

Propagation of Wildflowers from Wild collected Seeds or Cuttings[©]

Mack Thetford

University of Florida, WFEC, Department of Environmental Horticulture, Milton, Florida

Alison E. Heather, Hector E. Pérez

University of Florida, Dept. Environmental Horticulture, Gainesville, Florida

Sandra B. Wilson

University of Florida, IRREC, Dept. Environmental Horticulture, Fort Pierce, Florida

Email: thetford@ufl.edu

INTRODUCTION

Production of native plant species for ecological restoration is a growth segment of the nursery industry and offers a tremendous opportunity to utilize the expertise of plant propagators. For the producer of a native species of high interest and potential demand for which no formal production protocol has been published or is known the choice of beginning with seed or cuttings is of great importance. Basic to the production of native species is the development of seed or cutting propagation methods that respect the ecological and genetic integrity of native populations, maximize production potential of a given species and minimizing the time to production all at a minimal production costs. With this in mind the propagator must have a basic understanding of seed dormancy and vegetative propagation when in pursuit of economical seed and cutting propagation protocols. This paper outlines seed dormancy mechanisms and a basic approach to investigating the use of stem cutting propagation for wild collected plant species with potential for inclusion in the wildflower industry. This information is provided to assist the

propagator in designing small scale seed and stem cutting propagation trials for development of seed or cutting protocols of wild collected wildflowers.

PROPAGATION BY SEED

An initial step is to consider the life cycles of the plants in question and develop an understanding of the growth, dormancy, flowering, and fruiting characteristics for each plant. Annual and biennial species most often rely on development of seed production and germination protocols while perennial species may utilize both seed and vegetative propagation techniques. To estimate viability, a pre-germination Triphenyl tetrazolium chloride (TZ) test can be conducted on freshly harvested seeds. Triphenyl tetrazolium chloride is a redox indicator commonly used in biochemical experiments especially to indicate cellular respiration. The TZ test can be performed on seeds or embryos (depending on ease of extraction) incubated for 2 to 8 hr at 35°C in 0.1 to 1% tetrazolium (2, 3, 5-triphenyl tetrazolium chloride) solution.

While most propagators are aware of methods to determine seed viability these tests do not allow the propagator to know if the seeds have a dormancy mechanism which may delay germination of viable seed. Although germination in seeds is known to be delayed, little is known about the dormancy mechanisms of many wild plants. Dormancy delays germination of fresh, mature, viable seeds, and although important for the reproduction of plants in native communities, may interfere with propagation and production. Baskin and Baskin (2004) have written extensively on this subject and use the absence of radicle emergence in the timeline of four weeks in conditions favorable to germination to signify dormancy.

A basic germination test may be used to determine if fresh seeds will readily germinate or if the seeds have a type of seed dormancy. Seeds are germinated on moist blotters for 28 days with a 12 hour photoperiod treatment and a complete darkness treatment. Non-germinated seeds may

be subjected to a TZ test at the end of the germination trail to determine their viability.

Generally, non-germinated viable seeds represent the portion of the seed population which possesses some type of seed dormancy.

Five different types of seed dormancy are recognized and include: Physical dormancy (PY), Physiological dormancy (PD), Morphological dormancy (MD), Morpho-physiological dormancy (MPD), and Combinational dormancy (PY + PD). With an understanding of these five dormancy types, the propagator may employ specific germination tests to determine the presence of each of these dormancy types in order to develop a germination protocol for seeds of wild plants.

Additionally, Baskin and Baskin (2004) have developed a dichotomous key to distinguish between the 5 classes of seed dormancy and non-dormancy based on the developmental state or size of the embryo, the permeability of the seed or fruit coat and whether the seed will germinate within a 4 week period.

Physical dormancy (PY). This occurs when a seedcoat, or fruit coat is impermeable to water thus preventing imbibition; the first stage of seed germination. Physical dormancy is the second most common form of dormancy. The presence of a pericarp composed of one or more palisade layers of lignified cells or an endocarp (the innermost layer of the pericarp) that is impermeable to water is commonly the source of impermeability. Although PY is known to occur in at least 15 plant families such as the *Fabaceae*, *Mavaceae* and *Sapindaceae* (Baskin et al., 2000) PY is not present in all members of most of these families. An interesting adaptation with many species having PY is the presence of a specialized structure in the impermeable seed or fruit coat called the “waterplug” or “watergap”. These structures provide an entry point for water when they are dislodged or disrupted in response to environmental triggers such as fire, heat, or alternating temperatures.

PY may be overcome in seeds with the use of scarification treatments such as acid scarification, heat treatments, mechanical scarification, dipping in boiling water or exposure to dry heat. Baskin and Baskin (2004) warn however, that successful germination of some species varies or fails following exposure to dry heat or boiling water and the temperature regimes required to break PY vary with the species.

A basic imbibition test may be used to test for PY. The mass of 100 freshly harvested seeds will be determined (W_d). Seeds are placed on moist blotter paper within Petri dishes. Mass of seeds is determined again after 0.25, 0.5, 0.75, 1-8, and 24h (W_i). The amount of water taken up (percent change in weight) will be calculated using the formula $W_i = [(W_i - W_d) / W_d] \times 100$ where W_i and W_d = mass of imbibed and dry seeds respectively (Baskin).

Physiological dormancy (PD). This is the most common form of seed dormancy and results when the embryo is unable to grow large enough to break through the seed coat. Although the embryo is usually differentiated and developed, a physiological inhibition within the embryo will result in a lack of embryo growth. Baskin and Baskin (2004) report that differentiated, underdeveloped embryos usually occur in seed greater than 2.0 mm in length but also emphasize that fully developed embryos can occur in seeds that are less than or greater than 2.0 mm. This type of dormancy will generally require a combination of environmental signals such as cool wet, warm wet or warm dry conditions to overcome dormancy depending on the species and geographical region of origin. The presence of these environmental conditions will bring about a physiological change in the embryo of the seed (becomes fully non-dormant) which can then grow and push through all of the layers of seed or fruit coat surrounding it. Seeds should be imbibed for at least 24 hours at room temperatures prior to dissection to determine the developmental state of the embryo.

Seed treatments that relieve PD by alternating moisture and temperature regimes are commonly referred to as a form of stratification. PD may be broken via warm stratification (warm wet conditions), after-ripening (warm dry conditions) or cold stratification (cool wet conditions) depending on the species and geographical origin. Three levels of physiological dormancy (deep, intermediate, and non-deep) have been characterized relating to the number of weeks of cold stratification required to become non-dormant. In some cases a period of after-ripening may substitute for several weeks of cold stratification.

The temperature regime required to break dormancy in seeds with non-deep PD frequently is not the optimal temperature for germination. Interestingly, seeds with non-deep PD respond differently to a variety of germination conditions as they naturally progress from a dormant state through after-ripening to become non-dormant. For example, the range of temperatures at which the seed will germinate may be very narrow and differ in direct response to the degree of after-ripening the seed has achieved while the seed may germinate across a very broad range of temperatures once after-ripening is complete.

Simulation of natural habitat temperatures across different seasons of the year can be used to determine the independent requirements for breaking dormancy and germination. For seeds permeable to water, Baskin and Baskin (2003) describe a germination experiment called a “move-along” germination trial to discern the independent requirements for breaking dormancy and germination. A move-along germination trial simulates natural habitat temperatures across different seasons of the year. A typical experiment will consist of two move-along sequences that run concurrently and four temperature controls. Such experiments will determine if seeds require sequential temperatures (i.e. warm + cold + warm + cold stratification) to relieve dormancy.

Morphological dormancy (MD) This occurs in seeds where the embryo is undifferentiated or if differentiated it is very small or underdeveloped. For seeds with MD time is required for morphological changes or growth of the embryo to occur in order for germination to occur (after-ripening). Seed with only MD do not require any pretreatment and when placed on a moist substrate at appropriate temperatures and irradiance, embryo growth (after-ripening) and seed germination may occur within 4 weeks or less. (Baskin and Baskin, 1998).

Morpho-physiological dormancy (MPD) This is a combination of PD and MD and is commonly found in wildflowers of temperate deciduous forests. In seeds with both PD and an underdeveloped embryo, PD has to be broken, either prior to or after the embryo elongates, and the embryo must grow before the radicle can emerge. Examples of species for which this type of dormancy has been studied include larkspur (*Delphinium* spp.) and jack-in-the-pulpit (*Arisaema* spp.) (Baskin and Baskin, 2004). Seeds of plants with MPD will require some form of stratification (warm season stratification, cold season stratification or both) and a “move along” germination trial may be used to determine these requirements.

Eight levels of MPD have been identified based on temperature requirements for embryo growth, warm and/or cold stratification requirements for dormancy break, and effects of gibberellins (GA) such as GA₃ (Baskin and Baskin, 1998) on germination response (Baskin and Baskin 2004). The eight levels of morpho-physiological dormancy (MPD) are summarized in Table1 and include five levels of simple MPD dormancy (non-deep-, intermediate-, deep-, deep epicotyl- or deep double-) and three levels of complex MPD dormancy (non-deep-, intermediate- and deep-) (adapted from Baskin and Baskin, 2004). For many seeds with “simple” MPD the alternating temperatures between seed dispersal and spring germination will be sufficient to break dormancy. However, seeds with “complex’ MPD require multiple seasons of alternating

temperatures to promote embryo growth and radical emergence. For seeds with an intermediate MPD the application of gibberellins such as GA₃ will substitute for a cold stratification requirement.

Combinational dormancy (PY + PD) This occurs when the seed has a seedcoat, or fruit coat that is impermeable to water and a physiological inhibition in the embryo. In some species such as Geranium and Clover (*Trifolium sp.*), seeds must complete after-ripening to break PD before PY is broken whereas in other species such as Redbud and Basswood (*Tillia sp.*), PY must be broken (seed must be imbibed) before PD can be broken via cold stratification.

PROPAGATION BY CUTTINGS

Often a wildflower that has not previously been of interest to the nursery industry will have no published information available by which to guide the selection of propagation factors such as time of year for cutting collection, type of cutting material to be collected and evaluated and if there is a need for auxin, the appropriate auxin source, formulation, and concentration suitable for successful rooting. Practically, many nurseries already employ the use of basic propagation systems in which a new plant should be evaluated. A basic approach use by the authors is to evaluate spring, summer, or fall stem cuttings with four auxin treatments. A control consisting of no supplemental auxin and three rates (1,000, 2,500, and 5,000 ppm) of K-IBA (the potassium salt of Indole-butyric acid) dissolved in water. If these treatments do not result in successful propagation (70% or greater rooting with sufficient roots to produce a rootball) then an additional set of treatments will be included in subsequent experiments. These treatments will include K-NAA (The potassium salt of Naphthalene acetic acid) at rates of 1,000, 2,500, and 5,000 ppm and combination treatments of K-IBA and K-NAA at a ratio of 2:1. These combination treatments are generally derived from over-the-counter products such as Dip-N-

Grow since these products would be easy for any propagator to reproduce. Finally, if this full complement of treatments does not result in successful rooting of the stem cuttings at any of the seasons attempted a different approach of utilizing different propagules may be used or the propagation environment or propagation substrate may be altered.

An understanding of these five basic categories of seed dormancy and the use of TZ tests for seed viability will assist the propagator in determining the germination requirements of wild collected seed. While this information is meant to provide a basic understanding of seed dormancy, further study and additional germination trials may be required to determine requirements of seeds with more complex dormancy mechanisms where several factors interact to influence germination. In cases where germination requirements of wild collected seed remain difficult to determine the propagator may utilize the systematic cutting propagation protocols outlined herein.

LITERATURE CITED

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Table 1. Eight levels of morphological-physiological dormancy (MPD) based on temperature requirements for embryo growth, warm and/or cold stratification requirements for breaking dormancy, and effects of gibberellins (GA) such as GA ₃ .	
<u>SIMPLE MPD</u>	In general, the alternating temperatures between seed dispersal and spring germination will be sufficient to break dormancy.
Non-deep	Physical dormancy (PD) broken during summer (winter), embryo growth and germination occur in autumn (spring).
Intermediate	PD is completely broken during winter and seeds germinate in spring; GA ₃ can be used to substitute for the cold stratification (woody species).
Deep	PD partially broken in summer, embryo growth occurs in autumn; PD can also be completely broken during winter, and seeds germinate in spring.
Deep epicotyl	PD of seed is broken in summer; growth of radicle and emergence from the seed occur in autumn; PD of epicotyl is broken during winter, and cotyledons emerge in spring.
Deep double	PD of seed is broken during winter; radicle growth and emergence occur in spring; PD of the epicotyl is broken the following winter, and shoot growth occurs the second spring.
<u>COMPLEX MPD</u>	Cold stratification is required for embryo growth (embryo grows while seeds are being cold stratified)
Non-deep	Require summer (warm stratification) followed by winter (cold stratification) to break PD and promote growth of the embryo. Embryo growth occurs during winter, and seeds germinate in the spring.
Intermediate	Only cold stratification is required to break PD and promote embryo growth, but GA ₃ will substitute for the cold stratification requirement.
Deep	Only cold stratification is required to break PD and promote embryo growth. Seeds become non-dormant during winter and germinate in spring.