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RESEARCH OPINION

# Mistakes in germination ecology and how to avoid them

**Carol C. Baskin[1,2*], Ken Thompson[3] and Jerry M. Baskin[1]**

[1]Department of Biology, University of Kentucky, Lexington, KY 40506-0225 USA; [2]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546-0312 USA; [3]Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN UK

## Abstract

Frequently, in studies on seed germination ecology, mistakes are made that lead to problems in determining the kind of dormancy and in extrapolating data to the field situation. In this paper, we discuss 22 of these issues and offer suggestions on how to avoid them.

**Keywords: germination test procedures, seed dormancy, seed germination, seed germination ecology**

## Introduction

One of the major goals of seed germination ecology is to understand how dormancy (if present) is broken and how timing of germination is controlled in nature. Ideally, we can conduct studies in the laboratory/greenhouse/field and answer these questions. However, all too often, (seemingly small) mistakes lead to big problems in trying to germinate seeds and extrapolate data to the natural world. These problems sometimes result in manuscripts having to be revised or, even worse, rejected. The purpose of this paper is briefly to discuss various points related to research in germination ecology that potentially lead to problems, if appropriate actions are not taken. Some of these points are derived from the numerous questions asked by graduate students over the years, while others are problems frequently encountered by reviewers of manuscripts on germination biology.

Throughout this short paper we assume the aim is to understand how dormancy is broken and germination is controlled *in nature*, but other research aims are possible. It is frequently necessary to provide seedlings for research purposes, and here the aim is simply to produce seedlings by the quickest and most convenient route. Given that aim, treatments that seeds would never receive in nature may be applied (e.g. concentrated sulphuric acid, gibberellins). Note, however, that there is a grey area. Even if treatments that seeds *might* experience in nature (e.g. nitrates, smoke) are effective in promoting germination, it is not safe to assume that they play an important role in nature.

*Correspondence
Fax: +1 859 257 1717
Email: ccbask0@uky.edu

## Questions and discussion

### *1. What should I try to germinate?*

As far as possible, you should use the intact natural dispersal unit, because removal of parts from it may increase/decrease germination percentages. The 'intact natural dispersal unit' means the fruit or seed in the form in which it would normally occur in nature after dispersal. For fleshy fruits, this means removing the flesh, which often contains germination inhibitors. Unless it is likely to happen in the field, you should not attempt to remove structures such as the lemma and palea that remain tightly bound to many grass grains, even if their removal promotes germination.

### *2. What conditions should I use?*

It is best to test seeds/fruits over a range of alternating temperatures regimes, simulating those in the natural habitat during the growing season, and also place seeds/fruits in light and in darkness. If possible, constant temperatures and continuous light should be avoided; obviously, these conditions are not encountered frequently in nature, and in some species may decrease germination percentages. Also, high photon

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irradiance can inhibit germination. To answer this question, consider the sequence of environmental factors to which the seeds are exposed between maturity and germination. For example, if a seed is dormant at maturity in spring and germinates in autumn, it obviously does not need to be cold stratified.

### 3. Why should fresh seeds be used when determining if seeds are dormant?

If seeds have non-deep physiological dormancy, after-ripening (dormancy break) can occur during dry storage, especially at room temperatures. In a few species, drying can cause seeds to enter dormancy. If seeds have physical dormancy, drying may cause the water gap to open. The key point is that if your seeds have already received any 'treatment' (however inadvertent) before you try to germinate them, you cannot know what the effects of this treatment have been.

### 4. Is it OK to store seeds dry at low temperatures (e.g. 5°C or sub-zero) and test for presence of seed dormancy later?

Even at −20°C, after-ripening has been shown to occur in seeds of some species. If experiments cannot be started immediately, a preliminary germination test should be done before seeds are placed in dry storage at low temperatures. High germination percentages after seeds have been stored for a long period (at any temperature) does not prove that the fresh seeds were non-dormant.

### 5. When should a germination test be terminated?

Depending on the species, considerable dormancy break can occur in dry seeds at room temperatures within a few weeks; thus, we suggest that a test should be stopped within about 4 weeks. If you want to know if dormancy break and germination would eventually occur at a certain temperature regime, seeds could be checked at 2-week intervals and kept for years. Seeds at all test conditions should be given the same period of time for germination.

### 6. If fresh seeds germinate to a high percentage at only a portion of the test temperatures, are the seeds non-dormant?

Some freshly matured seeds are non-dormant, but they germinate only at high or at low temperatures, depending on the species. If the range of temperatures over which seeds will germinate cannot be increased, then seeds are non-dormant. However, in many species, the temperature range for germination will increase if seeds are given a dormancy-breaking treatment, e.g. cold stratification or exposure to high summer temperatures. If the temperature range increases, then the fresh seeds were conditionally dormant. The only way to know if fresh seeds are non-dormant (with a high or a low temperature requirement for germination) or conditionally dormant is to test them over a range of temperatures, give them a dormancy-breaking treatment and then test over the same range of temperatures. If the temperature range for germination increases, the fresh seeds were conditionally dormant. Try to make sure your test temperatures cover the whole range over which the seeds will germinate; even if your seeds germinate well at 25°C, test them at 30°C and 35°C before concluding that 25°C is the optimum temperature for germination.

### 7. How can I tell if seeds have physical dormancy?

Physical dormancy means the seed (or fruit) coat is water impermeable. To determine if diaspores are water impermeable, they should be weighed before and after a period of incubation (e.g. 24 h) on a wet substrate. Physical dormancy is currently known in the *Anacardiaceae, Bixaceae, Cannaceae, Cistaceae, Cochlospermaceae, Convolvulaceae* (including *Cuscutaceae*), *Curcurbitaceae, Dipterocarpaceae* (subfamilies *Monotoideae* and *Pakaraimoideae*, but not *Dipterocarpoideae*), *Fabaceae* (all three subfamilies), *Geraniaceae, Malvaceae* (including *Bombacaceae, Sterculiaceae* and *Tiliaceae*), *Nelumbonaceae, Rhamnaceae, Sarcolaenaceae, Sapindaceae and Surianaceae*. If your species is not in one of these families, it probably has water-permeable seeds/fruits. It should be noted that not all members of all of these families have water-impermeable seeds. For example, in *Anacardiaceae* only members of the '*Rhus* complex' have physical dormancy, and seeds of the other 70 or so genera in the family have either physiological dormancy or are non-dormant.

### 8. How can I tell if seeds have only physiological dormancy?

Seeds will imbibe water (sometimes no more than 20–30% increase in mass), they have an embryo that does not grow inside the seed prior to germination (although it may be small, i.e. low embryo length/seed length ratio, e.g. *Nymphaea*) and they require more than 4 weeks to germinate. In these seeds, the embryo does not have enough growth potential (push power) to overcome the mechanical restraint of the seed coat and

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endosperm, if present. After receiving appropriate dormancy-breaking treatments, the embryo can generate enough expansive force to break through the seed coat.

### 9. Do increases in germination percentages after treatment with acids (e.g. concentrated $H_2SO_4$) or mechanical scarification mean the seeds have physical dormancy?

No. To prove that seeds have physical dormancy, it is necessary to demonstrate that the seeds (or fruits) do not imbibe water.

### 10. If seeds are water permeable, why would mechanical or acid scarification promote germination?

Acid and mechanical scarification reduce the mechanical restriction of the seed/fruit coat. Thus, the radicle is able to emerge from the seed, although growth potential of the embryo has not been increased. Scarification also may overcome a light requirement for germination, reduce sensitivity to far-red light and increase sensitivity to gibberellic acid.

### 11. Is it OK to use laboratory-stored seeds rather than cold stratified ones to study the effects of salts (or other osmotica) on germination?

Cold stratification may increase the expansive force of the embryo, and thus allow seeds to germinate under higher osmotic stress than non-stratified seeds. Thus, if seeds are cold stratified in the field before they germinate, using non-stratified seeds may not give a true indication of the water potential at which seeds could germinate in the field.

### 12. How can I tell if seeds have morphological dormancy (MD)?

Seeds will imbibe water. They have a relatively small (low embryo length/seed length ratio) embryo in a relatively large seed with much endosperm. In MD, the embryo has to grow (inside the seed) before the seed germinates, and seeds germinate in about 4 weeks from the start of incubation. However, not every seed with a relatively small embryo has MD. To verify that seeds have MD, you need to demonstrate that the embryo grows inside the seed before the radicle emerges. To do this, measure the embryo in at least 15 freshly matured, imbibed seeds, and then measure the embryo in other seeds when the seed coat splits, i.e. just prior to radicle emergence.

### 13. How can I tell if seeds have morphophysiological dormancy?

Seeds will imbibe water. They have a relatively small embryo in a relatively large seed with much endosperm. Seeds do not germinate in 4 weeks, and may in fact require several months of dormancy-breaking treatments before the embryo grows and the radicle emerges. Seeds cannot germinate until both physiological dormancy is broken and embryo growth occurs. The conditions that permit embryo growth and that break dormancy may be very different. For example, in some temperate species a period of warm stratification is required for the former and cold for the latter.

### 14. Cold stratification is reported to break dormancy in my seeds. Thus, I put dry seeds in a paper bag in the refrigerator at 5°C for 12 weeks, but none of them germinated, regardless of the test temperature. Why?

The seeds did not germinate because they were still dormant. Seeds that require cold stratification must be imbibed for dormancy break to occur. In addition, it is not a wise general practice to store unimbibed seeds in paper bags at any temperature; doing so allows the seed moisture to change continually as a function of atmospheric relative humidity (RH). Any physiological change that is moisture content dependent (such as after-ripening) will be subject to the RH variability.

### 15. Cold stratification is reported to break dormancy in my seeds. Thus, I put imbibed seeds in the freezer for 12 weeks, but none of them germinated, regardless of the test temperature. Why?

Seeds cannot be cold stratified in the freezer because it is too cold (about −20°C in most freezers). A temperature between 0 and 10°C is suitable for most species, but some have more specific temperature requirements.

### 16. I gave the seeds cold stratification (and/or warm stratification), but only a low percentage of them germinated. Why?

The ungerminated seeds may not be viable. Thus, at the end of a germination test it is important to determine if ungerminated seeds are viable. Do the seeds have an embryo? Sometimes 'seeds' are empty, but you can not tell this by looking at them. Do the seeds have a firm, white embryo? Will the embryo stain pink if placed in a tetrazolium solution

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(indicating that the cells are metabolically active)? If seeds are not viable, the percentage of viability should be reported, and germination percentages should be calculated based on viable seeds present at the start of the experiment. If seeds are viable, they may need warm (≥15°C) stratification followed by cold stratification to break dormancy.

### *17. In my experiment, dark-incubated seeds were exposed to dim white (or room) light for about 2–3 min each day while germination was being checked. My results show that germination percentages were equally high in both light and darkness; thus, can I conclude that the seeds do not have a light requirement for germination?*

No. During the brief period of exposure to light, the light requirement for germination may have been fulfilled in the 'dark-incubated' seeds. Only a few seconds of light are required to fulfil the light requirement of seeds of some species.

### *18. How safe are green safe lights for checking germination of dark-incubated seeds?*

It depends on the species, time of year (i.e. stage of after-ripening) and quality of the light. Green light actually promotes seeds of some species to germinate, while seeds of other species are more sensitive to green light at some times of the year than at other times. The quality of the green light is important. It is not easy to get a narrow-band green safe light. Thus, at different stages of dormancy break, it is important to compare germination of seeds exposed to the green light with that of seeds not receiving any green light. A green safe light may not stimulate germination in the early stages of dormancy break, but may stimulate germination in the late stages of dormancy break, i.e. when the seeds are more sensitive to germination-stimulating factors.

### *19. Is it OK to collect the seeds, although they still look a little green?*

Testing green seeds may not give you a true picture about seed dormancy, but you might get seedlings (if that is your aim), depending on the species. One way to avoid the problem of physical dormancy in seeds of some species is to collect seeds/fruits before they dry on the mother plant, i.e. before the seed/fruit coat becomes water impermeable during the drying process. In a few species whose seeds have physiological dormancy, picking green seeds can also be a way of avoiding the problem of dormancy. However, some seeds with physiological dormancy may never germinate if they are not allowed to dry. Sometimes, seeds can be picked while slightly immature, and they will be normal with respect to dormancy. If seeds must be collected before they are fully ripe, it is best to leave the seeds attached to as much plant material as possible and to store them in a damp place where they will dry slowly.

### *20. My fresh seeds will germinate, but drying kills them. Does this mean they are recalcitrant (desiccation sensitive)?*

Not necessarily. All immature seeds are sensitive to desiccation, and in some species desiccation tolerance is acquired only very late in development. Before concluding that your seeds are recalcitrant, make sure that you are testing seeds that are completely mature.

### *21. If leaching in running water increases germination percentages, does this mean a chemical inhibitor was removed from the seeds?*

Maybe, but maybe not. Proving that a particular compound is present and that it prevents germination *in situ* is very difficult. Further, depending on the temperature and on length of the leaching period, seeds may have actually received a period of warm or cold stratification.

### *22. My mature seeds are dormant and/or can be stored in the dry state for long periods at low temperature without losing viability. Can I assume they will also be persistent in the soil seed bank?*

No. Seeds of many species are highly dormant and easy to store in the dry state, yet are short-lived in the soil. On the other hand, if dry seeds are difficult to store (and especially if they are recalcitrant), they are unlikely to persist in the soil for long either.

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