Available online at www.sciencedirect.com
ScienceDirect
Flora 204 (2009) 536–542
FLORA
www.elsevier.de/flora

The role of temperature in post-dispersal embryo growth and dormancy break in seeds of *Aconitum lycoctonum* L.

Filip Vandelook*, Jelle Lenaerts, A. Van Assche Jozef

Laboratory of Plant Ecology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 31, Box 2435, 3001 Leuven, Belgium

Received 6 June 2008; accepted 27 July 2008

Abstract

This research was performed to resolve temperature requirement for embryo growth, dormancy break and seed germination of *Aconitum lycoctonum*, an Eurasian perennial herb growing in deciduous forests. The dormancy strategy of *A. lycoctonum* was compared with that of other *Ranunculaceae* species growing in the temperate deciduous forest habitat. Seeds of *A. lycoctonum* germinate immediately after embryo growth is completed during winter and seedlings subsequently emerge in early spring. Experiments in controlled conditions revealed that (1) embryo growth and germination only occurred at low temperatures (<10 °C), (2) a high-temperature pre-treatment was not required for germination, and (3) application of gibberellic acid did not overcome the chilling requirement. Based on these results, seeds of *A. lycoctonum* can be classified as having deep complex morphophysiological dormancy. Dormancy breaking requirements of *A. lycoctonum* are very similar to related species studied before, suggesting stasis in seed dormancy traits has occurred in the *Aconitum–Delphinium* clade.

Keywords: Morphophysiological dormancy; Germination; Ranunculaceae; Seeds; Aconitum; Deciduous forest understory

Introduction

A study on the relative size and shape of embryo and endosperm revealed that seeds of numerous angiosperms contain an underdeveloped embryo at the moment of dispersal (Martin, 1946). Several authors have supported the hypothesis that an underdeveloped embryo is an ancestral character among seed plants (Forbis et al., 2002; Verdú, 2006). In accordance with this hypothesis, species of the Ranunculaceae considered basal in the Eudicots, in general have an underdeveloped embryo (Engell, 1995; Martin, 1946). Variation, however, exists in the degree of embryo development among Ranunculaceae species. Embryos in ripe seeds vary from rudimentary undifferentiated embryos, for example in *Aquilegia*, to well-differentiated embryos occupying a considerable amount of the seed volume, e.g. in *Delphinium* (Engell, 1995).

Despite the extensive knowledge on embryo morphology in Ranunculaceae species, data on requirements for embryo growth and dormancy break are still scarce for this family. In seeds with an underdeveloped embryo, the embryo has to grow to a critical length before germination can occur. The lag period resulting from the obligatory growth of the embryo has been termed as morphological dormancy (Nikolaeva, 1977). Studies have indicated that in some Ranunculaceae species there is no additional mechanism preventing embryo growth and seed germination (Baskin and Baskin, 1986;

*Corresponding author. Tel.: +32 16321522.
E-mail address: filip.vandelook@bio.kuleuven.be (F. Vandelook).

0367-2530/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2008.11.003

Bullowa and Ozeri, 1975). In seeds of many Ranunculaceae, however, an additional physiological mechanism delays embryo growth even in optimal conditions. These seeds are termed as morphophysiologically dormant (MPD), since they exhibit both morphological dormancy (MD) and physiological dormancy (PD). Usually an after-ripening period is required to lift the physiological block. Cold stratification is often the most effective treatment for breaking dormancy in Ranunculaceae growing in temperate and alpine climates (Baskin and Baskin, 1994; Forbis and Diggle, 2001; Frost-Christensen, 1974; Walck et al., 1999). Chemical stimulants such as gibberellic acid, nitrate and thiourea have also been shown to be effective in overcoming dormancy in Ranunculaceae seeds (Bungard et al., 1997; Hepher and Roberts, 1985; Probert et al., 1987).

To extend our knowledge on dormancy breaking mechanisms in Ranunculaceae, we performed a study on the ecophysiology of dormancy break and germination induction of *Aconitum lycoctonum* L. (syn. *Aconitum vulparia* Reichenb.). Although the genus *Aconitum* comprises about 400 species, including some ornamental and medicinal plants (Utelli et al., 2000), no extensive study on seed dormancy has been performed on a representative of this genus. It has been shown that some *Aconitum* species require chilling for dormancy break (Dosmann, 2002; Nichols, 1934) and that addition of GA stimulated germination of *Aconitum balfourii* (Pandey et al., 2000).

Based on phenology of embryo development and seedling emergence in natural conditions, a series of experiments was conducted in controlled conditions. Temperature conditions required for embryo elongation and dormancy break were tested. Finally, the ability of gibberellic acid to overcome dormancy was examined. The dormancy type based on the classification system of Baskin and Baskin (2004) was determined, enabling us to compare the results with other Ranunculaceae species. The results are discussed in an ecological and phylogenetic framework.

Materials and methods

Study species

A. lycoctonum is a herbaceous perennial restricted to montane and subalpine regions, where it grows mainly in moist open woodlands and forest ravines. It has a wide distribution range, extending from the Iberian peninsula up to Siberia and the Himalayan region (Hegi, 1975). After the last ice-age *A. lycoctonum* presumably recolonized the European continent from refugium populations in the Iberian peninsula and Eastern Europe (Utelli et al., 1999). Populations of *A. lycoctonum* distributed throughout Central and Southern Europe show high morphological variability. Nevertheless, since genetic diversity between populations throughout Europe is low, the *A. lycoctonum* complex is regarded as a single species (Utelli et al., 2000).

Seeds were collected from several plants of one *A. lycoctonum* population growing in a ravine forest along the Lesse river in Anseremme, Belgium (50°14′N, 4°54′E). Two separate batches of ripe seeds were collected from plants with opened fruits on 19 July 2006 and 9 July 2007. All experiments were started within 1 week after harvesting. Seeds that were visibly deficient were excluded from the experiments.

Phenology of embryo growth and seedling emergence

Phenology of seedling emergence of the 2007 seed batch was studied by sowing seeds of *A. lycoctonum* in a garden near Leuven (Belgium). On 12 July 2007, three replicates of 50 seeds were sown at a depth of 1 cm in plastic pots filled with potting soil. These pots were buried at soil level, at a site where a nearby building prevented direct sunlight from reaching the soil. The pots were covered with a net to avoid disturbance by birds and a molluscicide was applied regularly to avoid seedling damage by slugs. Emerged seedlings were counted and removed weekly during 1 year. Maximum and minimum soil temperature at a depth of 1 cm was recorded daily in the vicinity of the pots. This daily maximum and minimum was averaged over a 1-week period to obtain average weekly maximum and minimum soil temperature.

We examined growth of the embryo in natural conditions by burying seeds in the garden and exhuming them at set time intervals. Twenty-five nylon bags each were filled with 30 seeds of the 2007 seed batch and 10 g of white sand to simulate soil contact. On 12 July 2007 these bags were buried 5 cm deep at a shady site in the garden. Every 2 weeks a nylon bag was exhumed and the average embryo length to seed length ratio (E:S ratio) of 20 randomly selected seeds was determined. The E:S ratio was determined by cutting seeds into half under a dissection microscope and measuring embryo length and seed length using an ocular micrometer. The E:S ratio was not determined for seeds that had germinated; instead for germinated seeds the critical E:S ratio was applied. The critical E:S ratio was calculated as the average E:S ratio of 20 seeds with split seed coat, but no radicle protrusion.

Temperature requirements for embryo growth

An experiment in controlled conditions was performed to study the effect of after-ripening at low and high temperatures on embryo elongation. In this experiment, we used seeds that were collected in

July 2007. Seeds were placed on a filter paper (Macherey-Nagel 440) in 10 cm Petri-dishes and moistened with c. 10 mL distilled water. Effects of three different after-ripening conditions on embryo elongation were tested. A batch of 30 seeds was incubated at constant temperature of 23 °C for 32 weeks. At the end of this period 20 seeds were selected randomly and the average E:S ratio was determined as described above. One batch of 250 seeds was moved to an incubator constantly at 5 °C for 20 weeks and every 2 weeks the E:S ratio of 20 randomly selected seeds was determined. Another batch of 200 seeds was first placed at 23 °C for 16 weeks, after which they were transferred to 5 °C for another 16 weeks. Here again, during incubation at 5 °C the average E:S ratio of 20 seeds was determined every 2 weeks until the end of the experiment.

Effect of stratification on dormancy break

All further germination trials were performed using three replicates of 50 seeds collected in July 2006. Seeds were placed on a filter paper in Petri-dishes and moistened with distilled water. In extended germination trials filter papers were kept moist regularly by adding distilled water. Preliminary tests showed that effects of light on germination of *A. lycoctonum* are negligible. Therefore, all seeds were exposed to a 12-h photoperiod, whereby light (PAR = 52 μmol m^{-2} s^{-1}) was provided by fluorescent tubes (Philips LTD 80).

Temperature requirement for dormancy break and germination was tested by subjecting seeds to specific temperature conditions for an extended period of time. We incubated seeds at constant temperatures of 23, 10 and 5 °C and at daily fluctuating temperatures of 20/10 °C and 15/6 °C (12 h/12 h). The experiment lasted for 36 weeks, during which germinated seeds were counted and removed weekly.

A. lycoctonum seeds are dispersed during summer, suggesting that a period at high summer temperatures might be required to break dormancy. Therefore, we examined the effect of moist storage at elevated temperature on subsequent germination at lower temperatures. Seeds were stored in moist conditions at 23 °C for 4, 8 and 12 weeks, before being transferred to 20/10, 15/6, 10 and 5 °C for another 20 weeks. After transferring to lower temperatures, germinated seeds were counted and discarded every week. At the end of the experiment, we checked whether germinated seeds were still intact by squeezing them with tweezers. Seeds that were not firm were considered unviable and were not taken into account in the analyses. The effect of duration of pre-treatment at 23 °C on the final germination percentage was statistically analyzed using a one-way ANOVA followed by a Tukey multiple comparisons test (Statistica 6.0). Data were arcsine transformed prior to analysis, to stabilize variances.

Effect of GA_3 on seed germination

In seeds of certain species gibberellic acid (GA_3) can substitute the stratification requirement for dormancy break. The ability of GA_3 to overcome dormancy is being used as a criterion for classifying dormancy types (Baskin and Baskin, 2004). The effect of four different GA_3 concentrations on germination of *A. lycoctonum* was tested for seeds incubated at 10 and 23 °C. Three replicates of 50 seeds were placed in a Petri-dish and moistened with 0, 10, 100 or 1000 mg L^{-1} GA_3 solution. Seeds were incubated for 18 weeks, during which germinated seeds were counted and removed weekly. Effects of GA_3 concentration on final germination percentage was statistically analyzed using a one-way ANOVA followed by a Tukey multiple comparisons test. Data were arcsine transformed prior to analysis.

Results

Phenology of embryo growth and seedling emergence

Ripe seeds of *A. lycoctonum* have an initial E:S ratio of 0.30 ± 0.01 (mean ± SE). Embryos grew very little during the first 14 weeks of burial in the soil (Fig. 1). The first significant embryo growth was observed in seeds exhumed on 23 October 2007, when mean maximum and minimum soil temperatures in the preceding week were 11.8 and 8.8 °C, respectively. In most seeds, the embryo length increased gradually during winter until it had reached the critical E:S ratio 0.85 ± 0.02. In the bag exhumed on 29 January 2008, 28% of the seeds had grown to the critical E:S ratio and

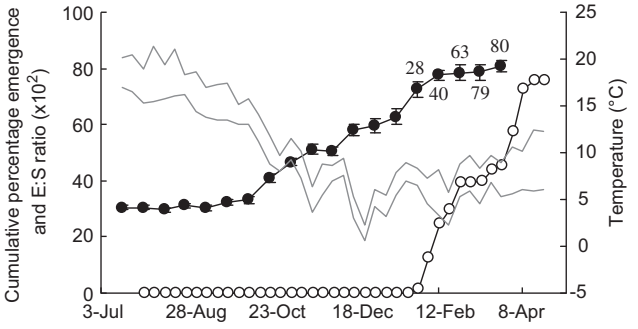

Fig. 1. Cumulative percentage of seedling emergence (open circles; $n = 3$) and phenology of embryo growth (closed circles; $n = 20$) of *A. lycoctonum* in natural conditions. Gray lines represent mean weekly maximum and minimum temperature at 1 cm depth. The numbers near the closed circles indicate the percentage of germinated seedlings in the respective bags. Vertical bars represent SE for embryo length.

had germinated. Additionally, on this date the first seedlings were observed to emerge above ground, while maximum and minimum temperature were as low as 8.4 and 6.5 °C. By 11 March 2008, 79% of the seeds buried in the nylon bags had germinated. About 76.7% of the seeds sown had emerged as seedlings during a 13 week period between 23 January and 23 April 2008. No seedlings were observed after this date. Seedling emergence was rather discontinuous, since this 13-week period encompassed 4 weeks (March) during which very few seedlings emerged.

Temperature requirements for embryo growth

None of the seeds that were incubated at 23 °C had reached the critical E:S ratio (0.85 ± 0.02) after 32 weeks of incubation. The average E:S ratio at the end of the incubation period was 0.36 ± 0.01. Growth of the embryo started immediately in seeds incubated at 5 °C (Fig. 2). The small standard error during the course of the experiment indicates that the embryo elongated gradually and at a similar rate in all seeds. After 18 weeks, 85% of the seeds had reached the critical E:S ratio and had germinated. The embryo growth rate was very similar in seeds that were moved to 5 °C following a 16-week pre-treatment at 23 °C. Of these seeds, 80% had reached the critical E:S ratio within 16 weeks after being transferred to 5 °C.

Effect of stratification on dormancy break

In the prolonged germination experiment, seeds only germinated when incubated at 5 or 10 °C (Fig. 3). No germination was recorded during 36 weeks at all other temperature conditions tested: 20/10, 15/6 and 23 °C. Seeds incubated at 5 °C germinated faster and to a higher percentage than seeds incubated at 10 °C. Germination started after 7 weeks of incubation at 5 °C and up to 98% of the seeds had germinated after 22 weeks. Seeds placed at 10 °C, however, required 34 weeks to reach 80% germination.

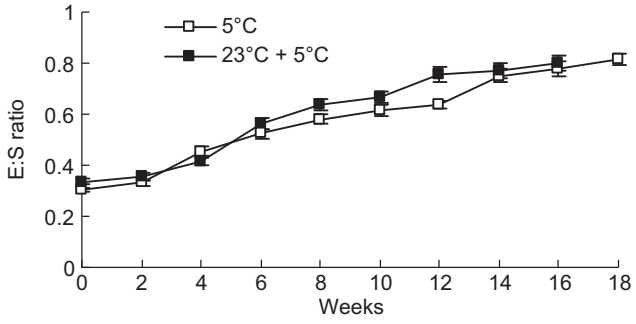

Fig. 2. Embryo growth of *A. lycoctonum* seeds incubated at 5 °C without pre-treatment (open squares) and at 5 °C following 16 weeks at 23 °C (closed squares). Vertical bars represent SE; $n = 20$.

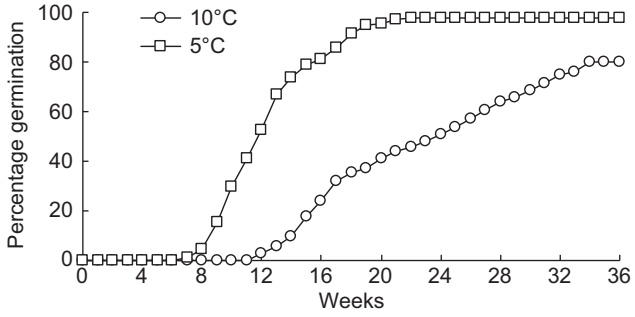

Fig. 3. Cumulative percentage germination of freshly collected seed of *A. lycoctonum* during 36 weeks at constant temperatures of 5 °C and 10 °C. $n = 3$.

Table 1. Final mean germination percentage and T50 of all viable *A. lycoctonum* seeds after pre-treatment at 23 °C..

Weeks at 23 °C	Percentage germination		T50 (weeks)
	10 °C	5 °C	5 °C
0	41.3 a	95.3 a	11.8 a
4	22.0 b	94.7 a	10.9 b
8	16.0 c	92.0 ab	12.1 a
12	12.0 c	88.0 bc	14.5 c
16	6.7 d	84.0 c	15.7 d

Means followed by the same letter within a temperature condition are not significantly different at the $P < 0.05$ level (Tukey multiple comparisons test).

Pre-treating the seeds at 23 °C did not result in germination during 20 weeks of incubation at 15/6 and 20/10 °C. Extending the pre-treatment period at 23 °C significantly ($P < 0.05$) reduced the final germination percentage of seeds placed at 5 and 10 °C (Table 1). Seeds that were placed at 10 °C without a pre-treatment had germinated to an average of 41.3% after 20 weeks, while only 6.7% of the seeds germinated at 10 °C when pre-treated at 23 °C for 16 weeks. Seeds incubated at 5 °C always germinated to more than 84%. The time required to reach 50% germination of all viable seeds (T50), however, was significantly higher for seeds that were pre-treated at 23 °C for 12 and 16 weeks.

Effect of GA_3 on seed germination

Seeds that were incubated at 10 °C germinated to a significantly higher percentage ($P < 0.05$) with increasing concentration of GA_3 (Table 2). Up to 74% of the seeds moistened with a $1000\,mg\,L^{-1}$ GA_3 solution had germinated after 18 weeks of incubation. None of the seeds germinated at any GA_3 concentration during incubation at 23 °C.

Table 2. Effect of GA_3 concentration on mean final germination percentage of *A. lycoctonum*.

Incubation temperature (°C)	Concentration GA_3 (mg L^{-1})			
	0	10	100	1000
10	35.3 a	38.0 a	48.7 b	74.0 c
23	0	0	0	0

Means followed by the same letter are not significantly different at the $P<0.05$ level (Tukey multiple comparisons test).

Discussion

The embryo occupies only a small part of the total seed volume in ripe seeds of *A. lycoctonum*. Before the seeds can germinate, the embryo has to grow to a critical size, indicating that *A. lycoctonum* has an underdeveloped embryo (Grushvitzky, 1967). Baskin and Baskin (1998) mentioned that seeds with an underdeveloped embryo germinated within 30 days in case no additional physiological mechanism delaying germination is present. Seeds of *A. lycoctonum* germinate without any pre-treatment at low-temperature conditions. However, even at 5 °C, the optimal temperature for germination, at least 7 weeks were required for the first seeds to germinate. Therefore, seeds of *A. lycoctonum* not only have morphological dormancy due to an underdeveloped embryo, but also an additional physiological dormancy mechanism, meaning the seeds are MPD.

Morphological and physiological dormancy in *A. lycoctonum* are broken simultaneously at low-temperature conditions of 5 and 10 °C. Seeds germinated immediately after elongation of the embryo was completed. Thus, according to the dormancy classification system of Baskin and Baskin (2004), seeds of *A. lycoctonum* have a complex type of MPD. Three types of complex MPD can be discerned: nondeep, intermediate and deep complex MPD. These can be differentiated by the requirement of a high-temperature pre-treatment before cold stratification and the ability of GA to overcome the cold stratification requirement. A high-temperature pre-treatment before cold stratification did not enhance the embryo growth rate nor germination rate of *A. lycoctonum* seeds (Fig. 2; Table 1). On the contrary, extending the duration of pre-treatment at 23 °C resulted in decreased final germination percentages and germination rates. Although application of a GA_3 solution had a positive effect on germination percentages at 10 °C, it did not overcome the chilling requirement in seeds incubated at 23 °C (Table 2). Since a high-temperature pre-treatment is not required and GA_3 is unable to overcome the chilling requirement, seeds of *A. lycoctonum* can be classified as having deep complex MPD.

From an ecological point of view, this dormancy strategy allows seedlings to emerge fairly early in spring. Spring is generally considered the optimal period for seedling emergence for many temperate woodland plants. By emerging in spring, seedling damage due to frost in winter is avoided and seedlings are able to grow in conditions of reduced competition (Grime, 2001). Although seeds of *A. lycoctonum* are dispersed in summer, germination in summer and autumn is prevented because of a chilling requirement for embryo growth and physiological dormancy break. Once embryo growth is completed in late winter/early spring seeds germinate at low temperatures. The ability to germinate at low temperatures enables seedlings to emerge in the very beginning of the growing season and to grow in optimal conditions for an extended period of time. Emerging even one or a few weeks earlier in the growing season can affect the seedlings future success (Verdú and Traveset, 2005).

Up to now, two other Ranunculaceae species, *Delphinium tricorne* and *Caltha leptosepala*, have been described with deep complex MPD seeds (Baskin and Baskin, 1994; Forbis and Diggle, 2001). This dormancy strategy, however, appears to be fairly common amongst temperate climate Apiaceae species. Six out of nine Apiaceae species with the early spring emergence strategy in Roberts' (1979, 1986) experiments on phenology of seedling emergence, presumably have deep complex MPD (Baskin et al., 2000; Grime et al., 1981; Stokes, 1952; Vandelook et al., 2007).

Eight types of MPD have been defined in the classification system of Baskin and Baskin (2004). Six out of these eight types have been observed in Ranunculaceae, indicating a wide variety of dormancy types in Ranunculaceae species exists (Baskin and Baskin, 1998). Forbis and Diggle (2001) suggested that requirements for dormancy break may result from an adaptation to specific habitat conditions. Studies on other Ranunculaceae species growing in temperate forests indicate that, among these species, a number of different dormancy types occur. Seeds of the North American woodland herb *Isopyrum biternatum*, for example, only exhibit MD and germinate in autumn (Baskin and Baskin, 1986). Nondeep complex MPD has been observed in seeds of the European perennials *Eranthis hiemalis* (Frost-Christensen, 1974), *Ranunculus auricomus* and *R. ficaria* (unpublished results). Yet another dormancy type is found in seeds of the European *Anemone nemorosa* and the North American *Hepatica acutiloba* and *Cimicifuga racemosa*, as these species show epicotyl dormancy (Ali et al., 2007; Baskin and Baskin, 1985). Interestingly, all these species occupy similar habitat types, but they do exhibit different dormancy breaking requirements.

These differences in dormancy strategy could result from selection in slightly different habitats. An alternative explanation was given by Angevine and Chabot (1979) stating that "a given pattern of emergence within a habitat can be achieved by a variety of physiological mechanisms".

Dormancy breaking requirements of only a few other *Aconitum* species have been tested, and to a certain extent resembled those of *A. lycoctonum*. A strict chilling requirement has been observed in *Aconitum uncinatum* growing in North America (Nichols, 1934) and *Aconitum sinomontanum* growing in China (Dosmann, 2002). A deep complex type of MPD has also been proposed for seeds of *A. sinomontanum*. The effect of GA on dormancy break was, however, not tested and seeds of *A. sinomontanum* might therefore also have intermediate complex MPD. Application of GA enhanced seed germination of *Aconitum heterophyllum*, but inhibited germination of *Aconitum balfourii* (Pandey et al., 2000). It is not clear whether seeds of these species had a chilling requirement. Moreover, since the effect of GA was tested in natural temperature conditions, these results cannot confirm whether GA overcomes a possible chilling requirement in these species. When looking at the genus level, both molecular and morphological data show high support for *Delphinium* as the sister genus of *Aconitum* (Hoot, 1995). Engell (1995) also observed that the embryos in *Delphinum* and *Aconitum* species are nearly identical at the moment the seeds are shed. Dormancy breaking requirements for the North American woodland perennial *D. tricorne* are very similar to those of *A. lycoctonum* and were also classified as deep complex MPD (Baskin and Baskin, 1994). Other *Delphinium* species also have been shown to have a chilling requirement for germination, but data are too limited to determine the dormancy type (Thompson, 1968; Williams and Cronin, 1968).

The presence of very similar dormancy breaking requirements in species of the sister genera *Aconitum* and *Delphinium*, prompts the question of evolutionary origin of their dormancy strategy. Further phylogenetic analysis at lower taxonomic levels and analysis of dormancy breaking requirements of other representatives of these genera should enable us to determine whether deep complex MPD is a plesiomorphic condition in this clade. If so, this could indicate that stasis of seed morphological and physiological traits has occurred over a considerable period of time in the *Aconitum–Delphinium* clade. On the other hand, more distantly related species of the Ranunculaceae with similar habitat preferences have developed different dormancy breaking requirements. This might be the result of independent evolutionary adaptations to the forest habitat within the Ranunculaceae, resulting in differing dormancy breaking requirements.

References

Ali, N., Probert, R., Hay, F., Davies, H., Stuppy, W., 2007. Post-dispersal embryo growth and acquisition of desiccation tolerance in *Anemone nemorosa* L. seeds. Seed Sci. Res. 17, 155–163.

Angevine, M.W., Chabot, B.F., 1979. Seed germination syndromes in higher plants. In: Solbrig, O.T., Jain, S., Johnson, G.B., Raven, P.H. (Eds.), Topics in Plant Population Biology. Columbia University Press, New York, pp. 188–206.

Baskin, J.M., Baskin, C.C., 1985. Epicotyl dormancy in seeds of *Cimicifuga racemosa* and *Hepatica acutiloba*. Bull. Torrey Bot. Club 112, 253–257.

Baskin, J.M., Baskin, C.C., 1986. Germination ecophysiology of the mesic deciduous forest herb *Isopyrum biternatum*. Bot. Gaz. 147, 152–155.

Baskin, C.C., Baskin, J.M., 1994. Deep complex morphophysiological dormancy in seeds of the mesic woodland herb *Delphinium tricorne* (Ranunculaceae). Int. J. Plant Sci. 155, 738–743.

Baskin, C.C., Baskin, J.M., 1998. Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, USA.

Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy. Seed Sci. Res. 14, 1–16.

Baskin, C.C., Milberg, P., Andersson, L., Baskin, J.M., 2000. Deep complex morphophysiological dormancy in seeds of *Anthriscus sylvestris* (Apiaceae). Flora 195, 245–251.

Bullowa, S., Ozeri, Y., 1975. Role of temperature and growth regulators in germination in *Anemone coronaria* L. Austr. J. Plant Physiol. 2, 91–100.

Bungard, R.A., McNeil, D., Morton, J.D., 1997. Effects of chilling, light and nitrogen-containing compounds on germination, rate of germination and seed imbibition of *Clematis vitalba* L. Ann. Bot. 79, 643–650.

Dosmann, M.S., 2002. Stratification improves and is likely required for germination of *Aconitum sinomontanum*. Hort. Technol. 12, 423–425.

Engell, K., 1995. Embryo morphology of the Ranunculaceae. Plant Syst. Evol. S 9, 207–216.

Forbis, T.A., Diggle, P.A., 2001. Subnivean embryo development in the alpine herb *Caltha leptosepala* (Ranunculaceae). Can. J. Bot. 79, 635–642.

Forbis, T.A., Floyd, S.K., De Queiroz, A., 2002. The evolution of embryo size in angiosperms and other seed plants: implications for the evolution of seed dormancy. Evolution 56, 2112–2125.

Frost-Christensen, H., 1974. Embryo development in ripe seeds of *Eranthis hiemalis* and its relation to gibberellic acid. Physiol. Plant. 30, 200–205.

Grime, J.P., 2001. Plant Strategies, Vegetation Processes, and Ecosystem Properties. Wiley, Chichester, UK.

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

Grushvitzky, I.V., 1967. After-ripening of seeds of primitive tribes of Angiosperms, conditions and peculiarities.

In: Borris, H. (Ed.), Physiologie, Ökologie und Biochemie der Keimung. Ernst–Moritz–Arndt Universität, Greifswald, Germany, pp. 329–336.

Hegi, G., 1975. Illustrierte Flora von Mittel-Europa. Dicotyledons. J.F. Lehmanns, München.

Hepher, A., Roberts, J.A., 1985. The control of seed germination in *Trollius ledebouri* – the breaking of dormancy. Planta 166, 314–320.

Hoot, S.B., 1995. Phylogeny of the Ranunculaceae based on preliminary *atp*B, *rbc*L and 18S nuclear ribosomal DNA sequences. Plant Syst. Evol. S 9, 241–251.

Martin, A.C., 1946. The comparative internal morphology of seeds. Am. Midl. Nat. 36, 513–660.

Nichols, G.E., 1934. The influence of exposure to winter temperatures upon seed germination in various native American plants. Ecology 15, 364–373.

Nikolaeva, M.G., 1977. Factors controlling the seed dormancy pattern. In: Khan, A.A. (Ed.), The Physiology and Biochemistry of Seed Dormancy and Germination. North-Holland, Amsterdam, pp. 51–74.

Pandey, K., Nandi, S.K., Nadeem, M., Palni, L.M.S., 2000. Chemical stimulation of seed germination in *Aconitum heterophyllum* wall. and *A. balfourii* Stapf.: important Himalayan species of medicinal value. Seed Sci. Technol. 28, 39–48.

Probert, R.J., Gajjar, K.H., Haslam, I.K., 1987. The interactive effects of phytochrome, nitrate and thiourea on the germination response to alternating temperatures in seeds of *Ranunculus sceleratus* L. – A quantal approach. J. Exp. Bot. 38, 1012–1025.

Roberts, H.A., 1979. Periodicity of seedling emergence and seed survival in some Umbelliferae. J. Appl. Ecol. 16, 195–201.

Roberts, H.A., 1986. Seed persistence in soil and seasonal emergence in plant species from different habitats. J. Appl. Ecol. 23, 639–656.

Stokes, P., 1952. A physiological study of embryo development in *Heracleum sphondylium* L. I. The effect of temperature on embryo development. Ann. Bot. 16, 441–447.

Thompson, P.A., 1968. Effects of after-ripening treatments and temperature on germination of some *Delphinium* species. Horticult. Res. 8, 62–66.

Utelli, A.B., Roy, B.A., Baltisberger, M., 1999. History can be more important than 'pollination syndrome' in determining the genetic structure of plant populations: the case of *Aconitum lycoctonum* (Ranunculaceae). Heredity 82, 574–584.

Utelli, A.B., Roy, B.A., Baltisberger, M., 2000. Molecular and morphological analyses of European *Aconitum* species (Ranunculaceae). Plant Syst. Evol. 224, 195–212.

Vandelook, F., Bolle, N., Van Assche, J.A., 2007. Seed dormancy and germination of the European *Chaerophyllum temulum* (Apiaceae), a member of a trans-Atlantic genus. Ann. Bot. 100, 233–239.

Verdú, M., 2006. Tempo, mode and phylogenetic associations of relative embryo size evolution in angiosperms. J. Evol. Biol. 19, 625–634.

Verdú, M., Traveset, A., 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology 86, 1385–1394.

Walck, J.L., Baskin, C.C., Baskin, J.M., 1999. Seeds of *Thalictrum mirabile* (Ranunculaceae) require cold stratification for loss of nondeep simple morphophysiological dormancy. Can. J. Bot. 77, 1769–1776.

Williams, M.C., Cronin, E.H., 1968. Dormancy, longevity and germination of seed of three larkspurs and western false hellebore. Weed Sci. 16, 381–384.