CHICAGO BOTANIC GARDEN

Germinating Native Seeds from the Dixon National Tallgrass Prairie Seed Bank

The earliest seed collections in the Dixon National Tallgrass Prairie Seed Bank have been stored in the seed vault at -20°C since 2003-2004.

Recently, a sample of a select group of these seeds was removed from their deep freeze, and placed in favorable growing conditions to see if they will germinate after more than 10 years of dormancy.

We project that the seeds of most of our Midwestern flora will survive long periods of freezing, but the only way to know for sure is to periodically remove a sample of seeds and test their viability. During the winter of 2015 and 2016 we acquired the assistance of two students from the Botanic Garden's graduate program in association with Northwestern University to perform the Seed Bank's first germination trials for some of its earliest seed collections.

Inside the seed vault at the Dixon National Tallgrass Prairie Seed Bank.

To successfully germinate seeds, it is helpful to understand what type of dormancy parameters apply to each species. A state of dormancy upon maturation is essential for a seed to survive periods that are inhospitable for growth (e.g., cold, drought). Understanding when a seed matures, disperses and germinates and the environmental conditions that exist before and during

germination holds clues to the type of dormancy a species exhibits and how to break its dormancy to allow germination.

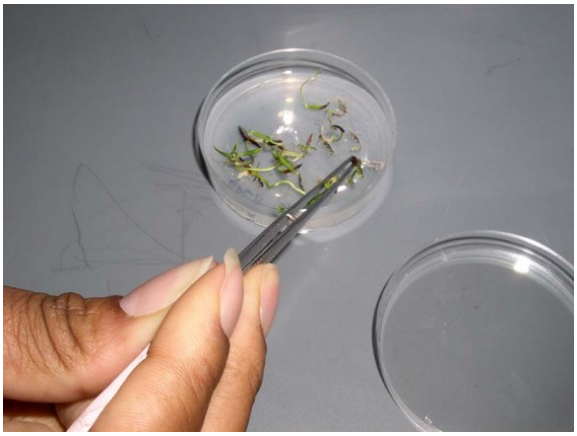
The seeds of many native perennial plants in the Midwest, that dominate the seed bank acquisitions, exhibit a type of dormancy called physiological dormancy. Seeds with physiological dormancy require a physiological cue to trigger germination. Often, that cue is a period of cold and moisture, typically called cold stratification. Seeds that mature and disperse in the fall and germinate in the spring often require cold stratification to break dormancy. Other types of dormancy include:

Morphological dormancy – seeds with undeveloped embryos upon dispersal which require additional time for their embryos to develop before they can germinate. A warm moist period is usually required.

Morphophysiological dormancy – seeds with a combination of the previous two dormancies that require a period of warm stratification, to ensure embryo development, followed by a period of cold stratification to break dormancy. Many spring blooming plants with seeds developing in spring or early summer can have this type of seed dormancy. There are many variations of morphophysiological dormancy exhibiting alternating warm and cold treatments.

Physical dormancy - seeds with hard seed coats that inhibit water absorption and, therefore, germination. When the seed coat is physically nicked or abraded in some way (scarified) allowing water absorption, germination will commence. Sometimes seeds with physical dormancy will also have physiological dormancy and will require cold stratification in addition to scarification before germinating.

Graduate student Alicia Foxx hard at work counting...



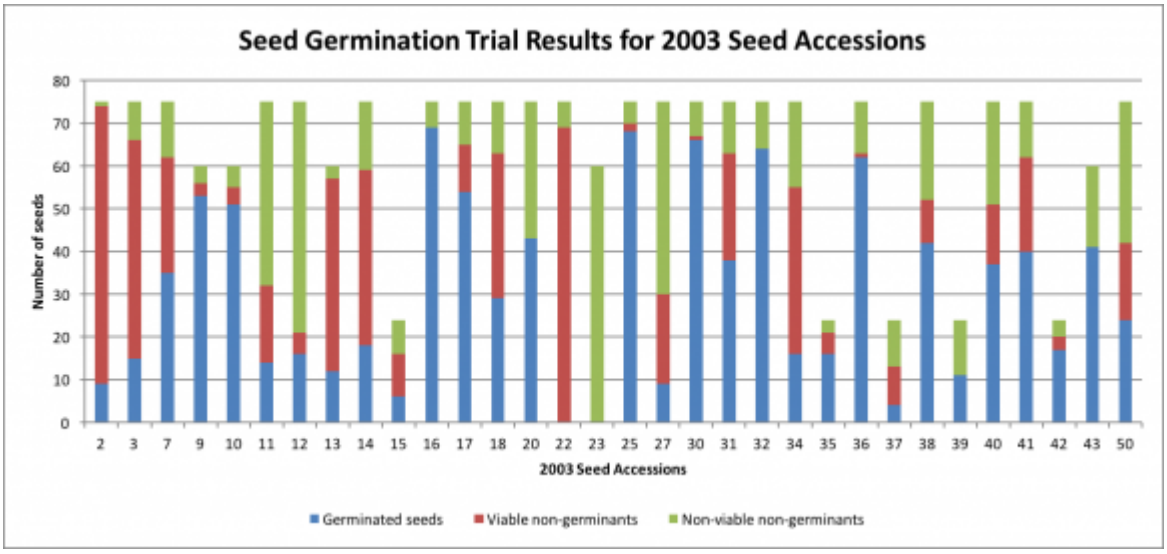
...and removing seeds that have germinated on an agar medium.

Knowing the type of seed dormancy exhibited by a particular species held in the seed bank is helpful in determining how to germinate those seeds, but providing periods of cold and warm stratification as needed is time consuming. Fortunately, there is a way to speed up the process.

The mechanism responsible for inducing dormancy and breaking dormancy is physiologically controlled by the presence or absence of two plant hormones. Dormancy is induced in seeds by the presence of the hormone abscisic acid and is broken by the presence of the hormone gibberellic acid. By soaking seeds in a 1% concentration of commercially produced gibberellic acid, dormancy can be broken for many seeds. For these trials, we pre-soaked seeds in gibberellic acid solution prior to placing them on agar filled petri dishes and then place them directly in a growth chamber calibrated to mimic environmental conditions suitable for germination, effectively bypassing cold stratification.

Trial results show that seeds of most species tested have germinated at a fairly high percentage. The seeds of species that did not germinate well under these conditions suggests that gibberellic acid may not be effective in breaking dormancy (some species exhibiting morphophysiological dormancy do not respond to gibberellic acid treatment) and additional treatments may be required.

In the graph below all seed accessions included in the trial are represented. Data was recorded on Monday and Friday of each week for 9 weeks. Seed were exposed to three day/night temperature increases inside a growth chamber during the trial period (20°/10°C, 25°/15°C, 30°/15°C respectively). The blue segment of each bar represents the number of seeds that germinated within that period. The red segment of the bars represents the number of seeds that did not germinate and were shown by x-ray examination to be “filled." Seeds that are recorded as filled suggest that they are potentially viable. The green segment of the bars represent seeds that did not germinate and were shown to be empty by x-ray and are considered not to be viable.



Seed sample sizes for trial were either 24, 60, or 75 seeds, depending on the number of seeds in the collection.

Species such as *Symphyotrichum novae-angliae* (9), *Apocynum cannabinum* (16), *Pycnanthemum virginianum* (25), *Veronica comosa* (30,) *Lycopus americanus* (36), and *Sorghastrum nutans* (42) that are represented in the bar graph with mostly blue and very little red show good germination with few viable (filled) seeds left over. Species such as *Spartina pectinata* (20) and *Liatris cylindracea* (43) also germinated well considering a good portion of their seeds in the trial sample

were empty. Species such as *Circaea lutetiana* (2), *Ceanothus americanus* (3), *Lespedeza capitata* (13), and *Cornus racemosa* (22) that are represented by mostly red suggest that we have either failed to break dormancy in those species and further treatment is warranted or those seeds have succumbed to the cold treatment and cannot be stored long-term in a seed bank. Further treatments to break dormancy are recommended for these species. For *Solidago juncea* (11), *Solidago canadensis* (12), *Sporobolus heterolepis* (23), and *Oligoneuron rigidum* (27) with bars dominated by the color green, much or all of the sample contained empty seeds. For these species, and all of the species for that matter, the green part of the bar does not reflect the accession's ability to survive in a seed bank because the seeds were never viable to begin with.

If we conclude from these results that live seeds are losing viability, we have two options if we wish to perpetuate those species that lose viability relatively quickly in the seed bank environment: 1. Sow seeds of the entire collection, grow them to maturity in nursery conditions, collect the seeds of the first generation and re-freeze them in the seed vault or, 2. Re-collect from the original population, if that population still exists.

Seeds that show good viability after 10 to 12 years in the seed vault will remain in the vault for another ten years before being tested again. It has been predicted that many of our native seeds held in seed bank conditions can survive hundreds of years in storage. We are in the process of testing that prediction for the species in our region.

