Effect of Gibberellic Acid *and* Standard Seed Treatments *on* Mountain Snowberry Germination

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ABSTRACT

Acid scarification, warm stratification, cold stratification, and soaks in gibberellic acid (GA_3) were effective in promoting germination in mountain snowberry (*Symphoricarpos oreophilus* Gray [Caprifoliaceae]) from New Mexico, but treatment levels and interactions were important. The combination of a 30-min acid soak, 21-d warm stratification treatment, and 84-d cold stratification treatment (the shortest duration evaluated) was highly effective in promoting germination. Increasing cold stratification from 84 to 168 d increased germination, as did incubation in all concentrations (250 to 1000 ppm) of GA_3, but the benefit of longer cold stratification and GA_3 incubation was reduced for acid-scarified seeds. Acid scarification breaks physiological dormancy of the embryo and may allow maturation of the embryo during cold stratification to begin sooner. Timing of GA_3 application was also important. For seeds undergoing acid scarification followed by warm stratification followed by cold stratification, application of GA_3 prior to warm stratification resulted in less germination compared to application following warm stratification. In snowberry, early GA_3 application may result in GA_3 catabolism during warm stratification, reducing the concentration available during cold stratification.

KEY WORDS: acid scarification, warm stratification, cold stratification, dormancy, GA_3

NOMENCLATURE: ITIS (2001)

Mountain snowberry (*Symphoricarpos oreophilus* Gray [Caprifoliaceae]) is an upright shrub occurring in numerous communities in montane regions of western North America (McMurray 1986). In New Mexico, mountain snowberry is found in ponderosa pine (*Pinus ponderosa* P. & C. Lawson [Pinaceae]) and mixed coniferous forests. This species occurs on open slopes and in the understory, occupies sites ranging from moist to dry, and grows on a wide range of soil pHs—characteristics that allow it to establish on disturbed sites (McMurray 1986). Mountain snowberry is a useful reclamation species, but propagation from seeds (technically, drupelets) is difficult.

A combination of mechanical, physiological, and morphological (embryo immaturity) mechanisms regulate snowberry seed dormancy. Initially, embryos are immature, requiring development that takes place during cold stratification (Flemion 1934). Restrictive seed coat fibers prevent embryo expansion and must be degraded before germination can occur (Flemion 1934; Pfeiffer 1934). In addition, maturation during cold stratification does not occur unless seed coats have previously been degraded by treatments such as acid scarification or prolonged (up to 70 d) warm stratification (Flemion 1934; Pfeiffer 1934). This requirement suggests that the seed coat exerts physiological control over the embryo, perhaps through growth-inhibiting substances.

Acid scarification, warm stratification, and cold stratification have been used successfully to overcome

TABLE 1

Mountain snowberry seed sources from New Mexico used in germination studies

Seed source	Latitude	Location	Elevation m (ft)	Collection date (1997)
Capulin	N 36° 42'	Questa	2987 (9800)	4 and 24 Sep
Cabin	N 36° 42'	Questa	2408 (7900)	2 Sep
Holman	N 36° 02'	Holman	2377 (7800)	7 Oct
Rociada	N 35° 50'	Rociada	2377 (7800)	17 Oct

dormancy in mountain snowberry and related species (Flemion 1934; Flemion and Parker 1942; Evans 1974; Rosner and others 2001). However, published literature regarding the use of gibberellic acid (GA) to promote germination of any snowberry species is lacking. Gibberellic acid has been found to substitute for both warm stratification and cold stratification requirements in numerous species (Baskin and Baskin 1998). This study was carried out to examine the relative effectiveness of acid scarification, warm stratification, cold stratification, and GA_3 treatments on mountain snowberry germination. In addition the importance of timing of GA_3 application was tested.

MATERIALS AND METHODS

We conducted 2 experiments. The first experiment tested combinations of acid scarification, GA_3, warm stratification, and cold stratification treatments applied in that order. The second experiment evaluated the timing of GA_3 incubation relative to the other treatments. For both experiments, seeds were collected from September through October 1997 at 4 locations (sources) in New Mexico (Table 1). Sources represented 2 locations at Molycorp Mine in Questa, New Mexico, and 2 locations in the Sangre de Cristo Mountains of northern New Mexico. Seeds were collected from 6+ plants at varying plant heights at each source. Fruits were soaked overnight in tap water, fermented for 48 h, mashed, and dried to facilitate cleaning. Seeds were dislodged from pulp in a rubbing box, separated from pulp in a South Dakota blower (Seedburo, Chicago, Illinois), and then stored at 5 °C (41°F) in paper envelopes for 1 to 2 y prior to testing.

Experiment 1

We used a completely randomized design with a factorial treatment structure. Factors were acid scarification (0 or 30 min), GA_3 incubation concentration (0, 250, 500, or 1000 parts per million [ppm]), warm stratification (0 or 21 d), and cold stratification (84 or 168 d). Each treatment combination was tested with four 100-seed replications.

Concentrated sulfuric acid (Reagent ACS, 95% to 98%) was used to impose acid scarification treatments. Each 100-seed sample was added to 10 ml acid in a 50-ml beaker and stirred vigorously for 30 s. Seeds then soaked undisturbed for the treatment duration. Following treatment, seeds were rinsed under running tap water for 1 min. Seeds underwent warm stratification mixed with moistened peat moss within self-sealing poly bags. Poly bags containing seeds and peat moss were stored in boxes in a laboratory, where air temperatures ranged from 21 to 24 °C

TABLE 2

Categorical analysis of variance table for mountain snowberry germination

Source of variability	df	Chi-square	Observed significance
Acid (A) [a]	1	299.6	< 0.001
Warm (W) [b]	1	246.1	< 0.001
Stratification (S) [c]	1	156.3	< 0.001
GA (G) [d]	3	71.0	< 0.001
A X W	1	11.0	< 0.001
A X S	1	51.5	< 0.001
A X G	3	20.3	< 0.001
W X S	1	12.5	< 0.001
W X G	3	5.4	0.15
S X G	3	10.9	0.01
A X W X S	1	2.4	0.12
A X W X G	3	3.6	0.31
A X S X G	3	5.8	0.12
W X S X G	3	2.1	0.56
A X W X S X G	3	0.6	0.9

[a] Acid = acid scarification
[b] Warm = warm stratification
[c] Stratification = cold stratification
[d] GA = gibberellic acid incubation

(69 to 75 °F). Gibberellic acid treatments involved submersing the seeds in 20 ml of the appropriate GA_3 solution (GA_3 90+%, Aldrich Chemical Company, Milwaukee, Wisconsin) for 24 h at room temperature. Seeds were cold stratified mixed with moistened peat moss in poly bags. Cold stratification temperatures fluctuated from an average daily low of –1 °C (29 °F) to an average daily high of 6 °C (41 °F).

Initially, seeds completing cold stratification were maintained within moistened peat moss in petri dishes for germination testing. Seedling emergence was to be the response variable. However, few seedlings emerged due to the fact that considerable germination occurred during cold stratification, and these germinants were weakened by pathogens and etiolation. The germination testing procedure was then changed, and samples tested prior to this change were dropped from the experiment. One replication of seeds undergoing 84 d of cold stratification in combination with all other treatments was dropped, and 2 additional replications of seeds undergoing 84 d of cold stratification and 0 d of warm stratification in combination and with all other treatments were dropped as well.

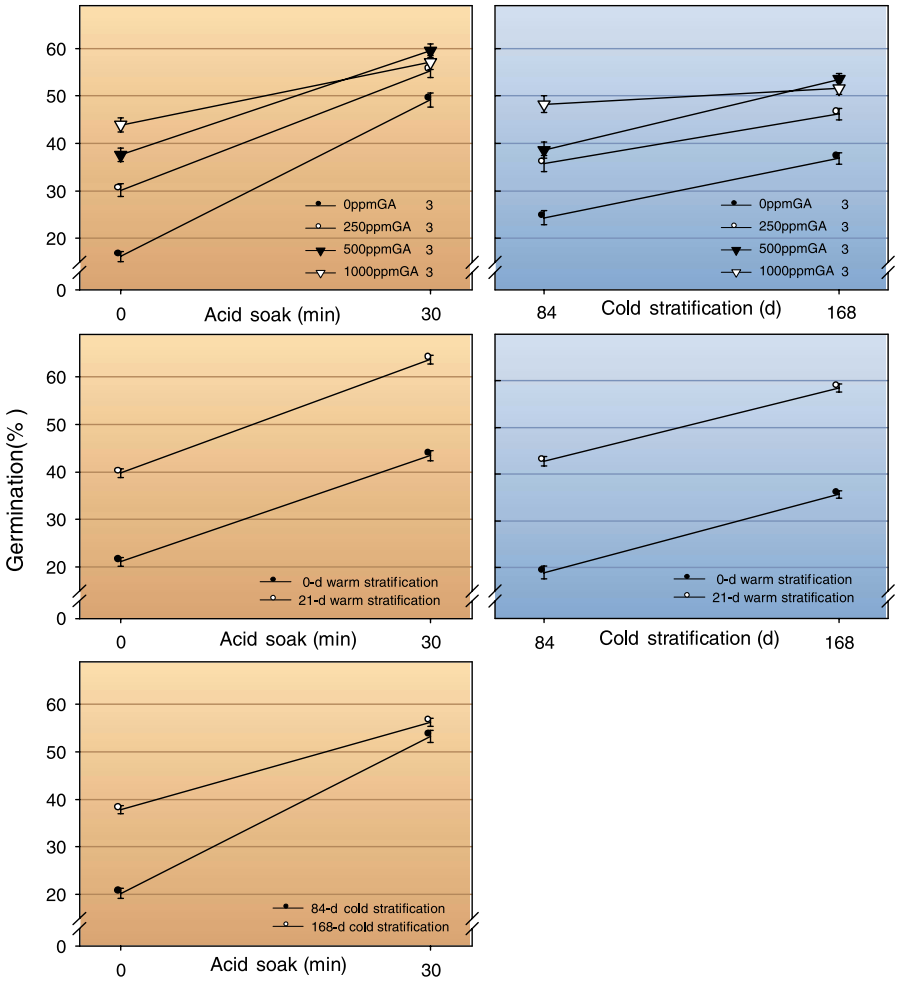

Figure 1 • *Significant (= 0.05) 2-factor interactions affecting mountain snowberry germination*

For remaining seed samples, a revised germination testing procedure was used. Seeds were separated from peat moss, and germinated seeds were counted. Germinated seeds were defined as having their radicle protruding through the seed coat. Non-germinated seeds were rinsed under running tap water for 30 s and placed between two #1 grade qualitative filter papers (Whatman, Clifton, New Jersey) in a 150 mm (5.9 in) petri dish. Filter papers were moistened with distilled water. The petri dishes were placed in 15 X 16 cm (5.9 X 6.3 in) self-sealing poly bags and placed on FloraCart (Grower's Supply Company, Dexter, Michigan) plant stands located in the laboratory. Two 40-watt Sylvania Grow Lux fluorescent bulbs were suspended 30 cm (11.8 in) over the petri dishes. The light cycle was a 10-h light period followed by a 14-h dark period. Air temperature immediately above the petri dishes ranged from an average daily low of 21 °C (69 °F) to an average daily high of 30 °C (85 °F). Germination was monitored after 7, 14, 21, and 28 days.

Experiment 2

The second experiment utilized a completely randomized design with a factorial arrangement of seed source (4) and GA_3 timing treatments (4) described below. Each treatment/source combination was replicated with four 100-seed samples. All seeds underwent acid scarification followed by warm stratification followed by cold stratification. Interposed among these treatments, seeds underwent incubation

TABLE 3

Main effects of acid scarification, warm stratification, cold stratification, and gibberellic acid incubation on mountain snowberry germination

Treatment level	Germination [a] (%)	Standard error	Improvement relative to control (%)
Acid scarification (min)			
0	32.0 b	0.7	
30	55.3 a	0.7	73
Warm stratification (d)			
0	32.3 b	0.7	
21	51.7 a	0.7	60
Cold stratification (d)			
84	36.8 b	0.8	
168	47.0 a	0.6	28
Gibberellic acid (ppm)			
0	32.7 c	1	
250	42.7 b	1	31
500	48.5 a	1	48
1000	50.5 a	1	54

[a] Germination percentages within treatments followed by the same letter are not significantly different at $\alpha = 0.05$ for acid scarification, warm stratification, and cold stratification, or $\alpha = 0.083$ ($\alpha = 0.05/6$) for gibberellic acid treatments.

in GA_3. Acid scarification (30 min), warm stratification (21 d), and cold stratification (168 d) treatment levels were standard for all seeds.

Acid scarification was imposed by the methods described above. Warm stratification treatment involved placing seed samples into 2 X 10 cm (0.8 X 3.9 in) synthetic screen pouches, which were inserted into moist peat moss within self-sealing poly bags. Seeds were placed in a single layer in the pouch, and the pouch was placed into the peat moss ensuring adequate seed–peat moss contact. Samples were maintained at room temperature, which fluctuated between mean daily lows of 22 +/- 0.1 °C (71 +/- 0.1 °F) and mean daily highs of 23 +/- 0.1°C (74 +/- 0.1 °F), for 21 d. Finally, seeds (still within screen pouches in peat moss) underwent 168 d of cold stratification in a walk-in cooler. Cooler temperatures fluctuated from an average daily low of -1 +/- 0.1 °C (30 +/- 0.1 °F) to an average daily high of 5 +/- 0.1 °C (42 +/- 0.1 °F). Gibberellic acid treatments involved submersing seeds in 20 ml of 500 parts per million (ppm GA_3 solution) for 24 h at room temperature. The 4 GA_3 timing treatments were: 1) acid scarification/ GA_3 incubation/ warm stratification/ cold stratification; 2) acid scarification/ warm stratification/ GA_3 incubation/ cold stratification; 3) acid scarification/ warm stratification/ 28-d cold stratification/ GA_3 incubation/ 140-d cold stratification; and 4) acid scarification/ warm stratification/ 56-d cold stratification/ 24-h GA_3 incubation/ 112-d cold stratification.

Following completion of treatments, germinated seeds were counted and non-germinated seeds were incubated to test for further germination. The filter paper method of germination testing described above was used and the conditions were the same.

Statistical Analysis

For both experiments, categorical analysis (SAS PROC CATMOD; SAS Institute 1989) was used to determine treatment differences in germination using factorial treatment structures. In addition, data was analyzed separately by seed source for the second experiment. Categorical analysis is a generalization of the chi-square (x^2) test of homogeneity, which uses the "logit"—the natural log of the ratio of germinated to non-germinated seed for each treatment—as the response. There is no need to transform germination data using this analysis. Due to some low cell counts, the generalized least square approach was used to calculate x^2 test statistics for the first experiment, whereas maximum-likelihood analysis was used in the second experiment. Observed significance levels less than α=0.05 were considered significant. Percentages and standard errors were calculated for main effects and interaction combinations. Approximate pairwise Z statistics were used to conduct comparisons of main effects having more than 2 treatment levels. A conservative alpha value of 0.05 divided by the number of comparisons was chosen (equivalent to Bonferroni multiple comparison method).

RESULTS

Experiment 1

Acid scarification, GA_3 incubation, warm stratification, and cold stratification affected germination (Table 2). For each main treatment effect, as treatment intensity increased, germination increased (Table 3). All first-order interactions among treatments (with the exception of the interaction between warm stratification and GA_3 incubation) impacted germination (Table 2). Certain 2-factor interactions such as warm stratification X cold stratification and warm stratification X acid scarification were statistically significant due to the large sample size resulting from averaging across the other 2 factors, but these differences were not practically significant. In all cases, the combination of 2 treatments (averaged over all levels of the other 2 factors) improved germination relative to each treatment individually (Figure 1). However, certain treatment combinations were only marginally more effective than the better treatment alone. For example, increasing cold stratification length

TABLE 4

Mean germination percentages and standard errors for mountain snowberry seeds undergoing combinations of acid scarification, gibberellic acid (GA_3) incubation, warm stratification, and cold stratification treatments

Acid soak (min)	Warm stratification (d)	Cold stratification (d)	Gibberellic acid (ppm)	Sample size (number of seeds)	Mean germination (%)	Standard error
0	0	84	0	100	2.0	1.4
0	0	84	250	100	6.0	2.4
0	0	84	500	100	3.0	1.7
0	0	84	1000	100	8.0	2.7
0	0	168	0	400	14.5	1.8
0	0	168	250	400	25.3	2.2
0	0	168	500	400	29.0	2.3
0	0	168	1000	400	32.0	2.3
0	21	84	0	300	10.7	1.8
0	21	84	250	300	22.3	2.4
0	21	84	500	300	24.7	2.5
0	21	84	1000	300	44.0	2.9
0	21	168	0	400	25.8	2.2
0	21	168	250	400	47.0	2.5
0	21	168	500	400	64.5	2.4
0	21	168	1000	400	64.8	2.4
30	0	84	0	100	17.0	3.8
30	0	84	250	100	36.0	4.8
30	0	84	500	100	42.0	4.9
30	0	84	1000	100	37.0	4.8
30	0	168	0	400	45.5	2.5
30	0	168	250	400	45.8	2.5
30	0	168	500	400	49.3	2.5
30	0	168	1000	400	43.8	2.5
30	21	84	0	300	48.0	2.9
30	21	84	250	300	59.0	2.8
30	21	84	500	300	63.3	2.8
30	21	84	1000	300	69.7	2.7
30	21	168	0	400	61.8	2.4
30	21	168	250	400	66.8	2.4
30	21	168	500	400	71.3	2.3
30	21	168	1000	400	66.0	2.4

TABLE 5

Categorical analysis of variance table for mountain snowberry germination response to timing of gibberellic acid incubation, seed source, and the interaction of both factors

Component	df	Chi-square	Observed significance
Seed source (S)	3	850.9	< 0.001
Treatment timing (T)	3	21.4	< 0.001
S X T	9	28.3	< 0.001

from 84 to 168 d nearly doubled the germination of non-scarified seeds but had little effect on germination of seeds that had undergone a 30-min acid soak. Likewise, gibberellic acid improved the germination of non-scarified seeds nearly threefold, but improvement was considerably less when this treatment was applied to acid-scarified seeds. Mean germination percentages and standard errors for all treatment combinations are presented in Table 4.

Experiment 2

Timing of gibberellic acid application affected germination response, but seed source had a much larger impact (Tables 5 and 6). The influence of timing of gibberellic acid treatment differed slightly among seed sources (Table 6). For all but the Capulin seed source, application of GA_3 prior to warm stratification treatment resulted in less germination compared to application at some point following warm stratification treatment. Seeds from the Capulin source germinated equally well regardless of timing of application.

DISCUSSION

Consistent with previous work on common snowberry (*Symphoricarpos albus* var. *albus* [L.] Blake, formerly *Symphoricarpos racemosus* Michx.) (Flemion 1934; Evans 1974), Indian currant snowberry (*Symphoricarpos orbiculatus* Moench) (Flemion and Parker 1942; Evans 1974), and mountain snowberry (Rosner and others 2001), acid scarification and warm stratification treatments were effective in promoting germination, and a combination of treatments was most effective. Both treatments degrade restrictive seed coat fibers and break physiological control over the embryo exerted by the seed coat (Flemion 1934; Pfeiffer 1934). Combined treatment is thought to allow more thorough seed coat degradation without the occurrence of embryo damage (Pfeiffer 1934).

Published literature on North American snowberry species recommends 120 to 180 d of cold stratification (Flemion 1934; Flemion and Parker 1942; Evans 1974). We found that increasing cold stratification duration from 84 to 168 d improved germination on average, but few acid-scarified seeds required cold stratification beyond 84 d. Acid scarification may allow embryo maturation to begin earlier in the cold stratification process, by breaking seed coat-imposed physiological control of the embryo.

Warm stratification, on the other hand, improved germination about as well in combination with 84- and 168-d cold stratification treatments. Published literature recommends 90- to 120-d treatment durations when warm stratification is used instead of acid scarification (Flemion 1934; Evans 1974), but shorter durations when used in conjunction with acid scarification (Flemion 1934; Flemion and Parker 1942). Short durations of warm stratification, such as 21 d used in this study, may be inadequate to thoroughly degrade restrictive seed coat fibers initially, but fungi infecting the seed coat during incubation at room temperature may continue to degrade seed coat fibers throughout the cold stratification process. In addition, short periods of warm stratification may stimulate some germination-promoting metabolic processes such as reduction in levels of abscisic acid (ABA) and alterations in cellular development (Chien and others 1998)

The role of gibberellic acids in promoting germination is highly variable among taxa (Li and Ross 1990; Karssen 1995; Chien and others 1998). Among some species with cold stratification requirements, ABA and GAs are thought to play antagonistic roles in the maintenance and breaking of dormancy (Nicolas and others 1996). Exogenous GA_3 application has been shown to substitute for cold stratification in some species (Powell 1987; Nicolas and others 1996; Baskin and Baskin 1998; Chien and others 1998).

GA_3 substituted for at least a portion of the cold stratification requirement in mountain snowberry in our study. Germination of GA_3-treated seeds undergoing 84 d of cold stratification was as high or higher (depending on treatment level) than germination of untreated seeds cold stratified for 168 d. Cold stratification is believed to activate the gibberellin-synthesizing mechanism (Powell 1987), but in some species cold stratification may also increase seed sensitivity to GAs (Chien and others 1998). In this study, exogenous GA_3 may have substituted for endogenous GAs typically produced later in the cold stratification period, or the concentration of applied GA_3 may have been high enough to overcome insensitivity to GAs. Gibberellic acid application also

improved germination of seeds cold stratified 168 d, suggesting that some seeds had a cold stratification requirement greater than 168 d, or that GA improved germination by some means other than as a substitute for cold stratification. Exogenous GA_3 application was less effective when used in conjunction with acid scarification. By reducing the necessary duration of cold stratification, acid scarification would be expected to reduce the efficacy of treatments substituting for cold stratification.

A risk with GA treatments is that some developmental processes taking place during cold stratification may be bypassed. During cold stratification, most aspects of metabolism are affected in some way (Mayer and Poljakoff-Mayber 1982). Also, high post-germination levels of GAs can interfere with proper seedling growth. GA_3 was used as a substitute for cold stratification in Judas tree (*Cercis siliquastrum* L. [Fabaceae]) and resulting seedlings had reduced root-to-shoot ratios and problems maintaining a favorable water balance (Rascio and others 1998). In that species, reserve mobilization normally begins after germination, but exogenous GA_3 treatment resulted in reserve mobilization prior to germination. Further research is needed to determine if all or only part of snowberry's cold stratification requirement can be bypassed by GA_3 treatment and to assess the effect of such treatment on the growth and development of the resulting seedling.

TABLE 6

Effect of timing of incubation in 500 parts per million gibberellic acid (GA_3) on mountain snowberry germination by seed source

Seed source	GA treatment [a] timing	Mean germination [b] (%)	Standard error
Capulin	1	50.0 a	2.5
	2	51.5 a	2.5
	3	43.8 a	2.5
	4	43.8 a	2.5
	pooled	48.5	1.2
Cabin	1	21.5 b	2.1
	2	30.0 a	2.3
	3	32.3 a	2.3
	4	24.8 ab	2.2
	pooled	27.1	1.1
Holman	1	62.8 b	2.4
	2	71.3 ab	2.3
	3	75.0 a	2.2
	4	73.5 a	2.2
	pooled	70.6	1.1
Rociada	1	68.8 b	2.3
	2	74.5 ab	2.2
	3	74.5 ab	2.2
	4	78.8 a	2.0
	pooled	74.1	1.1

[a] 1 = GA applied following acid scarificaiton.
2 = GA applied following warm stratification.
3 = GA applied following 28 d cold stratification.
4 = GA applied following 56 d cold stratification.

[b] Mean germination percentages within a source followed by the same letter are not significantly different at $\alpha = 0.083$ ($\alpha = 0.05/6$).

We found that GA_3 was most effective when applied at any time after completion of warm stratification for 3 of 4 seed sources. Gibberellic acid has been shown to break physiological dormancy related to warm stratification requirements in some species (Baskin and Baskin 1991; Baskin and Baskin 1998). In snowberry, however, the warm stratification requirement may be related to multiple dormancy mechanisms. Warm stratification facilitates degradation of the seed coat (Pfeiffer 1934) but may improve germination through other mechanisms—warm stratification improved germination of mountain snowberry seeds that had undergone supra-optimal acid scarification treatment (Rosner and others

2001). If the benefit of GA_3 treatment was related more to processes taking place during cold stratification than warm stratification, it would be expected that GA_3 application prior to warm stratification would be less effective than application made after warm stratification; GA_3 catabolism during warm stratification treatment would reduce the concentration available during cold stratification treatment.

PRACTICAL APPLICATIONS

Gibberellic acid treatment of mountain snowberry seeds can be used to avoid either sulfuric acid scarification or lengthy (> 84 d) cold stratification treatments but not both. To bypass acid scarification, seeds should be warm-stratified for 21 d, incubated in 500 or 1000 ppm GA_3 for 24 h, and stratified 168 d. When seeds are scarified for 30 min in sulfuric acid, warm stratified for 21 d, and then incubated in 250 to 1000 ppm GA_3 for 24 h, the cold stratification duration can be reduced to 84 days with little reduction in germination.

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