Some Procedures for Dormancy Break and Germination of Difficult Seeds

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Abstract

Seeds of a number of species we have studied over the past three decades are difficult to germinate. We have learned how to use information about the habitat and ecological life cycle of a species to plan effective strategies for breaking dormancy and promoting germination, e.g., when to use warm and/or cold stratification treatments. Also, we have become aware that temperature, light, substrate, and flooding (low oxygen) regimes during both the dormancy breaking and germination periods may influence germination percentages of a species. In this paper, some of the precautions and procedures we have discovered that help ensure high percentages of seed germination will be discussed.

Key words

cold stratification, flooding, light requirement, seed coat permeability, substrate, temperature, warm stratification

Introduction

Our experience with seeds comes from more than three decades of studies on how timing of germination is controlled in the field. To understand the seed germination ecology of a species, it is necessary to determine if fresh seeds are dormant and if so what kind of dormancy they have, when and how dormancy is broken in

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nature, and what environmental conditions are required for germination of nondormant seeds. In these studies, we have encountered a number of species whose seeds were difficult to germinate. For some species, high germination percentages were obtained when seeds were incubated at temperature (and to some extent soil moisture) regimes occurring in the habitat from the time of seed dispersal until the end of the natural germination season. However, even this approach did not result in high germination percentages in all species; consequently, changes in our experimental protocol for seeds of some species were required. The purpose of this paper is to briefly discuss some of the things we have learned about various species that help ensure high germination percentages.

Lessons We Have Learned

Filled seeds

In 1991, we buried approximately 156,000 seeds of Carex *lacustris* under flooded and under nonflooded conditions in a nonheated greenhouse in Lexington, KY. At monthly intervals, seeds from flooded and nonflooded conditions were exhumed and tested under nonflooded conditions in light and in darkness at five day/night alternating temperature regimes. After 7 mo, a grand total of only seven seeds had germinated in all germination tests. At this point, some seeds were cut open, and we discovered that only about 1% of them contained an embryo! Thus, we learned the hard way that just because seeds are large and feel firm to the touch both before and after imbibition does not mean they contain an embryo. Checking for presence of an embryo is always a good start for any seed germination study.

Dormancy break at high temperatures

Although cold stratification [moist, low temperature (about 0 to 10°C) conditions] breaks seed dormancy in many species, this treatment usually is ineffective in breaking dormancy in seeds of winter annuals and in those of autumn-germinating perennials. The best way to break dormancy in autumn-germinating seeds is exposure them to the temperature conditions of summer; the effective temperature range for dormancy loss is 15-35°C, with 20-30°C being optimal for many species. Cold stratification of autumn-germinating species actually can decrease germination. For example, fresh seeds of the redcedar (limestone) glade endemic

Delphinium carolinianum subsp. *calciphilum* germinated to 46 and 55% in light at 10 and 15°C, respectively, but after 2 mo of cold stratification germination was only 5 and 10%, respectively; after 2 mo of dry storage at 20-25°C, seeds germinated to 85 and 29%, respectively (Baskin and Baskin unpubl.). It should be noted, however, that seeds of the mesic woodland species *D. tricorne* require cold stratification for dormancy break (Baskin and Baskin 1994).

One problem in working with seeds that come out of dormancy during summer is deciding what moisture regime to use. Seeds of many winter annuals will come out of dormancy while stored dry at 20-25°C; this is called afterripening. Seeds of the woodland herbaceous perennial *Polemonium reptans* subjected to natural temperatures throughout the summer and watered daily germinated to 91%, while those stored dry at natural temperatures throughout the summer germinated to only 27% (Baskin and Baskin 1992a). (If seeds are moist during exposure to high temperatures, the treatment is called warm stratification.) On the other hand, seeds of the winter annual *Lesquerella filiformis,* a Missouri redcedar glade endemic, kept on continuously-moist sand at simulated summer temperatures (30/15°C day/night regime) for 3 mo did not come out of dormancy. The best moisture regime for dormancy break in seeds of *L. filiformis* was alternate wet (5 days) and dry (10 days) cycles throughout the summer (Baskin and Baskin 1998, unpubl.).

Dormancy break at low temperatures

Cold stratification frequently is effective in breaking seed dormancy of spring-germinating species in temperate regions; however, some species require warm followed by cold stratification (see below). Although 5°C often is reported in the literature as being the optimum temperature for cold stratification, it is not the optimum temperature for all species. In fact, 5°C may be too high to break dormancy in seeds of some species. For example, seeds of *Alliaria petiolata* stratified in darkness at 5°C germinated to I, 1, and 0% in darkness at 15/6, 20/10, and 25/15°C , respectively, while those statified in darkness at 1°C germinated to 60, 57, and 51%, respectively, in darkness (Baskin and Baskin 1992b). In *OsmorhiZa occidentalis,* the small (underdeveloped but differentiated) embryo grew while seeds were being cold stratified at 5°C, but seeds failed to germinate. In this species, the optimum temperature for embryo growth, dormancy break , and germination was 1°C (Baskin *et al.* 1995).

Warm followed by cold stratification

It is well known that warm followed by cold stratification is required to break dormancy in many species whose small (but fully differentiated) embryos also have physiological dormancy, *e.g., Erythronium albidum, Osmorhiza longistylis, Jeffersonia diphylla, Panax ginseng, Ilex opaca,* and *Taxus baccata* (Baskin and Baskin 1998). (In these species, the embryos must become fully elongated inside the seed before the radicle will emerge.) Although *Empetrum bermaphroditum* seeds have fully developed embryos, warm followed by cold stratification also is required to break dormancy in a high percentage of them (Baskin *et al.,* in press). In other species with fully developed embryos, *e.g., Florekea proserpinacoides* (Baskin et al. 1988) and *Cardamine concatenata* (Baskin and Baskin 1994), 12 wk of cold stratification were not effective in breaking dormancy, and 18 wk of cold stratification resulted in only about 50% germination. However, when seeds of *F proserpinacoides* and *C. concatenata* first were warm stratified for 4 wk, 100% of them germinated after 12 and 14 wk of cold stratification, respectively.

Permeability to water

Sometimes, seeds do not germinate because the seed or fruit coat is impermeable to water, and thus they fail to imbibe (swell) when placed on a moist substrate; this is called physical dormancy. The families known to have taxa with impermeable seed or fruit coats are the Anacardiaceae, Bixaceae, Cannaceae, Cistaceae, Cochlospermaceae, Convolvulaceae, Cucurbitaceae, Dipterocarpaceae, Geraniaceae, Leguminosae, Malvaceae [now also includes the Bombacaceae, Sterculiaceae, and Tiliaceae *(sensu* Bremer *et al.* 1999)], Nelumbonaceae, Rhamnaceae, Sapindaceae, and Sarcolaenaceae (Baskin *et al.* 2000). However, not all taxa in these families have physical dormancy. In fact, some tropical members of the Anacardiaceae, Bombacaceae, Cucurbitaceae, Leguminosae, Malvaceae, Sapindaceae, and Sterculiaceae have recalcitrant seeds, i.e. if seed moisture content declines below 15-45 %, depending on the species, the seed loses viability (Baskin and Baskin 1998). The way to determine if seeds are permeable or impermeable to water is to weigh them before and after they have been on a moist substrate for several hours. An increase in weight indicates that seeds are permeable to water and no increase that they are impermeable.

Some taxa in various families including the Anacardiaceae, Apocynaceae, Arecaceae, Betulaceae, Burseraceae, Caprifoliaceae, Cornaceae, Elaeagnaceae, Empetraceae, Ericaceae, Juglandaceae, Meliaceae, Menispermaceae, Moraceae, Nyssaceae, Oleaceae, Pandaceae, Rhamnaceae, Rosaceae, Sapotaceae, Styracaceae, and Zygophyllaceae have seeds covered by a hard or stony endocarp. However, water-impermeable endocarps in this group of families are known to occur only in *Rhus* and a few of its closely-related genera, in the Anacardiaceae (Baskin and Baskin, unpubl.). In dealing with seeds covered by stony endocarps, one is tempted to scarify them because they feel hard to the touch, but scarification may not improve germination. In fact, scarification could allow pathogenic organisms to invade and destroy the embryo. Thus, before scarifying stony endocarps, it is advisable to first determine if they are impermeable to water. In *Empetrum hermaphroditum* (Empetraceae in the strict sense), seeds are covered by stony endocarps, the endocarp is permeable to water, and a sequence of warm followed by cold stratification treatments is required to break dormancy in a high percentage of the seeds. Thus, warm stratification plays a role in breaking dormancy of the embryo and not in making the endocarp permeable to water, as has been speculated for some seeds with stony endocarps (Baskin *et al.,* in press).

Substrate effects

In some species, the problem of low germination percentages can be solved by changing the substrate used for seed incubation. Freshly matured seeds of *Campanula americana* germinated to 65, 87, 85, and 44% on soil in light at 15/6, 20/10, 25/15, and 30/15°C, respectively, but to only 5, 29, 76, and 55%, respectively, on sand (Baskin and Baskin 1984). Further, the substrate effect was accentuated following 12 wk of cold stratification in light at 5°C. Cold stratified seeds germinated to 89, 81, 83, and 62% on soil in light at 15/6, 20/10, 25/15, and 30/15°C, respectively, whereas those on sand germinated to only 2, 12, 35, and 39%, respectively (Baskin and Baskin 1984).

Following 16 wk of cold stratification in darkness at 1°C, seeds of *Alliaria petiolata* germinated to 60, 57, 51, and 35% on soil in darkness at 15/6, 20/10, 25/15, and 30/15°C, respectively, but none of those on sand germinated (Baskin and Baskin 1992b).

Temperatures for seed testing

It is possible to break seed dormancy but not to obtain seedlings because the appropriate conditions for germination were not provided. For example, seeds that have been warm- or cold-stratified may fail to germinate because temperatures are too high or too low, depending on the species. Thus, some species germinate best at low temperatures, others at high temperatures, and still others at intermediate temperatures. Nondormant seeds of the desert winter annual *Eriogonum abertianum* germinated to 86 and 79% in light at 15/6 and 20/10°C, respectively, but to only 3, I, and 0% at 25/15, 30/15, and 35/20°C, respectively (Baskin *et al.* 1993). Nondormant seeds of the herbaceous polycarpic perennial *RueIlia humilis,* on the other hand, germinated to 0 and 15% at 15/6 and 20/10°C, respectively, but to 98, 100, and 1_00% at 25/15, 30/15, and 35/20°C, respectively (Baskin and Baskin 1982). In contrast to both *E. abertianum* and *R. humilis,* nondormant seeds of the winter annual *Chaerophyllum tainturieri* germinated best at an intermediate temperature, *i.e., 7,* 39, 0, and 0% germination at 15/6, 20/10, 30/15, and 35/20°C, respectively, and 99% at 25/15°C (Baskin and Baskin 1990).

Another thing that might be helpful to know is that the temperature range for germination can widen as seeds of many species come out of dormancy. Being aware of this might allow you to obtain seedlings sooner than you would otherwise. For example, as seeds of the summer annual *Bidens polylepis* come out of dormancy during cold stratification, they exhibit a decrease in the minimum temperature at which they will germinate to 50% or more. After 2 months of burial in soil at natural winter temperatures in Lexington, KY, seeds germinated to about 95% at 30/15 and 35/20°C, but germination at 15/6°C did not exceed 50% until after seeds had been buried for 5 mo (Baskin *et al.* 1995). Thus, if we had been using only the 15/6°C temperature regime, we would not have known that seeds of this species are capable of germinating to high percentages after only 2 mo of exposure to winter conditions.

As seeds of the winter annual *Alopecurus carolinianus* come out of dormancy during warm stratification (buried in soil and exposed to summer conditions), they exhibited an increase in the maximum temperature at which they germinate to 50% or more. After I mo of burial, seeds germinated to about 85% in light at I 5/6°C, but germination at 30/15°C did not exceed 50% until after seeds had been buried for 4 mo (Baskin *et al.* 2000). Thus, if we had been using only the 30/15°C temperature regime, we would not have known that seeds of this species are capable of germinating to high percentages after only I mo of exposure to summer conditions.

Light requirement for germination

Another reason why seeds that have been sufficiently warm and/or cold stratified may not germinate is the lack of appropriate light or dark conditions. Although nondormant seeds of many species germinate equally well in light and darkness, it is not unusual for germination percentages to be higher in light than in darkness. Further, seeds of some species require light for germination, and those of a relatively few species require darkness (see chapter 10 in Baskin and Baskin 1998).

Seeds with an absolute light requirement for germination vary with regard to the time when the light requirement can be fulfilled. Seeds of *Solidago altissima* and *S. nemoralis* exposed to light during a 12-wk cold stratification period at 5°C subsequently germinated 82 and 99%, respectively, in darkness at 20/10°C; seeds cold stratified in darkness and incubated in darkness germinated to 0 and 1%, respectively (Walck *et al.* 1997). Thus, the light requirement could be fulfilled during stratification, and seeds could germinate in darkness at simulated spring temperatures. On the other hand, seeds of Cyprus *squarrosus* (syn: *C. aristatus,* C. *inflexus)* require cold stratification and light for germination, but the light requirement for germination can not be fulfilled during cold stratification. Thus, light is required for germination of nondormant seeds during incubation at suitable spring-summer germination temperatures (Baskin and Baskin 1971). For many species, light during both the dormancy breaking and germination periods results in higher germination percentages than when light is given during only one of the periods, *e.g.,* seeds of *Echinacea angustifolia,* which require cold stratification for germination (Baskin *et al.* 1992).

Effect of flooding

Although flooding, which results in low oxygen availability, may inhibit dormancy break in some species (Baskin *et al.* 1994), it has no inhibitory effect on dormancy loss in seeds of some wetland species (Baskin *et al.* 1996). However, maximum germination may be obtained in some wetland species by cold-stratifying seeds under nonflooded conditions and then germinating them in light under flooded conditions, *e.g., Schoenoplectus purshianus,* a summer annul occurring on wet mud adjacent to depression flooded during summer (Baskin *et al.* 2000). Thus, for species growing in wet habitats, it is important to determine the time of year when seeds are flooded.

Conclusions

Some of the difficulties in germinating seeds, especially those for which no previous research data are available, can be avoided by (I) making sure the seeds have an embryo, (2) determining if seeds imbibe water, (3) using a range of test temperature regimes, (4) incubating seeds in both light and darkness, and (5) simulating warm and/or cold stratification treatments and substrate moisture conditions that seeds would be exposed to in the field from dispersal to germination. Thus, the more one knows about the natural habitat and ecological life cycle of the species, the easier it will be for him/her to plan effective strategies to obtain high germination percentages.

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