Temperature Effects on Seed Germination of Lenten Rose (*Helleborus ×hybridus*) L.[1©]

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Lenten rose is well suited to dry shade gardens in the Southeastern U.S. but faces significant production difficulties due to poor seed germination. Higher and more synchronous germination would result in more efficient propagation. The objectives of these experiments were to determine the number of weeks of warm followed by cold stratification needed to increase germination percentage, shorten time to germination, and increase synchronous germination in Lenten rose 'Red Hybrids'. Study 1 treatments consisted of 4, 6, 8, or 10 weeks of warm at 25 °C (77 °F) followed by 4, 6, 8, or 10 weeks of cold at 4 °C (39 °F) stratification. Study 2 treatments consisted 10 weeks of warm at 25 °C (77 °F) followed by 1, 2, 3, 4, or 5 weeks of cold at 4 °C (39 °F) stratification. Ten weeks of warm duration followed by one week of cold duration produced the highest germination percentage. The shortest number of days to radicle emergence occurred after 4 weeks of warm followed by 4 weeks of cold in study 1 and after 1 week of cold in study 2. Germination percentages indicated that this seed lot of Lenten rose exhibited nondeep, simple morphophysiological dormancy.

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[1]First Place – Graduate Student Research Paper Competition

INTRODUCTION

Lenten rose (*Helleborus* ×*hybridus*) is an evergreen, perennial member of the Ranunculaceae family with striking winter to early-spring flowers in an array of colors. Flowers appear as single, semi-double, or double forms and last up to two months (Rice and Strangman, 1993). Lenten rose is easy to cultivate in the garden, long-lived, deer resistant, tolerant of dry conditions, and is well suited to the climate of the southeastern United States (Burrell and Tyler, 2006; Rice and Strangman, 1993). It was named the 2005 Perennial Plant of the Year by the Perennial Plant Association (Perennial Plant Association, 2008).

An obstacle to large scale production of Lenten rose is inefficient propagation methods. Lenten rose is commonly propagated by seed; however, seed dormancy results in slow, uneven germination with low germination rates (Niimi et al., 2006; Burrell and Tyler, 2006). The erratic and unpredictable nature of Lenten rose germination is undesirable to commercial growers who desire higher and more synchronous germination.

Few reports are available on the germination of any *Helleborus* species (Niimi et al., 2006); however, it is well known that temperature plays an important role in the germination of many species. As an environmental signal, temperature has a strong effect on the dormancy status within seeds. Temperature impacts a seeds ability to germinate and the rate at which germination occurs (Bewley and Black, 1994). Warm followed by cold stratification is traditionally recommended for breaking dormancy in *Helleborus* species (Rice and Strangman, 1993).

Lenten rose is thought to have morphophysiological dormancy, a combinational dormancy requiring a specific sequence of warm and/or cold stratification to break dormancy and induce germination (Burell and Tyler, 2006). The morphological component is characteristic of seeds with underdeveloped embryos at dispersal requiring warm temperatures for embryo growth before germination can occur (Baskin and Baskin, 2004; Geneve, 2003). The physiological dormancy component is attributed to restraints caused by the seed coverings, blocking the radicle from escaping the seed covering and is relieved by moist, cold stratification in nature (Geneve, 2003).

Previous work by Niimi et al. (2006) on Christmas rose (*Helleborus niger*) indicated that treatment at 25 °C (77 °F) for 8 weeks allowed the underdeveloped embryo to mature and cold stratification for at least 8 weeks at 4 °C (39 °F) was needed to break physiological dormancy. Based on the finding by Niimi et al. and characteristics of other members of the genus, it is most likely that Lenten rose has deep, simple morpho-physiological dormancy (Burell and Tyler, 2006).

The objectives of these experiments were to determine the number of weeks of warm followed by cold stratification needed to increase germination percentage, shorten time to germination, and increase synchronous germination in Lenten rose ‘Red Hybrids’.

MATERIALS AND METHODS

Study 1. Seeds of Lenten rose ‘Red Hybrids’ were purchased from Jelitto Perennial Seed (Schwarmstedt, Germany). Treatments consisted of warm at 25 °C (77 °F) for 4, 6, 8, or 10 weeks followed by cold stratification at 4 °C (39 °F) for 4, 6, 8, or 10 weeks and a control receiving no stratification. Each treatment and the control were replicated four times with 16 seeds per replication.

On 15 February 2007, seeds were allowed to imbibe in aerated, distilled water for 8 h at room temperature followed by surface sterilization in a 15% bleach (6% sodium hypochlorite) and distilled water solution. Seeds were sown in containers, clear slim compact disc cases, containing germination paper moistened with distilled water. This was later changed to a 100 ppm solution of Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, CA) after fungal infection was observed on the seeds. All treatments were sown on 16 February 2007. Treatments were placed in a PC-70 germination chamber (Pro-Grow Supply Corp., Brookfield, WI) with a temperature set point of 25 °C (77 °F). The control was placed in a 10 °C (50 °F) cooler.

After completion of the prescribed duration of warm stratification, treatments were individually moved to a cooler set at 4 °C (39 °F); after the prescribed duration of cold stratification, treatments were individually moved to a cooler set at 10 °C (50 °F), until germination occurred. Treatments were kept in darkness except when examined, and were examined approximately every 3 days to record the date of germination, defined as the day the radicle reached 3 mm in length. The range of germination was determined from the start of the experiment on 16 February 2007. Germination date and range were continuously recorded until the study was terminated on 30 September 2007.

The data were analyzed as a completely randomized design using SAS version 9.1.3 (SAS Institute, Cary, NC) with the warm and cold stratification in a partial factorial treatment arrangement. The normality assumption for ANOVA was tested using the tests for normality statistics in PROC UNIVARIATE. The number of days to germination and germination range were analyzed with PROC GLM. The number of seed germinated out of the total number sown per treatment combination was analyzed with PROC GENMOD using the binomial probability distribution. Single degree of freedom orthogonal contrasts were used to test linear and quadratic treatment trends at $\alpha = 0.05$.

Study 2. The results of Study 1 were inconclusive for the effect of cold temperature duration on germination. The results indicated that a shorter cold duration might be effective; however, the shortest cold duration studied was 4 weeks. Therefore, study 2 was conducted from 19 November 2007 to 9 May 2008 with the following changes.

Treatments consisted of 1, 2, 3, 4, or 5 weeks of cold stratification and a control. Each treatment and the control were replicated four times with 20 seeds per replication. After 10 wks of warm treatment at 25 °C (77 °F) on benches in a climate controlled room, treatments 1 through 5 were moved to a cooler set at 4 °C (39 °F). The control was moved directly to a cooler set at 10 °C (50 °F). After completion of the respective cold stratification period, treatments were moved to the 10°C (50° F) cooler until germination occurred.

RESULTS

Study 1. There was an interaction of warm and cold duration for days to germination (Table 1). There were linear increases in days to germination for both cold and warm with increasing duration. Four weeks of warm followed by 4 weeks of cold resulted in the fewest days to germination, while 10 weeks of warm followed by 10 weeks of cold resulted in the greatest. There was a warm by cold duration interaction for germination range. Six weeks of warm resulted in a linear increase in the germination range with increasing cold duration. Germination range after 4, 8, and 10 weeks of cold resulted in a linear increase, while 6 weeks cold resulted in a quadratic change with the narrowest range at 4 weeks warm. The narrowest germination range was at 4 weeks warm and 10 weeks cold. However, this result may be misleading because only 17% of seed germinated

Only the main effect, warm duration, was significant for percent germination (Table 2). There was a linear increase in percent germination with increasing warm duration. It was a general pattern with this seed lot that the fewest days to germination occurred with the shorter durations of warm and cold. The results for percent germination indicate that 10 weeks of warm duration produced the highest germination rate with no effect of cold duration on percent germination.

Study 2. There was a cubic response to cold duration in percent germination with the highest germination occurring after one week of cold (Table 3). There was a linear increase in days to germination with increasing cold, with the fewest days to germination occurring after 1 week of cold stratification. Concurrently, there was a linear decrease in germination range with increasing cold with the shortest range occurring after 3 weeks and the longest range after 0 weeks cold stratification. This study indicated that after 10 weeks of warm duration, very little cold stratification was needed for germination in this seed lot of Lenten rose, only 1 week. Although treatments with longer periods of cold had a shorter range of germination, this can be explained by the small germination percentages. In the longer stratification periods, seeds began to germinate during treatment, and then quickly stopped within 1 to 2 weeks, but in the more effective stratification periods, seeds were able to continue germination to achieve a higher overall percent germination.

DISCUSSION

The requirement for cold stratification following a warm period reported in these studies supports previous research by Niimi et al. (2006) that members of the *Helleborus* genus have morphophysiological dormancy. This is typical of other members of the Ranunculaceae family (Atwater, 1980). However, the short cold stratification required by this seed lot is contrary to work on Christmas rose, which required much longer cold stratification. The results do not indicate that Lenten rose has a level of dormancy as extreme as the Christmas rose, which has a deep, simple morphological dormancy (Niimi, 2006). The physiological component of dormancy determines the dormancy level based on cold stratification requirements and the response to external gibberellic acid applications (Geneve, 2003). Based on the results of these studies, this seed lot of Lenten rose exhibited non-deep, simple morphophysiological dormancy due to the highest germination occurring after 10 weeks of warm in study 1 and the highest germination and the shortest days to germination occurring after 1 week of cold stratification in study 2. These results are consistent with reports that Lenten rose is quicker to germinate than other Hellebore species (Burrell and Tyler, 2006).

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Table 1. Effect of warm 25 °C (77 °F) followed by cold 4 °C (39 °F) stratification duration on days to and range of germination in Lenten rose 'Red Hybrid' seeds[z].

Cold	Days to germination[y]					Germination range (days)				
	Warm (weeks)					Warm (weeks)				
(weeks)	4	6	8	10	Sign.[x]	4	6	8	10	Sign.
4	89	103	121	136	L***	8	18	21	26	L**
6	94	108	128	141	L***	14	32	21	16	Q**
8	105	114	133	149	L***	9	10	21	25	L**
10	108	116	140	157	L***	2	13	12	18	L*
Sign.	L***	L***	L***	L***		NS	L*	NS	NS	

[z]Control not included in analysis due to insufficient germination numbers to complete analysis.

[y]The warm by cold duration interaction was significant at α=0.05. Germination was when radicles were 3mm long.

[x]Non-significant(NS), linear, or quadratic (Q) at α=0.05(*), 0.01(**), or 0.001 (***) using orthogonal contrasts.

Table 2. Effect of warm 25 °C (77 °F) followed by cold 4 °C (39 °F) stratification duration on percent germination in Lenten rose Red Hybrid seeds.

	Warm (weeks)					
	0	4	6	8	10	Sign.
Germination (%)[z]	6	16	53	50	63	L***

[z]The warm duration main effect was significant at α=0.05.

Table 3. Effect of cold 4C (39F) stratification duration on the germination of Lenten rose Red Hybrid seeds.

Treatments	Percent germination	Days to germination	Germination range (days)
Control	41	130	30
1	58	128	27
2	30	135	21
3	15	138	7
4	19	137	14
5	21	139	15
Sign.[z]	C**	L***	L***

[z]Non-significant(NS), linear, or cubic (C) at α=0.05(*), 0.01(**), or 0.001 (***) using orthogonal contrasts.