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## Effects of storage on seed dormancy and survivorship in black cohosh (*Actaea racemosa* L.) and goldenseal (*Hydrastis canadensis* L.)

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### Summary

Medicinal herbs indigenous to eastern deciduous forests are increasingly cultivated in forest gardens for economic and cultural purposes, yet little information is available on how post-harvest seed storage effects survivorship and germination. In this study, seeds of the medicinal woodland herbs, black cohosh (*Actaea racemosa* L.) and goldenseal (*Hydrastis canadensis* L.), were subjected to a factorial combination of storage conditions over a 360 d period to quantify seed survivorship and dormancy levels. Our results corroborate a prior study that indicates seeds of both species are morphologically dormant (MPD) and require a sequence of warm -4 cold temperatures for complete dormancy-break. Laboratory-stored seed populations removed at six different storage intervals and germinated over a range of thermoperiods in light and darkness failed to germinate, indicating that no seeds after-ripened in ambient (23°C) and cold (5°C) temperature storage. Seed germination percentages (80-90%) in *H. canadensis* remained relatively unchanged when laboratory-stored seed populations were moved through temperature sequences that broke dormancy with fresh seed. By contrast, storage at ambient and cold temperature for > 270 d induced a deeper dormancy in viable seed populations of *A. racemosa*. Seed populations of *H. canadensis* lost viability by 360 d when stored at either ambient or cold temperatures whereas approximately 30% of *A. racemosa* seeds survived dry-storage for 360 d.

### Introduction

Black cohosh (*Actaea racemosa* L.) and goldenseal (*Hydrastis canadensis* L.) are prized medicinal herbs indigenous to deciduous forests of eastern North America (Foster and Duke, 1991). Rhizomes of these species are harvested increasingly from the wild for sale in a lucrative botanical medicines industry (Robbins, 1999, 2000). Although a culturally important practice, there is increasing concern that harvest of subterranean structures may be contributing to the depletion of these non-timber based resources (Robbins, 2000). Consequently, proprietors of privately owned woodlands are now encouraged to propagate these species as an alternative to generating income from conventional timber-based activities, and/or harvest of these species from the wild (Teel and Buck, 2002; Garrett, 2003). Traditionally, medicinal woodland herbs are propagated vegetatively from material sourced from the wild, but there is an increasing push to use seeds for propagation efforts in order to mitigate the long-term effects of harvesting on wild populations. However, conservation and management strategies are hampered by a poor understanding of their seed germination biology.

Like most members of the Ranunculaceae, newly dispersed seeds of *A. racemosa* and *H. canadensis* contain rudimentary embryos that are morphophysiological dormant (MPD) (Baskin and Baskin, 1985; Baskin and Baskin, 2001). Newly dispersed seed of *A. racemosa* exhibits deep simple epicotyl MPD (Baskin and Baskin, 1985), whereas *H. canadensis* exhibits dormancy characteristics that appear intermediate between deep simple and deep simple epicotyl MPD (Baskin and Baskin, 2001). Thus, both species require a warm --> cold temperature sequence to break primary dormancy and are characterized as strict spring germinators (Baskin and Baskin, 2001).

A common practice among growers and propagators, to temporarily store freshly collected seed at room temperature or in cold refrigeration until outplanting in forest plots, frequently results in sporadic germination rates (Davis, 1999; Cech, 2002). Germination protocols for *H. canadensis* recommend that fresh seed be sowed immediately, as short-periods of dry-storing seed purportedly results in loss of seed viability and/or germinability (Hus, 1907; Deno, 1993; Davis, 1999; Cech, 2002). In contrast, Deno (1993) reported that dry storage at ambient laboratory temperatures for 6 months maximized germination of *A. racemosa* seed. To date, however, no studies have quantified the inter-relationship between storage conditions and seed survivorship.

Variation in germination rates may result from an interaction between length and temperature of storage conditions (Roberts, 1973). Seeds can experience a wide variety of changes in dry storage including accelerated dormancy loss (Priestly, 1986), induction of a deeper dormancy (Edwards and El-Kassaby, 1988), relief of germination requirements imposed on seeds at the time of dispersal (Probert *et al.*, 1985; see also review by Probert, 2000) and/or loss of viability (Murdoch and Ellis, 2000).

In order to clarify, their response to artificial storage, we subjected seeds of *A. racemosa* and *H. canadensis* to a factorial combination of storage conditions in a controlled laboratory study to understand how storage temperature and duration affect seed survivorship and dormancy levels.

## Material and methods

Ripe fruits were collected from cultivated populations of *A. racemosa* and *H. canadensis* growing on an experimental research farm in southeast Ohio during the autumn (September) and summer (July) of 2003, respectively. *Actaea racemosa* was growing beneath an artificial shade structure in an old-field whereas *H. canadensis* was growing in an adjacent second-growth (> 60 yr) mixed-mesophytic forest. All seed germplasm originated from plants that were previously growing wild in the Appalachian region but were transplanted for conservation purposes.

Post-harvest seed handling varied according to interspecific differences in fruit types and followed the general methods recommended for growers (Davis, 1999; Cech, 2002). *A. racemosa* follicles were allowed to dry at room temperature for 2 d after collection; seeds were then removed from follicles by gently tapping the infructescence against the sides of paper bags. Only brown, firm seeds were used for germination studies. *H. canadensis* seeds were depulped by soaking berries in distilled water for 24 h to loosen the fleshy

pericarp. Fruits were then macerated by hand and only seeds that sank in water were used for experimental study, since seeds that float are considered nonviable (Cech, 2002). In a companion study, we found that seeds originating from these populations exhibited morphophysiological dormancy that was broken by a sequence of 12 wk at 15/6°C → 12 wk at 5°C, a result consistent with previous studies (Baskin and Baskin, 1985; Baskin and Baskin, 2001). Based on a random sampling consisting of four batches of 50 seeds each,  $96.0 \pm 1.4\%$  (mean  $\pm$  1SE) and  $91.5 \pm 1.3\%$  of the *A. racemosa* and *H. canadensis* seeds, respectively, were viable. Seed moisture content, expressed as a difference between mass of 100 fresh seeds before and after being oven-dried at 105°C for 24 h, was 6.8% and 10.5% for *A. racemosa* and *H. canadensis*, respectively.

Seeds of each species were air-dried in the laboratory for 14 days and placed into glass jars sealed with a screw-cap lid. Equal numbers of jars were stored in a cold storage incubator (5°C, relative humidity 50%) and in ambient laboratory storage (23°C, relative humidity 15%) for 30, 60, 90, 180, 270 and 360 days. We chose these storage conditions in order to mimic how seed may be commonly stored prior to outplanting. At each storage interval, seeds were removed from storage treatments and placed onto a 1:1 mixture of moistened sand and potting soil, in glass Petri dishes (9-cm diameter). Germination tests were conducted in light-controlled germinators equipped with 20 W cool white fluorescent tubes. For dark germination treatments, Petri dishes were placed into steel canisters wrapped in aluminum foil. Each treatment condition consisted of 4 replicates of 50 seeds each for *A. racemosa* and 4 replicates of 30 seeds each for *H. canadensis*. Primary root emergence was the criterion for germination.

In the first experiment, we quantified survivorship rates of laboratory stored seeds and checked for after-ripening that may have occurred during storage. After each time interval, seeds were removed from storage and germinated in light (12 h photoperiod) and complete darkness at each of the following thermoperiods: 5, 15/6, 20/10 and 30/15°C. After 30 d, seeds were removed from the germinators and the number of germinates was counted. A control consisted of incubating fresh seed (14 d old) at each of the thermoperiods in light and darkness for 30 d. At the end of each experiment, nongerminated seeds were scored as alive or dead based on a Tetrazolium stain test (Cottrell, 1947).

In the second experiment, the effects of storage temperature and duration on seed germinability were evaluated. At each time interval, seeds were removed from storage and then transferred through the corresponding temperature sequence that alleviates dormancy in fresh seed of *A. racemosa* (12 wk at 15/6°C → 12 wk at 5°C) and *H. canadensis* (12 wk at 15/6°C → 12 wk at 5°C → 6 wk at 15/6°C; Baskin and Baskin 2001). Germination tests were conducted only in a simulated diurnal 12 h photoperiod, due to a limitation in the quantity of seed germplasm available for study. The experimental control consisted of transferring fresh seed (14 d old) of each species through its corresponding temperature sequence that breaks MPD.

Germination and survival data were analyzed using a generalized linear model (PROC GENMOD, SAS 2001) with a logit link function and binomial error structure, as the data were not normally distributed. Separate tests were conducted for each species. Germination fractions were calculated as follows:  $G = V - D$ , where  $V$  represents the total number of viable seeds at the end of the incubation period and  $D$  is the number of dormant or

ungerminated seeds. Overdispersion was accounted for by dividing the likelihood ratio  $\times 2$  by the Pearson  $\chi^2 / df$  (pscale option in SAS), resulting in an analogous ANOVA F-test (Littell *et al.*, 2002). Single degree of freedom contrasts were used to test specific hypotheses when main effects were significant (Littell, Stroup and Freund, 2002).

## Results

Fresh seed of *A. racemosa* and *H. canadensis* failed to germinate over a range of thermoperiods in light or darkness after a 30 d incubation period, confirming that seeds were dormant at maturity (*sensu* Baskin and Baskin, 2004). For both species, no germination was observed with seeds stored dry at ambient and cold temperatures and then tested over a range of thermoperiods in both light and darkness during the 30 - 360 d storage period (data not shown).

Seeds stored dry at ambient and cold temperature conditions exhibited differential survival rates within and among the two species (figure 1). Overall, seeds of both species survived at significantly greater rates in ambient storage relative to cold storage conditions (table 1). For *H. canadensis*, seed survival varied in the storage treatments over time (significant temperature  $\times$  time interaction; table 1). After 90 d, seed survival rates were similar, as 66% and 61% of *H. canadensis* seeds stored in ambient laboratory and cold temperature conditions, respectively, were viable. After 360 d, no *H. canadensis* seeds remained viable at ambient laboratory storage and < 2% of seeds survived cold temperature storage.

Germination fractions of *A. racemosa* seeds stored in ambient laboratory and cold temperature conditions declined significantly over time (table 1). Overall, germination fractions in *A. racemosa* seed were lower after storage compared to those of freshly dispersed seed (contrast fresh vs. stored,  $P = 0.009$ ), whereas no differences were detected for *H. canadensis* (contrast fresh vs. stored,  $P = 0.89$ ). In both species, freshly dispersed

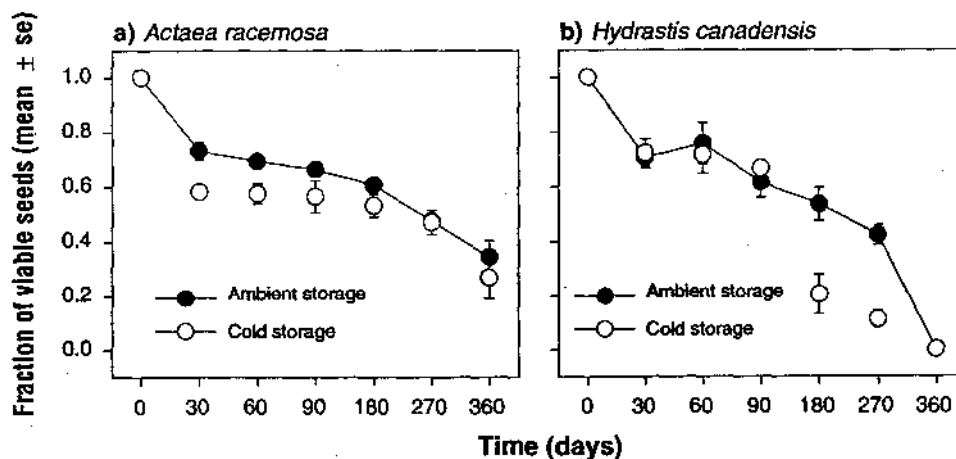


Figure 1. Seed survival of (a) *Actaea racemosa* and (b) *Hydrastis canadensis* in ambient laboratory (23°C) and cold (5°C) storage conditions.

seed transferred through the dormancy-breaking temperature sequences germinated to > 80%. *A. racemosa* seeds stored in ambient laboratory and cold temperature conditions for > 270 d exhibited significantly lower germination percentages than seeds stored for < 180 d (contrast 270 d vs. < 180 d,  $P = 0.02$ ; contrast 360 d vs. < 180 d,  $P = 0.0001$ ), suggesting that storage induced a 'deeper' dormancy (figure 2). Germination fractions of *H. canadensis* seeds were relative constant over time (figure 2), although seeds stored at cold temperatures germinated to slightly greater rates than seeds stored at ambient laboratory conditions (contrast ambient vs. cold,  $P = 0.05$ ).

Table 1. Generalized linear model results for the effects of storage temperature and duration on a) survival and b) germination of *Actaea racemosa* and *Hydrastis canadensis* seeds after incubation in a temperature sequence that breaks primary root dormancy (see Methods).

Effect	df	<i>Actaea racemosa</i>		<i>Hydrastis canadensis</i>	
		F	P	F	P
a) Survival fraction					
Temperature	1,36	12.98	0.0009	11.10	0.002
Time	6,36	18.64	0.0001	75.13	0.0001
Temperature × Time	6,36	0.71	0.62	6.42	0.0002
b) Germination fraction					
Temperature	1,36	0.69	0.41	6.68	0.01
Time	6,36	16.65	0.0001	0.45	0.84
Temperature × Time	6,36	2.03	0.10	1.19	0.33

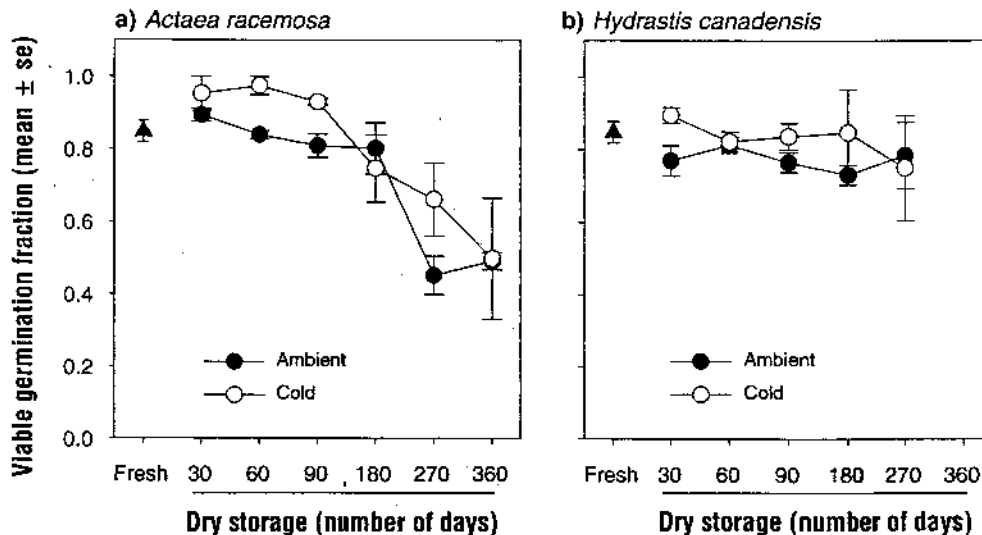


Figure 2. Fraction of dormant seeds of (a) *Actaea racemosa* and (b) *Hydrastis canadensis* following dry-storage at ambient laboratory (23°C) and cold temperature (5°C) conditions. Viable seeds that failed to germinate after dormancy-breaking temperature sequences were considered dormant.

## Discussion

*A. racemosa* and *H. canadensis* seed remained in primary dormancy throughout the 360 d storage period, as seeds failed to germinate when removed from each of the six storage intervals (spread over a 360 d period) and then subjected to the 30 d germination test over a range of constant thermoperiods in both light and darkness. Similarly, Hidayati *et al.* (2000; 2002) observed little after-ripening over a 12 and 24 month period, respectively, for MPD seeds of the temperate forest shrubs *Lonicera fragrantissima* and *Diervilla lonicera*. Dormant seed of the perennial woodland herbs, *Dioscorea villosa* and *Collinsonia canadensis* also did not after-ripen after six months in dry storage (Albrecht and McCarthy, 2006). During storage, seeds of these temperate species appear to maintain primary dormancy that they acquired while ripening on mother plants. Thus, storing seeds at ambient (23°C) and cold (5°C) temperatures prior to sowing are not appropriate pre-treatment methods for accelerating dormancy-break.

In both species, viable germination fractions of fresh seed moved through the dormancy-breaking temperature sequences were comparatively similar to those obtained with seed populations stored for short-periods (< 90 d) in ambient and cold temperatures. This further suggests that short-term storage does not change dormancy-levels or the temperature requirements for dormancy-break in these species. This lack of physiological change in seeds may be related to their deep physiological dormancies, as only seeds with nondeep physiological dormancy should after-ripen in ambient dry storage conditions (Nikolaeva, 1977).

Prolonged storage (> 180 d) in cold and ambient conditions reduced viable germination fractions in *A. racemosa* but not *H. canadensis* seed populations. This study was unable to determine if dormant *A. racemosa* seed subjected to prolonged storage (>180 d) gained an additional requirement to break dormancy, or whether seeds entered a 'delayed' dormancy, whereby seeds simply needed more than one exposure to a warm - cold temperature sequence for effective dormancy-break. Previous studies with newly dispersed seed (Baskin and Baskin, 1985) showed that germination in a small portion of a single seed crop is spread over multiple seasons, indicating that some seeds innately require at least two (sometimes three) warm - cold temperature sequences for dormancy-break. Also, a previous study showed that dormancy-break occurs independently of light, even if seeds are subjected to complete darkness immediately following dispersal (M.A. Albrecht, unpublished data). Thus, it is unlikely that changes in photoperiod requirements were responsible for altering viable germination fractions. Although the mechanism is not entirely clear, dry-storage has been shown to induce and/or prolong dormancy in other species, including *Eucalyptus pauciflora* (Beardsell and Mullet, 1984) and *Pseudotsuga menziesii* (Edwards and El-Kassaby, 1988).

Survival rates for *H. canadensis* seed populations that were dry-stored in ambient laboratory and cold temperature conditions were lower than those of *A. racemosa*. After 360 d of storage, > 97% of the *H. canadensis* seeds lost viability, indicating that *H. canadensis* seeds are relatively short-lived compared to orthodox seeds of other perennial herbs that reportedly can survive for several years at ambient laboratory temperatures (Priestly, 1986; Walck

*canadensis* seeds be sown immediately and never allowed to dry out, presumably because seeds lose viability when removed from moist environments (Davis, 1999). Data presented here support this supposition, as approximately 30% of the *H. canadensis* seed population lost viability after only 30 d in both storage treatments. This apparent sensitivity of seeds to dry conditions may be one factor limiting *H. canadensis* distribution to moist microhabitats in closed-canopy deciduous forests (Sanders and McGraw, 2005).

Because this study was designed to mimic common methods of short-term seed storage used by growers, seed moisture levels were not manipulated, making it impossible to appropriately classify post-harvest seed storage behavior as intermediate, orthodox, or recalcitrant (Roberts, 1973; Hong and Ellis, 1996). According to predictions based on the association between seed storage behavior and seed moisture content at maturity, however, *A. racemosa* and *H. canadensis* seeds likely exhibit orthodox seed storage behavior (Hong and Ellis, 1997).

From a propagation perspective, MPD represents a dual constraint, because morphological (MD) and physiological dormancy (PD) each impose a distinctive requirement that must be satisfied for seeds to completely germinate. This type of dormancy certainly imposes -constraints on agronomic production of these species because seeds must first experience specific temperatures that break morphological dormancy, in this case fluctuating warm (15°C) and cold (5°C) temperatures (Baskin and Baskin, 1985; Baskin and Baskin, 2001, Albrecht and McCarthy, unpublished data), prior to at least 8 wks of cold-stratification necessary for breaking PD. During this relatively long dormancy-break period seeds must be imbibed, making them more susceptible to attack by microorganisms (Bewley and Black, 1982; Deno, 1993). Further, our results also indicate that storage at ambient and cold conditions does not overcome the specific temperature requirements for dormancy-break, or accelerate the rates at which seeds lose dormancy. Thus, based on current methods, germinating seeds of these medicinal woodland herbs is a relatively slow process even in controlled conditions. Due to the loss of viability over time, storage methods employed in this study are unacceptable for long-term storage (> 180 d), particularly for *H. canadensis* seeds. Given that seed germplasm is often in limited supply for these species, sowing seeds immediately following dispersal in the field would maximize resources and germination success. Future studies that explore alternative handling and storage methods are needed to prolong the longevity of seeds of these medicinal woodland herbs.

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