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Variation in dormancy and germination in three co-occurring perennial forest herbs

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Abstract Mesic deciduous forest herbs often disperse seed with morphophysiological dormancy (MPD) that prevents germination during unfavorable periods for seedling survival. However, for seeds of some species with MPD, seasonal separation of root and shoot emergence and variation in dormancy levels can complicate interpretation of seedling emergence timing in the field. We tested whether dormancy-break and germination requirements differed among co-occurring perennial forest herbs, *Actaea racemosa*, *Hydrastis canadensis*, and *Sanguinaria canadensis*, which are wild-harvested for their medicinal properties and known to have MPD. Seeds of all species exhibited a summer → autumn → winter requirement for seedling emergence in spring. However, species differed in seed-bank persistence due to variation in primary dormancy levels and stratification requirement of seeds. *A. racemosa* and *H. canadensis* can form short-term persistent seed bank, whereas *S. canadensis* can form a long-term persistent seed-bank, regardless of whether elaiosomes were removed from seeds prior to burial. *A. racemosa* seeds are dispersed in autumn with weak physiological dormancy, as seeds germinated to high rates at 15/6°C after 8 weeks. In contrast, most seeds of the summer dispersed species, *H. canadensis* and *S. canadensis*, require summer temperatures to overcome physiological dormancy. Consequently, seedling emergence is reduced and delayed by 1 year if seeds are not sown immediately following the period of natural dispersal. Seedling emergence was much lower in the field than in controlled conditions for all species, especially in the small-seeded *A. racemosa*. Interspecific variation in dormancy levels and germination traits must be considered when establishing populations for conservation purposes and in understanding recruitment limitation in perennial forest herbs.

Keywords *Actaea racemosa* · *Hydrastis canadensis* · Phenology · *Sanguinaria canadensis* · Seed bank · Temperature

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Introduction

Seed dormancy, defined as a seed characteristic that determines what environmental conditions are required for germination (Vleeshouwers et al. 1995), plays a central role in plant life-histories because seeds represent the essential link for species persistence across space and time (Harper 1977). The

primary function of seed dormancy is to prevent germination in seasons unfavorable for seedling establishment (Vleeshouwers et al. 1995; Fenner and Thompson 2005). Although not a requirement for seed bank persistence (Thompson et al. 2003), seed dormancy can also spread germination across years by allowing a fraction of the seed crop to "carry-over," even when environmental cues for germination are favorable (Baskin and Baskin 2001; Walck et al. 2005).

Dormancy-breaking and germination requirements are often species specific, making it difficult to classify germination strategies according to life-history traits and habitat (Schütz and Rave 1999; Vandelook et al. 2008). One exception may be mesic deciduous forests where many perennial herbs disperse seeds with morphophysiological dormancy (MPD), a class of dormancy in which embryos are both underdeveloped and physiologically dormant at the time of dispersal (Baskin and Baskin 2001). Seeds with MPD often exhibit pronounced seasonal germination patterns, mediated primarily by shifts in temperature. This ensures seedlings emerge during a brief period in spring, just prior to canopy closure (Baskin and Baskin 1988; Vandelook and Van Assche 2008). In seeds with epicotyl dormancy, including the focal ones studied here, a considerable delay exists between root and shoot emergence, as each event requires different temperatures to overcome physiological dormancy (Baskin and Baskin 2001). In seeds with MPD, dispersal phenology must be considered in context with seasonal temperature shifts to understand seed-bank persistence and how dormancy-break relates to seedling emergence timing (Karlsson et al. 2005; Walck et al. 2005).

In this study, we experimentally examined seed dormancy and germination in three forest herbs that co-occur throughout much of eastern North America: *Actaea racemosa* L. (Ranunculaceae), *Hydrastis canadensis* L. (Ranunculaceae), and *Sanguinaria canadensis* L. (Papaveraceae). The roots and rhizomes of these long-lived perennials are harvested from the wild for their medicinal properties. Due to concern over the sustainability of wild-collection, there is increasing interest in understanding the role of seedling recruitment in the regeneration of populations following disturbance (Van der Voort et al. 2003; Sanders and McGraw 2005a; Albrecht and McCarthy 2006) and how to establish populations for conservation and agroforestry purposes (Sinclair and Catling 2001, 2004; Sanders and McGraw 2005b; Burkart and Jacobson 2009). Understanding how temperatures govern the rates and timing of seedling emergence is critical for restoring populations and identifying how early life-history stages influence population dynamics (Albrecht and McCarthy 2009; Christensen and Gorchov 2010).

Seedlings are rarely observed in natural populations of these perennial forest herbs (Drayton and Primack 2000; Sanders and McGraw 2005a; Sinclair et al. 2005); suggesting seedling emergence is a cryptic life-cycle event. Studies conducted on seeds of these species reported highly variable rates and timing of seedling emergence, ranging from delayed germination of one to several years after sowing and emergence rates from 0 to 90% (Hus 1907; Davis 2000; Cech 2002). However, in these studies, seeds were either subjected to temperatures not necessarily representative of those that seeds experience following dispersal, failed to distinguish between mortality and dormancy in non-germinated seeds, and/or did not account for the possibility that germination traits might be altered by dispersal agents (e.g., ants in *S. canadensis*; Lobstein and Rockwood 1993), thereby limiting ecological interpretations of seed dormancy and germination in nature (Baskin et al. 2006).

We subjected seeds of the focal species to natural temperature shifts simulated in the laboratory and factorial field experiments to resolve dormancy-breaking and germination requirements of these medicinally important forest herbs. Our study starts with the assumption that seeds of all three species exhibit some level of MPD (Table 1), with a warm (W) + cold (C) stratification requirement for seedling emergence. However, some seeds of *S. canadensis* might also exhibit double dormancy and require a C + W + C for dormancy-break (Barton 1944), with seedlings emerging 2 years after dispersal. We asked the following questions: How do seasonal temperatures influence dormancy-break and the timing of root and cotyledon emergence among the three species? What fraction of the seed population delays germination over time? How does germinability change when seeds are not sown immediately following the period of natural dispersal? Are seeds that delay emergence in primary dormancy?

We hypothesized that the summer dispersed species would require warm temperatures for dormancy-

Table 1 Seed ecology traits of the three mesic forest herbs used in this experiment

	Actaea racemosa	*Hydrastis canadensis*	*Sanguinaria canadensis*
Dispersal season[a]	September–November	July	May–June
Dispersal mode[a]	Gravity	Vertebrates	Ants
Fresh seed mass (mg, mean ± SE)[a]	2.7 ± 0.06	24.8 ± 1.0	10.6 ± 0.2
Morphophysiological dormancy level[b,c]	Deep simple epicotyl[b]	Transition between deep simple and deep simple epicotyl[d]	Deep simple epicotyl and/or deep simple double (?)
Root emergence season[c]	October	March–April	October or March–April (?)
Shoot emergence season[c]	March–April	March–April	March–April

[a] Albrecht and McCarthy (2009); [b] Baskin and Baskin (1985); [c] Baskin and Baskin (2001); [d] a special type of deep simple MPD has been used to describe *Hydrastis canadensis* seeds because the seed coats split open in autumn following embryo growth, but roots do not fully penetrate the endosperm covering until spring (Baskin and Baskin 2001)

break. Thus, delayed planting of seeds might increase the fraction of seeds that carry-over in the seed-bank because seeds would not experience summer temperatures which are required for dormancy-break, perhaps, in part, explaining germination patterns reported previously. Because the study species exhibit similar life-cycles and habitat distributions, we hypothesized they would differ in germination traits due to variation in dormancy levels that would regulate the timing of root and/or shoot emergence among the species.

Methods

Study species and seed collection

The three study species are shade-adapted, perennial herbs that often co-occur in mesic deciduous forests (Hutchinson et al. 1999). These species all disperse seeds with MPD but represent a range of reproductive traits including seed size, dispersal phenology, and dispersal agents that might influence seed dormancy and germination (Table 1).

During the summers of 2002 and 2003, fruits of the three species were collected from multiple ramets in 2–3 wild populations growing under similar forest types and topographies; most stands were second-growth (>80 years) mixed-oak forests in Athens and Meigs Co., Ohio. Seeds were extracted from all fruits, mixed prior to the experiments, and germination studies were initiated within 30 days of collections. Differences in seed availability among species precluded using equal sample sizes in all experiments. A tetrazolium stain test on a random sub-sample of 50 fresh seeds indicated that ≥92% of seeds were viable for all species. In 2002, we experimentally created two seed populations of *S. canadensis* by carefully removing the elaiosome with forceps on half of the seeds (hereafter: −elaiosome and +elaiosome), because a previous study indicated that elaiosome removal enhances germination rates (Lobstein and Rockwood 1993). For all subsequent analysis, we included each *S. canadensis* seed type (±elaiosome) as a separate level when testing the main effect of "species."

General laboratory experiments

Laboratory studies were conducted in incubators set at a 12-h photoperiod (illuminated by cool white fluorescent tubes) and at daily alternating temperature regimes that simulates mean daily maximum and minimum seasonal temperatures in the central Appalachian region: 15/6°C (early spring/late autumn), 20/10°C (late spring/early autumn), 30/15°C (summer), and a constant temperature of 5°C (winter). Each treatment combination consisted of four replicates comprised 20 seeds per dish for *H. canadensis* and *S. canadensis* and 50 seeds per dish for *A. racemosa*. Seeds were placed in glass Petri dishes (9 cm diameter) on top of a mix (1:1:1) consisting of peat moss, sand, and field soil collected from the sites where seeds were collected. Field soil was sieved (2 mm) prior the experiments to remove propagules from the resident seed bank. Seeds remained constantly imbibed throughout the study by adding 2 ml of distilled water to Petri dishes as needed. Petri

dishes were checked every 7 days for germinates until the completion of each laboratory experiment, when all non-germinated seeds were subsequently dissected and examined beneath a microscope; seeds with white and firm embryos were scored as viable.

Move-along experiment

A move-along experiment (*sensu* Baskin and Baskin 2004) was used to clarify the season of root and cotyledon emergence for each species and determine the dependency of seeds on temperature sequences for dormancy-break. Fresh seed (collected in 2002) of each species were started at either a summer (30/15°C) or winter (5°C) sequence and moved through each sequence twice (see caption in Fig. 2 for temperature sequence). Emergence of roots (hereafter germination) and cotyledons (hereafter seedling emergence) was recorded every 7 days, and the number viable seeds (firm when pinched with forceps) recorded at the end of each sequence. Controls were fresh seed (2002), kept continuously moist in light for 80 weeks at each of the four thermoperiods.

Field experiment

We used a field experiment to compare germination timing and rates with the laboratory experiments and to test whether germination traits were altered when seeds (collected in 2003) were stored at varying temperatures (cold vs. ambient) and lengths of time (30 vs. 90 days) prior to sowing. For the cold stratification treatment, seeds were placed in plastic bags filled with a moistened soil mix described previously and placed in the dark in a 5°C incubator. The plastic bags remained open during the cold stratification period to allow air exchange, and bags were moistened as needed. Seeds stored dry at ambient temperatures were placed in open glass jars and stored in the dark in ambient laboratory conditions (20°C).

After 30 and 90 days, seeds were removed from storage and sown in field plots arranged as a randomized complete block factorial design (six blocks) at the Appalachian Forest Resource Center in Meigs County, OH (39°5′N, 82°9′W), a 20-ha second-growth deciduous forest preserve. Each block was divided into 0.5 × 0.5 m plots for each species by treatment combination, including a control that consisted of fresh sown seed (2 × 2 factorial design with a separate control) with one exception. For *A. racemosa*, we only established control plots for comparison with the laboratory experiment since this species disperses seed in autumn and seeds are typically not stored for more than 30 days prior to sowing. Each plot received 100 seeds buried ~1 cm in the soil.

Seed-bank study

Burial experiments commenced immediately following the collection of ripe seed in 2002: 7–June for *S. canadensis*, 3–July for *H. canadensis*, and 19–September for *A. racemosa*. For each species, seeds were placed into packets constructed of 10 × 10 cm nylon mesh squares sewn closed on all sides (*A. racemosa*: $n = 40$ packets, 50 seeds/packet; *H. canadensis*: $n = 24$ packets, 30 seeds/packet; *S. canadensis:* $n = 32$ packets, 15 seeds/packet). In 2002, a total of fifteen 4-m transects ($n = 5$ per species) were established in a ravine that ran parallel to the contour of an E-SE-facing slope in a ravine at the Ridges Land Lab (39°19′N, 82°07′W), a second-growth mixed oak forest located in Athens County, Ohio, USA. At every 0.5 m on each transect, a seed packet was buried at a depth of ~2 cm and tied to a metal stake with nylon string to facilitate recovery. Every 90 days from the initial burial date of each species, seeds packets (*A. racemosa*: $n = 5$ packets; *H. canadensis*: $n = 3$ packets; *S. canadensis*: $n = 4$ packets) were retrieved by selecting at random one packet from each transect. On each retrieval date, seed packets were placed into paper bags and brought back to the laboratory, where each seed was placed into one of the following categories: (1) germinated, (2) empty or unrecovered, and (3) non-germinated. Non-germinated seeds that were firm when pinched were placed into Petri dishes (up to 30 seeds per dish) and incubated in light for 30 days at temperatures optimum for germination of each species (*A. racemosa* and *H. canadensis* = 15/6°C; *S. canadensis* = 20/10°C). All non-germinated seeds that were viable after a cut test (embryos were white and firm) following the 30-day germination test were considered to be dormant.

Statistical analyses

For the move-along experiment, we calculated the following response variables for each species:

emergence fraction (total number seeds with emerged cotyledons/number of seeds initiated), mortality fraction (number of seeds that died before producing cotyledons/number of seeds with emerged roots [for *A. racemosa* and *S. canadensis*] or split seed coats [for *H. canadensis*]), and dormant fraction (number of viable non-germinated seeds at end of the treatment/number of seeds initiated). The consequences of species and temperature sequence on each response variable were tested using generalized linear model (GLM), with a logit link function and quasibinomial error distribution to account for overdispersion using R 2.6.2 (R Development Core Team 2008). Single-degree of freedom contrast tests were used to test for differences within and among species when main effects were significant.

Seed longevity among species was compared by calculating the dormant fraction of buried seed at each sampling time according to the formula: $N_t = N_0 e^{kt}$, where N_t = viable seed fraction in packets at time t, N_0 = initial number of viable seeds, and k is the estimated decay rate of buried seeds. We tested whether rates of loss (caused by either loss of viability or germination) of dormant seeds differed among species over time using an accelerated failure-time analysis (LIFEREG procedure; log-logistic distribution; SAS Institute 2001) with species, time (days), and their interactions as fixed-effect covariates. Although we report root and cotyledon emergence phenologies, these were not subjected to statistical analysis because we could not discern whether a seed had germinated in the bag and then subsequently died or had decayed from natural causes. Due to technical error in methodology, we do not report data from the last sampling time for *S. canadensis* seeds (+elaiosome).

In the field experiment, we tested whether cumulative (across 3 years) seedling emergence fractions differed among species and treatments using two separate GLM models. First, we ran a three-way GLM to test whether emergence fractions differed among species, storage treatments, and storage time as fixed-effects. Second, we ran a one-way GLM separately for each species, coding storage and time interactions as a single treatment factor (five levels including control). We then used orthogonal contrasts to test the a priori hypothesis that seedling emergence fractions should be greater with fresh seed than with stored seed. Models were initially run as mixed GLMs with a random block effect, but variance components for the block effect were small; therefore, we only report parameter estimates and P values from models without the block effect. We report species effects in statistical analyses, but limit our discussion on its relevance, given our primary focus is on the response of MPD seeds to seasonal temperatures and seed-bank persistence.

Results

Move-along experiment

Fresh seed of the three forest herbs failed to germinate over a range of thermoperiods during a 30-day incubation period, confirming that seeds were in primary dormancy (Fig. 1). Most seeds remained dormant in summer (30/15°C) and winter (5°C) temperatures but species differed in their response to autumn/spring temperatures (Fig. 1). At 15/6°C, roots began emerging sooner (8 weeks) in *A. racemosa* seeds than in *H. canadensis* seeds (35 weeks), and at significantly ($F_{1,6} = 24.83$, $P = 0.003$) greater fractions after 48 weeks (*A. racemosa* = 75%, *H. canadensis* = 16%). At 20/10°C, roots of *A. racemosa* and *S. canadensis* seeds began emerging at 16 and 20 weeks, respectively, whereas only 1% of roots emerged from *H. canadensis* seeds.

In the seasonal temperature cycle, all species exhibited pronounced seasonal patterns of root and cotyledon emergence (Fig. 2). In the winter and summer sequence, root emergence in *A. racemosa* and *S. canadensis* seeds was confined to autumn and winter, and roots did not emerge in other seasons (Fig. 2). Splitting of the seed coat (embryo growth) in *H. canadensis* seeds began in the autumn after seeds experienced summer temperatures and then continued into winter. For all species, cotyledons did not emerge until seeds first experienced a summer → autumn → winter temperature sequence.

The seasonal sequence did not significantly affect emergence, mortality, or dormancy fractions (all P values > 0.10). Mortality fractions differed among species ($F_{1,24} = 5.94$, $P = 0.004$). Mortality fractions of *S. canadensis* seeds (averaged across elaiosome treatments) were significantly lower than in *A. racemosa* ($P < 0.005$) and *H. canadensis* ($P < 0.005$; Table 2). Elaiosome removal had no

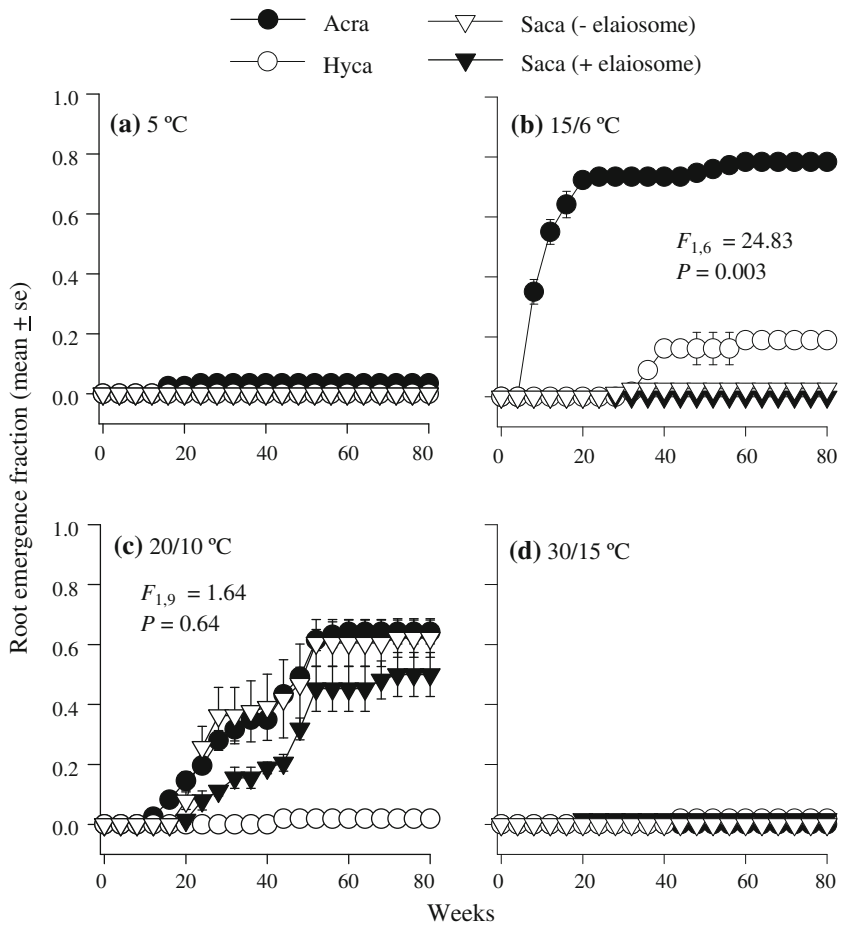

Fig. 1 Cumulative root emergence fractions (mean ± 1SE) of seeds for three mesic forest herbs maintained continuously in a 12 h/12 h alternating photoperiod for 50 weeks at **a** 5°C, **b** 15/6°C, **c** 20/10°C, and **d** 30/15°C. Seeds of *Sanguinaria canadensis* were tested with (+) and without (−) elaiosomes. Generalized linear models were used to test for differences among species in cumulative root emergence fraction. No tests were conducted on seeds at 5°C and 30/15°C since germination was negligible. Acra = *Actaea racemosa*; Hyca = *Hydrastis canadensis*; Saca = *Sanguinaria canadensis*

effect on *S. canadensis* mortality fractions ($P = 0.89$), but increased dormant fractions in the winter sequence relative to summer sequence ($P = 0.03$). At the end of the move-along experiment, a moderate fraction of *S. canadensis* remained dormant, but no viable non-germinated seeds remained for *A. racemosa* and *H. canadensis* (Table 2).

Seed-bank study

Germination phenologies of buried seed populations were consistent with the laboratory experiments (Fig. 3). For *S. canadensis*, seeds with emerged roots were observed in autumn and winter, and fully developed seedlings (roots + cotyledons) were observed only in spring. However, in the second summer retrieval date, *S. canadensis* roots emerged in the laboratory germination test (+elaiosomes = 45%; −elaiosomes = 15%), although no seeds with roots emerged were found in seed packets at this retrieval (Fig. 3).

Species diverged significantly in their rates of loss (either to germination or death) from the buried seed pool (Fig. 4; Species: Wald's $\chi^2 = 618.90$, df = 3, $P < 0.0001$; Species × Time: Wald's $\chi^2 = 157.24$, df = 20, $P < 0.0001$). After two germination seasons, *H. canadensis* seed populations were depleted in the burial study whereas ~10% of *A. racemosa* seeds remained dormant. Decay rates were much lower for *S. canadensis*, with >50% of buried seed (pooled across elaiosome treatments) remaining viable and dormant after two germination seasons (Fig. 4). In *S. canadensis*, elaiosome removal did not significantly influence the loss of dormant seed from the seed-bank (elaiosome vs. no elaiosome: $\chi^2 = 0.43$, df = 1, $P = 0.51$).

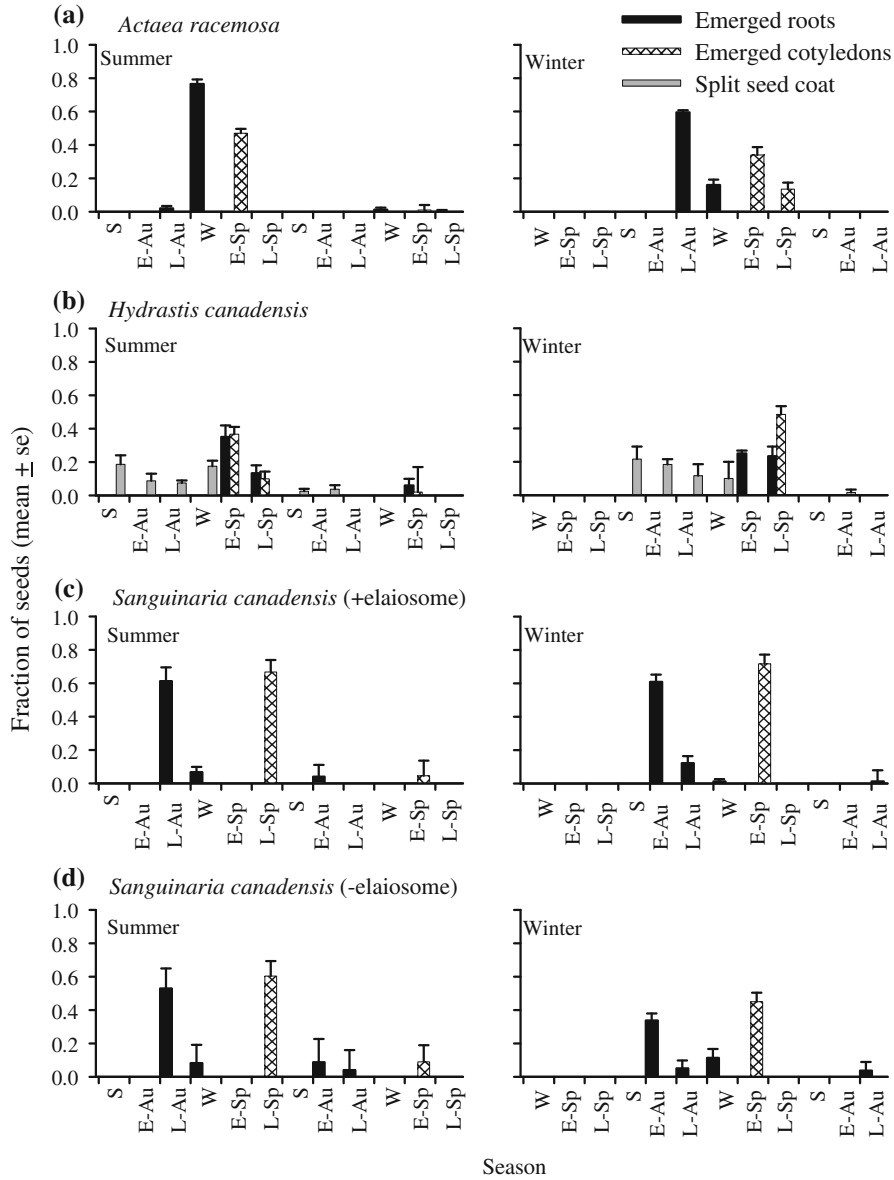

Fig. 2 Fraction (mean ± 1SE) of seeds with split seed coats (only *H. canadensis*), and emerged roots and cotyledons for three forest herbs transferred twice through the annual temperature cycle that started at summer (30/15°C) or winter (5°C). Seeds started in the summer treatment were moved through the following sequence: summer (12 weeks at 30/15°C) → early autumn (4 weeks at 20/10°C) → late autumn (4 weeks at 15/6°C) → winter (12 weeks at 5°C) → early spring (4 weeks at the 15/6°C) → late spring (4 weeks at 20/10°C). Seeds started in the winter treatment were moved through the following sequence: winter (12 weeks at 5°C) → early spring (4 weeks at the 15/6°C) → late spring (4 weeks at 20/10°C) → summer (12 weeks at 30/15°C) → early autumn (4 weeks at 20/10°C) → late autumn (4 weeks at 15/6°C). *S* summer, *E-Sp* early spring, *L-Sp* late spring, *W* winter, *E-Au* early autumn, *L-Au* late autumn. Seeds of *Sanguinaria canadensis* were tested with (+) and without (−) elaiosomes

Field study

For all species, seedlings emerged only in spring (March–April). Cumulative seedling emergence fractions significantly differed among species (one-way GLM, $F = 13.38$, $P < 0.0001$). Seedlings of *A. racemosa* emerged at lower fractions (mean ± 1SE, 0.08 ± 0.02) than *S. canadensis* (0.23 ± 0.02)

Table 2 Summary statistics (mean ± SE) after moving seeds of three mesic forest herbs through summer and winter treatments of a move-along experiment

Species	Treatment	Emergence fraction	Mortality fraction[a]	Dormant fraction
Actaea racemosa	Summer	0.60 ± 0.07	0.26 ± 0.07	0 ± 0
	Winter	0.47 ± 0.04	0.37 ± 0.04	0 ± 0
Hydrastis canadensis	Summer	0.47 ± 0.11	0.35 ± 0.11	0 ± 0
	Winter	0.48 ± 0.05	0.34 ± 0.10	0 ± 0
Sanguinaria canadensis (+elaiosome)	Summer	0.58 ± 0.06	0.18 ± 0.07	0.22 ± 0.08
	Winter	0.72 ± 0.06	0.03 ± 0.02	0.10 ± 0.03
Sanguinaria canadensis (−elaiosome)	Summer	0.44 ± 0.09	0.11 ± 0.05	0.18 ± 0.07
	Winter	0.54 ± 0.03	0.03 ± 0.03	0.36 ± 0.06

See Fig. 2 for treatment descriptions

[a] Mortality fraction = Fraction of seeds with emerged roots (*A. racemosa* and *S. canadensis*) or split seed coats (*H. canadensis*) that died before producing cotyledons

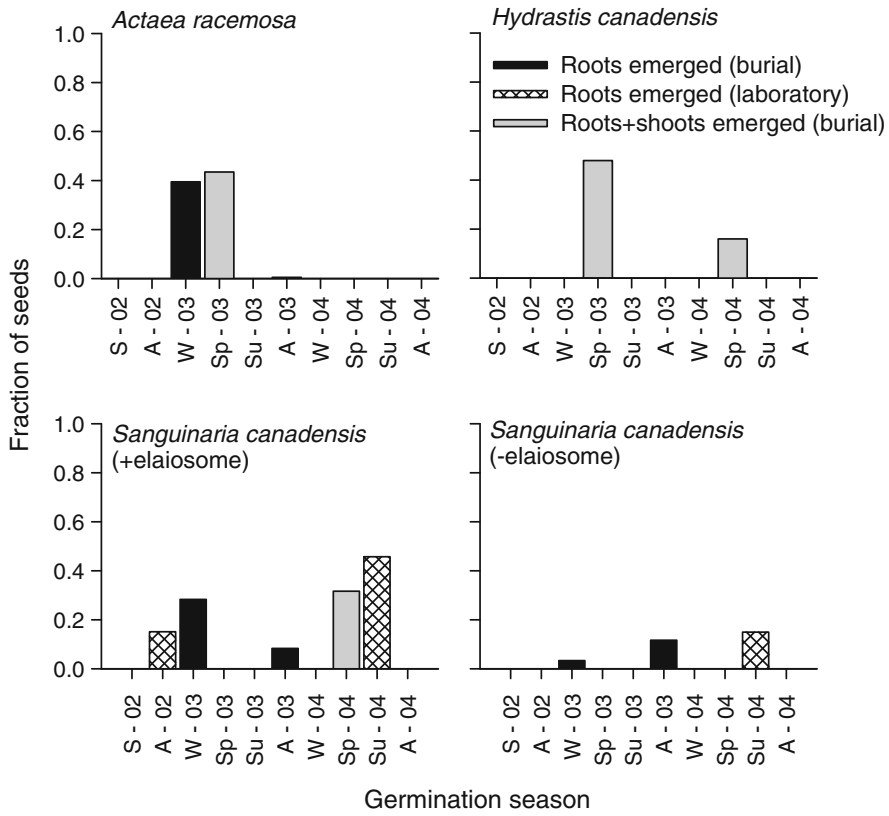

Fig. 3 Fraction of seeds with emerged roots and roots + cotyledons in mesh bags recovered from burial and in the 30-day germination test of non-germinated seeds in the laboratory. In the laboratory test, no cotyledons emerged

and *H. canadensis* (0.25 ± 0.04). Species also differed in their ability to spread germination over time, with *A. racemosa* and *S. canadensis* seedlings emerging across 3 years and *H. canadensis* emerging in the first year only.

Seedling emergence fractions differed among *H. canadensis* and *S. canadensis* in response to storage time and temperature, but the three-way interaction was non-significant (Table 3). Overall, seedling emergence fractions were greater with fresh sown

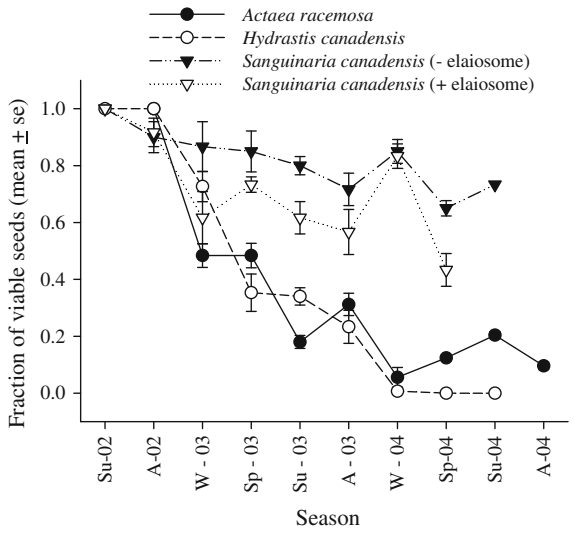

Fig. 4 Fraction of viable non-germinated (dormant) seeds of the three mesic forest herbs that were buried at a depth of 2 cm in a mixed-hardwood forest over a two-year period. Seeds of *Sanguinaria canadensis* were tested with (+) and without (−) elaiosomes. *X*-axis represents the season, and year seeds were excavated from the soil. *A* autumn, *S* summer, *Sp* spring, *W* winter

Table 3 Analysis of deviance on the effects of storing seeds for varying lengths of time (30 vs. 90 days) and in varying temperatures (cold vs. ambient) prior to sowing in field plots on seedling emergence fractions

Term	df	Deviance	*F*	*P*
Temperature (*T*)	1	17.58	7.63	0.009
Days (*D*)	1	0.55	0.24	0.62
Species (*S*)	1	0.40	0.17	0.68
$T \times D$	1	45.66	19.81	<0.0001
$S \times D$	1	161.45	70.06	<0.0001
$S \times T$	1	18.16	7.88	0.007

Hydrastis canadensis and *Sanguinaria candensis* were included in the model but not *Actaea racemosa* (see "Methods")

seed than with stored seed (contrast: control vs. treatments) for *H. canadensis* ($P < 0.0001$) and *S. canadensis* ($P < 0.0001$) (Fig. 5). Storage for 90 days in either ambient or cold temperatures delayed emergence of most *S. canadensis* seedlings until 2005 (Fig. 5). However, cumulative emergence fractions of *S. canadensis* seedlings were greater with 90 days cold stratified seed than all other treatments (Fig. 5, all *P* values < 0.001). In contrast,

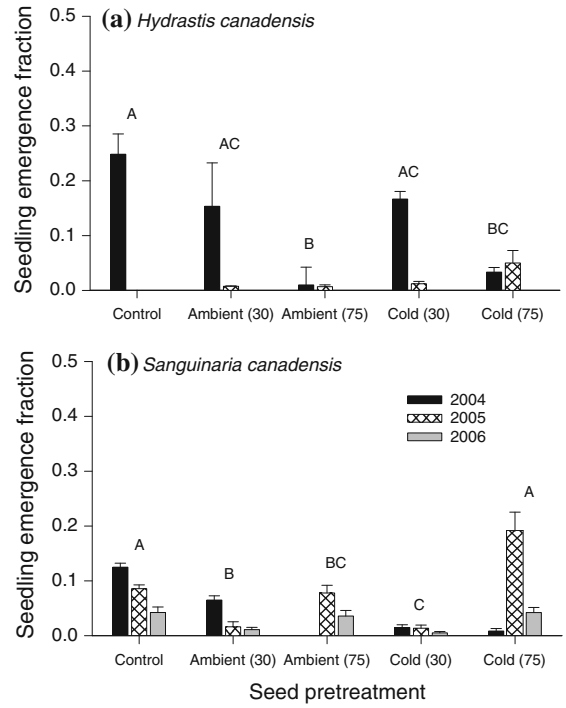

Fig. 5 Seedling emergence fractions of **a** *Hydrastis canadensis* and **b** *Sanguinaria canadensis* in field experiments using seeds stored at ambient (20°C) and cold (5°C) temperatures for varying lengths of time. Numbers in parentheses on the *x*-axis are the number of days seeds were held before sowing. Emergence fractions in 2005 and 2006 are conditional on emergence fractions in 2004. Treatments with *different letters* are significantly different based on post hoc pairwise *t* tests with Holm adjusted *P* values on cumulative emergence fractions

H. canadensis seed stored for 90 days reduced seedling emergence relative to seed stored for 30 days but only significantly in ambient temperature storage (Fig. 5).

Discussion

Seeds of all three species are dormant at dispersal and those that started in the winter treatment sequence did not emerge as seedlings until the second spring following dispersal (54–57 weeks after sowing), after seeds experienced a summer → autumn → winter sequence. In contrast, seeds started in the summer sequence began germinating in autumn and cotyledons emerged the following spring after winter chilling (34–37 weeks after sowing). In the field burial study, root emergence (or splitting of the seed

coat in *Hydrastis canadenis*) was confined similarly to autumn or winter, and cotyledon emergence was confined to spring. Seeds therefore must first experience warm temperatures before they become responsive to cool alternating temperatures and confirms that the order in which seeds must experience seasonal temperatures for dormancy-break is not interchangeable (Baskin and Baskin 2001; Vandelook and Van Assche 2008).

In central Appalachian populations, seeds of *S. canadensis* are dispersed in early-June, and those seeds that emerge the following spring experience a summer → autumn → winter → spring temperature sequence. In early work by Barton (1944), high seedling emergence rates (90%) were obtained by moving *S. canadensis* seeds through a temperature cycle of cold (5°C for 12 weeks) → warm (21°C for 24 weeks) → cold (5°C for 12 weeks). Seeds with a C + W + C stratification requirement would be double dormant, thus roots should emerge in spring or summer after the first winter chilling, PD would be broken after the second winter chilling, and seedlings would emerge the second spring (Baskin and Baskin 2001). In the move-along sequence, however, roots only emerged in *S. canadensis* seeds in autumn after seeds experienced summer temperatures. Seeds with emerged roots were similarly observed only in seed packets excavated during fall and winter, but never in spring or summer. Thus, when subjected to the seasonal temperature cycle, all *S. canadensis* seeds (±elaiosome) exhibited a phenology pattern consistent with deep simple epicotyl MPD rather than deep simple double MPD.

Despite the predictability of seasonal temperature shifts, variation in dormancy levels within seed populations ensures that some seeds are nonresponsive to the first annual temperature cycle resulting in carry-over in the seed bank. However, the fraction of buried seeds that remains dormant varied widely among the three species. Most fresh seed of *H. canadensis* can be expected to emerge the first spring following dispersal, with little or no carry-over beyond the second emergence season. A small fraction of *A. racemosa* seed can be expected to persist beyond the second emergence season, consistent with a previous study where emergence was spread over three spring seasons (Baskin and Baskin 1985). According to Walck et al.'s (2005) scheme for seed-banking strategies of species with pronounced seasonal dormancy, *A. racemosa* and *H. canadensis* both exhibit short-term (<6 spring emergence seasons) persistent seed bank strategies. In contrast, using parameter estimates (decay rate) from the regression model, the estimated half-life for an *S. canadensis* seed was ~6 years, suggesting potential formation of a long-term persistent seed bank (Walck et al. 2005). Seeds collected from another *S. canadensis* population and subjected to the annual temperature cycle spread germination over eight seasons (Baskin and Baskin 2001), indicating that the results observed here are not exceptional.

Differences among seeds in the number of annual temperature cycles required for dormancy-break appears to be a mechanism that ensures carry-over and formation of a persistent seed bank (Baskin and Baskin 2001; Fenner and Thompson 2005). Results suggest that MPD was not broken in buried seeds of *A. racemosa* and *H. canadensis* that were unresponsive to the first annual temperature cycle. Seeds retrieved from burial only germinated during seasons when germination was observed in the move-along experiment, suggesting that seeds that did not germinate after the first annual temperature cycle were in primary dormancy and that seeds do not experience dormancy cycling.

In contrast, some *S. canadensis* seeds that were unresponsive to the first seasonal temperature cycle germinated upon retrieval the second summer after dispersal. This suggests MPD was broken in some seeds during burial, but germination was inhibited by lack of appropriate germination cues, most likely spring temperatures. These results are somewhat consistent with Barton's (1944) prediction that some seeds require cold stratification to overcome root dormancy. In the field study, cumulative emergence rates of *S. canadensis* seeds cold-stored for 90 days before planting were similar to those of fresh sown seed, although emergence was delayed until the second spring and we do not know whether roots emerged the following spring after seeds experienced a C + W + C sequence. Different stratification requirements among seeds seem to function as a mechanism that ensures carry-over through time, yet some *S. canadensis* seeds that persist in the seed bank may be non-dormant or cycle in and out of dormancy. More extensive burial studies are needed to understand the mechanisms (e.g., dormancy cycling) that promote seed-bank persistence in forest herbs with MPD.

Germination timing and seed-bank carry-over can be altered when field sowing is delayed and seeds do not experience the natural temperature cycle. For *H. canadensis* and *S. canadensis*, emergence was delayed in ~50% and >95% of seeds until the second emergence season, respectively. For these summer dispersed species, seeds stored for 90 days before planting do not experience summer temperatures, which is an apparent requirement for overcoming dormancy. In the laboratory experiments, seeds of both species germinated rapidly at autumn temperatures but only after they first experienced summer temperatures. Thus, seeds were largely unresponsive to autumn temperatures when planted after 90 days storage because they did not first experience summer stratification. Similar dependency on summer temperatures for dormancy-break has also been reported in other co-occurring perennial mesic herbs, *Asarum canadense* (Baskin and Baskin 1986) and *Hydrophyllum macrophyllum* (Baskin and Baskin 1983). However, the germination of fresh *A. racemosa* seed at 15/6°C suggest this species does not require summer temperatures for dormancy-break, rather it suggests seeds are in a weaker state of physiological dormancy than *H. canadensis* and *S. canadensis*. Similar patterns were observed in temperate grasslands where spring–summer dispersed species exhibited greater stratification requirements for dormancy-break than autumn dispersed species (Washtani and Masuda 1990).

Variation in germination patterns described previously for these forest herbs can be explained by the specific seasonal temperature shifts required for dormancy-break. Davis (2000) reported that when fresh *H. canadensis* seeds were (1) stored in moist sand at 21°C for 30 days, (2) transferred to 5°C, and (3) then outplanted in spring, emergence did not occur until the second spring season after dispersal. In this instance, emergence was probably delayed until the second spring season because, during the first year, seeds never experienced the summer → autumn → winter temperature sequence that would have probably broken dormancy in most seeds. Similarly, Cech (2002) reported that cold-stored *H. canadensis* seed sown in February 2001 germinated 414 days later, in April 2002, whereas fresh seed of *H. canadensis* sown in September 2001 germinated 192 days later in April 2002. Deno (1993) reported that *S. canadensis* and *H. canadensis* seeds germinated to low percentages (<6%) when transferred through several cycles of a temperature sequence (90 days at 21°C → 90 days at 5°C) that did not include fluctuating autumn temperatures preceded by warm summer temperatures, which are necessary for dormancy-break. Henkel and Klugh (1908) sowed *H. canadensis* seed populations outdoors immediately following dispersal (July) and then the following spring, presumably after storing seeds in dry ambient conditions. Seeds stored indoors and then planted outdoors the following spring did not germinate, because seeds never experienced the warm → cold temperature sequence, whereas 30% of the seeds planted in July germinated the following spring (Henkel and Klugh 1908). In our field study and previous work (Albrecht and McCarthy 2007), overall lower emergence fractions relative to fresh seed also suggests germinability is reduced when not planting fresh seed.

While temperature is the primary factor that mediates dormancy-break and germination, other factors might also influence germination traits. Birds could play a role in dispersal of *H. canadensis* seeds (Sinclair et al. 2000), but it is unknown how bird ingestion influences seed viability and germination in this species. Ants are expected to increase germination rates in *S. canadensis* seeds (Lobstein and Rockwood 1993), but our results were somewhat equivocal on this point. We found elaiosome effects to be highly variable and detected statistically significant differences between *S. canadensis* seed populations (±elaiosome) in only one instance, perhaps because we considered seedling emergence across the entire annual temperature cycle and accounted for mortality and dormancy in seed populations (also see Christian and Stanton 2004; Martins et al. 2009). Although light was not evaluated in this study, photoblastic germination is unlikely since seedlings emerged in packets buried belowground, and the seed mass for each species exceeded the threshold (1.5 mg) for light-dependent germination observed in other temperate forest herbs (Jankowska-Blaszczuk and Daws 2007).

At present, these species are rare at the edges of their geographic ranges and are threatened rangewide by a combination of factors including habitat loss, overexploitation by wildcrafters, and browsing by an overabundant deer population. Our results have important implications for establishing populations of mesic forest herbs with MPD for conservation and

sustainable forest management. For summer dispersed species, fresh seed should be sown soon after natural dispersal, so seeds experience the entire seasonal temperature sequence. Otherwise, seedling emergence fractions are reduced and delayed until the second spring after dispersal. For *A. racemosa*, delayed planting until winter would similarly result in seedling emergence delays until the second spring after dispersal since seeds need to experience autumn temperatures before winter. However, much lower seedling emergence fractions in the field than in controlled conditions indicate strong seedling recruitment limitation in these species, especially for the small-seeded *A. racemosa*, in which many seeds failed to develop into seedlings. Consequently, microenvironmental variation in post-dispersal processes also drives seedling recruitment variation in these species and should therefore be accounted for when restoring and conserving perennial forest herbs (Albrecht and McCarthy 2009; Baeten et al. 2009).

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