Seed dormancy and germination vary within and among species of milkweeds

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Abstract. Pollinators in general and monarch butterflies in particular are in decline due to habitat loss. Efforts to restore habitats for insects that rely on specific plant groups as larvae or adults depend on the ability of practitioners to grow and produce these plants. Monarch larvae feed exclusively on milkweed species, primarily in the genus Asclepias, making propagation and restoration of these plants crucial for habitat restoration. Seed germination protocols for milkweeds are not well established, in part due to the large number of milkweed species and conflicting reports of seed dormancy in the genus. We tested for seed dormancy and the optimum period of cold stratification in 15 populations of A. speciosa and 1–2 populations of five additional species, including A. asperula, A. fascicularis, A. subulata, A. subverticillata and A. syriaca. We exposed seeds to cold (5 °C) moist conditions for 0, 2, 4, 6 and 8 weeks and then moved them to 15 °C/25 °C alternating temperatures. In A. speciosa, dormancy was detected in eight populations, and this dormancy was broken by 2–4 weeks of cold stratification. The remaining seven populations showed no dormancy. Seed dormancy was also detected in two populations of A. fascicularis (broken by 4–6 weeks of cold stratification) and a single population of A. syriaca (broken by 2 weeks of cold stratification). No dormancy was detected in A. asperula, A. subulata or A. subverticillata. Seed dormancy appears to be widespread in the genus (confirmed in 15 species) but can vary between populations even within the same species. Variation in seed dormancy and cold stratification requirements within and among Asclepias species suggests local adaptation and maternal environments may drive seedling ecology, and that growers should watch for low germination and use cold stratification as needed to maximize seed germination and retain genetic variability in restored populations.

Keywords: Cold stratification; conservation biology; habitat restoration; monarch butterfly; plant propagation; pollinator conservation.

Introduction
Declines in insect pollinator abundance and diversity have been documented over the past decade on regional and global scales (Ghazoul 2005; Steffan-Dewenter et al. 2005; Biesmeijer et al. 2006; Williams and Osborne 2009; Cameron et al. 2011). The loss of these pollinators may have serious impacts on ecologic function and economic stability (Potts et al. 2010) because pollinators are important or essential for 65 % of wild flowering plants (Ollerton et al. 2011) and 75 % of domestic food crops (Klein et al. 2007). Monarch butterflies (Danaus plexippus) in particular have declined up to 90 % over the past
15 years (Jepson et al. 2015), due in part to loss of milkweed in their summer breeding habitat from increased use of herbicides in intensive farming practices (Brower et al. 2012).

Plants in the milkweed family (Asclepiadaceae) are the exclusive food source for monarch butterfly larvae. Monarchs in North America feed on at least 27 species in the genus Asclepias and a few closely related genera (Malcolm and Brower 1986). Milkweed populations have suffered considerable declines in habitat in the central USA due mainly to the use of genetically modified, glyphosate resistant crops, and the placement of an additional 10 million hectares of herbicide-tolerant corn into production since 2007 (Pleasants and Oberhauser 2012). Restoring milkweed habitat is crucial to the recovery of the monarch butterfly and will require planting large numbers of milkweeds throughout its geographic range (Fallon et al. 2015; Jepsen et al. 2015; Luna and Dumroese 2013). Milkweeds also benefit many other insects and pollinators, such as milkweed beetles in the Cerambycidae (Evans 2014), bumblebees (Bombus spp.), honey bees (Apis mellifera), and other bees and lepidoptera of various body sizes (e.g. Fishbein and Venable 1996), so conservation of these plants has cascading positive effects on ecosystem function and multiple groups of organisms (Buckley and Nabhan 2016; Dumroese et al. 2016).

To produce milkweeds for habitat conservation at a large scale, reliable propagation protocols must be available. One method of milkweed propagation is by vegetative rhizome cutting, which can be an effective method for growing and establishing several species of milkweeds in gardens and habitat restoration sites (Ecker and Barzilay 1993; Landis 2014; Landis and Dumroese 2014). Although cuttings of this type can produce large plants relatively quickly, the process generally results in clones of fewer genotypes, and thus lower genetic diversity and fitness in restored populations, than propagation from seed (Williams 2001). Germination is the first step in producing plants from seed and requires an understanding of dormancy and germination requirements for efficient and successful plant propagation at a large scale. But seed germination protocols for Asclepias species are relatively poorly developed, despite the large number of species in the genus. Over 100 species of Asclepias are known to occur in North and Central America (Mabberley 1997) with ~76 species in the USA and Canada (USDA, NRCS 2017). Related species, and populations within species, are often assumed to have the same germination requirements because of phylogenetic constraints (Seglias et al. 2018). It is generally unknown if seed dormancy and cold stratification requirements vary across Asclepias species or populations. In some cases, suggested germination protocols for Asclepias species are inconsistent or even contradictory (Borland 1987). For example, cold stratification has been recommended to release seeds of A. speciosa from dormancy but some practitioners report that no cold stratification is necessary for large-scale propagation (Stevens 2000).

Here we present the results of research on seed dormancy and germination in six Asclepias species. In particular, we examine variation in dormancy and cold stratification requirements among populations of A. speciosa as well as between five other species. We test the hypothesis that dormancy varies among populations of A. speciosa from across the species’ geographic range, and populations of the five additional species. We predict that dormancy varies substantially, thus explaining conflicting reports of the importance of cold stratification in this genus. We also compile and review the primary literature and unpublished reports on dormancy and germination of several additional milkweed species.

**Methods**

**Germination and viability tests**

We obtained seed from 15 populations of A. speciosa (Fig. 1) and one or two populations of five additional North American species, including A. asperula, A. fascicularis, A. subulata, A. subverticillata and A. syriaca (Table 1) from multiple locations in the USA (Fig. 2). These seeds had either been collected during the summer immediately prior to germination testing, or had been in dry, cold (<0 °C) storage at the US Forest Service Bend Seed Extradatory or the US Agricultural Research Service, National Plant Germplasm System.

To determine if seeds from each species and source population required a period of cold stratification to release them from dormancy, we exposed seeds to a range of cold, moist periods. Dormancy was defined here as the inability of a seed to germinate in a specified period of time under combinations of normal physical factors (e.g. temperature, light, etc.) that are otherwise favourable for germination, following Baskin and Baskin (2004). Seeds were stratified for 0, 2, 4, 6 or 8 weeks at 5 °C at the Oregon State University Seed Lab. We used four or five replicates (depending on seed availability, Table 1) of 50 seeds from each seed source. For all replicates, seeds were placed on moistened germination paper in 15 cm × 15 cm × 3 cm transparent plastic boxes with fitted lids. The paper was moistened weekly as needed with distilled water. After stratification treatments were applied, seeds were placed in a growth chamber with 15 °C/25 °C alternating temperatures, with 8 h of darkness at 15 °C and 16 h of fluorescent light.
at 25 °C. We defined germination as emergence of the radicle at least 3 mm, and counted germinated seeds after 10 days (Baskin and Baskin 2001). In preliminary trials (not shown) we found that 95 % or more seeds germinated within this period of time, and any additional germination took many weeks to complete. Seed

![Figure 1. Asclepias speciosa (A) in flower and (B) dehiscing fruit with seeds. Plant photographed in Willamette Valley, OR.](https://academic.oup.com/aobpla/article-abstract/10/2/ply018/4917378)

**Table 1.** Asclepias species and populations included in dormancy and germination tests, with seed sources, samples (50 seeds each) per treatment and years in storage. †BSE indicates the US Forest Service, Bend Seed Extractory; GRIN indicates the US National Germplasm Resources Information Network.

| Species            | Population          | Seed source† (accession number)                  | Samples per treatment | Years in storage |
|--------------------|---------------------|-------------------------------------------------|-----------------------|------------------|
| *Asclepias speciosa* | Denver, CO          | BSE (CO932-306-Jefferson-12)                    | 4                     | 5                |
|                    | Montrose, CO        | Colorado Plateau Native Plant Program           | 4                     | 2                |
|                    | Minidoka, ID        | US Fish and Wildlife Service                    | 5                     | 0                |
|                    | Treasure Valley, ID | Idaho State University                          | 5                     | 0                |
|                    | Navajo Dam, NM      | BSE (NM930N-86-SanJuan-12)                      | 4                     | 5                |
|                    | Vernal, NM          | BSE (SOS-NM930N-12-10)                          | 4                     | 7                |
|                    | Galice, OR          | BSE (SOS-OR110-904-Josephine-15)                | 4                     | 2                |
|                    | Malheur, OR         | US Fish and Wildlife Service                    | 5                     | 0                |
|                    | Ontario, OR         | Institute for Applied Ecology                   | 5                     | 0                |
|                    | Tub Springs, OR     | BSE (OR110-637-Jackson-13)                      | 4                     | 4                |
|                    | Willamette Valley, OR | Heritage Seedlings, Inc.                       | 5                     | 0                |
|                    | Escalante, UT       | BSE (UT030-256-Garfield-15)                     | 4                     | 2                |
|                    | Maeser, UT          | BSE (SOS-UT080-140 UINTAH-13)                   | 4                     | 4                |
|                    | Little Pend Oreille, WA | US Fish and Wildlife Service              | 5                     | 0                |
|                    | Kane, WY            | BSE (SOS-WY020-11-11)                           | 4                     | 10               |
| *Asclepias asperula*          | Santa Cruz, AZ     | GRIN (W6-48232)                                 | 4                     | 4                |
| *Asclepias fascicularis*      | Agate Reservoir, OR | BSE (SOS-OR110-608-Jackson-13)                 | 4                     | 4                |
| *Asclepias subulata*          | Lake Havasu City, CA | GRIN (W6-46777)                           | 4                     | 5                |
| *Asclepias subverticillata*   | Anvil Points, CO   | BSE (SOS-CO932-160-08)                          | 4                     | 9                |
| *Asclepias syriaca*           | UT                  | GRIN (W6-36792)                                 | 4                     | 10               |
|                    |                    | GRIN (W6-48817)                                 | 4                     | 4                |
viability was tested with tetrazolium (TZ) by the Oregon State University Seed Lab using standard procedures (Elias et al. 2012). The TZ test was used to estimate the percentage of live and dead seeds in each seed source, regardless of dormancy level (Baskin and Baskin 2001). Seeds were cut longitudinally to expose interior tissues and facilitate the entrance of TZ solution, and incubated in a 1% TZ solution for 24 h at 35 °C. Seeds were inspected for TZ staining, specifically the pattern and intensity of red colour in live tissues in seeds. Seed viability was over 90 % in most Asclepias populations as tested with TZ, with the exception of two populations with lower viability, Willamette Valley, OR (73 ± 6.0 %) and Malheur, OR (28 ± 6.1 %). In populations with substantial dormancy, <60 % of seeds germinated without cold stratification, but with 2 or more weeks of cold stratification germination increased to the levels of seed viability, or nearly so. For example, without cold stratification 32 ± 5 % of seeds from Little Pend Oreille, WA, germinated, but after 2 weeks of cold stratification germination rose to 91 ± 5 %, close to the viability estimate of 96 ± 2.6 % (Fig. 3A). Seed from Malheur, OR, required 4 weeks of cold stratification to fully break dormancy, while germination of seeds from Willamette Valley, OR, did not germinated to the level of their estimated viability. Populations with largely non-dormant seeds (Fig. 3B) germinated to very high rates at or near their viability estimate without any cold stratification. Seed germination was not higher after 8 weeks of cold stratification than 6 weeks in any population tested, so this treatment is not shown (Fig. 3).

Interspecific differences in dormancy and germination

Dormancy and germination varied among species of Asclepias examined here. Again there was a significant interaction between cold stratification treatment and population for the five taxa examined (F = 40.29, df = 24;105, P < 0.0001), with two species showing positive effects on germination of cold stratification of 2 or more weeks, but the remaining taxa possessing
little or no dormancy. Without cold stratification, both populations of *Asclepias fascicularis* that were sampled had germination of only 17 ± 5.1 % and 35 ± 5.1 %, despite viability estimates of 93 ± 3.5 % and 90 ± 4.2 %, respectively, indicating substantial dormancy. Both of these populations required 6 weeks of cold stratification to achieve maximum germination of 80 ± 5.1 % and 82.5 ± 5.1 %, respectively (Fig. 4). Eight weeks of cold stratification (not shown) did not improve seed germination any further in *A. fascicularis*.

*Asclepias syriaca* also had partial dormancy, with only 38.5 % of seeds germinating without cold stratification, despite viability estimated at 95 ± 3.0 %. After 2 weeks of cold stratification of this sample, germination increased to 92 ± 5.1 % (Fig. 4). The remaining species, *A. asperula*, *A. subulata* and *A. subverticillata*, germinated to levels close to (or within 95 % confidence intervals) of their seed viability as estimated by TZ even without cold stratification. Again, cold stratification for 8 weeks (not shown) did not increase seed germination over the 6-week period (Fig. 4).

**Discussion**

**Seed dormancy and viability**

We found that seed dormancy varied widely within and among *Asclepias* species, and that the period of time in cold stratification needed to break dormancy varied as well. In *A. speciosa* in particular, seeds from seven source populations had essentially no dormancy, while seeds from eight other populations generally needed 2 weeks (but up to 6 weeks) of cold stratification to reach germination levels similar to viability. Of the remaining species tested here, the seeds of three (*A. asperula*, *A. subulata* and *A. subverticillata*) were non-dormant, while two (*A. fascicularis* and *A. syriaca*) required cold stratification to break dormancy. Seed germination tests in *A. subulata* have also found no dormancy in populations from California (Everett 2012). Previous reports have suggested little or no dormancy in *A. speciosa* (Bartow 2006; Skinner 2008), but our results indicate that the benefits of cold stratification differ substantially among source populations.
populations. Even in populations where seed germination was enhanced by cold stratification, many seeds germinated without a cold treatment, suggesting that when dormancy was present, it typically affected only a portion of the seeds from a given location. This was true when dormancy was present in *A. speciosa* as well as *A. fascicularis* and *A. syriaca*, which had 3% to 54% germination even without cold stratification.

Seed viability was typically very high (over 85%) in our samples, with two exceptions out of the 22 populations we tested. High seed viability has also been reported in *A. cordifolia* (Barner 2009) and *A. syriaca* (Bhowmik 1978). Unpublished records of seed viability and dormancy in *Asclepias* species tested by the US Department of Agriculture National Laboratory for Genetic Resources Preservation are compiled in Table 2. Their records from germination tests of small seed samples (typically two replicates of 50 seeds, but in one case only 44 seeds) with specific periods of cold stratification ranging from 0 to 21 days suggest wide variation in viability and dormancy within and among populations of *Asclepias* species. For example, among eight populations of *A. fascicularis*, seed viability ranged from 68 to 98%, and seven of the source populations possessed no seed dormancy (defined here as <80% germination of viable seeds) when no cold stratification was provided. This is in contrast to our findings of substantial dormancy in both populations of *A. fascicularis* that we examined. In *A. speciosa*, seed viability ranged from 65 to 98% among 13 populations, and six out of nine populations were dormant without cold stratification (Table 2), a pattern that agrees with our observation of wide variation in dormancy in that species.

**Cold stratification**

Baskin and Baskin (2001) suggest that *Asclepias* seeds may have physiological dormancy, which is typically broken by cold stratification, seed coat removal or chemical inducements, all of which improve germination in *A. syriaca* (Oegema and Fletcher 1972). Our review of published and unpublished accounts of seed germination and dormancy in 17 species of *Asclepias* suggests that variation in the period of cold stratification needed to break dormancy appears to be common within and among milkweed species (Table 3). For example, Green and Curtis (1950) examined five species of milkweed from Wisconsin and found a range of periods of cold stratification needed to completely release seeds from dormancy, from none at all to 5 months. Most researchers use cold stratification temperatures of 4°C to 5°C (Luna and Dumroese 2013), although some placed seeds outdoors in winter to expose seeds to ambient cold conditions (e.g. Green and Curtis 1950).

Species with non-dormant seeds (i.e. in which no benefit of cold stratification has been reported) include *A. erosa* (Everett 2012), *A. meadii* (Bowles et al. 1998), *A. perennis* (Edwards et al. 1994), *A. speciosa* (Bartow 2006; Skinner 2008), *A. syriaca* (Radijovic et al. 2016) and *A. tuberosa* (Green and Curtis 1950; Phillips 1985). However, in some of these same species cold stratification has been found to improve germination in studies from other populations, and the optimal period of cold stratification varies as well. For example, *A. syriaca* and *A. tuberosa* are important for monarch butterfly populations (Seiber et al. 1986; Borders and Lee-Mader 2014) and have received the most attention for their...
Germination requirements, and both vary widely in cold stratification needs. *Asclepias syriaca* populations with seed dormancy may require 56 days or more of cold stratification (Green and Curtis 1950; Oegema and Fletcher 1972), or as little as 7 days (Evetts and Burnside 1972; Farmer et al. 1986). We found 2 weeks of cold stratification was sufficient to release dormancy in this species, but we did not try a shorter period. In *A. tuberosa*, as much as 90–120 days of cold stratification (Salac and Hesse 1975; Cullina 2000; Blessman and Flood 2001) or as little as 21 days (AOSA 2016) may be needed for optimal germination of dormant seed lots. Similarly, 30 days (Vandevender and Lester 2014a) down to 1 week or less (Lincoln 1983; Schultz et al. 2001a) of cold stratification may be needed to improve germination in *A. incarnata*. Among species tested by the National Laboratory for Genetic Resources Preservation, most populations that received cold stratification of 7–21 days were released from dormancy (Table 2).

### Factors that affect dormancy and germination in *Asclepias*

Seed dormancy can vary among species, populations (Keith and Myerscough 2016; Siles et al. 2017), collections from the same population but different years (Green and Curtis 1950; Kaye 1999) and among individual mother plants (Andersson and Milberg 1998). Seed dormancy can affect interactions within and among species by determining the seasonal timing of germination, seedbank dynamics, and exposure of seeds and seedlings to hazards and competition for resources (Harper 1977; Baskin and Baskin 2001). Dormancy has been shown to be under genetic control in some species, often in response to natural selection, such as in *Digitaria* (Hacker 1984), *Arabidopsis* (Alonso-Blanco et al. 2003; Bentsink et al. 2010) and *Oryza* (Gu et al. 2004), or it can result from conditions during seed maturation in the environment of the maternal plant and zygote (Bodrone et al. 2017; Penfield and MacGregor 2017), or both (Postma and Agren 2015). To our knowledge, neither genetic nor environmental controls on dormancy have been documented in *Asclepias*. Despite the widespread presence of seed dormancy in *Asclepias* species, persistent seed banks have not been detected even when milkweeds are present in the above-ground vegetation (Smith and Kadlec 1983; Johnson and Anderson 1986). Seedling emergence of *A. syriaca* exceeds 80% for seeds buried 0.5–4 cm (Yenish et al. 1996) leaving only a small proportion of seeds in the soil unaccounted for and which could contribute to a seedbank or succumb to mortality. Germination of *Asclepias* seeds can also be affected by light, scarification, substrate,

### Table 2. Summary of seed dormancy in *Asclepias* species tested at the USDA/ARS National Laboratory for Genetic Resources Preservation, Seed Quality Lab, with duration of cold stratification (if any), number of populations sampled and number of populations with and without dormancy. Germination is relative to viability, and is shown as the amount or range determined for each period of stratification. Populations were classified as possessing seed dormancy if <80% of viable seeds germinated. Each population represents a separate seed collection.

| Species         | Stratification | No. of populations sampled | Viability    | Populations without dormancy | Populations with dormancy |
|-----------------|----------------|---------------------------|--------------|------------------------------|----------------------------|
|                 |                |                           |              | No. of populations | Germination | No. of populations | Germination |
| A. asperula     | 7 days         | 1                         | 100%         | 1                            | 100%         | 0                    | –          |
| A. fascicularis | 0 day          | 8                         | 68–98%       | 7                            | 92–100%      | 1                    | 37%        |
| A. hirtella     | 0 day          | 1                         | 100%         | 0                            | –            | 1                    | 33%        |
| A. latifolia    | 0 day          | 1                         | 100%         | 1                            | 100%         | 0                    | –          |
| A. speciosa     | 0 day          | 9                         | 73–98%       | 3                            | 89–100%      | 6                    | 8–78%      |
|                 | 7 days         | 4                         | 65–95%       | 4                            | 93–100%      | 0                    | –          |
|                 | 14 days        | 1                         | 98%          | 1                            | 100%         | 0                    | –          |
| A. subulata     | 0 day          | 1                         | 100%         | 1                            | 100%         | 0                    | –          |
| A. subverticillata | 0 day     | 2                         | 92–96%       | 2                            | 100%         | 0                    | –          |
|                 | 14 days        | 1                         | 96%          | 1                            | 100%         | 0                    | –          |
| A. syriaca      | 0 day          | 8                         | 92–100%      | 0                            | –            | 8                    | 10–67%     |
| A. tuberosa     | 0 day          | 3                         | 96–100%      | 1                            | 88%          | 2                    | 50–72%     |
|                 | 7 days         | 1                         | 55%          | 1                            | 93%          | 0                    | –          |
|                 | 14 days        | 1                         | 98%          | 0                            | –            | 1                    | 50%        |
|                 | 21 days        | 3                         | 50–76%       | 3                            | 100%         | 0                    | –          |
Table 3. Duration of cold stratification needed to break dormancy in *Asclepias* species from published sources, with post stratification temperatures used or recommended for germination, where reported.

| Species                | Period of cold stratification needed to break dormancy | Germination temperature | References |
|------------------------|--------------------------------------------------------|--------------------------|------------|
| *Asclepias amplexicaulis* | 60 days                                                 | Heon and Larsen (1999)   |            |
| *Asclepias erosa*       | 0 day                                                  | Everett (2012)           |            |
| *Asclepias exaltata*    | 0–60 days                                              | Heon and Larsen (1999)   |            |
| *Asclepias floridiana*  | 120 days                                               | 18–21 °C                 | Green and Curtis (1950) |
| *Asclepias incarnata*   | 0–60 days                                              | 27 °C/16 °C              | Heon and Larsen (1999) |
|                        | 5 days                                                 | 18 °C                    | Lincoln (1983) |
|                        | 7 days                                                 |                          | Schultz et al. (2001a) |
|                        | 30 days                                                |                           | Vandevender and Lester (2014a) |
|                        | 90 days                                                |                           | Cullina (2000) |
| *Asclepias hirtella*    | 0–60 days                                              | Heon and Larsen (1999)   |            |
| *Asclepias meadii*      | 0 day                                                  | Bowles et al. (1998)     |            |
|                        | 70 days                                                | Betz (1989)              |            |
| *Asclepias ovalifolia*  | 120 days                                               | Green and Curtis (1950)   |            |
| *Asclepias perennis*    | 0 day                                                  | Edwards et al. (1994)    |            |
| *Asclepias purpurascens*| 0–60 days                                              | Heon and Larsen (1999)   |            |
| *Asclepias speciosa*    | 0 day                                                  | Bartow (2006)            |            |
|                        | 0 day                                                  | Skinner (2008)           |            |
| *Asclepias subulata*    | 0 day                                                  | Everett (2012)           |            |
| *Asclepias sullivantii* | 0–60 days                                              | Heon and Larsen (1999)   |            |
|                        | 90–120 days                                            | 21–27 °C/18–24 °C        | Blessman et al. (2002) |
|                        | 150 days                                               | 21–27 °C/18–24 °C        | Green and Curtis (1950) |
| *Asclepias syriaca*     | 0 day                                                  | 26 °C/21 °C              | Radijovic et al. (2016) |
|                        | 0–60 days                                              | Heon and Larsen (1999)   |            |
|                        | 7 days                                                 | 20 °C/30 °C              | Evetts and Burnside (1972) |
|                        | 7 days                                                 | 20 °C/30 °C              | Farmer et al. (1986) |
|                        | 14–21 days                                             | 30 °C/15 °C              | Baskin and Baskin (1977) |
|                        | 21 days                                                | 25 °C                    | Jeffery and Robison (1971) |
|                        | 30 days                                                | 18 °C                    | Schultz et al. (2001b) |
|                        | 30 days                                                | 20 °C/30 °C              | Lincoln (1976) |
|                        | 56 days                                                | 26 °C                    | Oegema and Fletcher (1972) |
|                        | 60 days                                                |                           | Green and Curtis (1950) |
| *Asclepias tuberosa*    | 0 day                                                  | Phillips (1985)          |            |
|                        | 0 day                                                  | Green and Curtis (1950)   |            |
|                        | 0–60 days                                              | Heon and Larsen (1999)   |            |
|                        | 21 days                                                | 30 °C/10 °C              | AOSA (2016) |
|                        | 30 days                                                |                           | Vandevender and Lester (2014b) |
|                        | 60 days                                                |                           | Bir (1986) |
|                        | 60–90 days                                             |                           | Grabowski (1996) |
|                        | 71 days                                                |                           | Nichols (1934) |

(Continued)
temperatures, after-ripening and other factors. Light may be required for germination in some Asclepias species, such as A. incarnata (Lincoln 1976; Deno 1993), or have no effect on others, including A. tuberosa (Mitchell 1926; Deno 1993). Light has little or no effect on germination of A. syriaca once adequate (≥2 weeks) cold stratification has been provided (Baskin and Baskin 1977; Lincoln 1983). Mechanical scarification of the seed coat improves germination in A. syriaca (Oegema and Fletcher 1972; Evetts and Burnside 1972). Smoke treatments that mimic wildfire smoke can improve germination of A. syriaca as well (Mojzes and Kalapos 2015), but burning and soil disturbance reduced seedling emergence in A. meadii (Roels 2013). Experiments with temperature and substrate have found that A. syriaca germinates best under alternating temperatures of 20 °C/30 °C on clay or clay mixed with peat (Farmer et al. 1986), or 21 °C/26 °C on sand or loam (Radivojevic et al. 2016). Temperatures that promote successful germination after cold stratification of Asclepias also vary considerably, and most reports recommend an alternating temperature regime. For example, a temperature cycle of 10 °C/30 °C is effective for A. perennis (Edwards et al. 1994) and the standard for seed testing in A. tuberosa (AOSA 2016). Alternating 16 °C/27 °C (Lincoln 1983) has been successfully used for A. incarnata, and cycles of 15 °C/30 °C (Baskin and Baskin 1977) and 20 °C/30 °C (Evetts and Burnside 1972; Lincoln 1976; Farmer et al. 1986) have been recommended for A. syriaca. Bhowmik found germination in A. syriaca increased with increasing temperature from 10 °C to 27 °C. We used alternating 15 °C/25 °C in our trials and this cycle was generally effective for germination of the species and populations we examined. In the case of the A. speciosa population from Malheur, OR, we also used a cycle of 20 °C/30 °C but found germination to be reduced by about 30 % compared to the 15 °C/25 °C cycle (data not shown).

After-ripening, a period of time after seed dispersal in which changes in the seeds affect their ability to germinate, can affect some species of Asclepias. For example, Baskin and Baskin (1977) suggest that with after-ripening A. syriaca seeds can germinate at lower temperatures and after shorter periods of cold stratification, and Bhowmik (1978) found that germination increased gradually with time in storage from 1 to 11 months. Seed dormancy in A. speciosa populations included in our experiments may have been affected by the amount of time seeds were in storage as well as local environmental conditions. We used fresh seeds that had been collected in the same year of the experiment and had been stored for ~3 months, as well as seeds that had been in dry, cold (<0 °C) storage for up to 6 years. Fresh seeds from six populations had >40 % dormancy, while seeds stored for 2, 5 and 6 years had <15 % dormancy. But seeds stored for 4 years from two populations had dormancy levels similar to fresh seeds, suggesting that dormancy can persist even in stored seeds and may involve other factors beyond storage. Further confounding the effects of storage time was that most of our fresh seeds came from latitudes north of 40° and all of the stored seeds came from farther south, and no seed dormancy was found in the southern populations; in other words, most non-dormant seeds had been stored and were from southern latitudes. Therefore, it is not possible for us to separate the effects of storage time from local environments, but we speculate that both seed storage and local conditions (Seglias et al. 2018) could influence seed dormancy in A. speciosa and possibly other Asclepias species.

Conclusions
Growers of Asclepias species should consider cold stratification to break seed dormancy when it is encountered. We found widespread evidence of seed dormancy in the genus through germination tests reported here as well as published and unpublished reports. Seed dormancy has been detected in at least some populations of 15 species in the genus, including A. amplexicaulis, A. exaltata, A. fascicularis, A. floridiana, A. incarnata,
A. hirtella, A. meadii, A. ovalifolia, A. purpurascens, A. speciosa, A. sullivantii, A. syriaca, A. tuberosa, A. verticillata and A. viridiflora (Table 3). Cold stratification is a relatively simple method to break this dormancy and increase seed germination substantially, and can shorten germination time (Salac and Hesse 1975) and even out differences in germination across populations (Milberg and Andersson 1998). For native plant specialists, commercial growers and managers who want to grow milkweed to support habitat restoration for monarch butterflies (Borders and Lee-Mader 2015) and pollinators, the use of cold stratification treatments may be integral to successful large-scale production. For most milkweed species and populations, some germination of seeds is likely to occur even in the absence of cold stratification, but incomplete germination could result in loss of genetic variability in plants or harvested seeds and reduced adaptive potential of restored populations in the long term (Basey et al. 2015). Variation in seed dormancy and cold stratification requirements among Asclepias species and populations suggests that growers should watch for low seedling emergence and use cold stratification as needed to maximize seed germination. Seed dormancy in Asclepias may be under the control of multiple factors, including local adaptation, maternal response to specific environments, as well as storage conditions, with implications for plant propagation and ecological interactions across this genus of flowering plants.

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Contributions by the Authors
The germination experiments were designed by T.N.K. and implemented by T.N.K., I.J.S. and M.A.B. T.N.K. conducted the data analysis. All authors contributed to the literature review and writing.

Conflict of Interest
None declared.

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Literature Cited
Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H, Koornneef M. 2003. Analysis of natural allelic variation at seed dormancy loci of Arabidopsis thaliana. Genetics 164:711–729.
Andersson L, Milberg P. 1998. Variation in seed dormancy among mather plants, populations and years of seed collection. Seed Science Research 8:29–38.
AOSA. 2016. Rules for testing seeds, Vol. 1. Principles and procedures. Washington, DC: Association of Official Seed Analysts.
Barner J. 2009. Propagation protocol for production of propagules (seeds, cuttings, poles, etc.) Asclepias cordifolia (Benth.) Jeps. seeds USDA FS – R6 Bend Seed Extractory Bend, Oregon. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).
Bartow AL 2006. Propagation protocol for production of Container (plug) Asclepias speciosa Torrey plants plugs; USDA NRCS - Corvallis Plant Materials Center Corvallis, Oregon. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).
Basey AC, Font JB, Kramer AT. 2015. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. Native Plants Journal 16:37–53.
Baskin JM, Baskin CC. 1977. Germination of common milkweed (Asclepias syriaca L) seeds. Bulletin of the Torrey Botanical Club 104:167–170.
Baskin CC, Baskin JM. 2001. Seeds: ecology, biogeography and evolution in dormancy and germination. San Diego, CA: Academic Press.
Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. Seed Science Research 14:1–16.
Bentsink L, Hanson J, Hanhart CJ, Blankestijn-de Vries H, Coltrane C, Keizer P, El-Lithy M, Alonso-Blanco C, de Andrés MT, Reymond M, van Eeuwijk F. 2010. Natural variation for seed dormancy in Arabidopsis is regulated by additive genetic and molecular
Johnson RG, Anderson RC. 1986. The seed bank of a tallgrass prairie in Illinois. The American Midland Naturalist 115:123–130.

Kaye TN. 1999. Propagation of endangered species: variable germination of pink sand verbena from Pacific Coast beaches. Combined Proceedings of the International Plant Propagators Society 43:617–621.

Keith DA, Myerscough PJ. 2016. Population variation in germination traits and its implications for responses to climate change in a fire-prone plant species complex. Plant Ecology 217:781–788.

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society of London B: Biological Sciences 274:303–313.

Landis TD. 2014. Monarch waystations: propagating native plants to create travel corridors for migrating monarch butterflies. Native Plants Journal 15:5–16.

Landis TD, Dumroese RK. 2014. Propagating native milkweeds for restoring monarch butterfly habitat. Proceedings of the 2014 Annual Meeting of the International Plant Propagators Society 1085:299–307.

Lincoln WC Jr. 1976. Germination of Asclepias syriaca, common milkweed. Newsletter of the Association of Official Seed Analysts 50:17–18.

Lincoln WC Jr. 1983. Laboratory germination methods of some native herbaceous plant species – preliminary findings. Newsletter of the Association of Official Seed Analysts 57:29–31.

Luna T, Dumroese RK. 2013. Monarchs (Danaus plexippus) and milkweeds (Asclepias species): the current situation and methods for propagating milkweeds. Native Plants Journal 16:5–15.

Mabberley DJ. 1997. The plant-book, 2nd edn. Bath, UK: The Bath Press.

Malcolm SB, Brower LP. 1986. Selective oviposition by monarch butterflies (Danaus plexippus L.) in a mixed stand of Asclepias curassavica L. and A. incarnata L. in southern Florida. Journal of the Lepidopterists Society 40:255–263.

Milberg P, Andersson L. 1998. Does cold stratification level out differences in seed germinability between populations? Plant Ecology, 134:225–234.

Mitchell E. 1926. Germination of seeds of plants native to Dutchess County, New York. Botanical Gazette 81:108–112.

Mojzes A, Kalapos T. 2015. Plant-derived smoke enhances germination of the invasive common milkweed (Asclepias syriaca L.). Polish Journal of Ecology 63:280–285.

Nichols GE. 1934. The influence of exposure to winter temperatures upon seed germination in various native American plants. Ecology 15:364–373.

Oegema T, Fletcher RA. 1972. Factors that influence dormancy in milkweed seeds. Canadian Journal of Botany 50:713–718.

Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals? Oikos 120:321–326.

Penfield S, MacGregor DR. 2017. Effects of environmental variation during seed production on seed dormancy and germination. Journal of Experimental Botany 68:819–825.

Pleasants JM, Oberhauser KS. 2012. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity 6:135–144.

Phillips HR. 1985. Growing and propagating wild flowers. Chapel Hill, NC: University of North Carolina Press.

Postma FM, Ågren J. 2015. Maternal environment affects the genetic basis of seed dormancy in Arabidopsis thaliana. Molecular Ecology 24:785–797.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25:345–353.

Radivojevic L, Saric-Krsmanovic M, Umljilendic JG, Bozic D, Santric L. 2016. The impacts of temperature, soil type and soil herbicides on seed germination and early establishment of common milkweed (Asclepias syriaca L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 44:291–295.

Roels SM. 2013. Influence of seed characteristics and site conditions on establishment of the threatened prairie milkweed Asclepias meadii. The American Midland Naturalist 170:370–381.

Salac SS, Hesse MC. 1975. Effects and storage and germination conditions on the germination of four species of wild flowers. Journal of the American Society of Horticultural Science 100:359–361.

Schultz J, Beyer P, Williams J. 2001a. Propagation protocol for production of container Asclepias incarnata L. plants. Hiawatha National Forest, Marquette, MI. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).

Schultz J, Beyer P, Williams J. 2001b. Propagation protocol for production of container Asclepias syriaca L. plants. Hiawatha National Forest, Marquette, MI. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).

Seglas AE, Williams E, Bilge A, Kramer AT. 2018. Phylogeny and source climate impact seed dormancy and germination of restoration-relevant forb species. PLoS One 13:e0191931.

Seiber JN, Brower LP, Lee SM, McChesney MM, Cheung HT, Nelson CJ, Watson TR. 1986. Cardenolide connection between overwintering monarch butterflies from Mexico and their larval food plant, Asclepias syriaca. Journal of Chemical Ecology 12:1157–1170.

Siles L, Müller M, Cela J, Hernández I, Alegre L, Munné-Bosch S. 2017. Marked differences in seed dormancy in two populations of the Mediterranean shrub, Cistus albidus L. Plant Ecology & Diversity 10:231–240.

Skinner DM. 2008. Propagation protocol for production of Container (plug) Asclepias speciosa Torr. plants 10 cu. in. USDA NRCS - Pullman Plant Materials Center Pullman, WA. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).

Smith LM, Kadlec JA. 1983. Seed banks and their role during drawdown of a North American marsh. Journal of Applied Ecology 20:673–684.

Steffan-Dewenter I, Potts SG, Packer L. 2005. Pollinator diversity and crop pollination services are at risk. Trends in Ecology and Evolution 20:1–2.

Stevens M. 2000. Plant guide for showy milkweed (Asclepias speciosa). USDA-Natural Resources Conservation Service, National Plant Data Center. https://plants.usda.gov/plantguide/pdf/pg_assp.pdf (16 June 2016).

USDA, NRCS. 2017. The PLANTS database. National Plant Data Team, Greensboro, NC. http://plants.usda.gov (20 September 2017).
Vandevender J, Lester R. 2014a. Propagation protocol for production of Container (plug) Asclepias incarnata L. plants USDA NRCS - Appalachian Plant Materials Center Alderson, West Virginia. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).

Vandevender J, Lester R. 2014b. Propagation protocol for production of Container (plug) Asclepias tuberosa L. plants USDA NRCS - Appalachian Plant Materials Center Alderson, West Virginia. US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources. http://NativePlantNetwork.org (16 June 2016).

Williams SL. 2001. Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. Ecological Applications 11:1472–1488.

Williams PH, Osborne JL. 2009. Bumblebee vulnerability and conservation world-wide. Apidologie 40:367–387.

Yenish JP, Fry TA, Durgan BR, Wyse DL. 1996. Tillage effects on seed distribution and common milkweed (Asclepias syriaca) establishment. Weed Science 44:815–820.