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Non-deep Physiological Dormancy in Seeds of Two *Polygonella* Species with Horticultural Potential

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Abstract. **The perennial nature, prolific white to pink racemes, and attractive foliage and form of october flower [*Polygonella polygama* (Vent.) Engelm. & A. Gray] and sandhill wireweed [*Polygonella robusta* (Small) G.L. Nesom & V.M. Bates] suggest that these wildflowers could have significant ornamental and landscape potential if an effective propagation method could be developed. However, a paucity of seed biology information exists for these species. Two- to 4-month-old seeds of both species were tested for viability using triphenyltetrazolium chloride (TZ) before germination experiments. Initial viability of seed lots was 77.4% ± 6.3% and 53.5% ± 6.8% for october flower and sandhill wireweed, respectively. Initial germination tests showed that both species had the highest number of germinated seeds in cool temperatures (22/11 °C day/night), but a portion of the seed population remained dormant. Germination of both species at simulated seasonal temperatures indicated that seeds require a warm, moist period (warm stratification) before germination starts at cooler temperatures. Germination of both species also increased when gibberellic acid (GA_3) was applied at the highest rate of 1000 ppm. We conclude that seeds of both species exhibit non-deep physiological dormancy.**

Dormancy is a seed trait that prevents germination when conditions are favorable for germination yet the probability of seedling survival is low (Baskin and Baskin, 2001; Fenner and Thompson, 2005). Five kinds of dormancy are currently recognized in seeds. Physical dormancy results from water-impermeable seed coats or fruit coats. Seeds with embryos that are undifferentiated or underdeveloped at shedding and require time for further development before germination are considered morphologically dormant. Physiological dormancy is characterized by low growth potential of embryos or the inability of embryos to rupture covering structures such as testa, endosperm, perisperm, and/or pericarp. Combinations of morphological and physiological (i.e., morphophysiological dormancy) and physical and physiological (i.e., combinational dormancy) have been reported (Baskin and Baskin, 2004; Bradbeer, 1988). Dormancy is common in seeds of native wildflowers and may last anywhere from several weeks to many months. Often species possess more than one type of dormancy mechanism (Baskin and Baskin, 2004; Bradbeer, 1988). Although seed dormancy is important from an ecological perspective, it can hinder propagation and production of wild species with potential in horticultural markets. In the southeastern United States, a native wildflower industry is emerging (Pérez et al., 2009), but limited information exists on dormancy mechanisms or germination requirements of wildflowers.

October flower (*Polygonella polygama*) and sandhill wireweed (*Polygonella robusta*) are Florida native wildflowers in the family Polygonaceae that have limited commercial availability. Attempts to propagate these species through seeds have been met with limited success as a result of unpredictable collection times and unknown seed storage and germination requirements (N. Bissett, The Natives Nursery, personal communication). Haehle and Brookwell (1999) note the aesthetic appeal of the genus. Both species have attractive form and floral characteristics that differentiate them from other commercially available species, making them excellent options for consumers seeking unique native wildflowers. Currently, no information regarding the seed storage and germination requirements or dormancy mechanisms of these species has been published. The objective of this research was to enhance seed propagation efforts by determining if seed dormancy is present in october flower and sandhill wireweed and, if so, which kind(s) of dormancy exists.

Materials and Methods

Seed collection and storage. Achenes, referred to as seeds, of october flower were collected from a native population in Brooksville, FL, on 15 Feb. 2008. Sandhill wireweed seeds were collected on 10 Apr. 2008 in Hobe Sound, FL. After collection, the seeds were manually cleaned of excess floral parts and other debris with a screen and stored in sealed plastic bags under ambient laboratory conditions (22.8 °C, 27.5% relative humidity) for 2 to 4 months before undergoing testing.

Pre-germination viability assay. Seed viability was examined using a TZ staining test before germination experiments started. Viability testing procedures were adapted from Peters (2000) using protocols developed for other members of Polygoneaceae. Seeds were scarified at the distal end by nicking with a scalpel. Seeds were randomly assigned to one of four 25-seed replicates. Each replicate was placed in one of four beakers containing 5 mL of 1% TZ solution (pH 7) and all were incubated in the dark at 35 °C for 48 h. Seeds were bisected longitudinally and embryo staining patterns were examined under 10× magnification. Seeds without embryos and embryos that were unstained or black were considered non-viable. Embryos stained light or dark pink were considered viable.

Initial germination tests. Seeds of october flower (n = 400) and sandhill wireweed (n = 400) were surface-sterilized in a 1.2% sodium hypochlorite solution for 10 min and then triple-rinsed with distilled deionized water. After being placed on moistened blotter paper in petri dishes, seeds were exposed to one of four simulated Florida seasonal diurnal temperatures (22/11, 27/15, 29/19, or 33/24 °C) derived from monthly maximum and minimum temperatures collected by the National Climatic Data Center at sites across the state. Four 25-seed replicates were used for each treatment. Each replicate was exposed to a 12-h daily photoperiod (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$, cool white fluorescent light) with the warmer temperature occurring during the light period. An additional 400 seeds of each species, also surface-sterilized, were incubated in the dark by covering petri dishes with two layers of aluminum foil. Germination counts for seeds in light were taken weekly and only at Week 4 for dark-incubated seeds. After 4 weeks, any remaining non-germinated seeds were assayed for viability as described previously; seeds that molded were counted as non-viable.

Water uptake by intact and scarified seeds. Imbibition was measured for scarified and non-scarified seeds. Half of the pericarps

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of both species were nicked with a scalpel on the proximal end (scarified treatment), whereas the other half were left intact (non-scarified treatment). The initial dry mass (W_n) of four 25-seed replicates was measured gravimetrically. After initial mass determinations, seeds were placed into dishes containing two sheets of moistened blotter paper. After 0.25 h, seeds were removed from the dishes, lightly blotted with a paper towel, and fresh weight was determined. Seeds were returned to their respective dishes, repeating this measurement at 0.5, 0.75, 1 to 12, 24, and 48 h. Fresh weight increase was calculated using the formula $[(W_i - W_n)/ W_n] \times 100$, in which W_i was the mass of imbibed tissue.

Seed anatomy and morphology. Seeds of both species were excised from exocarps under a dissecting microscope using a scalpel and then nicked on the proximal and distal ends. Separated seeds (n = 10) were fixed, dehydrated, and then set into resin using procedures adapted from Bozzola and Russell (1999). Thick sections (≈680 nm) were taken and stained with toluidine blue. Tissues were examined under a light microscope at 40× magnification. Images were captured using a digital camera (Olympus BH2-RFCA, Tokyo, Japan). Images of longitudinal sections, obtained from light microscopy studies, were used to calculate embryo length-to-seed-length ratios. Images were uploaded into Adobe Photoshop (Adobe Systems Inc., San Jose, CA) and the lengths of embryos (n = 10) and their testa were calculated using the software's measurement feature.

Requirements for warm or cold stratification. A move-along experiment (Baskin and Baskin, 2003) adapted to seasonal temperatures in Florida was used to determine whether seeds required warm or cold stratification (Table 1). Four 25-seed replicates of both species were surface-sterilized and placed on blue blotter papers in petri dishes. The treatments consisted of four control chambers set to 22/11, 27/15, 29/19, or 33/24 °C and two move-along treatments that began with the summer temperature (33/24 °C) or the winter temperature (22/11 °C). Seeds in the move-along treatments spent 4 or 12 weeks at each temperature to mimic the seasonal changes (Table 1). Germination data were collected weekly for 1 year.

Gibberellic acid soak. Four 25-seed replicates of both species were placed on blue blotter papers moistened with GA_3 solutions of 0, 1, 10, 100, or 1000 mg·L^{-1} for 24 h. After imbibition, seeds were surface-sterilized with 20% bleach solution for 10 min. All seeds were then incubated at 22/11 °C with a 12-h photoperiod. Germination data were collected weekly for 4 weeks.

Data analysis. A randomized complete block design was used for all germination experiments. Seeds were recorded as germinated when the emerged radicle was at least 2 mm in length. Final percent germination was adjusted by removing contaminated seeds from the calculation. Mean germination time (MGT) was estimated as described by Pérez et al. (2009). Germination percentages were transformed using the arcsine of the square root when appropriate (Little and Hills, 1978). Non-transformed means are presented. Data were analyzed using the GLM procedures in SAS Version 9.1 (SAS Institute, Cary, NC). Post hoc mean separation was performed using Duncan's multiple range test at $\alpha = 0.05$.

Results

Pre-germination viability assay and initial germination tests. Staining with TZ indicated the initial seed viability of october flower and sandhill wireweed to be 77% ± 6.3% and 53% ± 6.8%, respectively (data not presented). All viable seeds of october flower and sandhill wireweed germinated to 100% when incubated at simulated winter temperatures (i.e., 22/11 °C); but as temperature increased, germination decreased (Table 2). Final germination at simulated summer temperatures (i.e., 33/24 °C) was less than 15% (Table 2). Germination percent at simulated winter temperatures was higher than percent germination at summer and late fall/early spring seasonal temperatures for october flower. No germination was observed at any simulated seasonal temperature for seeds of either species incubated in the dark (data not shown). However, according to TZ tests carried out after the 4 weeks, ≈5% and 12% of october flower and sandhill wireweed seeds, respectively, incubated in the dark remained viable. The mean germination time of seeds of october flower ranged from 7 to 14 d with faster germination occurring at 27/15 °C (Table 2). MGT of sandhill wireweed ranged between 7 and 10 d and was shorter when seeds were incubated at 33/24 °C (Table 2).

Water uptake by intact and scarified seeds. Fresh weight increased regardless of species and scarification treatments, indicating uptake of water (Fig. 1). Initially, scarified october flower seeds (Fig. 1A) displayed a more rapid increase in fresh weight; however, by the end of the 48 h, scarified seeds increased in fresh weight by 61.4% ± 3.8% compared with 74.2% ± 1.1% for non-scarified seeds. This difference in final fresh weight was statistically significant ($F_{1, 3} = 12.9$; $P = 0.04$). Sandhill wireweed displayed a similar pattern, in which scarified seeds initially had a greater fresh weight increase. In contrast to october flower, after the 48-h experimental period, the difference between final fresh weight percentage of scarified and non-scarified seeds of sandhill wireweed was not significant ($F_{1, 3} = 2.59$; $P = 0.21$).

Seed morphology. The average length of october flower embryos and seeds was 1.6 ± 0.1 mm and 1.7 ± 0.1 mm, respectively (data not presented). Embryos of sandhill wireweed averaged 1.5 ± 0.2 mm, and the seeds were 1.6 ± 0.2 mm (data not presented). Therefore, embryo:seed size ratios were 0.95 ± 0.01 and 0.94 ± 0.01 for october flower and sandhill wireweed, respectively. Moreover, fully developed embryos (i.e., cotyledons, embryonic axis, and radicle) were clearly distinguishable in longitudinal sections (Fig. 2).

Requirements for warm or cold stratification. After 1 year, seeds of october flower incubated in control chambers showed reduced germination compared with the move-along treatments (Fig. 3A–B). For both move-along treatments, a sharp increase in the slope of germination was visible when incubators were changed from warmer to cooler temperatures (illustrated in Figure 3 by downward-pointing arrows). The lowest germination percentages occurred for seeds incubated at 29/19 and 33/24 °C, in which germination reached 18.8% ± 7.9% and 0.0% ± 0.0%, respectively (Table 3). Mean germination time was shortest for seeds in the 22/11 °C treatment (115 ± 11.1 d) and longest for 27/15 °C (236 ± 12.9 d) (Table 3).

Total germination of sandhill wireweed seeds at summer and winter move-alongs reached 52.0% ± 9.9% and 56.0% ± 6.3%, respectively (Fig. 3D). Again, seeds incubated under both move-along treatments had an increase in percent germination when temperatures were moved from warmer to cooler temperatures. However, the increase in germination for sandhill wireweed was not as dramatic as was observed for october flower. In the control chambers, seeds performed the poorest when kept at a constant 33/24 °C,

Table 1. The move-along procedure (Baskin and Baskin, 2003) adapted to seasonal temperatures throughout Florida.[z]

Weeks at temp.	Move-along treatments		Control treatments			
12	22/11 °C Winter ↓	33/24 °C Summer↓	22/11 °C↓	27/15 °C ↓	29/19 °C ↓	33/24 °C ↓
4	27/15 °C Early Spring ↓	29/19 °C Early Fall↓	22/11 °C ↓	27/15 °C ↓	29/19 °C ↓	33/24 °C ↓
4	29/19 °C Late Spring ↓	27/15 °C Late Fall↓	22/11 °C ↓	27/15 °C ↓	29/19 °C ↓	33/24 °C ↓
12	33/24 °C Summer ↓	22/11 °C Winter↓	22/11 °C ↓	27/15 °C ↓	29/19 °C ↓	33/24 °C ↓
4	29/19 °C Early Fall ↓	27/15 °C Early Spring↓	22/11 °C ↓	27/15 °C ↓	29/19 °C ↓	33/24 °C ↓
4	27/15 °C Late Fall ↓	29/19 °C Late Spring ↓	22/11 °C ↓	27/15 °C ↓	29/19 °C ↓	33/24 °C ↓
12	22/11 °C Winter	33/24 °C Summer	22/11 °C	27/15 °C	29/19 °C	33/24 °C

[z]Seeds were incubated under simulated winter or summer conditions and then moved to the next simulated temperatures after the appropriate time had elapsed; or seeds were maintained at the same temperature regime for the duration of the experiment. The move-along procedure is used to determine the influence of seasonal temperature change on germination.

Table 2. Germination and viability of october flower and sandhill wireweed seeds.

Temperature (°C)	Germination ± SE (%)	Dormant ± SE (%)	Total viable ± SE (%)[z]	Final germination (%)[x]	MGT (d)[w]
October flower					
22/11	30.1 ± 5.3	0.0 ± 0.0	30.1 ± 5.3	98.6 a[y]	14.4 a
27/15	1.4 ± 1.4	20.0 ± 6.4	21.4 ± 6.3	6.3 b	7.0 a
29/19	17.2 ± 3.0	5.8 ± 5.8	23.0 ± 8.1	87.5 a	12.3 a
33/24	0.0 ± 0.0	33.5 ± 12.2	33.5 ± 12.2	0.0 b	—
Sandhill wireweed					
22/11	59.4 ± 17.6	0.0 ± 0.0	59.4 ± 17.6	100.0 a	9.3 a
27/15	55.9 ± 9.2	32.1 ± 5.7	88.0 ± 7.4	63.6 b	9.6 a
29/19	26.1 ± 11.5	17.1 ± 10.2	43.2 ± 20.1	73.3 ab	8.2 a
33/24	8.3 ± 8.3	22.2 ± 15.7	30.6 ± 17.8	15.0 c	7.0 a

[z]Total viability percentage was determined by adding the percentage of germinated seeds from the standard germination test and percentage of remaining non-germinated, dormant seeds as determined by triphenyltetrazolium chloride staining.
[y]Final germination percent represents the germination percentage adjusted for viability.
[x]Columns with the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test.
[w]Mean germination time.

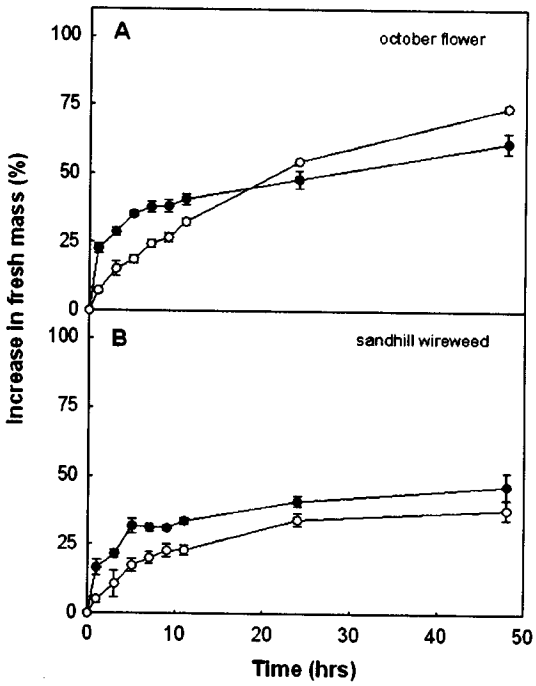

Fig. 1. Percentage increase in fresh weight of mechanically scarified (solid circles) or non-scarified (open circles) seeds of october flower (**A**) or sandhill wireweed (**B**) allowed to imbibe on germination blotter. Error bars represent SE of the mean.

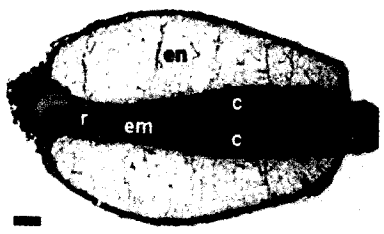

Fig. 2. Longitudinal section of october flower seed showing fully developed embryo (em) with cotyledons (c) and radicle (r) surrounded by the endosperm (en). Magnification = 20× with bar representing 0.1 mm.

reaching a final germination of less than 20% (Table 3). Germination rates were faster for seeds incubated at a constant 22/11 and 33/24 °C (less than 20 d) as compared with both move-along treatments (90 d) or incubation at 27/15 or 29/19 °C (Table 3).

Gibberellic acid soak. October flower seeds treated with 1000 mg·L^{-1} GA_3 reached a final germination of 32.5% ± 0.8%, which was nearly double that of the control (Fig. 4). The trend in germination was quadratic over the increasing GA_3 concentrations ($F_{2, 19} = 11.29$, $P = 0.001$; $y = 14.91 - 0.04x + 0.00006x^2$, $r^2 = 0.57$). Average germination rate ranged from 9 to 21 d with germination occurring fastest when treated with 10 mg·L^{-1}. There was no trend observed in MGT. Gibberellic acid concentrations of 100 and 1000 mg·L^{-1} had a positive effect on the germination percent of sandhill wireweed seeds (Fig. 4). The trend observed in these data were linear over increasing GA_3 concentrations ($F_{1, 19} = 4.74$, $P = 0.043$; $y = 41.05 + 0.02x$, $r^2 = 0.21$). Seeds that germinated did so in an average of 9 to 11 d with the fastest germination occurring at 100 mg·L^{-1}. However, no trend in MGT was observed.

Discussion

Physical dormancy does not occur in seeds of october flower or sandhill wireweed because both intact and scarified seeds imbibed water at similar rates and to similar final fresh weight percentages. Seeds with physical dormancy do not regularly imbibe water. For example Pérez et al. (2009) noted a sharp fresh weight increase reaching 142% over an 8-h period when seeds of summer farewell (*Dalea pinnata*, Fabaceae), a species with physical dormancy, were scarified compared with only a 12% increase when seeds were not scarified. Scarification with hot water of six physically dormant genera of Rhamnaceae also resulted in an increase in fresh weight reaching nearly 75%, whereas fresh weight of non-treated seeds was less than 16% over a 96-h period (Turner et al., 2007). Likewise, morphological dormancy is not present for either species. The embryo: seed size ratio indicated fully mature embryos that did not require additional time to develop within the seed before germination (Baskin and Baskin, 2004).

Despite the lack of physical and morphological dormancies, dormancy has long been known to occur in some taxa of Polygonaceae (Ransom, 1935). Viability and initial germination studies indicate that physiological dormancy is present in seeds of both species after harvest and subsequent storage. For example, over 20% (data not reported) of october flower seeds remained unstained during pre-germination viability testing, but these embryos appeared intact, which, in addition to suggesting nonviable seeds, may also point toward a portion of the seed population being dormant (Baskin and Baskin, 2004; Norcini et al., 2006; Peters, 2000). Furthermore, ≈6% to 34% of seeds that had not germinated at the conclusion of initial germination tests stained positively for viability. The occurrence of physiological dormancy is also supported by the increase in germination percent and/or rate after periods of warm stratification (summer in move-along) and exposure to GA_3 (Baskin and Baskin, 2001). Seeds of wild buckwheat (*Polygonum convolvulus*) collected from populations occurring in a continental climate region of the United States (USDA Zone 3) had a similar increase in germination percent and rate in response to stratification at low temperatures (10 °C or less) followed by incubation at 25 °C (Metzger, 1992). From an ecological perspective, the increase in germination with a decrease in

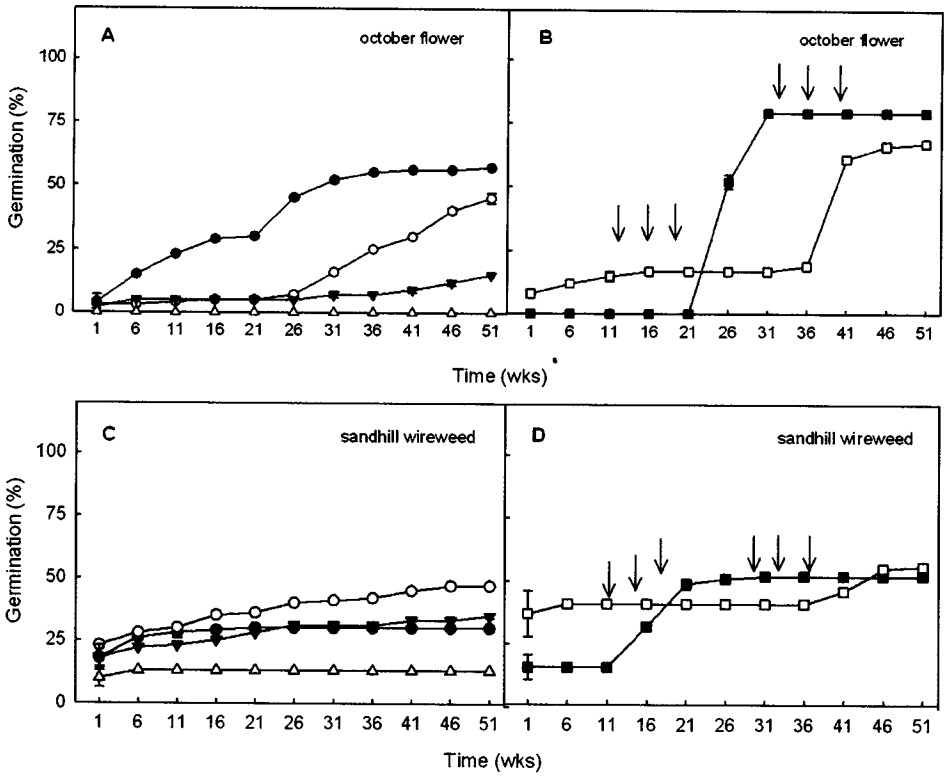

Fig. 3. Cumulative germination of october flower at (**A**) simulated seasonal temperatures of: 22/11 °C (winter; closed circle), 27/15 °C (late fall, early spring; open circle), 29/19 °C (early fall, late spring; closed down triangle), or 33/24 °C (summer; open up triangle). Seeds remained at these temperatures for 1 year. (**B**) Germination of october flower under move-along treatments beginning at summer (closed square) or winter (open square) temperatures. Germination of sandhill wireweed at (**C**) simulated seasonal temperatures and (**D**) move-along treatments. Arrows indicate when temperatures were adjusted for the move-along treatments.

Table 3. Germination of october flower and sandhill wireweed seeds under the move-along experiment.[z]

Treatment	Final germination (%)[y]	MGT (d)[x]
October flower		
22/11 °C	62.1 b[w]	114.9 b
27/15 °C	52.3 b	236.2 a
29/19 °C	18.8 c	235.5 a
33/24 °C	0.0 d	—
Summer move-along	86.0 a	179.9 a
Winter move-along	80.8 a	213.1 a
Sandhill wireweed		
22/11 °C	41.3 bc	18.8 b
27/15 °C	68.6 ab	74.0 a
29/19 °C	50.4 abc	74.0 a
33/24 °C	19.9 c	9.3 b
Summer move-along	66.5 ab	89.8 a
Winter move-along	73.1 a	90.0 a

[z]Seeds were incubated under simulated winter or summer conditions and then moved to the next simulated temperatures after the appropriate time had elapsed; or seeds were maintained at the same temperature regime for the duration of the experiment.
[y]Final germination percent represents the germination percentage adjusted for viability.
[x]Mean germination time.
[w]Columns with the same letters are not significantly different at $\propto = 0.05$ level according to Duncan's multiple range test.

temperature indicates a requirement for a period of warmth followed by relatively cool temperatures for maximum germination in the field. This type of environmental shift in temperatures occurs during the succession of summer to fall to winter in Florida.

The issue that persists for this study is that seeds were stored in the laboratory for 2 to 4 months before experimentation. Harvested seeds of october flower and sandhill wireweed are routinely stored within paper bags in non-climate-controlled sheds before propagation (A. Heather, personal observation). Although other members of Polygonaceae have had substantial success in maintaining viability during storage under dry conditions at room temperature (Forman and Kesseli, 2003), the effects of controlled or uncontrolled storage conditions on the viability and germination of october flower and sandhill wireweed are unknown. Seeds of both species may have been initially more dormant as observed with most species possessing non-deep physiological dormancy (Baskin and Baskin, 2004). Seeds with non-deep physiological dormancy cannot germinate or will only germinate at a small range of temperatures (Baskin and Baskin, 2004). Fewer seeds would be able to germinate initially over a range of simulated seasonal temperatures, but conditions for germination become broader as dormancy is alleviated (Baskin and Baskin, 2004). The effect of air-dry storage, usually at ambient room temperatures, has been reported to alleviate dormancy and promote germination through after-ripening in many flowering genera, including *Achyranthes*, *Cerastium*, *Hymenoxys*, and *Striga* (Baskin and Baskin, 2001; Bewley and Black, 1994). As after-ripening proceeds, seeds become more germinable over a wider range of conditions (Bewley and Black, 1994) and display greater sensitivity to chemical treatments such as GA_3 (Foley, 2001). The seeds in this study displayed a capacity to germinate over a wide range of temperatures and some sensitivity to exogenous GA_3, although a proportion of the seed population remained dormant. This implies a possible after-ripening effect that may be driving stored seeds to non-dormancy in this study. Seeds with non-deep physiological dormancy are the most responsive to after-ripening treatments (Baskin and Baskin, 2001, 2004).

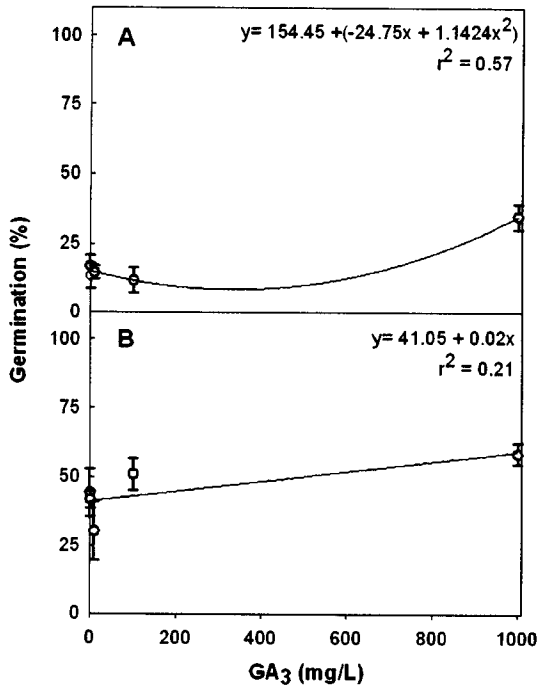

Fig. 4. Final germination percent for (**A**) october flower and (**B**) sandhill wireweed seeds after soaking for 24 h in varying concentrations of gibberellic acid and subsequent incubation at the constant 22/11 °C for 28 d.

We conclude that at least some seeds of october flower and sandhill wireweed possess non-deep physiological dormancy, the level of which may be affected by subsequent storage after harvest. Rather than expending resources exploring other dormancy-breaking techniques to enhance germination, it is suggested that methods to promote germination in these species should focus on refining treatments such as warm stratification and after-ripening. Application of GA_3 also showed improved germination, although an increase in the range of concentrations should be tested. We believe a more robust production plan can then be created for seed producers and propagators when these methods are coupled with investigations dealing with the effects of storage temperature, relative humidity and duration on viability, dormancy, and germination of mature october flower and sandhill wireweed seeds.

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