When Breaking Seed Dormancy Is a Problem
Try a Move-along Experiment

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ABSTRACT

The move-along experiment is a double germination phenology study that is easy to use, does not require large numbers of seeds, and allows one to determine if summer only, winter only, or a summer-winter sequence of temperatures is required for dormancy break in seeds with water-permeable seed (or fruit) coats. Two temperature profiles (simulating 1-y cycles proceeding from winter to winter or summer to summer) and control treatments (seeds kept continuously at each temperature regime) are run concurrently. For most species, the combination of dormancy-breaking temperatures required for germination can be determined in 1 y with this technique.

KEY WORDS
seed germination, cold stratification

NOMENCLATURE
Kartesz and Meacham (1999)

Seeds of *Echinacea angustifolia* DC. var. *angustifolia* (Asteraceae) require cold stratification for dormancy break; consequently, they become nondormant during winter and germinate in spring.

Photo by Thomas G Barnes

Temperature is the major environmental factor responsible for changes in dormancy states of seeds (Baskin and Baskin 1998). Germinating seeds of native species in temperate regions of the world may require exposure to summer and/or winter temperatures to break dormancy. Unless one has some previous experience with the study taxon, however, or a close relative of it, or has information from colleagues or the literature, it is difficult to decide which treatment(s) may have the best possibility of breaking seed dormancy. For example, seeds of some species (such as the limestone cedar glade endemic *Lesquerella lyrata* Rollins (Brassicaceae) [Baskin and Baskin 2000]) require high summer temperatures for dormancy break, but if one does not know this and only gives fresh seeds a cold stratification (moist, low temperature [0.5 to 10 °C (33 to 50 °F)]) treatment, dormancy break does not occur. However, if seeds of such species are exposed to summer temperatures (ambient room temperatures usually are fine), they come out of dormancy and will germinate in autumn.

Seeds of many species require cold stratification for dormancy break; consequently, they become nondormant during winter and germinate in spring (for example, the prairie forb *Echinacea angustifolia* DC. var. *angustifolia* (Asteraceae) [Baskin and others 1992]). In still other species, seeds require exposure to high summer temperatures followed by low winter temperatures before they will germinate at spring temperatures. This may apply to seeds with small underdeveloped embryos (for example, the mesic deciduous forest herb *Erythronium albidum* Nutt. (Liliaceae) [Baskin and Baskin 1985]) as well as to those with large fully developed embryos (for example, the mesic deciduous forest herb *Cardamine concatenata* (Michx.) O. Schwarz. (Brassicaceae) [Baskin and Baskin 1995] and the circumboreal shrub *Empetrum hermaphroditum* Hagerup (Empetraceae) [Baskin and others 2002]). Seeds of a few species require a sequence of summer ⟶ autumn⟶winter temperatures to break dormancy, and then they will germinate in spring, as is the case with the mesic deciduous forest herb *Jeffersonia diphylla* (L.) Pers. (Berberidaceae) (Baskin and Baskin 1989).

Without using thousands of seeds (which can be a problem if the study species is rare, uncommon, or does not set many seeds) and/or experimenting over several years, how can one determine whether to give seeds warm and/or cold treatments to break dormancy? About 15 y ago, we devised a method for obtaining basic information on the temperature requirements for dormancy break of seeds, and it has been used with good success on a number of species that are difficult to germinate, such as the shrub *Symphoricarpos orbiculatus* Moench (Caprifoliaceae) (Hidayati and others 2001). We call our method the "move-along experiment," but double-germination phenology technique is a more scientifically accurate term. This method is easy to use, does not require large numbers of seeds, and works just as well for seeds with small underdeveloped embryos as for those with large fully developed embryos. We have used this method, however, primarily for seeds that are permeable to water and not for those with water-impermeable seed (or fruit) coats (physical dormancy). The account of the move-along experiment given below is extracted (and modified) from a previous description of it (Baskin and Baskin forthcoming).

CONDUCTING A MOVE-ALONG EXPERIMENT

The move-along experiment is designed to determine the temperature, or temperature sequence, for seed dormancy break in seeds that are permeable to water. Thus, before initiating this experiment, we determine if the intact seeds or other germination units are permeable and if they are dormant. To determine if the seed (or fruit) coat is water permeable, weigh seeds (or fruits) on an analytical balance before and after they have been incubated (ambient room temperature is okay for this) on a moist substrate for 1 to several days. (Note: the fleshy part of the fruit should be removed prior to checking for water uptake.) If seed mass (weight) does not increase, then the seed (or fruit, for example, endocarp + true seed in *Rhus* [Anacardiaceae] and some Caprifoliaceae) coat is impermeable to water. Conversely, if seed mass increases ≥ 20% based on air-dry seed or fruit mass, then one can assume that the seed (or fruit) coat is permeable to water. To determine if permeable seeds are dormant, incubate them for 2 to 4 wk at several temperature regimes. If seeds fail to germinate, they are likely dormant and could benefit from a move-along experiment.

TEMPERATURE KEY

°C	°F
5	41
5/1	41/33
15/6	59/43
20/10	68/50
25/15	77/59
30/15	86/59

For a move-along experiment, we use 5 °C (or sometimes 5/1 °C) to simulate winter stratifying temperatures, 25/15 °C (or sometimes 30/15 °C) for summer, 20/10 °C for early autumn and for late spring, and 15/6 °C for late autumn and for early spring; these are 12-h day temperatures alternated with 12-h night temperatures. These temperatures were chosen using US Weather Bureau maximum and minimum air temperature data for Kentucky and adjacent areas, thus you may need to adjust these temperatures to those occurring in your study region. Because most seeds germinate best in light, or at least equally well in light and in darkness, we incubate seeds at a 14-h daily photoperiod of cool white fluorescent light (irradiance equal to about 2% of visible portion of full sunlight in summer). Seeds

TABLE 1

Outline for the move-along experiment [a]. The blue column shows progression of temperatures (°C) for seeds starting in "winter" and the green column shows the "summer" progression. The shaded areas may be deleted if the number of seeds or incubators is limited.

Weeks at treatment temperature	Move along		Controls			
12	5° [b] winter	25/15° summer	5°	15/6°	20/10°	25/15°
4 [c]	15/6° [d] early spring	20/10° early autumn	5°	15/6°	20/10°	25/15°
4 [c]	20/10° late spring	15/6° late autumn	5°	15/6°	20/10°	25/15°
12	25/15° summer	5° winter	5°	15/6°	20/10°	25/15°
4 [c]	20/10° early autumn	15/6° early spring	5°	15/6°	20/10°	25/15°
4 [c]	15/6° late autumn	20/10° late spring	5°	15/6°	20/10°	25/15°
12	5° winter	25/15° summer	5°	15/6°	20/10°	25/15°

[a] Modified from Baskin and Baskin (forthcoming).

[b] Conversion: ([°C • 1.79] + 32) = °F

[c] If the late autumn/early spring periods are deleted, increase the lengths of the 20/10 °C treatment periods (early autumn/late spring) from 4 to 8 wk.

[d] The first temperature is provided during 12 h of the 14 h light period each day and alternated with the second temperature during the other 12 h.

are at the high temperature for 12 h each day and at the low temperature for 12 h each day. The lights come on in the incubator 1 h before the high temperature begins and remain on 1 h after the low temperature begins. If number of seeds and/or incubators are limited, 15/6 °C can be dropped from the design and 20/10 °C can be used to simulate the entire autumn and spring. Thus, if 15/6 °C is dropped, the move-along seeds should remain at 20/10 °C for 8 wk during a simulated autumn and for 8 wk during a simulated spring. A long (that is, 8-wk) autumn can be especially important for some seeds, for example, *Jeffersonia diphylla* (Baskin and Baskin 1989) because this is when the small underdeveloped embryo grows. Also, a long (8-wk) spring is needed to give seeds an opportunity to germinate before they are exposed to high summer temperatures, which may reduce the percentage of seeds that germinate, as is the case for the mesic deciduous forest herb *Delphinium tricorne* Nutt. (Ranunculaceae) (Baskin and Baskin 1994).

To use the move-along technique, we prepare 18 petri dishes of seeds sown on moist soil, sand, or filter paper. We prefer to use 50 seeds per dish, but for rare species we sometimes use 25 per dish. Three dishes each are placed in light at 5 (or 5/1), 15/6, 20/10, and 25/15 (or 30/15) °C to serve as controls. Control seeds remain at their respective temperature regime for the duration of the experiment, and they should be checked at 2-wk (or shorter) intervals for germinated seeds (which should be removed from the dishes) and the substrate watered, if needed. The move-along portion of the experiment consists of 2 treatments of 3 dishes each. One treatment is started at 5 °C and the other at 25/15 °C. Thus, when we first start the experiment we have 6 dishes at 5 °C, six at 25/15 °C, and three each at 20/10 and 15/6 °C.

Seeds started at 5 °C (winter) subsequently are moved through spring, summer, autumn, and so on, while those started at 25/15 °C subsequently are moved through autumn, winter, spring, and so on (Table 1). If seeds have not germinated after 1 y, we recommend a continuation of the move-along experiment. For example, seeds of the mesic deciduous forest herb *Medeola virginiana* L. (Liliaceae) started at 25/15 °C in September 1986 did not germinate until July 1988 (23 mo later), after being moved through the following sequence of temperatures: 25/15 ⟶ 20/10 ⟶ 15/6 ⟶ 5 ⟶ 15/6 ⟶ 20/10 ⟶ 25/15 ⟶ 20/10 ⟶ 15/6 ⟶ 5 °C (Baskin and Baskin 1998, unpublished data). When seeds of *M. virginiana* started germinating in July 1988 they had been at 5 °C for almost 12 wk, but this was the second time they had received a cold stratification treatment! In the case of *M. virginiana*, dormancy was not broken during the first period at 5 °C, although seeds previously had received a 12-wk warm period; we do not know why. Thus, seeds of *M. virginiana* germinated after 2 warm periods and 2 cold periods, so it pays not to give up too soon.

INTERPRETING RESULTS

If seeds require only summer temperatures for dormancy break, the move-along seeds that start at 25/15 °C will germinate to high percentages when moved to autumn temperatures (20/10 and/or 15/6 °C), but little or no germination will occur in control seeds kept continuously at any of the 4 control temperatures, or in the seeds started at 5° after they are moved to spring temperatures (15/6 and/or 20/10 °C). Conversely, if seeds require only winter temperatures for dormancy break, the move-along seeds that start at 5 °C will germinate to high percentages when moved to spring temperatures (15/6 and/or 20/10 °C), but little or no germination will occur in control seeds kept continuously at any of the 4 temperatures, or in the seeds started at 25/15 °C after they are moved to autumn temperatures (20/10 and/or 15/6 °C).

For seeds that require the summer through winter temperature sequence (or warm followed by cold) to come out of dormancy, the move-along seeds that start at 5 °C will not germinate when moved to spring temperatures (20/10 and/or 15/6 °C), but those that start at 25/15 °C will germinate to high percentages when they are moved to spring temperatures; the controls at 15/6 and 20/10 °C do not germinate. Further, seeds started at 5 °C and subsequently moved from spring ⟶ summer ⟶ autumn ⟶ winter ⟶ spring will germinate in the second spring. That is, by the second spring the seeds would have received summer followed by winter temperatures, which are required for dormancy break.

When seeds of many species are incubated at 5, 15/6, 20/10, and 25/15 (or 30/15) °C, little or no germination occurs after 4 to 6 wk, and the conclusion is made (and correctly so) that they are dormant. In a few species, however, we have found that some of the control seeds germinate to high percentages at one of the incubation temperatures after extended periods of time. For example, seeds of the woodland shrub *Callicarpa americana* L. (Verbenaceae) incubated at 5, 15/6, 20/10, and 30/15 °C had germinated to 0%, 0%, 1%, and 75%, respectively, after 12 wk (Baskin and Baskin unpublished data), indicating that dormancy break and germination had occurred at the high temperature. In another example, seeds of the mesic to wet-mesic deciduous forest herb *Mertensia virginiana* (L.) Pers. (Boraginaceae) incubated at 5, 15/6, 20/10, and 25/15 °C germinated to 91%, 1%, 0%, and 0%, respectively, after 20 wk (Baskin and Baskin unpublished data), indicating that dormancy break and germination occurred at the low-temperature regime.

CONCLUSIONS

We have found the move-along experiment to be particularly useful for breaking dormancy in water-permeable seeds that are difficult to germinate and/or in cases where the number of seeds available to work with is limited. The procedure, as outlined in this paper, applies broadly to seeds of species in the temperate region. However, the move-along experiment easily could be modified to accommodate seeds of species from other climatic-vegetation types worldwide, such as subtropical montane or semievergreen tropical forest. For some species, consideration may need to be given to natural environmental factors other than temperature in the dormancy-breaking protocol. For example, dormancy break in seeds of *Lesquerella filiformis* Rollins (Brassicaceae), a rare winter annual mustard endemic to limestone glades of the Ozarks of southwestern Missouri, is facilitated considerably by alternate wetting and drying during summer (Baskin and Baskin 2001). Thus, to get the best germination results for seeds of certain species it may be necessary to modify our "continuously wet" move-along procedure to include alternate wetting and drying during summer. The idea of the move-along experiment, then, would be to subject dormant seeds not only to the sequence of temperatures they experience between seed dispersal and germination in nature but also to simulate soil moisture conditions during the year. Although we have not explored use of the move-along experiment to break physical dormancy in seeds in depth, there is no reason why one could not add other factors to the simulated yearly cycle of environmental factors known to break physical dormancy in some species, for example, high fluctuating soil temperatures (McKeon and Mott 1982) and fire (Baskin and Baskin 1997).

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