A STUDY OF GERMINATION TECHINQUES FOR *HELLEBORUS NIGER*

by

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Introduction

Helleborus niger, Christmas rose is a herbaceous perennial native to cool, moist woodland areas of Central and Southern Europe and the Alps. Hellebores, members of the Ranunculaceae, are a favorite perennial garden selection and are forced as a cutflower crop for the Christmas season (3,12,19,20,27). Its evergreen foliage is palmately lobed, leathery dark green and attractive. Solitary flowers measure 6.4 cm in diameter and are white flushed with pink. These plants reach a height of 15-30 cm and in their native habitat will flower anytime from November to April (3,11). Due to lower flowering temperature requirements in comparison to traditionally grown florist crops (27) and due to the fact that hellebores are native to alkaline soils, this crop has potential for Kansas greenhouse production.

Seed production of plants insures the widest utilization of plants and the least loss. This, along with lower initial production costs, indicates the need to focus on germination techniques.

Mechanisms that control germination are triggered by external conditions such as temperature, light, water tension or gas content of the surrounding soil which can provide physical or chemical stimuli to which the seed can respond. Seed response to external stimuli is often modified as time passes by internal changes occurring within the seed itself. Thus germination is dependent upon a combination of external stimuli and internal conditions. Germination can be precisely

controlled to occur only in response to particular conditions during certain seasons. Adaptation of a species to a geographical area depends on the stimuli which promote germination operating in such a way that when germination occurs, it does so at a season which promises a high chance of survival and maturation for the plant species (37,40). Therefore it is reasonable to assume that in order to better understand the behavior of a species, the climate of its native habitat must be considered. This knowledge would provide valuable guidelines to the conditions needed for germination of seed. Some of the dominant features of southern Europe are dry summers and mild winters with mean minimum temperatures of 3-5°C (37). The main emphasis of this study will be to determine germination procedures for Helleborus niger, Christmas rose.

Fig. 1. Helleborus niger L. (Ranunculaceae). A, petal. B, stamen. C, carpels. D, vertical section of carpel. E, same in fruit. F, seed. G, vertical section of seed. (taken from Families of Flowering Plants, by Hutchinson vol. 1 pg. 401).

Plant Description

Helleborus niger, Christmas rose, is a herbaceous, evergreen perennial admired for its very early flowers and attractive leaves. These plants are native to alkaline soils of the woodlands of Central and Southern Europe and the Alps. They grow best in a cool, moist, partially shaded situation.

Underground rhizomes, from which the plant receives the specific name niger, are black in color. Erect, dark green, palmate leaves are divided into seven leaflets and are sometimes spiny on the margin. Solitary flowers nearly 6.4 cm in diameter, are actinomorphic, bisexual and white with pink shading. They have five, large petal-like sepals that surround

a showy center of yellow stamens. Flowering may occur anytime between November and April with the plant blooming as the snow recedes. The flowers are superior with 5 carpels.

This plant is a hardy, slow growing perennial. It reaches a mature height between 15- 30 cm and a spread of 38 cm.

The seeds are angular, winged or ridged, with abundant endosperm. The embryo is small and linear with two cotyledons. The embryo is often hard to locate because of its size and similarity to endosperm.

Propagation is by seed or division. Hellebores are planted in the garden either during the spring or late August to September in well drained soils high in organic matter. The old foliage should be removed each spring.

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A Study of Germination Techniques for *Helleborus niger*

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Abstract. Seed germination of *Helleborus niger* is very slow due to a basal rudimentary embryo. Potassium nitrate and GA_3 soaks were ineffective in promoting embryo growth and development. Scarification treatments of NaOCl and H_2SO_4 did not improve the efficacy of chemical treatments. Germination temperature (4-16°C) and storage treatments (duration of 0-105 days and temperature of 0-25°C) showed no significant germination differences and did not hasten germination.

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Seed dormancy may be due to immaturity of the embryo, impermeability of the seed coat to water or gases, prevention of embryo development due to mechanical causes, special requirements for temperature or light, or the presence of substances inhibiting germination (21).

Little is known about germinating hellebores, but articles about this species indicate that germination is a difficult and slow process (3,20). Maurer and Dickel (20) report the best germination results were obtained when imbibed Helleborus niger seeds were kept warm for 2 to 3 months and then kept cold for an equal length of time. They found GA_3 applied at 100 and 1000 ppm was ineffective in stimulating germination; however, seeds did swell a few hours after imbibition and were therefore not hard shelled.

Mayer and Poljakoff-Mayber (21) and Crocker (7) indicated that in some Ranunculus species immature embryos are the cause of dormancy. They stated that seeds having immature embryos must complete their development before germination can begin and that the period required for such embryos to reach maturity varied from a few days to several months. Atwater (2), in a review, stated seed structure is remarkably similar within plant families and their close relatives. She listed members of the Ranunculaceae as having basal rudimentary embryos. This type of dormancy was also confirmed by Bullowa et al. (5) citing that seed germination in the Ranunculaceae is commonly delayed since their mature, dry dispersal units contain immature embryos. Immature embryos are also common in other

families such as the Orchidaceae, Umbelliferae, and in species such as Fraxinus excelsior in the Oleaceae family (5).

The principal block to germination in this dormancy category is the delay in maturation of the embryo before germination. In many species this delay can be enhanced by the presence of inhibitors in the endosperm which need to be leached or neutralized. Stratification or an alkaline soak is effective as well as the addition of gibberellic acid to hasten embryo growth. According to Atwater (2), seed of families described as having basal rudimentary embryos were treated with a 0.2% KNO_3 solution to promote germination. Bullowa's research on Anemone coronaria, also in the Ranunculaceae, shows the optimal temperature range for germination of achenes was 10-20°C. In the same study it was also found that gibberellic acid promoted germination but only at supraoptimal temperatures (25°) (5). Since hellebores are in the Ranunculaceae, immature embryos could be a possible germination problem.

Many reports have been published describing the use of gibberellins to promote seed germination under naturally unfavorable conditions or in some cases where the precise natural conditions leading to germination were unknown. Thompson stimulated germination in several species of Labiatae with diverse requirements for germination at a constant temperature of 25°C by using a gibberellic acid treatment (36).

Kallio and Piiroinen (16), Spicer and Dionne (30), Miller and Halcomb (24) and Corns (6) have all reported promotion of seed germination in various species following applications of

gibberellic acid. Treatment with gibberellic acid during the period of seed maturation on the plant may prevent the onset of dormancy (4) and treatment of imbibed seed may satisfy after-ripening requirements which normally would require either periods of chilling treatment (10) or fluctuating temperature cycles (33,39).

Gibberellic acid was completely without effect on some plant species if the seeds were not first pricked with a needle or scarified with either H_2SO_4 (22,23,25,33,38) or NaOCl (8,9,13,14). However, prolonged treatment of NaOCl or H_2SO_4 scarification may impair germination by damaging seed tissues (13,14,38).

Salac and Hesse (28), in their study of 4 wildflower species, investigated conditions that best overcame dormancy problems and promoted maximum germination. Their research included storage conditions of room temperature, 4°C dry, and 4° moist storage. The length of storage periods were 0, 21, 42, 63, 84, and 105 days. They found that seeds of all species germinated best and most rapidly when stratified at 4° prior to germination although response to length of storage was generally dependent upon species.

The objective of the study was to determine the stratification and germination requirements of Christmas Rose.

Materials and Methods

Experiment 1: Acid scarification and gibberellic acid seed treatment. The objectives of this experiment were to determine if gibberellic acid (GA_3) would promote germination of Helleborus niger seed and to evaluate whether sulfuric acid (H_2SO_4) scarification would increase the seeds' sensitivity to GA_3. Helleborus niger seed was obtained from Herbst Brothers Seedmen, Inc. and subjected to 12 different seed treatments which were GA_3 soaks at 10, 30, 100, 300, or 1000 mg/l; concentrated H_2SO_4 scarification; H_2SO_4 followed by gibberellic acid soaks at 10, 30, 100, 300, or 1000 mg/l; and a control receiving no chemical treatment.

All seeds were surface sterilized in a 0.5% NaOCl solution for 5 min. Seeds receiving H_2SO_4 and/or GA_3 treatments were then soaked for 5 min and 24 hr, respectively. Treated seeds were wrapped in cheesecloth bags, placed in glass vials, covered with moist sand, and then were stratified for 0, 21, 42, 63, 84, and 105 days at 3 stratification temperatures 0, 5 and 25.5°C. A recording thermograph (Speedomax Recorder, Leeds and Northrup) was used to monitor temperatures.

After the respective storage periods, seeds were removed from the vials, placed on moistened filter paper in 10 cm petri dishes, and placed in a Freas-816 Precision Scientific germinator at 16°C in the dark.

Germination counts were taken once every 3 days after seeds were placed under germination temperatures and percent germination was calculated.

This experiment began 15 April 1983 and was concluded 30 August 1983. The experiment was a completely randomized design using a total of 36 treatments, 3 replications, and 20 seeds/replication/treatment.

Experiment 2: Potassium nitrate seed treatment. The objectives of this experiment were to test whether an alkaline soak would enhance germination of Helleborus niger and to examine the effects of temperature cycles on germination.

A 5 min 0.5% NaOCl soak was used to surface sterilize Helleborus niger seed. Seeds were soaked for 0 min, 15 min, 1 hr, 8 hr, and 24 hr in a 0.2% KNO_3 solution. Seeds were placed immediately in petri dishes and stored at either 4° or 16°C, for 6 storage periods of 0, 21, 42, 63, 84, and 105 days. After seeds had been stored at one temperature for the respective storage periods, they were transfered to the other temperature regime for germination.

Data taken included percent germination. Embryo development was examined by taking seed samples every 21 days and fixing in FAA killing solution followed by dehydration and preparation for sectioning and staining with safranin O and fast green (Appendix I).

This study began 26 August 1983 and was concluded 23 April 1984. The experiment was completely randomized using 10 treatments, 3 replications, and 20 seeds/treatment/replication.

Experiment 3: Sodium hypochlorite seed treatment with continual potassium nitrate and gibberellic acid treatment. Considering the results of the first two experiments with regard to temperature and chemical treatments, Expt. 3 was designed with the following objectives: 1) to determine if a NaOCl soak enhances germination by increasing the penetration of KNO_3 and GA_3 into the seed; 2) to observe if KNO_3 and GA_3 when used concurrently would have a synergistic effect on germination; 3) to examine the effect of fluctuating temperature on germination; and 4) to determine if continual chemical applications increase their effect on seed germination.

Seeds were surface treated with Thiram 50 WP (Arasan) to control fungal growth. Twenty seeds were placed on filter paper in 10 cm petri dishes. Five chemical treatments were administered to the seeds: 1) Control - distilled water; 2) 0.2% KNO_3; 3) 0.2% KNO_3 + 10 mg/l GA_3; 4) NaOCl soak + 0.2% KNO_3; and 5) NaOCl soak + 0.2% KNO_3 + 10 mg/l GA_3.

The KNO_3 and KNO_3 + GA_3 solutions were applied continually throughout the experiment, distilled water was substituted in the control plates for the chemical treatment. Those treatments receiving NaOCl were treated for 4 hr in 5.25% NaOCl (Clorox, commercial product of the Clorox Co., Oakland, Calif.) prior to receiving either KNO_3 or KNO_3 + GA_3. Seven storage conditions were used: 1) Continual storage at 4°C; 2) 50 days at 4° then to 10°; 3) 70 days at 4° then to 10°; 4) Continual storage at 10°; 5) 50 days at 10° then to 4°; 6) 70 days at 10°

then to 4°; and 7) diurnal temperature 4 - 10°.

Each storage condition contained 3 replications of the 5 chemical treatments. This experiment was started on 3 January 1984 and concluded 9 September 1984 with the experimental design being a randomized complete block.

Germination counts were taken every week and percent germination calculated. Embryo development was studied on seed samples taken at 0, 50 and 70 days of storage. Embryo sections were prepared using the fixing, dehydration and staining procedures in as in Expt. 2 (Appendix I).

Results and Discussion

Expt 1: Stratification temperature Stratification temperature had a significant influence on germination (Table 1). When seeds were stored in sealed vials containing moist sand at room temperature, no germination was observed between 21 and 105 days of storage (Table 2). Seeds receiving this temperature treatment had severely rotted after approximately 1 week of storage.

When seeds were stored at 5°C, they could be stored up to 63 days before intense decay occurred (Table 2). By lowering the storage temperature to 0°, seeds could be stored up to 105 days with no detrimental effects.

These results indicate that hellebore seeds should not be stored moist for any length of time at 25.5°C. Both Steinbauer and Thompson found similar results in their work with related species (31,37). Steinbauer (31) found that placing seeds of Fraxinus pennsylvanica, F. pennsylvanica var. lanceolata and F. americana (red, green, and white ash) in germinators at or near room temperature was ineffective in producing uniform germination. Thompson also found little or no germination at constant temperatures near 25° and made the general statement that species of the Mediterranean region germinate best at lower temperatures (37).

Storage period. Germination was the greatest with seeds placed directly in 16°C germination temperature and decreased with prolonged storage (Table 1).

The trend at 5° and 25.5°C was for germination to decrease

as storage period increased (Table 2). This can be related to the high incidence of decay at these temperatures. However, at 0° there were no differences in germination among storage periods (Table 2).

Chemical treatments. Seeds in the control treatment had significantly higher germination than seeds receiving GA_3 (Table 1). In comparing GA_3 treatments, the best germination was obtained at the low concentrations of 10 and 30 mg/l. This agrees with work by Maurer and Dickel (20) who showed that treating hellebores with 100 to 1000 ppm GA was ineffective.

Karssen points out that processes unique to germination, such as alpha-amylase synthesis in wheat, do not proceed in immature seed despite the presence of GA's (17). Immature embryos are common among members of the Ranunculaceae and the fact that hellebores seed appear to contain an immature embryo could be the reason GA_3 is ineffective in enhancing germination (Fig. 1).

Scarification treatments with concentrated H_2SO_4 for 5 min. resulted in severe injury to the seed. Seeds receiving this pretreatment quickly became infected by fungi and failed to germinate regardless of stratification temperature. Similar results with Oryzopsis hymenoides, Indian ricegrass, receiving prolonged treatment with H_2SO_4 are found in the literature (38).

According to McDonald (22), due to the location of the aleurone layer immediately beneath the seed testa and its role in the early stages of hydrolytic enzyme synthesis, any damage

to this tissue caused by acid scarification would clearly retard early hydrolysis of endospermic reserves and could thus, affect the nutritional status of the growing embryo. Scarification of hellebores with H_2SO_4 resulted in irreparable damage to either the embryo or the tissue responsible for initiating endosperm hydrolysis.

In this experiment, germination was extremely low and seed with radicle emergence failed to develop any further. This can be attributed to the method of seed storage. Moist storage of Helleborus niger seed for long periods of time encouraged decay and fungal growth. Many seeds were completely destroyed prior to being removed from storage.

Table 1. Influence of stratification temperature, length of storage and GA_3 treatment on Helleborus niger seed germination at 16°C.

Main effects	No. seed germinated
Stratification temperature[z]	
0°C	69
5°C	49
25.5°C	26
X^2 Test of independence	19.7
Critical X^2, 5% level	6.0
Storage period (days)[y]	
0	63
21	27
42	17
63	17
84	10
105	10
X^2 Test of independence	86.1
Critical X^2, 5% level	11.1
GA_3 Treatment[x]	
Control	55
10 mg/l	23
30 mg/l	25
100 mg/l	18
300 mg/l	13
1000 mg/l	10
X^2 Test of independence	56.1
Critical X^2, 5% level	11.1

[z]Total number of seed per stratification temperature = 2160.
[y]Total number of seed per storage period = 1080.
[x]Total number of seed per treatment = 1080.

Table 2. Effects of length of storage and temperature on germination of *Helleborus niger*.

Storage period[z] (days)	No. of seed germinated at each storage temperature		
	25.5°C	5°C	0°C
0	26	22	15
21	--	20	7
42	--	4	13
63	--	2	15
84	--	1	9
105	--	--	10
X^2 Test of independence		56.5	5.0
Critical X^2, 5% level		11.1	11.1

[z]Total number of seed at each stratification temperature and storage period = 360. Blanks indicate seed lost due to decay.

Expt. 2. In expt. 2, germination was very low. This is evident by slow development of the rudimentary embryo found in the seed (Fig. 2). Slow development was compounded by infection of a wide range of fungal pathogens which could not be effectively controlled by NaOCl. If germination is to be improved, a more effective control of fungi attack must be achieved.

There were no significant differences among stratification temperatures or storage periods in the germination study (Table 3) or in the embryo study. However, germination appears to be highest when seeds are stored for approximately 63 days before being placed in germination temperatures (Table 3). At 160 days, germination of seeds stored at 16°C and then transfered to 4° was slightly greater than seeds stored at the cooler temperature. However, at 250 days this trend is reversed (Table 3). According to Thompson, species similar to Helleborus which contain morphologically immature embryos when the seed is shed, require warm conditions for the completion of embryo development before treatments leading to germination are started (37). These trends in Helleborus require further data for confirmation of stratification requirements. However, the trend occurring at 160 days is similar to the results obtained by Maurer and Dickel (20) working with Helleborus. They obtained close to 90% germination when seeds were kept warm for 10 to 20 weeks in their swollen condition followed by a cold treatment also of 60 to 80 days. Steinbauer also showed that stratification of Fraxinus at 5° should be proceeded by at

least a two month storage period at temperatures near 20° to allow the embryos to become fully enlarged (31).

In expt. 2, germination was very low. This is documented by slow embryo development (Fig. 2). The seeds were quite susceptible to a wide range of fungal pathogens which could not be effectively controlled by NaOCl. If germination is to be improved a more effective control of fungi attack must be achieved.

Germination of hellebores seed treated with KNO_3 was not significantly different than the control nor were there noticeable differences among the KNO_3 treatments (Table 3). In addition, no differences among KNO_3 treatments and the control were detected in the embryo development study. These results contrast those obtained by Atwater (2) who states that stratification or alkaline soak (KNO_3) is effective as well as the addition of GA_3 to hasten embryo growth in the Ranunculaceae.

Conversely, Steinbauer (31) found when Fraxinus seeds were soaked in ranges of concentrations of KNO_3, glutathione, thiourea, ethylene chlorhydrin, and GA, no germination resulted. However, he also stated it was possible that the substances were not penetrating the seeds and therefore were ineffective in stimulating germination.

In this experiment total germination was calculated on 31 January after approximately 160 days and again on 23 April after 250 days. Additional germination was observed among seed stored at 4°C for 42 and 63 days at 250 days under germination

conditions (Table 4). In contrast to expt. 1 where seedlings failed to develop after germination, some seed showed continued growth upon germination in expt. 2.

Table 3. Influence of stratification temperature, length of storage and KNO_3 treatment on Helleborus niger seed germination at 16°C.

Main effects		No. seed germinated 160 days	250 days
Temperature[z]			
Stratification	Germination		
16°C	4°C	47	55
4°C	16°C	41	59
X^2 Test of independence		0.4	0.1
Critical X^2, 5% level		3.8	3.8
Storage period (days)[y]			
0		63	16
21		27	18
42		17	17
63		17	29
84		10	11
105		10	23
X^2 Test of independence		10.6	10.5
Critical X^2, 5% level		11.1	11.1
KNO_3 Treatment[x]			
Control		16	21
15 min KNO_3		23	24
1 hr KNO_3		17	24
8 hr KNO_3		15	19
24 hr KNO_3		17	26
X^2 Test of independence		2.3	1.4
Critical X^2, 5% level		9.5	9.5

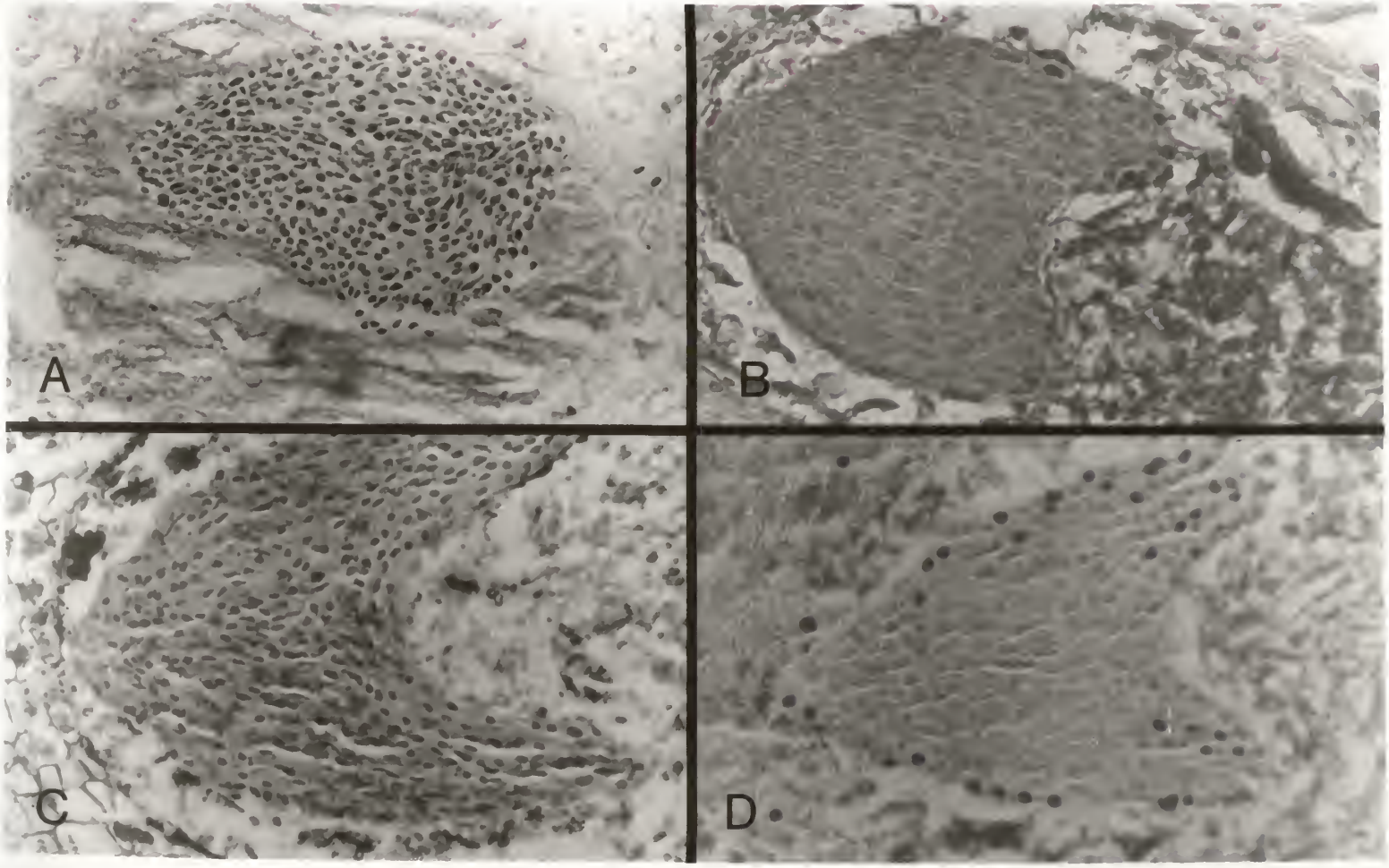
[z]Total number of seed per stratification temperature = 1800.
[y]Total number of seed per storage period = 600.
[x]Total number of seed per treatment = 720.

Table 4. Storage period and temperature affects on Helleborus niger germination 160 and 250 post-storage.

Storage period[z] (days)	Days post-storage	No. of seed germinated Storage temperature 4°C	16°C
0	160	6	9
	250	6	10
21	160	5	13
	250	5	13
42	160	5	3
	250	13	4
63	160	8	13
	250	14	15
84	160	6	2
	250	7	4
105	160	11	7
	250	14	9
X^2 Test of Independence		4.0	14.9
Critical X^2, 5% level		11.1	11.1

[z]Total number of seed at each stratification temperature and storage period = 300.

Figure 2. *Helleborus niger* embryos during stratification. A. 21 days; B. 42 days; C. 63 days; D. 84 days of stratification (magnification approximately 400 X).


A
B
C
D

Expt. 3. There are no significant germination differences among seeds stratified at 4° and 10°C (Table 5: Temperature). A diurnal temperature cycle showed no advantage over the continual or 50 and 70 day storage periods (Table 6). The trend at 250 days agrees with expt. 2 where germination is greater at 4°.

Germination was significantly greater for seeds held at a constant stratification/germination temperature in comparison to seed which were moved to different temperatures after either 50 or 70 days of stratification (Table 5: Storage period). The major contribution to this trend is from greater germination at constant 4°C (Table 7).

Chemical seed treatment of Helleborus niger with KNO_3 and GA_3 solutions did not enhance germination (Table 5). These results are similar to expt. 2 where KNO_3 was found to be ineffective. Hsiao (13), found that in wild oats the pericarp and testa were the main barriers to the exchange of gases and exogenous GA_3. Treatment of these seeds with H_2SO_4 or NaOCl improved germination. Contrary to Hsiao's results, NaOCl did not improve germination of Helleborus. However, unlike H_2SO_4, scarification with NaOCl did not injure hellebores seed.

The ineffectiveness of scarification on promoting germination was also confirmed by Villiers (41). He found that treatment of the testa of Fraxinus excelsior in various concentrations of acid or physical removal of the testa, did not promote germination. He confirmed that scarification and acid treatment were ineffective due to the permeability of the

testa and endosperm to oxygen.

Like expt. 2, results obtained from the embryo study show a slow rate of embryo growth. No apparent differences in embryo development could be seen at 4° or 10°C or with diurnal temperatures. In addition there are no noticable chemical treatment effects at 50 or 70 days storage.

As in the previous experiments, germination was low. However, in contrast to NaOCl surface sterilization, Thiram gave a markable improvement on fungal pathogen control.

Table 5. Influence of Stratification temperature, length of storage and chemical treatment on Helleborus niger seed germination.

Main effects		No. seed germinated 250 days
Temperature[z]		
Stratification	Germination	
4°C	10°C	10
10°C	4°C	3
X^2 Test of independence		3.8
Critical X^2, 5% level		3.8
Storage period (days)[y]		
0		9
50		2
70		2
X^2 Test of independence		7.7
Critical X^2, 5% level		6.0
Chemical Treatment[x]		
Control		3
KNO_3		3
KNO_3 + GA_3		2
NaOCl + KNO_3		1
NaOCl + KNO_3 + GA_3		5
X^2 Test of independence		3.2
Critical X^2, 5% level		9.5

[z]Total number of seed per stratification temperature = 300.
[y]Total number of seed per storage period = 600.
[x]Total number of seed per treatment = 420.

Table 6. Comparison of constant and diurnal germination temperature and storage period on Helleborus niger seed germination.

Storage period (days)	Germination Temperature[z]	No. seed germinated (250 days)
0	4°C	7
	10°C	2
	Diurnal	1
X^2 Test of independence		6.3
Critical X^2, 5% level		6.0
50	4°C	1
	10°C	1
	Diurnal	1
X^2 Test of independence		0
Critical X^2, 5% level		6.0
70	4°C	2
	10°C	0
	Diurnal	1
X^2 Test of independence		2.0
Critical X^2, 5% level		6.0

[z]Total number of seed per stratification temperature = 300.

Table 7. Effect of stratification temperature and length of storage on Helleborus niger seed germination.

Storage period[z]	Stratification temperature (No. seed germinated 250 days)	
	4°C	10°C
0	7	2
50	1	1
70	2	0
X^2 Test of independence	6.3	2.0
Critical X^2, 5% level	6.0	6.0

[z]Total number of seed per storage period = 600.

Conclusions

Germination of *Helleborus niger* is difficult at best. From these studies it appears that this species possesses a physiologically deep dormancy of the embryo. None of the traditional seed treatments appeared capable of inducing growth and development of the rudimentary embryo. Perhaps a prolonged warm-dry period greater than 105 days is needed for development of the embryo. This to be followed by a prolonged cool-moist period to remove any internal inhibitors prior to germination.

Even though the species has many attractive morphological features which would lend itself to pot culture, production would need to be initiated by other means such as rhizome divisions or embryo culture.

Literature Cited

1. Amen, R. A. 1968. A model of seed dormancy. Bot Rev. 34:1-31.

2. Atwater, B. R. 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. Seed Sci. and Technol. 8:523-573.

3. Bailey, L. H. 1914. The standard encyclopedia of horticulture. 3:1454-1455.

4. Black, M. and J. M. Naylor. 1959. Prevention of the onset of seed dormancy by gibberellic acid. Nature 184:468-469.

5. Bullowa, S., M. Negbi, and Y. Ozeri. 1975. Role of temperature light and growth regulators in germination in Anemone coronaria L.. Aust. J. Pl. Phys. 2:91-100.

6. Corns, W. G. 1960. Effects of gibberellin treatments on germination of various species of weed seeds. Can. J. Plant Sci. 40:47-51.

7. Crocker, W. 1916. Mechanics of dormancy in seeds. Am. J. Bot. 3:99-120.

8. Fieldhouse, D. J. and M. Sasser. 1975. Stimulation of pepper seed germination by sodium hypochlorite treatment. HortScience 10:622.

9. Frank, A. B. and K. L. Larson. 1970. Influence of oxygen, sodium hypochlorite, and dehulling on germination of Green Needlegrass seed (Stipa viridula Trin.). Crop Sci. 10:679-682.

10. Frankland, B. 1961. Effect of gibberellic acid, kinetin and other substances on seed dormancy. Nature 192:678-679.

11. Franklin Park Conservatory and Garden Center. 1983. Plant of the Week. Columbus Dispatch 113:(178) pg. K5.

12. Hahn, E. 1976. Helleborus-Kulturen fur den Schnitt bei Haller in Burstadt. Gartenwelt 24:497.

13. Hsiao, A. I. 1979. The effect of sodium hypochlorite and gibberellic acid on seed dormancy and germination of Wild Oats (Avena fatua). Can. J. Bot. 57:1729-1734.

14. Hsiao, A. I. 1979. The effect of sodium hypochlorite, gibberellic acid, and light on seed dormancy and germination of Wild Buckwheat (Polygonum convolvulus) and Cow Cockle (Saponaria vaccaria). Can. J. Bot. 57:1735-1739.

15. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Company Inc. New York.

16. Kallio, P. and P. Piiroinen. 1959. Effect of gibberellin on the termination of dormancy in some seeds. Nature 183:1830-1831.

17. Karssen, C. M. 1982. The role of endogenous hormones during seed development and the onset of primary dormancy. In Plant Growth Substances, P. F. Wareing (ed.), Academic Press Proc. of the 11th International Conference on Plant Growth Substances, Aberystwyth, Wales. pgs. 623-632.

18. Koller, D., A. M. Mayer, A. Poljakoff-Mayber, and S. Klein. 1962. Seed Germination. Ann. Rev. Pl. Phys. 13:437-464.

19. Mathew, B. 1981. A survey of Hellebores. Plantsman 3:1-10.

20. Maurer, M. and B. Dickel. 1970. Helleborus-Hybriden. Gb+Gw 79:166-167.

21. Mayer, A. M. and A. Poljakoff-Mayber. 1975. Germination of seeds. 2nd edition, Pergamon Press, New York.

22. McDonald, M. B. Jr. and A. A. Khan. 1978. Changes in Indian Ricegrass seeds in relation to coat-imposed and embryonal dormancy. Argon. J. 70:829-834.

23. McDonald, M. B. Jr. and A. A. Khan. 1983. Acid scarification and protein synthesis during seed germination. Agron. J. 75:111-114.

24. Miller, E. A. and E. J. Holcomb. 1982. Effect of GA_3 on germination of Primula vulgaris Huds. and Primula x polyantha Hort.. HortScience 17:814-815.

25. Nagao, M. A., K. Kanegawa, and W. S. Sakai. 1980. Accelerating palm seed germination with gibberellic acid, scarification, and bottom heat. HortScience 15:200-201.

26. Pizzetti, I. and H. Cocker. 1975. Flowers - A guide for your garden. Harry N. Abram, Inc., Publishers, New York pgs. 576-579.

27. Richter, P., A. Mugge, w. Kieker, and K. Kubitz. 1980. Helleborus-eine schnittblume mit geringem Energiebedarf, aber mit speziellen Standortanspruchen. Gartenbau 27:372-373.

28. Salac, S. S. and M. C. Hesse. 1975. Effects of storage and germination conditions on the germination of four species of wildflowers. J. Amer. Soc. Hort. Sci. 100:359-361.

29. Sass, J. E. 1940. Elements of Botanical Microtechnique. McGraw-Hill Book Company, Inc. New York.

30. Spicer, P. B. and L. A. Dionne. 1961. Use of gibberellin to hasten germination of Solanum seed. Nature 189:327-328.

31. Steinbauer, G. P. 1937. Dormancy and germination of Fraxinus seeds. Pl. Physiol. 12:813-824.

32. Stuart, P. 1981. Helleborus niger 'White Magic'. R. New Zealand Inst. of Hortic. Ann. J. 6:122-124.

33. Tager, J. M. and B. Clarke. 1961. Replacement of an alternating temperature requirement for germination by gibberellic acid. Nature 192:83-84.

34. Taylorson, R. B. and S. B. Hendericks. 1977. Dormancy in seeds. Ann. Rev. Pl. Phys. 28:331-354.

35. Thompson, P. A. 1969. Comparative effects of gibberellins A_3 and A_4 on the germination of seeds of several different species. Hort. Res. 9:130-138.

36. Thompson, P. A. 1969. Germination of species of Labiatae in response to gibberellins. Physiologia Plantarum 22:575-586.

37. Thompson, P. A. 1971. Research into seed dormancy and germination. Proc. Int. Pl. Prop. Soc. 21:211-228.

38. Toole, V. K. 1940. The germination of seed of Oryzopsis hymenoides. Agron. J. 32:33-41.

39. Trelawny, J. G. S. and D. J. Ballantyne. 1963. The effect of gibberellin and temperature on the germination of seed of Bells of Ireland. Can. J. Plant Sci. 43:522-527.

40. Vegis, A. 1964. Dormancy in higher plants. Ann. Rev. Pl. Phys. 15:185-218.

41. Villiers, T. A. and P. F. Wareing. 1964. Dormancy in fruits of Fraxinus excelsior L.. J. Exp. Bot. 44:359-367.

42. Wareing, P. F. and P. F. Saunders. 1971. Hormones and dormancy. Ann. Rev. Pl. Phys. 22:261-288.

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Appendix I

Seed samples were taken and fixed in FAA killing solution for at least 24 hr. The seeds were then taken through the following dehydration series:

50% Ethanol

60% Ethanol

70% Ethanol

80% Ethanol

95% Ethanol

100% Ethanol

75 parts 100% Ethanol to 25 parts Xylene

50 parts 100% Ethanol to 50 parts Xylene

25 parts 100% Ethanol to 75 parts Xylene

100% Xylene

Ethanol solutions were changed every 12 hr and the xylene solutions were changed every 3 hr. Paraffin shavings were added to vials containing 100% xylene solution until saturation was reached at room temperature. The vials were moved to a hot plate (41°C) and more shavings added until the solution once again reached saturation. The vials were then moved to a 56°C paraffin oven (Elconap, Electric Heat Control Apparatus Co.) for 6 hr. One-third of the paraffin solution was removed and replaced with melted paraffin and the vials were returned to the oven for 12 hr, after which time two-thirds of the mixture was removed and replaced with melted paraffin. After 24 hr in the oven, all of the mixture was poured off and replaced with

melted paraffin. This was repeated two more times, once every 24 hr. Seeds were then embedded in paraffin blocks.

Longitudinal sections were made using a rotary microtome (Spenser Lens Co., Buffalo N.Y. No. 3768) at 12-16 microns. The paraffin ribbon sections were attached to slides using Adhesive III as described by Sass (29).

The slides were then stained using the following staining procedure with the slides remaining in each solution only about 15-20 sec:

100% Xylene
100% Xylene
95% Ethanol
80% Ethanol
70% Ethanol
Safranin O - 2 hr
Wash distilled water
70% Ethanol
80% Ethanol
95% Ethanol
Fast Green - 15 sec
95% Ethanol
90 parts 100% Xylene to 10 parts 100 Ethanol
100% Xylene
100% Xylene

Cover slips were fastened using Canadian Balsam and cured in a 53°C oven for 24 hr. The slides were then examined.

A STUDY OF GERMINATION TECHNIQUES FOR *HELLEBORUS NIGER*

by

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AN ABSTRACT OF A MASTER'S THESIS

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Abstract

Seed germination of Helleborus niger is slow due to the delay in maturation of their basal rudimentary embryo. Three separate experiments were conducted to determine possible germination techniques.

The objective of expt. 1 was to determine if GA_3 would promote germination of hellebores seed. In expt. 1 half the seeds were scarified for 5 min in H_2SO_4. Seeds were then soaked for 24 hr in concentrations of 10, 30, 100, 300 or 1000 mg/l GA_3. Treated seeds were stored for 0, 21, 42, 63, 84 and 105 days at 3 stratification temperatures 0, 5 and 25.5°C After storage seeds were placed in a germinator at 16° and germination counts taken.

Experiment 2 was used to test whether an alkaline soak would enhance germination and to examine the effect of temperature cycles on germination. Seeds were soaked for 0 min, 15 min, 1 hr, 8 hr, and 24 hr in a 0.2% KNO_3 solution and stored at either 4 or 16°C. Seeds were stored for periods ranging from 0-105 days and germination counts taken after each respective storage period.

The purpose of expt. 3 was to determine if NaOCl scarification would enhance germination; to determine if there is a synergistic effect on germination when using KNO_3 and GA_3 concurrently; to examine the effect of fluctuating temperature on germination; and to determine

if continual chemical applications increase their effect on germination. Five chemical treatments were administered: control, 0.2% KNO_3, 0.2% KNO_3 + 10 mg/l GA_3, NaOCl scarification + 0.2% KNO_3 and NaOCl scarification + 0.2% KNO_3 + 10 mg/l GA_3. The KNO_3 and KNO_3 + GA_3 solutions were applied continually throughout the experiment. Germination temperatures of 4°, 10° and diurnal 4-10°C along with storage periods of 0, 50 and 70 days were examined.

Potassium nitrate and GA_3 soaks were ineffective in promoting embryo growth and development. Scarification treatments of NaOCl or H_2SO_4 did not improve the effect KNO_3 and GA_3 and in the later case damaged seed tissues. Germination temperature and storage treatments showed no significant germination differences and did not hasten germination.