the soil and germination is spread over several years. Combinational dormancy might have evolved in Mediterranean climates, where cool and wet winters are safe seasons for survival of the germlings. It is possible that winter annuals in temperate climates originated in Mediterranean climates and retained this life history after migration to regions with a temperate climate.

Our experiments show that some seeds of *M. arabica* will swell but do not germinate at the higher temperatures after summer rains. Afterwards the soil dries during summer, seeds may dehydrate, but they tolerate desiccation and even several cycles of hydration and dehydration. Seeds of *M. arabica* can remain in a swollen state for up to 4 weeks and still be tolerant to desiccation. This is in contrast to seeds of *Lotus corniculatus* that became sensitive to desiccation afters 24 h of hydration (McKersie and Stinson, 1980). Imbibed seeds of several species maintain viability if dehydrated prior to radicle emergence. However, if seedlings are dehydrated after radical emergence, seedlings are damaged and germination reduced (Berrie and Drennan, 1971; Hegarty, 1978).

According to the current view, seeds of most *Fabaceae* remain impermeable during dry storage (Baskin and Baskin, 1998). We found that seeds of

https://doi.org/10.1017/S0960258510000218 Published online by Cambridge University Press

only one species, *V. sativa*, become permeable during dry storage. Drying during summer, resulting in breaking of physical dormancy, may be a supplementary cue for germination in autumn, as shown in several *Geraniaceae* (Van Assche and Vandelook, 2006). We conclude that seeds of *Fabaceae* regulate germination via a great diversity of mechanisms (or combination thereof) and by convergent evolution show ecological adaptations similar to those of other plant families.

References

Ballard, L.A.T. (1958) Studies of dormancy in the seeds of subterranean clover (*Trifolium subterraneum* L.). 1. Breaking of dormancy by carbon dioxide and by activated carbon. *Australian Journal of Biological Sciences* **11**, 246–260.

Baskin, C.C. (2003) Breaking physical dormancy in seeds – focussing on the lens. *New Phytologist* **158**, 229–232.

Baskin, C.C. and Baskin, J.M. (1998) *Seeds. Ecology, biogeography and evolution of dormancy and germination*. San Diego, Academic Press.

Baskin, J.M. and Baskin, C.C. (1974) Some eco-physiological aspects of seed dormancy in *Geranium carolinianum* L. from Central Tennessee. *Oecologia* **16**, 209–219.

Baskin, J.M. and Baskin, C.C. (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1–16.

Baskin, J.M., Baskin, C.C. and Li, X.J. (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology* **15**, 139–152.

Berrie, A.M.M. and Drennan, D.S.H. (1971) The effect of hydration–dehydration on seed germination. *New Phytologist* **70**, 135–142.

Berry, J.A. and Raison, J.K. (1981) Responses of macrophytes to temperature. pp. 277–338 *in* Lange, O.L.; Nobel, P.S.; Osmond, C.B.; Ziegler, H. (Eds) *Physiological plant ecology*, Vol. 12A. Berlin, Springer-Verlag.

Bewley, J.D. and Black, M. (1994) *Seeds, physiology of development and germination* (2nd edition). New York, Plenum Press.

Borris, H. (1940) Über die inneren Vorgänge bei der Samenkeimung und ihre Beeinflussung durch Aussenfaktoren. *Jahrbuch für Wissenschaftliche Botanik* **89**, 254–339.

Geneve, R.L. (1991) Seed dormancy in eastern redbud (*Cercis canadensis*). *Journal of American Society of Horticultural Science* **116**, 85–88.

Grant Lipp, A.E. and Ballard, L.A.T. (1959) The breaking of seed dormancy of some legumes by carbon dioxide. *Australian Journal of Agricultural Research* **10**, 495–499.

Grime, J.P., Mason, G., Curtis, A.V., Rodman, J., Band, S.R., Mowforth, M.A.G., Neal, A.M. and Shaw, S. (1981) A comparative study of germination characteristics in a local flora. *Journal of Ecology* **69**, 1017–1059.

Hegarty, T.W. (1978) The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Plant Cell and Environment* **1**, 101–119.

Jansen, P.I. and Ison, R.L. (1994) Hydration–dehydration and subsequent storage effects on seed of the self-regenerating annuals *Trifolium balansae* and *T. resupinatum*. *Seed Science and Technology* **22**, 435–447.

Katznelson, J. and Carpenter, J.A. (1972) Germination of subterranean clover in a Mediterranean summer. *Israel Journal of Botany* **21**, 228–242.

Kigel, J. (1995) Seed germination in arid and semi-arid regions. pp. 645–700 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

McKersie, B.D. and Stinson, R.H. (1980) Effect of dehydration on leakage and membrane structure in *Lotus corniculatus* L. seeds. *Plant Physiology* **66**, 316–320.

Meulebrouck, K., Ameloot, E., Van Assche, J.A., Verheyen, K., Hermy, M. and Baskin, C.C. (2008) Germination ecology of the holoparasite *Cuscuta epithymum*. *Seed Science Research* **18**, 25–34.

Morrison, D.A., Auld, T.D., Rish, S., Porter, C. and McClay, K. (1992) Patterns of testa-imposed seed dormancy in native Australian legumes. *Annals of Botany* **70**, 157–163.

Norman, H.C., Cocks, P.S., Smith, F.P. and Nutt, B.J. (1998) Reproductive strategies in Mediterranean annual clovers: germination and hardseededness. *Australian Journal of Agricultural Research* **49**, 973–982.

Profumo, P. and Gastaldo, P. (1977) Ulteriori richerche sulla germinazione di *Cercis siliquastrum* L. Azione dell'acido gibberellico e ruolo dell'endosperma nel fenomeno della dormienza. *Giornale Botanico Italiano* **111**, 211–218.

Taylor, G.B. (1981) Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. *Australian Journal of Plant Physiology* **8**, 547–558.

Thomson, J.R. (1965) Breaking dormancy in germination tests of *Trifolium* spp. *Proceedings of the International Seed Testing Association* **30**, 905–909.

Van Assche, J.A. and Vandelook, F.E.A. (2006) Germination ecology of eleven species of Geraniaceae and Malvaceae, with special reference to the effects of drying seeds. *Seed Science Research* **16**, 283–290.

Van Assche, J.A., Debucquoy, K.L.A. and Rommens, W.A.F. (2003) Seasonal cycles in the germination capacity of buried seeds of some Leguminosae (Fabaceae). *New Phytologist* **158**, 315–323.

https://doi.org/10.1017/S0960258510000218 Published online by Cambridge University Press