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Stratification Improves and Is Likely Required for Germination of *Aconitum sinomontanum*

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SUMMARY. ***Aconitum sinomontanum*** **is a robust perennial monkshood native to China that shows promise as a cultivated ornamental. However, nothing has been reported about the germination requirements of the species, and little is known about the requirements of the genus as a whole. The objective of this study was to test the influence of stratification (moist prechilling) on germination of *A. sinomontanum* seeds. The seeds were from wild-collected plants of identical provenance growing at the Arnold Arboretum (Jamaica Plain, Mass.). After harvest and before stratification, seeds were stored dry at 38 °F (3.3 °C) and percentage germination was assessed after seeds were stratified, also at 38 °F, for 0, 21, 42, or 84 days. It is likely that stratification is required for seeds of this species to germinate, as unstratified seeds failed to germinate through the duration of the experiment (73 days). The highest level of germination (90.8%) was achieved after 84 days of stratification, and as length of stratification increased, so did percentage germination and indices of peak value and germination value. Days to maximum germination decreased with additional days of chilling. Growers wishing to germinate seed of this species should stratify seed for 3 months to achieve the highest level of germination.**

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Herbaceous perennial plants have increased in popularity and use in the ornamental landscape, particularly in the past 2 decades. Coincident with the surge of interest, many promising, new herbaceous species have been released, some successfully brought into cultivation from the wild for the first time, such as several clones of the blue corydalis (*Corydalis flexuosa*) from Sichuan, China (Rix, 1993).

Species of monkshood (*Aconitum* spp.) are valued in the horticultural trade for their late summer and autumn dark blue to purple floral displays and finely textured, divided leaves reminiscent of delphinium (*Delphinium*) also in the Ranunculaceae. While the genus contains about 100 species of annual, biennial and perennial herbaceous plants, only a few are found in cultivation. Native to Europe, the common monkshood (*A. napellus*) may be the most frequently grown of the species, having the general attributes and characteristics of the genus and reaching about 4 ft (1.2 m) in height. Others encountered include the asian azure monkshood (*A. carmichaelii*), the european yellow wolfsbane (*A. lamarckii*) and the hybrid bicolor monkshood (*A.* ×*cammarum*).

In September 1994, during a North America China Plant Exploration Consortium (NACPEC) expedition, several plants of *A. sinomontanum* were found on Wudang Shan, Hubei Province (lat. 32°23'43" N, long.111°00'13" E), at an altitude of 4272 ft (1302 m), growing among wild english or persian walnut (*Juglans regia*), chinese chestnut (*Castanea mollissima*), and *Lindera obtusiloba*. As the fruit had not yet matured, several young plants from the same population were collected under the number WD 067. Although this species was described in 1935 (Nakai, 1935), this collection likely represents its only introduction to cultivation.

Some of these original plants (394-95 MASS) are growing at the Arnold Arboretum of Harvard University. The species shows potential for introduction as a cultivated ornamental, particularly after further evaluation and selection. While plants on Wudang Shan were observed to be only 3.3 ft (1 m) in height, plants under cultivation at the Arnold Arboretum ultimately produce erect stems up to 7.2 ft (2.2

Table 1. Percentage germination (PG), days to complete germination (DG), and log-transformed indices of peak value (PV+1) and germination value (GV+1) of *Aconitum sinomontanum* seeds germinated after 0, 21, 42, or 84 d of stratification (moist prechilling) at 38 °F (3.3 °C). Each value is the mean of 16 replications.

Stratification period (d)	Dependent variable			
	PG (%)	DG (d)	log (PV[z]+1)	log (GV[y]+1)
0	0.0	---	---	---
21	41.5	20.9	0.506	0.73
42	72.8	14.4	0.891	1.55
84	90.8	11.0	1.863	2.80
Significance[x]	0.0001	0.0001	0.0001	0.0001

[z]Peak value = the maximum ratio of cumulative germination percentage and the number of days from the beginning of the germination test.
[y]Germination value = the product of the peak value and the mean daily germination percentage.
[x]Levels of significance for observations from analysis of variance.

m). Because of their size, the cultivated plants should be provided with support, just as the plants in the wild are supported by neighboring shrubs and herbs. The finely textured, dark green vegetation provides ornamental interest through spring and summer. Anthesis begins in late August or early September and floral development proceeds acropetally through November. Flowers are deep blue to purple and are produced in multiple racemes up to 7.9 inches (20 cm) in length.

Recommendations for propagation of this species are unknown, although propagation of other species of monkshood is generally through division of roots. Germination of monkshood seed is reported to be difficult (Still, 1994). Armitage (1997) attributes this difficulty to the onset of deep dormancy during ripening, and he recommended sowing seed immediately after collection. Stratification (moist prechilling) at 41 °F (5 °C) or lower for 6 weeks has been recommended to break such dormancy (Hartmann et al., 1997). In other cases, germination of several Himalayan species of monkshood (*A. heterophyllum* and *A. balfourii*) was improved or stimulated after treatment with gibberellic acid (GA_3) (Pandey et al., 2000; Prasad, 1999; Singh et al., 2000). The objective of this study was to determine the response of germination to stratification in *A. sinomontanum*.

Materials and methods

Seed was collected from a uniform, single-accession (394-95 MASS) planting of three *A. sinomontanum* plants of growing at the Arnold Arboretum. After flowering, follicles develop rapidly to dehisce and expel seeds, and the indeterminate shoots bear both ripened fruit and flowers at the same time. Therefore, to obtain sufficient mature seeds for the study, collections were made (from mature, basal-most follicles) on four dates in 2000: 11 Oct., 27 Oct., 7 Nov., and 28 Nov. After seeds were shaken loose of the follicles, they were placed in sealed containers and stored in a refrigerator maintained at 38 °F.

On 28 Nov. 2000, 64 individual plastic containers [3.5 × 3.5 × 3.25 inches (8.9 × 8.9 × 8.3 cm)], with drainage holes, were filled with substrate [Fafard Germination Mix (super fine), Agawam, Mass.], saturated with tap water, and placed on a greenhouse bench to dry overnight. On 29 Nov., 25 seeds were sown on the surface of each container. A thin layer of about 0.12 in (about 3 mm) of milled Sphagnum peat moss was placed over the surface of each container, and tap water was gently applied overhead to wet the peat moss and seeds. There were four stratification treatments: 0, 21, 42 and 84 d. Units in the unstratified treatment were placed directly on a greenhouse bench that received supplemental light using 100-W incandescent bulbs to extend the daily photoperiod to 12 h. The remaining units were sealed in plastic bags (to prevent desiccation) and placed in a walk-in refrigerator set at 38 °F. At the completion of each stratification treatment, the appropriate units were removed from the refrigerator and randomly placed on the same greenhouse bench. Water was supplied daily by overhead irrigation with tap water. Ambient temperatures in the greenhouse ranged from 63 to 92 °F (17.2 to 33.3 °C), and the mean temperature was 74 ± 5.5 °F (23.3 ± 3.1 °C).

Percentage germination (PG), defined as the percentage of seeds that showed emergence of either a radicle or hypocotyl, was recorded every 3 to 5 d for each unit (container). At the conclusion of the experiment (9 Feb. 2001) final PG and number of days until maximum germination was determined for each container. Peak value (PV) and germination value (GV) for each container were also calculated after Czabator (1962). For each day of recording, cumulative PG is divided by the number of days of germination. The maximum value obtained during the course of the experiment is selected as the PV, and is an index of germination vigor. The mean daily germination (MDG) is an expression of vigor over time. It is the average number of seeds germinated per day over the test period (ending on the last day of germination). The germination value combines these two values (GV = PV×MDG) into a single index. Both PV and GV are useful in assessing treatment effects because multiple components of germination can be characterized at once (Couvillon, 2002; Dosmann et al., 2000). Before analysis and interpretation, PV and GV were transformed on a log scale, but because some values of PV and GV were zero, it was necessary to add the value of 1 to each indexed value before log transformation. Thus, the values represent log (PV+1) and log (GV+1). Analysis of variance (ANOVA), based on a completely randomized design, was performed on the data, and linear and nonlinear regression were fitted as appropriate.

Results and discussion

There was a significant effect of stratification treatment for all dependent variables (Table 1). None of the seeds in any of the unstratified containers germinated, despite observation for 73 d. While it is likely that periods of stratification less than 21 d would be sufficient to fulfill some of the seeds' dormancy requirements, longer periods improved both PG and time to germination. Extending the duration of chilling from 21 d to 84 d increased germination from 41.5% to 90.8%, and also decreased the number of days to maximum germination from 20.9 d to 11.0 d. Stratification period

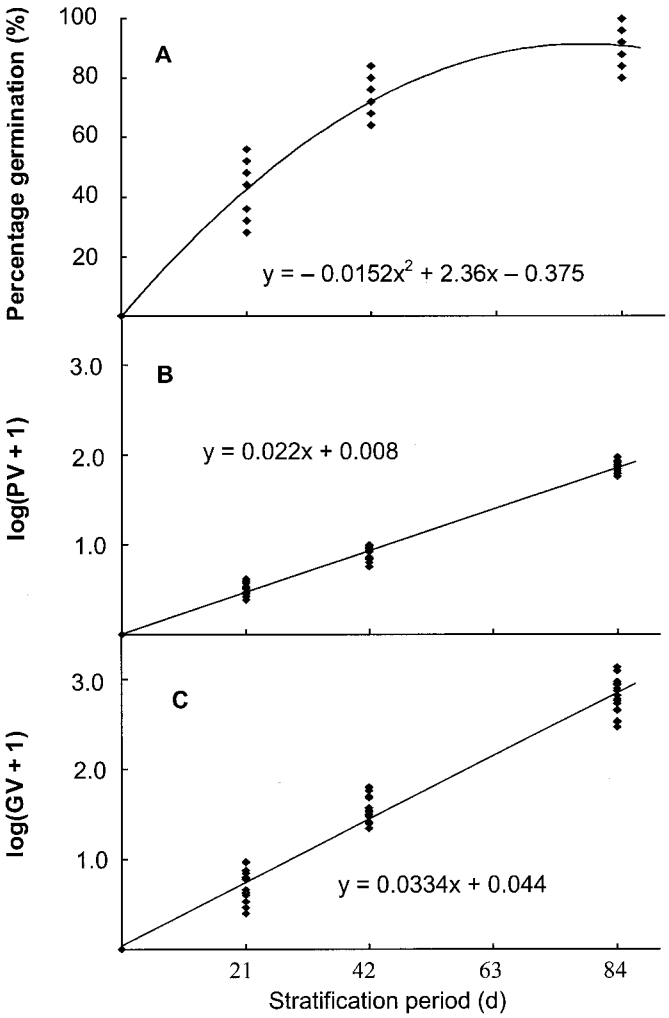

Fig. 1. Influence of 0, 21, 42, or 84 d stratification (moist prechilling) at 38°F (3.3°C) on (A) percentage germination, (B) log-transformed peak value [log(PV+1)] and (C) log-transformed germination value [log(GV+1)] of germinated *Aconitum sinomontanum* seeds. Peak value is the maximum ratio of cumulative germination percentage and the number of days from the beginning of the germination test. Germination value is the product of the peak value and the mean daily germination percentage. Stratification was significant at $P \leq 0.001$ for final germination percentage ($R^2 = 0.963$), log(PV+1) ($R^2 = 0.990$), and log(GV+1) ($R^2 = 0.976$). Each point represents a single value.

and the log-transformed PV and GV indices were clearly characterized by a linear relationship with length of stratification, the longest stratification periods having the greatest values (Fig. 1). However, longer periods of stratification are unlikely to significantly increase PG (Fig. 1) and they are unlikely to decrease days to maximum germination much further. Thus, periods of stratification longer than those tested here seem unnecessary for most commercial or research propagation systems, as obtaining over 90% germination in less than 2 weeks may be adequate.

According to Baskin and Baskin (1998), seeds of Ranunculaceae have rudimentary embryos and morphophysiological dormancy (MPD). Seeds with intermediate complex MPD require only cold stratification to remove dormancy, although gibberellic acid (GA) can substitute for the chilling requirement. Cold stratification also removes dormancy in seeds with deep complex MPD, however the application of GA has no effect. Based upon the results of this study, seeds of *A. sinomontanum* can be classified as having either intermediate or deep complex MPD; the exact classification would depend on their response to GA. Because seed dormancy in other species of monkshood can be removed with GA (Pandey et al., 2000; Prasad, 1999; Singh et al., 2000), and thus classified as having intermediate complex MPD, further research may show that *A. sinomontanum* has the same type of dormancy.

Growers and researchers wishing to propagate this species by seed can do so with best results obtained after 84 d (3 months) of stratification (moist chilling). Though useful, there are some limits to the interpretation of these data. *Aconitum sinomontanum* is not in cultivation and other provenances of this species were not available for this study. Because these results are based a single population which is assumed to be genetically uniform, seeds of different provenances may respond differently.

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