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Seed germination and dormancy in the medicinal woodland herbs *Collinsonia canadensis* L. (Lamiaceae) and *Dioscorea villosa* L. (Dioscoreaceae)

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Abstract

We used a double germination phenology or "move-along" experiment (*sensu* Baskin and Baskin, 2003) to characterize seed dormancy in two medicinal woodland herbs, *Collinsonia canadensis* L. (Lamiaceae) and *Dioscorea villosa* L. (Dioscoreaceae). Imbibed seeds of both species were moved through the following two sequences of simulated thermoperiods: (a) 30/15 °C → 20/10 °C → 15/6 °C → 5 °C → 15/6 °C → 20/10 °C → 30/15 °C, and (b) 5 °C → 15/6 °C → 20/10 °C → 30/15 °C → 20/10 °C → 15/6 °C → 5 °C. In each sequence, seeds of both species germinated to high rates (>85%) at cool temperatures (15/6 and 20/10 °C) only if seeds were previously exposed to cold temperatures (5 °C). Seeds kept at four control thermoperiods (5, 15/6, 20/10, 30/15 °C) for 30 d showed little or no germination. Seeds of both species, therefore, have physiological dormancy that is broken by 12 weeks of cold (5 °C) stratification. Morphological studies indicated that embryos of *C. canadensis* have "investing" embryos at maturity (morphological dormancy absent), whereas embryos of *D. villosa* are undeveloped at maturity (morphological dormancy present). Because warm temperatures are required for embryo growth and cold stratification breaks physiological dormancy, *D. villosa* seeds have non-deep simple morphophysiological dormancy (MPD). Neither species afterripened in a 6-month dry storage treatment. Cold stratification treatments of 4 and 8 weeks alleviated dormancy in both species but *C. canadensis* seeds germinated at slower speeds and lower rates compared to seeds given 12 weeks of cold stratification. In their natural habitat, both species disperse seeds in mid- to late autumn and germinate in the spring after cold winter temperatures alleviate endogenous dormancy.

Keywords: *Dioscorea*; *Collinsonia*; Seed dormancy; Seed germination

Introduction

Collinsonia canadensis L. (Lamiaceae) and *Dioscorea villosa* L. (Dioscoreaceae), commonly known as stone-root and wild yam, respectively, are medicinal woodland herbs native to the temperate forests of eastern North America. The rhizomes and roots of *D. villosa* contain diosgenin, a biochemical precursor in the synthetic production of progesterone and other corticosteroids, while the dried rootstocks of *C. canadensis* are used as a diuretic, and the leaves may improve capillary function which aids in the healing of skin wounds (Foster and Duke, 1991). Because of their popularity in Europe and North America as botanical dietary supplements, these

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species are wild-harvested from public forestlands (Robbins, 1999), and/or cultivated at small-scales under a forest canopy by private landowners to supply herb companies which manufacture value-added medicinal products from rootstock material (Krochmal, 1968).

With the ever-escalating global demand for botanical forest products (Foster, 1997; Freese, 1998; Vance, 1995), the cultivation of shade-requiring native medicinal herbs under forest canopies is an important conservation management and development strategy in temperate woodlands (Teel and Buck, 2002). Medicinal plant management programs encourage the planting of seed and/or root material to either augment preexisting forest populations, or establish new populations in forests where they are locally absent. Yet reliable information on the population ecology of many botanical forest products is recurrently cited as an impediment to conservation management efforts (Duchesne and Wetzel, 2002; Robbins, 2000; Vance, 2002). Thus, the purpose of this study was to investigate seed germination and dormancy in the increasingly important medicinal forest herbs *D. villosa* and *C. canadensis*.

Although the dormancy-breaking and germination requirements for many polycarpic woodland herbs are well documented (Baskin and Baskin, 2001), little is known concerning the seed germination biology of *C. canadensis* and *D. villosa*. Neither species are cited in reviews on the seed germination ecology of temperate forest herbs (Baskin and Baskin, 1988, 2001). Martin (1946) observed that *C. canadensis* seeds had large, functionally developed embryos, whereas seeds of *D. villosa* had small, functionally underdeveloped, capitate-shaped embryos. When embryos are underdeveloped at maturity, seeds are considered morphologically dormant (MD) because they require some pretreatment for embryos to grow to some critical threshold for full germination (Baskin and Baskin, 2001). Terui and Okagami (1993) reported that *D. villosa* seed air-dried for 50 d germinated at rates $<50\%$ at a constant temperature of 25 °C, whereas seeds that were cold stratified for 120 d germinated to $>95\%$ over a range of warm constant temperatures (20–25 °C). However, previous studies with *D. villosa* seeds investigated germination behavior under constant laboratory temperatures. Dormancy-breaking and seed germination conditions in nature involve daily temperature oscillations and the natural progression of seasonal temperature changes (Baskin and Baskin, 2001; Probert, 2000; Thompson and Grime, 1983). Consequently, it is essential to evaluate seed germination behavior over a range of simulated thermoperiods and alternating temperature regimes in controlled conditions, and concurrently conduct field experiments to fully characterize dormancy-break (Baskin and Baskin, 2001; Thompson and Grime, 1983).

We experimentally investigated dormancy-break in *D. villosa* and *C. canadensis* by employing a double germination phenology study or "move-along" experiment (Baskin and Baskin, 2003). Based on prior information, we were able to eliminate morphological dormancy (i.e., underdeveloped embryos) as a potential dormancy type in *C. canadensis*. Although fresh seed could be non-dormant (i.e., seed would germinate within 30 d), we hypothesized that *C. canadensis* seeds, which are dispersed in mid-autumn, would have a cold stratification requirement (i.e., physiological dormancy) that would delay germination until favorable spring conditions. Because *D. villosa* embryos are underdeveloped at maturity and appear to require cold stratification for full germination, we hypothesized that seeds would have some form of morphophysiological dormancy (MPD) (i.e., seeds would require some critical temperature for embryo growth and seeds would require some dormancy-breaking pretreatment to germinate). By transferring imbibed seeds through a sequence of thermoperiods that simulate seasonal temperatures in eastern temperate forests, the move-along experimental template would test our hypotheses while also determining the optimum temperatures required for dormancy-break.

Material and methods

Species

Collinsonia is a genus of five species restricted to eastern North America (Gleason and Cronquist, 1991). *C. canadensis* has the broadest distribution within the genus, occurring in early to late-succesional woodlands from Quebec to western Missouri, south to Louisiana and northern Florida, and east to New Hampshire (Gleason and Cronquist, 1991). In early spring, an aerial stem (sometimes two or more) bearing opposite leaves arises from a hard, knotty perennating rhizome (hence the common name stoneroot). Aerial shoots tend to branch near the apex and flowering ramets terminate in divided panicles that bear hermaphroditic flowers from July through September. Flowers are yellow, lemon-scented and obligate xenogamous (Skinner, 1976). Skinner (1976) observed that Bumble Bees (*Bombus* spp.) were the primary pollinators in a naturally occurring population in an Ohio mixed-hardwood forest. The fruit is a four-seeded nutlet that matures during leaf drop in mid-autumn. Seeds are gravity dispersed (Beattie and Culver, 1981).

Dioscorea is a largely pantropical genus with six species occurring in eastern North America, the majority of which are restricted to the southeastern United States. The fleshy tubers and rhizomes of

subtropical members of the genus *Dioscorea* are an economically important food crop. *D. villosa* is a dioecious, herbaceous vine that inhabits thickets, hammocks, and moist and dry woodlands from Connecticut to Wisconsin, west to Oklahoma, and south to the Florida panhandle (Al-Shehbaz and Schubert, 1989). Unlike *Dioscorea polystachya,* a non-indigenous sympatric congener, *D. villosa* never produces vegetative propagules (i.e., bulbils) in its leaf axils (Raz, 2003). In spring, aerial shoots arise from a perennating rhizome and reproductive ramets develop inflorescences in the leaf axils from June through July. Within a population, sex ratios tend to favor staminate plants, which typically outnumber carpellate plants by three to five times (Al-Shehbaz and Schubert, 1989). Staminate plants produce branched panicles that contain several small, sweetly scented flowers. In members of the genus *Dioscorea*, the stamens produce glutinous pollen (Al-Shehbaz and Schubert, 1989) which Coursey (1967) hypothesized was transported to carpelate plants by nocturnal insects. Carpelate plants produce small, solitary flowers at each node on a short spike (Al-Shehbaz and Schubert, 1989). The fruit matures in mid-autumn and is a membranous, three-valved loculicidal capsule with one or two winged seeds (diaspores) occupying each locule; Al-Shehbaz and Schubert (1989) hypothesized that diaspores in members of the genus *Dioscorea* are wind dispersed.

Plant material

We collected ripe seeds of *C. canadensis* from populations growing in mixed-oak second-growth forest in Vinton County, Ohio, on 23 Oct. 2003, and at a roadside adjacent to a second-growth forest edge in Perry County, Ohio, on 25 Oct. 2003. Germination studies commenced on 29 Oct. 2003. Since *D. villosa* populations in southern Ohio are patchily distributed and occur at low density, we were unable to collect enough seed from local populations for meaningful laboratory studies. Thus, in October 2002 we purchased seed from a reputable commercial supplier that propagates native plants (Horizon Herbs Inc., Williams, Oregon). Purchased seed originated from plants that were originally sourced from wild regional populations and are currently growing in an agroforestry system (R. Czech, Horizon Herbs, pers. comm.). These plants have been under no artificial selection or breeding program.

Germination experiments

Initially, we checked for imbibition by comparing the mass of fresh seed to the mass of seed after incubation in moist conditions. Twenty seeds for each species were placed on moistened filter paper in a petri dish wrapped with plastic film for 24 h. Seeds were then removed from the dish, blotted dry with a paper towel, and weighed. Changes in seed mass was calculated by subtracting the mass of fresh seed after 24 h exposure to moist conditions from the initial mass of fresh seed, and then dividing by the mass of initial seed.

Fresh seeds were placed on top of a 3:1 (v/v) mixture of potting soil and sand moistened with distilled water in 9 cm (diameter) plastic petri dishes. For each species, treatments consisted of four replicates of 30 seeds each ($N = 120$ seeds/treatment). To determine if fresh seed of either species would germinate over a range of temperature conditions, a set of replicates was incubated in germinators with a 14 h photoperiod (white fluorescent light) and 12 h/12 h alternating thermoperiods of 30/15, 20/10, and 15/6 °C, and a constant temperature of 5 °C. All petri dishes were removed after a 4-week incubation period and germination percentages were calculated. Seeds that had completely germinated (radicle and shoot emergence) were removed from the dish. Dishes were then placed back in the germinators for an additional 40 weeks and checked every 7 d for germination. Dishes were watered as needed so that seeds remained imbibed throughout the study.

A move-along experiment is particularly valuable when large sample sizes for extensive germination studies are difficult to obtain (Baskin and Baskin, 2003). According to the move-along experimental template we simultaneously conducted the following two treatments, hereafter referred to as "warm treatment" and "cold treatment." In the warm treatment, a set of four replicate dishes for each species was incubated in the following sequence: 12 weeks at 30/15 °C→4 weeks at 20/10 °C→4 weeks at 15/6 °C→12 weeks at 5 °C→4 weeks at 15/6 °C→4 weeks at 20/10 °C→12 weeks at 30/15 °C. In the cold treatment, a set of replicates for each species was incubated in the following sequence: 12 weeks at 5 °C→4 weeks at 15/6 °C→4 weeks at 20/10 °C→12 weeks at 30/15 °C→4 weeks at 20/10 °C→4 weeks at 15/6 °C→12 weeks at 5 °C. The cold treatment would determine if seeds required cold stratification only or a cold followed by warm stratification, whereas the warm treatment would determine if seeds required a period of warm stratification or warm followed by cold stratification. The chosen thermoperiods represent average daily maximum and minimum air temperatures for our study region; 30/15 °C for summer, 20/10 °C for early autumn and late spring, 15/6 °C for late autumn and early spring, and 5 °C for winter (Baskin and Baskin, 2003). To complement the move-along experiment, we conducted a germination phenology only with *D. villosa* since we were unable to collect enough *C. canadensis* seeds for a meaningful study. We sowed 50 seeds in each of four flats filled with potting soil and covered with a thin layer of mixed-oak leaf litter to prevent dessication. Flats were placed in a wood shadehouse covered with lattice

located at the Ohio University West State Street Research Gardens (Athens, Ohio). The shadehouse was located within a fenced area where seeds were protected from predators but exposed to natural temperatures. Flats were watered as needed to maintain constant imbibition. Germination was checked on weekly basis for 2 years.

We also tested the response of seeds of both species to different periods of cold stratification (5 °C). A set of replicates for each species was incubated for 4 weeks at 5 °C and 8 weeks at 5 °C, and then placed in warm/cool temperatures (20/10 °C). Mean and standard errors for final germination percentages were calculated from each set of replicates (i.e., four petri dishes, 30 seeds/dish).

To determine whether an extended period of dry storage could alleviate dormancy for either species, 480 fresh seeds for each species were air-dried for 2 weeks and then stored in closed glass jars for 6 months at ambient laboratory conditions (ca. 21 °C). At 6 months, seeds were removed from storage and placed on a moist germination mixture at each of the four thermoperiods ($N = 120$ seeds for each species at each thermoperiod). Seeds were checked for germination after 28 d and results were compared with mean percentage of fresh seeds germinated after 4 weeks at each thermoperiod with a one-way ANOVA.

Results

In the imbibition test, seed mass of both species increased by >50% after a 24 h period in moist petri dishes; thus, we concluded that seeds did not possess impermeable seed coats (i.e., physical dormancy).

Seeds of *D. villosa* exposed to natural temperatures in the shadehouse reached peak germination percentages the third week of April, when daily maximum and minimum temperatures ranged from 15–20 °C to 3–10 °C, respectively (Fig. 1). No seeds germinated the following spring (2004).

In the move-along experiment, seeds initially started in the cold treatment (12 weeks at 5 °C) germinated after transfer to warmer temperatures (Fig. 2a). *D. villosa* germination peaked at 15/6 °C, with 97.4% ± 2.6 (mean ± 1 SE) of seeds germinating before they were transferred to 20/10 °C, where the remaining viable seeds germinated. Conversely, 41% ± 3.5 of *C. candensis* seeds germinated at the end of the 4-week sequence at 15/6 °C and 34% of seeds germinated after 3 weeks at 20/10 °C (total germination: 97.5% ± 2.5; Fig. 2a). For both species the germination mode was hypogeous, yet the radicle preceded shoot emergence by several days in *D. villosa*, whereas the radicle and shoot emerged concurrently in *C. canandensis*.

For *D. villosa*, seeds placed initially in the warm treatment did not germinate until transferred through

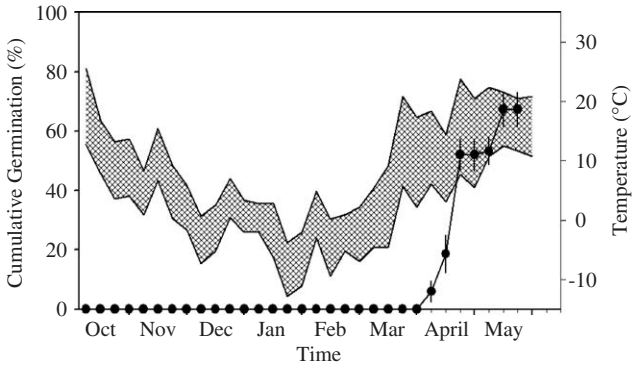

Fig. 1. Cumulative germination percentages (mean ± 1 SE) for 200 seeds (four replicates of 50 seeds) of *Dioscorea villosa* sown in fall 2002. Flats were placed in a wood shadehouse exposed to natural temperature conditions at the Ohio University West State Street Research Garden. Mean weekly maximum and minimum air temperatures (hatched areas) for 2002–2003 were obtained from a National Climate Data Center weather observation station located in Athens, Ohio (39°21′N/82°06′W) (NOAA, 2004).

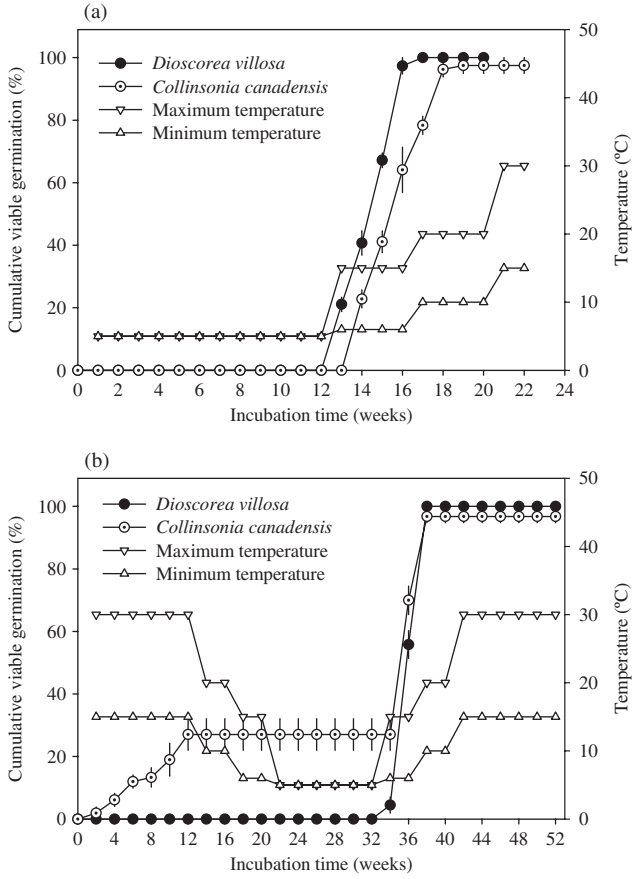

Fig. 2. Cumulative viable germination percentages (mean ± 1 SE) for *Collinsonia canadensis* and *Dioscorea villosa* in the (a) cold treatment sequence and (b) warm treatment sequence of a move-along experiment.

the following sequence of temperatures: 12 weeks at 5 °C→4 weeks at 15/6 °C→4 weeks at 20/10 °C, seeds then germinated to 100% at 20/10 °C (Fig. 2b). In contrast, 27.0% ± 5.0 of *C. canadensis* seeds germinated during the initial 12 weeks at 30/15 °C but germination was subsequently suppressed when seeds were transferred through the following temperature sequence: 4 weeks at 20/10 °C→4 weeks at 15/6 °C→12 weeks at 5 °C; seeds then germinated to 96.8% ± 1.9 after their second exposure to 15/6 and 20/10 °C (Fig. 2b).

Neither species germinated within 4 weeks in any of the control thermoperiods. However, both species germinated after a protracted time period in the controls but the thermoperiods at which dormancy-break occurred differed between the two species. After 8 weeks at the 20/10 °C thermoperiod, *D. villosa* seeds began germinating, although the speed of germination was slow and after 18 weeks germination stabilized at 61.9 ± 7.9% (Fig. 3a). *D. villosa* seeds in the 15/6 °C thermoperiod began germinating after 10 weeks and germinated to high rates (80%) after a 24-week period (Fig. 3a). No *D. villosa* seeds germinated at 5 or 30/15 °C. In contrast, *C. canadensis* seeds began germinating after 4 weeks at 30/15 °C and after 22 weeks at 5 °C (Fig. 3b). Seeds also began germinating after 22 weeks at 15/6 °C but seed germination rates were <20% (Fig. 3b). Seed germination was negligible at 20/10 °C (Fig. 3b).

Each species responded differently to varying lengths of cold stratification. *C. canadensis* seeds maintained at 5 °C for 8 weeks germinated to higher percentages at 20/10 °C than seeds maintained at 5 °C for 4 weeks, although germination was slow and did not begin until 8 weeks (Fig. 4a). In contrast, *D. villosa* seeds germinated to similar percentages when held for 4 or 8 weeks at 5 °C and then moved to 20/10 °C (Fig. 4b).

In the 6-month dry storage treatment, no seeds for either species germinated after 28 d inbetween of the

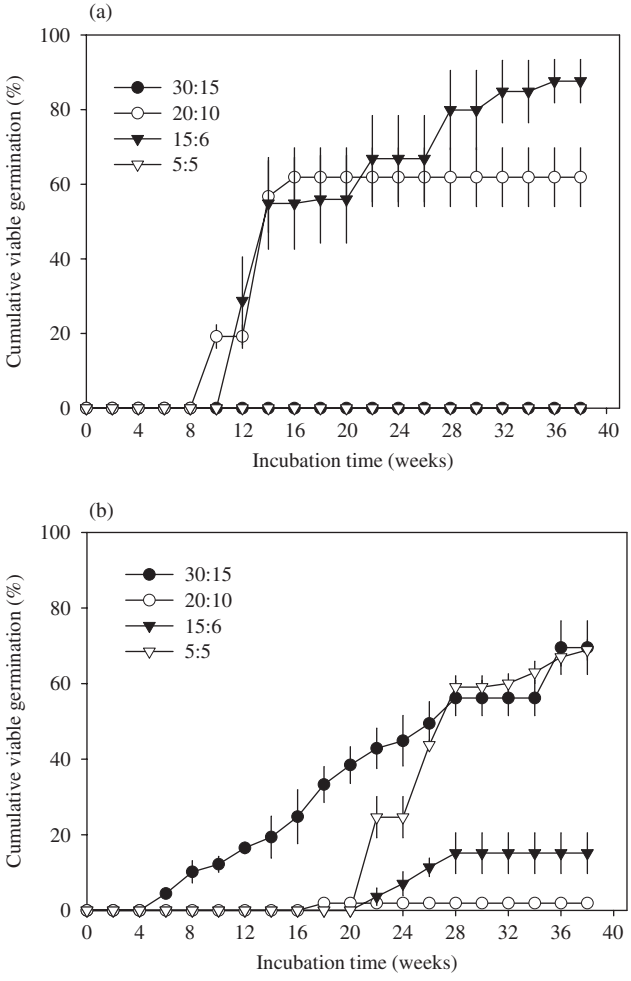

Fig. 3. Cumulative germination percentages (mean ± 1 SE) for seeds of (a) *Dioscorea villosa* and (b) *Collinsonia canadensis* after incubation at four temperature regimes for 40 weeks.

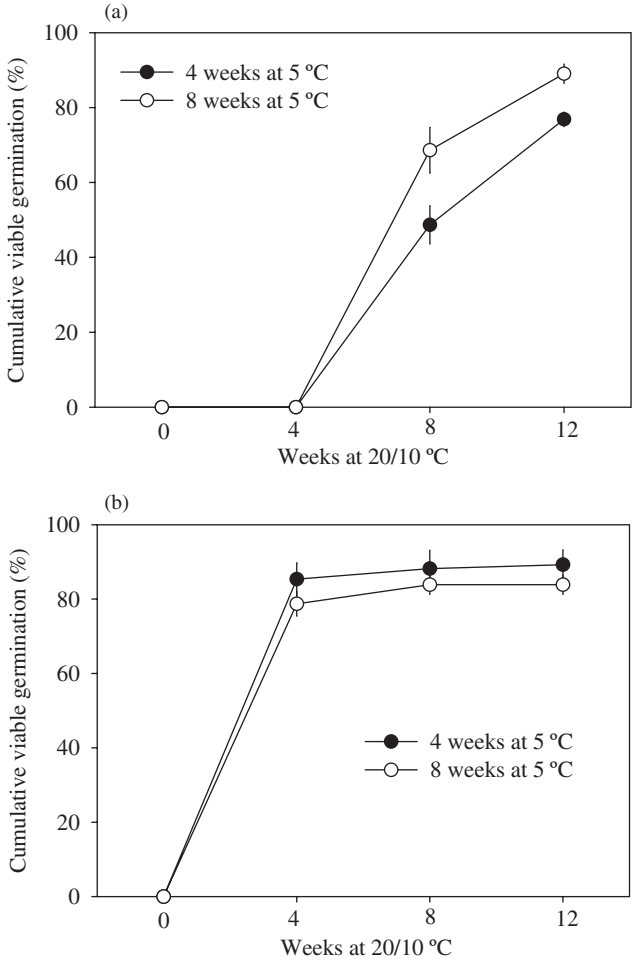

Fig. 4. Cumulative viable germination percentages (mean ± 1 SE) for (a) *Collinsonia canadensis* and (b) *Dioscorea villosa* seeds given 4 and 8 weeks of cold stratification at 5 °C and then transferred to warm temperatures (20/10 °C), where cumulative germination was observed for 4, 8, and 12 weeks.

thermoperiods. For *D. villosa*, the mean percentage of viable seeds was significantly (one-way ANOVA, $F_{1,30} = 79.38$, $P < 0.0001$) lower at study termination with 6 month dry stored seed (39.3% ± 2.7) compared at our control thermoperiod experiment with fresh imbibed seed (70.8% ± 2.0). For *C. canadensis*, the mean percentage of viable seeds was significantly (one-way ANOVA, $F_{1,30} = 178.45$, $P < 0.0001$) lower at study termination with 6 month dry stored seed (24.1% ± 2.4) compared at our control thermoperiod experiment with fresh imbibed seed (75.5% ± 2.6).

Discussion

Dormancy classification

Over a range of simulated thermoperiods fresh seed of *D. villosa* and *C. canadensis* failed to germinate after incubation for 30 d (or 4 weeks), indicating that seeds were dormant at maturity (Baskin and Baskin, 2001). Since effective dormancy-break in *D. villosa* and *C. canadensis* occurred following cold stratification, results from the move-along experiment supports the hypothesis that both species require cold stratification to alleviate physiological dormancy. Martin's (1946) and our own observations from longitudinal cross-sections of seeds indicate that *D. villosa* has small, underdeveloped embryos at maturity. If species with underdeveloped embryos require some time period for embryos to grow to some critical threshold before germination occurs they are also MD (Baskin and Baskin, 2001). Thus, the move-along experiment and shadehouse study supports our initial hypothesis and confirms prior laboratory studies (Terui and Okagami, 1993) that *D. villosa* seeds are morphophysiologically dormant (MPD).

Baskin and Baskin (1998) described eight levels of MPD based on the response of seeds to three parameters: the effectiveness of gibberellic acid (GA_3) at breaking dormancy, temperatures required for dormancy-break, and temperatures required for embryo growth. Although we did not explicitly measure embryo growth in this study, Terui and Okagami (1993) found that when embryos were isolated from *D. villosa* seeds and placed in warm temperatures (14–32 °C) they germinated to high rates (>80%), indicating that warm temperatures promote embryo growth and that after embryo growth occurs, cold stratification is not required for germination. In the three complex types of MPD (non-deep complex, intermediate complex, and deep complex), cold temperatures (5 °C) are required for embryo growth, whereas embryo growth proceeds at warm temperatures for the five simple types of MPD (non-deep simple, intermediate simple, deep simple, deep simple epicotyl, and deep simple double; Baskin and Baskin, 2004). In four of the five simple levels of MPD, embryo growth occurs at warm temperatures but a sequence of warm followed by cold stratification is needed for full germination (radicle and shoot emergence) (Baskin and Baskin, 2001, 2004). Since cold stratification breaks dormancy without a prior warm stratification treatment, and embryo growth appears to be delayed until warm temperatures occur in the spring, we conclude that *D. villosa* has non-deep simple MPD. This level of MPD has also been observed in other polycarpic perennials, such as *Chamaelirium luteum* (Baskin et al., 2001) and *Thalictrum mirabile* (Walck et al., 1999), that show similar phenological patterns in the field (i.e., seeds disperse in autumn and germinate in spring) and dormancy-break in the laboratory.

Dormancy-break: laboratory and field conditions

In *D. villosa*, effective dormancy-break can occur over a prolonged time period when 12 h of cold stratification (at 6 or 10 °C) alternates with 12 h of cool stratification (15 or 20 °C). Conversely, these alternating temperatures were ineffective at breaking dormancy in *C. canadensis*, suggesting that temperatures higher than 5 °C are ineffective at breaking physiological dormancy in this species. Although 5 °C is often the optimum cold stratification temperature that alleviates dormancy in temperate woodland herbs (Baskin and Baskin, 2001), temperatures ranging from 0 to 10 °C may also be effective at overcoming dormancy (Bewley and Black, 1982). For example, in temperate congeners, *D. japonica* and *D. septemloba*, effective dormancy-break occurred at 0 °C whereas no or little germination was observed at 5 °C (Okagami and Kawai, 1982). Baskin et al. (2001) observed that with MPD seeds of the woodland herb *C. luteum*, dormancy-break occurred after 8 weeks at 15/6 and 20/10 °C thermoperiods. They hypothesized that cold temperatures (6–10 °C) alternating with cool temperatures (15–20 °C) alleviate physiological dormancy while simultaneously promoting slow growth of the underdeveloped embryo. This type of dormancy-break can be understood by examining the number of hours seeds were cold stratified. For example, with *D. villosa*, seeds would have been exposed to 840 h of cold stratification (or 12 h of cold stratification per day) when they began germinating after 70 d in the 15/6 and 20/10 °C thermoperiods. When seeds were given a constant cold stratification for a period of 4 weeks (or 672 h) and then moved into warmer temperatures (20/10 °C), dormancy was broken and seeds germinated to high rates (>80%) after 28 d. This suggests that once the critical time period of cold stratification (5–10 °C) demanded by *D. villosa* seeds is achieved, seeds are capable of germinating in temperatures >15 °C.

An ecological interpretation of our move-along experiment suggests that fresh seed of both species overcomes dormancy during winter and seeds rapidly germinate after exposure to warm days (15–20 °C) and cold nights (6–10 °C). This pattern of dormancy loss is typical of woodland herbs that naturally disperse seeds in mid-autumn, since delaying germination until spring ensures favorable conditions for seedling establishment (Probert, 2000). Our phenology study with *D. villosa* in the shadehouse confirmed the results of the move-along experiment since no dormancy-break occurred in *D. villosa* seeds during the autumn months. We also found no additional *D. villosa* germination after 2 years in the shadehouse (spring 2004), a germination behavior that corresponds with Thompson and Grime's (1979) Type II transient seed bank.

A common feature of species that display physiological dormancy is germination occuring at temperatures uncharacteristic to those they would be experiencing in their natural habitat (Baskin and Baskin, 2001). This is consistent with our results with fresh *C. canadensis* seed that began germinating at a slow velocity in warm (30/15 °C) conditions after 4 weeks. However, warm stratification tends to be more effective at breaking dormancy in obligate winter annuals than polycarpic woodland herbs (Baskin and Baskin, 2001). One possible reason for dormancy-break at 30/15 °C is this thermoperiod has a larger daily temperature amplitude than the 20/10 and 15/6 °C thermoperiods; large amplitudes have been found to trigger germination in other temperate herbs (Thompson and Grime, 1983). However, in field conditions, the results from our simulated mid- to late-autumn temperature (20/10 and 15/6 °C) treatments indicate that seeds would undergo conditional dormancy at the time of dispersal (mid-autumn), thus preventing epicotyl emergence in circumstances otherwise unfavorable for seedling establishment, i.e., late-autumn and winter freezing.

Cultivation recommendations

Dry storage may overcome the cold stratification requirement for physiological dormancy loss in some herbaceous species (Baskin and Baskin, 2001). Our results indicate that neither species afterripened (overcame dormancy) in the 6-month (~180 d of storage) dry storage treatment since no seeds germinated in 4 weeks at any of the tested thermoperiods. While dry storage tends to alleviate primary dormancy in seeds that require warm stratification for overcoming PIM, as is often the case in winter annuals, it tends to be less effective at overcoming a cold stratification requirement in polycarpic perennials (Baskin and Baskin, 2001). Alternatively, dry storage may induce seeds into protracted dormancies, although our investigation would have not detected this. A 6-month dry storage interval simulates a possible sequence for propagating plants from seed in forest cultivation systems: (1) fresh seed collected in October; (2) seed dry-stored in unchilled conditions during the winter; and (3) seed planted the following spring (April). If this sequence were followed in the field, based on our results from the move-along experiment it appears that *D. villosa* seed germination would not occur until the following spring (18 months after seed was collected) after seeds were exposed to a winter chilling period, an apparent requirement for full germination. However, *D. villosa* seeds are capable of germinating following only 4 weeks of cold stratification suggesting that seeds sown in late winter or early spring may provide a long enough cold stratification period for full germination, although dry-storing seeds would result in a smaller viable seed population. While 4 and 8 weeks of cold stratification did alleviate dormancy in *C. canadensis* seeds, seeds germinated at a slower speed and overall lower rates compared to the 12-week cold stratification treatment in the move-along experiment, suggesting that longer periods of cold stratification may be more effective at breaking dormancy. From a forest cultivation perspective, we recommend that seeds of both species be sown shortly after dispersal. This would preclude viability loss incurred during dry-storage and ensure a long chilling period that is most favorable for full germination.

In conclusion, our study is the first description of physiological dormancy in *C. canadensis* and non-deep simple MPD in *D. villosa*. The temperatures requirements for dormancy-break identified in this study can guide direct seeding in the field and thereby facilitate conservation management strategies with often limited seed germplasm.

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