ENHANCEMENT OF SEED GERMINATION AND SEEDLING

GROWTH IN LENTEN ROSE

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ENHANCEMENT OF SEED GERMINATION AND SEEDLING GROWTH IN LENTEN ROSE

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ENHANCEMENT OF SEED GERMINATION AND SEEDLING

GROWTH IN LENTEN ROSE

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VITA

Laura Michelle McElhannon was born July 9, 1982 in Spokane, Washington. She is the daughter of Jerry and Debra Moore. Michelle graduated from the University of Georgia in 2004 with a Bachelor of Science in Agriculture. She married Charles McElhannon in June 2006 and they had their first child, Liam Charles McElhannon, in September 2007. Michelle began attending graduate school at Auburn University in January 2006 and studied under Dr. Luther Waters Jr., Professor. She graduated with her Master's of Science degree in August 2008.

THESIS ABSTRACT

ENHANCEMENT OF SEED GERMINATION AND SEEDLING GROWTH IN LENTEN ROSE

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The purpose of these studies was to develop methods to increase germination percentage, shorten germination time, provide more synchronous germination, and result in more efficient seed propagation techniques for Lenten rose (*Helleborus* × *hybridus*). Chapter two investigated the number of weeks of warm followed by cold stratification needed for germination. Seeds received warm 25C (77F) durations for 4, 6, 8, or 10 weeks followed by cold 4C (39F) durations for 4, 6, 8, or 10 weeks plus a control. The fewest days to radicle, hypocotyl, and first true leaf emergence generally occurred with shorter durations of warm and cold. Ten weeks of warm duration produced the highest radicle, hypocotyl, and true leaf counts. Four weeks of cold duration produced the highest true leaf count, but results were inconclusive for the effect of cold duration on germination rate, time to germination, and range of germination. Average plant width

decreased with increasing cold and warm durations, while plant height decreased with increasing warm durations.

Chapter three investigated the effects of density sorting on seed germination and seedling growth in Lenten rose. Treatments consisted of four seed density groups and an unsorted control. The highest radicle, hypocotyl, and first true leaf counts were found in the unsorted control, while the lowest were found in the lowest density. The lowest density was greatly impacted by high disease incidence. In chapter four, seeds of Lenten rose were treated with three GA_3 concentrations at 200 ppm, 400 ppm, or 800 ppm with three application times prior to warm stratification, between warm and cold stratification or after cold stratification and a control. Treatment of seeds with 200 ppm, 400 ppm, and 800 ppm GA_3 prior to warm stratification increased germination rates by 42% to 58%.

The results of Chapter two indicated that a short cold duration might be desirable in producing the highest true leaf counts in Lenten rose; however, the shortest cold duration studied was 4 weeks. Therefore, Chapter five was conducted to further investigate the appropriate weeks of cold stratification for germination of Lenten rose. Treatments consisted of 1, 2, 3, 4 or 5 weeks of cold 4C (39F) durations plus a control. The highest percent radicle emergence and the fewest days to radicle emergence occurred after 1 week cold stratification. The shortest range of radicle emergence occurred after 3 weeks and the longest range after 0 weeks cold stratification. Based on the results with regard to temperature requirements and the ability of GA_3 to promote germination, this seed lot of Lenten rose exhibits nondeep, simple morphophysiological dormancy.

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Style manual used

Journal of Environmental Horticulture

Computer software used

Microsoft Word 2007, Microsoft Excel 2007, SAS 9.1

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CHAPTER I

LITERATURE REVIEW

Helleborus

Hellebores are perennial members of the Ranunculaceae, or buttercup, family offering impressive winter to early-spring flowers in an assortment of colors. They are popular as ornamental, cut flower, and medicinal plants in Europe and many species are well suited to the southeastern United States (20, 24). Hellebores are easy to cultivate in the garden and have enjoyed increasing popularity in the United States since the 1980s. Lenten rose (*Helleborus* × *hybridus*) was named the 2005 Perennial Plant of the Year by the Perennial Plant Association (8).

Hellebores are native to Europe and Asia, with a range from western Britain, excluding Ireland, east through central Europe and into Russia (24). Only one species, *Helleborus thibetanus* Franchet, is native to China (8). The majority of *Helleborus* species are concentrated in the Balkan region (24). The *Helleborus* genus consists of 18 to 20 species divided into two groups, caulescent and acaulescent species, based on the arrangement of their stems and leaves. Caulescent species have leaves on the flowering stems, while acaulescent species have leaves and flowers that arise from the rhizome. The leaves are fan-shaped, leathery in texture, and ternately, palmately, or pedately

divided (2, 24). Leaflets are oval to lance shaped and commonly toothed. Plants can be deciduous or evergreen, dependent upon moisture and nutrient conditions (8). Hellebores have a compact rhizome with new leaves and stems arising from the rhizome tips. The root system is large and penetrates deep into the soil. This allows the plants to withstand drought well, but can make transplanting difficult (24).

Hellebores are loved by gardeners for their early flowering, beginning as early as December in some species. Flowers last for 2 to 3 months and come in a multitude of colors from whites, yellows, and greens to pinks, plums, purples, blue-blacks and even spotted forms. Flowers appear in single, semi-double, or double forms. Unlike most plants, the petals of hellebores are not showy, but are modified into short nectarines. To attract insect pollinators, most commonly bees, the five sepals are enlarged and appear as petals with only the outer two functioning to protect the rest of the flower (19, 24). The sepals are persistent and fade in color after the flowers bloom, but remain to actively photosynthesize and support seed development (24).

Lenten Rose

The two most popular *Helleborus* species in the United States are the Christmas rose (*H. niger* Linn.) and the Lenten rose (*H. orientalis* Lamarck). The majority of the hellebores currently grown as *H. orientalis* are actually hybrids of this species and several other Helleborus species (24). These have previously been known as "orientalis hybrids" but have now been given the newer botanical name of *Helleborus* × *hybridus* (19, 24). Both the true species, now rarely found, and the hybrids are known as Lenten rose (24).

Lenten rose is easy to cultivate, hardy, long-lived, tolerant of dry conditions, evergreen, deer resistant, and is considered to have the showiest flowers of the hellebore species (8, 22, 24). Flowers come in many shades, are up to 3 inches across, and generally nodding forms, however, upward facing forms are occasionally found (8). Lenten rose accepts a wide range of soil conditions, but prefers not to have poor drainage in the winter. It grows in alkaline, semi-shade conditions in its native habitat (19) and is adaptable to USDA hardiness zones 4 to 9 (22) and partial to full shade. Plants grow 18-24 in (45-61 cm) in height and 24-30 in (61-76 in) in width and have pedately divided foliage with seven to eleven coarsely toothed, thick leaflets which add a nice texture to the garden (22, 24).

Hellebores, although much loved in the garden, are difficult to propagate in both a nursery and laboratory setting (20, 24). Seed, division, and in vitro propagation are methods used in propagation. Propagation by cuttings is not an option for the acaulescent Helleborus species (24). Division of rhizomes can be used when identical offspring are required, such as named selections, but this process is time consuming and often results in a slow recovery time (19, 20, 24). Some growers, such as Pine Knot Farms, have had success with division by heeling plants in sterile, bagged medium prior to planting (8).

In vitro propagation has the advantage of producing identical plants, thus allowing consumer selection of flower color prior to flowering, but some hellebore species, such as *Helleborus* × *hybridus*, have proven to be difficult to produce profitably (8). Recent studies have provided methods for in vitro propagation of several Helleborus species, such as *H. argustifolius* Viviani, *H. foetidus* Linn., *H. niger*, and *H. orientalis* (9, 26).

However, better protocols are needed to develop an efficient mass production system to result in increased survival and multiplication rates (9).

As a result of low survival and multiplication rates with in vitro propagation and the time required for division, hellebores are commonly propagated by seed when identical plants are not necessary or for the production of the named seed strains (19, 20). Propagation by seed has the advantage of producing a substantial number of seeds from one plant with 50 or more seeds produced by a single flower (19), but many months are needed for the seeds to germinate after they have been released from the mother plant (20). Hellebore seeds are not necessarily difficult to germinate, a substantial number of seedlings can be found under a mature hellebore plant in the garden (24); however, a long germination period is undesirable to commercial growers who require higher and more synchronous germination.

Hellebore seed ripening and release generally occurs by mid-summer (24). Seeds of hellebores are round to kidney-shaped and black to orange brown in color when ripe. Seeds vary in size by species with seeds of *Helleborus* × *hybridus* being around 3/16 in (5mm) long and 1/8 in (3.2mm) in diameter. Often a white appendage, known as an elaiosome, is visible. The elaiosome contains fats and sugars to attract ants that disperse the seeds. Germination usually occurs by late December for most species. The earliest plants flower in just over a year after germination, but most will take two to three years (8).

Germination of Hellebores

Hellebore seeds germinate best when sown immediately upon ripening because seed stored dry for more than a few weeks will germinate poorly. Seeds can be stored, in dampened peat moss (19) and subsequently imbibed in warm water overnight before sowing (8). Seed sowing can be done at any time, but waiting until after summer or fall will delay germination until the following fall or winter season (19). Commercially, seeds are sown in in-ground seed beds and transplanted after germination or are sown in deep flats filled with well-drained, sterile media and covered with expanded slate or sand (8). Seedlings often emerge after the first few frosts indicating that little or no cold period is needed for germination (19). Lenten rose begins to germinate in December and continues to germinate throughout the spring (8), taking up to 18 months. Germination is uneven forcing growers to transplant seedlings over several weeks or months until germination has concluded. Seedlings are transplanted in the cotyledon stage and take 6 to 8 months to reach a finished size (23).

Hellebore seeds often fail to germinate even under environmental conditions suitable for germination (20). This is thought to be attributed to a combination of endogenous dormancies, both morphological and physiological (8). Morphological dormancy is attributed to a rudimentary embryo that must mature after the seed ripens and has been reported in other members of the Ranunculaceae family (8, 18, 20). This dormancy can be overcome by moist, warm stratification for 6 to 12 weeks (8, 14). Physiological dormancy is believed to be caused by a chemical inhibitor that suppresses germination, known as physiological inhibition (8). Physiological inhibitors must be

leached or neutralized from a fully developed embryo to allow germination to occur (18). Chilling, or cold stratification, is traditionally used to remove physiological inhibitors (8, 14). Potassium nitrate and gibberellic acid soaks have been used, but were ineffective, in Christmas rose (18). The presence or absence of light is not known to effect dormancy or germination in hellebore seeds (1, 27). Once dormancy is overcome, fresh hellebore seed generally germinate at temperatures between 4C (40F) and 10C (50F) (14). This replicates the seeds natural temperature environment during dormancy release and germination (8).

Niimi et al. reported in a 2006 study on embryo development and seed germination of Christmas rose that there were few previous reports available on seed germination of *Helleborus* species. Observation of embryo development in this study indicated that Christmas rose has an underdeveloped, rudimentary embryo at seed dispersal. The embryo transforms from a heart shape to a torpedo shape followed by a cotyledon shape before radicle emergence, but continuation through the developmental stages only occurred in seeds that were transferred from one temperature regime to another. Treatment at 25C for 8 weeks allowed the embryo to mature from the heart to the torpedo stage and chilling for at least 8 weeks at 4C was needed to break dormancy. Similar results of changing temperature requirements for each embryo developmental stage have been reported in other members of the Ranunculaceae family. The researchers concluded from these results that seeds of Christmas rose have deep, simple morphophysiological dormancy, a combinational dormancy resulting from the immature embryo and a physiological dormancy (20).

Seed Dormancy and Germination

The primary function of a seed is to establish a new plant. The seed contains the embryo, food reserves, and is structurally and physiologically capable of being the dissemination unit for a new plant. Seed dormancy is described as a barrier to germination of an intact viable seed under favorable environmental conditions (6). It is an ecologically adaptive mechanism to give plants the best possible germination over time in a seed population (13). Quick germination and subsequent growth are desired in ornamental and agricultural crops making dormancy an unfavorable characteristic (6). Although scientists and propagators have long realized that dormancy mechanisms exist (13), reviews of the literature have shown that dormancy is one of the least understood processes in seed biology (15). Recently, there has been a significant increase in the number of publications investigating seed dormancy, thus enhancing understanding of these processes (12).

Defining germination can be difficult because the only way to assess germination is by the lack of it (15). Germination begins with water uptake by the inactive, dry seed causing an abrupt renewal of metabolic activity. This results in embryo expansion, elongation of the radicle, and is concluded when the radicle punctures the surrounding tissues. This process consists of three phases; phase I, imbibition, is a rapid early water uptake, followed by a plateau period, or Phase II. In Phase III, water uptake once again rapidly increases causing the radicle to elongate and break through the outer layers to conclude germination. It is important to note that dormant seeds can only undergo Phases I and II; they cannot enter Phase III until they become nondormant and complete

germination. Most angiosperm embryos are protected by two covering layers, the inner endosperm and the outer testa, or seed coat. Cell elongation is required for radicle protrusion through the covering layers, which is considered the sign of visible germination (6).

Nondormant seeds can germinate over a wide range of physical and environmental conditions for their specific genotype. Some of the basic requirements for germination are water, oxygen, and appropriate temperature. Seeds can also be sensitive to other inputs such as light or chemicals (4, 5, 13). The threshold stimulus of these requirements needed for germination varies greatly among seeds even in an individual population, causing germination to be a non-synchronous event (6).

Seed dormancy and its ability to block germination have evolved differently in each species as an adaptation to the natural environment. This allows the germinating seed the best likelihood of establishment and reproduction (4, 6, 15). Given a wide range of environmental conditions, it is no surprise that there is an equally wide range of naturally occurring blocks to germination, or dormancy mechanisms.

Dormancy is not only a lack of germination, but is a characteristic of the seed that decides the conditions needed for germination (11, 28). Baskin and Baskin have provided a modern definition of seed dormancy: "a dormant seed does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favorable for its germination, i.e. after the seed becomes nondormant" (4). Any environmental factor that modifies the conditions

required for germination is by definition modifying dormancy and when the seed ceases to require precise environmental cues it is nondormant (12).

The event that determines the end of seed germination and the start of seedling growth is radicle protrusion through structures surrounding the embryo. Radicle protrusion is driven by turgor pressure and may or may not involve cell division. There are three possible methods that control radicle protrusion. One method involves osmotic potential ($\psi\pi$); as solutes collect in the radicle towards the end of the germination process, the radicle cells develop a negative osmotic potential causing increased water uptake and cell extension. A second method is that the capacity of radicle cells to expand by cell wall loosening is great enough to allow them to penetrate surrounding tissue. Another possibility is the tissues surrounding the radicle must be weakened, possibly by growth regulators or other chemicals, before protrusion can occur. This last method is a much more common occurrence and has been studied in depth (6).

Key Factors in the Maintenance of Dormancy and Initiation of Germination

Dormant, imbibed seeds are metabolically active and are capable of receiving external stimuli that can release dormancy and initiate germination. These stimuli can include chemical or hormonal signals or environmental signals such as temperature, soil nitrate levels, or light (6). Abscisic acid (ABA) and gibberellic acid (GA) have a considerable influence on dormancy and germination in seeds. ABA and GA act antagonistically during seed maturation, dormancy, and germination (12). ABA is a principal controller of dormancy induction and maintenance (6, 17). There is overwhelming evidence to support ABA as an essential factor in the dormancy control of

many species (6). Seed deficient in ABA during development lack primary dormancy once dispersed. Conversely, an over expression of ABA synthesis genes during seed development enhances dormancy and hinders germination (12). Only ABA produced by the seed during development can provide a prolonged dormancy period (17).

ABA acts by preventing radicle protrusion. The effect is so strong that ABA can even inhibit protrusion late in the germination process, possibly by preventing the cell wall loosening process from occurring (6). In nondormant *Brassica napus* L. embryos, complete germination was prevented when ABA inhibited radicle protrusion by preventing cell wall loosening (25).

GA is an important component of dormancy release and the stimulation of seed germination (12). GA promotes germination, reduces many of the environmental requirements for germination, and has a role in counteracting the inhibitory effects of ABA. It is most active after inhibition of germination by ABA has been suppressed (6, 7). The importance of GA to germination has been demonstrated through the study of GA-deficient mutants of *Arabidopsis* and tomato; both required an external source of GA to conclude germination (6).

An increase in GA concentration and/or a reduction in ABA synthesis are necessary for dormancy release to occur, resulting in a shift to low ABA:GA ratios (17). This shift causes an increase in embryo growth potential and weakens the tissues surrounding the radicle, thus eliminating mechanical restraints (6, 17).

As an environmental signal, temperature has an intense effect on the dormancy and germination status within seeds (7, 16, 17). Temperature controls dormancy cycling

in the soil. Seeds have the ability to 'store' soil temperature information to monitor seasonal change for proper germination time. Light stimulates germination and terminates dormancy in many species (6, 16). Light is perceived by seeds through phytochrome receptors and has been shown increase bioactive GA concentration (16). Light acts with GA to release dormancy and promote germination in numerous seeds with seed coat dormancy (17).

Types of Dormancy

Seed dormancy is broken into two main categories, primary and secondary dormancy. Primary dormancy is invoked during seed development by the production of ABA in the mother plant, the osmotic environment, or both (6, 15). High ABA levels and a poor osmotic environment are easily overcome in nondormant seeds by drying during maturation and dispersal by the mother plant (6). Secondary dormancy is generally induced when changing environmental conditions cause undesirable germination conditions, such as unfavorable temperature, extended light or darkness, water stress, or lack of oxygen (7, 13). Secondary dormancy is usually related to seasonal dormancy cycles and can be lost and re-introduced repetitively until the necessary germination conditions are met. Secondary dormancy is thought of as conditional dormancy because germination is limited by a narrow range of accommodating environmental factors. These factors can change rapidly inducing seed into a deeper state of dormancy at any point during dormancy loss (5). Seeds with primary dormancy are dispersed in a dormant state from the mother plant; however, seeds with secondary dormancy can be dispersed in a dormant or nondormant state (6).

Primary dormancy is the main focus of modern dormancy research (15) and much work has been conducted to further classify primary dormancy based upon the morphological and physiological requirements needed to break dormancy and induce seed germination (12). Primary dormancy can be exogenous, endogenous, or combinational (13). Marianna G. Nikolaeva created a dormancy classification system in 1967 (12, 21). Based on Nikolaeva's system, Baskin and Baskin proposed a classification system using five classes of seed dormancy, where each class is broken down into specific levels and types. Baskin and Baskin have recommended that this system be used to classify dormancy (4, 12).

Physiological dormancy is the most predominant class of dormancy and is common in gymnosperms and angiosperms (4). It is attributed to the restraints caused by the seed coverings that block the radicle from emerging from the coverings (13). Many widely researched species exhibit physiological dormancy as their primary form of dormancy; therefore much research has been conducted on this dormancy class. Moist cold stratification relieves physiological dormancy in nature. Treatment of seeds with gibberellic acid, scarification, after-ripening in dry storage, cold or warm stratification, light, or darkness can be used to break dormancy, depending on the species (4, 13). Many small seeded species with nondeep, physiological dormancy require light or darkness for germination to occur. This sensitivity to light is mediated by phytochrome. There is often an interaction between light and temperature allowing the light requirement to be compensated for by cool or alternating temperatures (13).

Physiological dormancy is divided into nondeep, intermediate, and deep levels, separated by the length of stratification time required to relieve dormancy. Short periods, days to a few months, are needed to break nondeep dormancy while intermediate dormancy requires at least 2 months of cold stratification and responds to external applications of GA as a substitute for chilling (13). Deep physiological dormancy is divided into subtype "a", requiring cold stratification before germination can occur, and subtype "b", requiring warm stratification before germination can occur. Several months of stratification is required for germination and gibberellic acid treatment does not break dormancy in seeds with deep physiological dormancy (12).

The second class of dormancy, morphological, is characteristic of seeds with embryos that are immature at dispersal (4). Seeds are thought to have morphological dormancy if the embryo fills less than half of the seed at dispersal (5). The embryos are not truly dormant, but require growth before germination can occur. Embryo growth occurs after the seeds have imbibed, but before germination initiates. There are three types of morphological dormancy in herbaceous crops, rudimentary, linear, and undifferentiated, based on embryo type (13).

Rudimentary embryos are minute embryos nested in substantial endosperm tissue. These are common in many families including Ranunculaceae, Papaveraceae, and Araliaceae. Linear embryos are torpedo in shape and fill up to half of the seed. These are common in members of Apiaceae, Ericaceae, Primulaceae, and Gentianaceae. Undifferentiated embryos occur in the Orchidaceae family and lack abundant seed storage materials (3).

The third class of dormancy, morphophysiological, is a combinational dormancy; seeds have immature embryos and a physiological requirement to break dormancy (13). Seeds with this class of dormancy usually require a specific combination of warm and/or cold stratification treatments to break dormancy (4). All conditions inhibiting germination must be removed in proper order to induce germination (13). There are eight known levels of morphophysiological dormancy (4), with two types of horticultural interest, simple and epicotyl (13).

Warm conditions, above 15C, followed by cold, 1 to 10C, are required for breaking simple, morphophysiological dormancy. Seeds have an immature, linear, embryo that develops in the warm temperature period. Once the embryo reaches mature size, physiological dormancy can be released during the chilling period. Seeds with epicotyl dormancy have different dormancy breaking requirements for the radicle and the epicotyl (13).

The physical dormancy class is attributed to water resistant layers of cells in the seed coat (testa) or endocarp that control water movement into the seed (4). Imbibition of embryo water uptake is prevented by palisade cells in these layers. In nature, physical dormancy is broken by changing temperatures. For horticultural and agronomic purposes it can easily be relieved by mechanical abrasion, chemical scarification, or submersion in hot water (13). The fifth class of dormancy, combinational dormancy, is simply the combination of physiological and physical dormancy (4).

Release of Nondeep Physiological Dormancy

The most widespread dormancy class in Baskin and Baskin's classification system is physiological dormancy (12). The predominance of this dormancy class means that the release of nondeep physiological dormancy has been extensively studied, revealing that this type of dormancy consists of an embryo and/or a coat component. Embryo dormancy refers to a block that prevents the growth and extension of the embryo. Excised embryos will not grow until this block is removed. Coat dormancy is caused by the mechanical resistance of the embryo against the testa, endosperm, and/or pericarp (6, 15).

Embryo dormancy is attributed to a high ABA:GA ratio, while dormancy release is attributed to a decrease in ABA sensitivity and an increase in GA sensitivity, or a decreasing ABA:GA ratio. Once an embryo is released from dormancy there is an increase in growth potential and cell extension growth and the capability to bring on the release of coat dormancy (12).

Coat dormancy is caused by the combination of low growth potential and mechanical restraints by the testa or endosperm tissue (6). Coat dormancy is different from hard seed coat induced physical dormancy (12). The testa is made up of mostly dead tissue and its characteristics are influenced during seed development by ABA. Rupture of the testa is assisted by enzymes released from the endosperm and/or radicle. The endosperm is living tissue and can actively regulate the ABA:GA ratio. ABA to some degree inhibits endosperm weakening, while GA increases embryo growth potential and assists in endosperm weakening, thus helping to break coat dormancy (6, 12, 16).

Recent research has proposed that cell-wall modifying proteins, such as endo-β-l,4-mannanases and endo-β-1,3-glucanases, cause endosperm weakening and coat dormancy loss. The β-1,3-glucanases regulate cell to cell movement (6, 12, 16, 17) and are hypothesized to cause endosperm weakening by breaking intercellular adhesion, causing cell separation (12).

Due to the widespread geographical distribution of physiological dormancy, the initiation and loss of dormancy can be brought on by many physiological means (5). Predispersal environmental conditions, such as light quality, photoperiod, and temperature, and postdispersal conditions, such as temperature, light, and soil nutrients (10) can all affect physiological dormancy. The conditions needed to remove physiological dormancy can vary greatly even within the same species, but are often related to seasonal changes or disturbances in the soil (5). This is often an ongoing process as the temperature range and seed sensitivity to light or other factors expands and narrows continuously (4).

Conclusion

Lenten rose is a highly desired plant by gardeners, but an extended germination period causes production to be expensive. The processes of seed dormancy and germination are poorly understood. Dormant seeds are sensitive to chemical, hormonal, and environmental signals. Seed dormancy is classified based on morphological and physiological seed properties (Table 1.1). Lenten rose is thought to have morphophysiological dormancy, a combinational dormancy attributed to a rudimentary embryo and a chemical inhibitor that requires leaching or neutralization by cold

stratification. This dormancy and the mechanisms that break it and induce germination need further study.

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Table 1.1. Classification and characteristics of primary seed dormancy (from information in Baskin and Baskin, 2004 and Geneve, 2003).

1. Physiological
   Factors within the embryo inhibit germination.
      Levels
         Deep
            Requires several months of cold stratification for dormancy release
            GA does not promote germination
         Intermediate
            Requires 2-3 months cold stratification for dormancy release
            Some species respond to external GA applications
         Non-deep
            Requires short periods of cold or warm stratification to break dormancy
            GA promotes germination
            Types (of non-deep)
               Embryo dormancy
               Coat dormancy
2. Morphological
   Embryos are underdeveloped at seed dispersal.
      Types
         Rudimentary embryo
         Linear embryo
         Undifferentiated embryo
3. Morphophysiological (physiological + morphological)
   Seeds have immature embryos and a physiological requirement to break dormancy.
      Levels
         Non-deep simple
         Intermediate simple
         Deep simple
         Deep simple epicotyl
         Deep simple double
         Non-deep complex
         Intermediate complex
         Deep complex
4. Physical
   Water-impermeable seed coat.
5. Combinational (physiological + physical)
   Water-impermeable seed coat and a physiologically dormant embryo.

CHAPTER II

TEMPERATURE EFFECTS ON THE SEED GERMINATION AND SEEDLING GROWTH OF LENTEN ROSE

Abstract

Seed production of Lenten rose (*Helleborus* × *hybridus*) allows for lower propagation costs but significant difficulties occur due to poor germination. These difficulties are due to some form of morphophysiological dormancy requiring a specific combination of warm and/or cold stratification to break dormancy. The objectives of this experiment were to determine the number of weeks of warm followed cold stratification to increase germination rate, shorten time to germination, and increase synchronous germination in Lenten rose. Treatments consisted of 4, 6, 8, or 10 weeks of warm 25 (77F) durations followed by 4, 6, 8, or 10 weeks of cold 4C (39F) durations plus a control. The fewest days to radicle, hypocotyl, and first true leaf emergence generally occurred with the shorter durations of warm and cold. Ten weeks of warm duration produced the highest radicle, hypocotyl, and true leaf counts with no effect of cold duration on radicle or hypocotyl counts. Four weeks of cold duration produced the highest true leaf count. Average width decreased with increasing durations of cold and warm. While height decreased with increasing warm durations, the best cold durations were found at 6 and 8 weeks.

Index words: *Helleborus* × *hybridus*, dormancy, morphophysiological dormancy, seed propagation, Hellebore, stratification

Species used in this study: *Helleborus* × *hybridus* (Lenten rose)

Significance to Industry

Lenten rose is well suited to dry shade gardens in the southeastern United States but has significant production difficulties due to complex dormancy and poor germination. An increase in germination rate, decrease in time to germination, and more synchronous germination would result in more efficient propagation. In this study, the fewest days to radicle, hypocotyl, and first true leaf emergence generally occurred with the shorter durations of warm and cold stratification. Ten weeks of warm duration produced the highest radicle, hypocotyl, and true leaf counts while 4 weeks of cold duration produced the highest true leaf counts. Plant height and average width indicate that shorter durations of warm and cold produced larger plants in this seed lot, but any method that decreases time to emergence and allows plants to begin growth earlier will benefit plant size. There is not a single warm and cold duration that provided the best emergence and count of radicle, hypocotyl, and first true leaves or the overall best seedling performance based on height and width in this particular seed lot. It is up to the grower to select which characteristics are most important to their production system.

Introduction

Lenten rose (*Helleborus* × *hybridus*) is a perennial member of the Ranunculaceae, or buttercup, family with striking winter to early-spring flowers in an array of colors. It

is a popular ornamental, cut flower, and medicinal plant in Europe (14, 17). It is well suited to the climate of the southeastern United States and was named the 2005 Perennial Plant of the Year by the Perennial Plant Association (6, 15).

Lenten rose has many attractive features; it is easy to cultivate, hardy, long-lived, tolerant of dry conditions, evergreen, and deer resistant (6, 15, 17). Gardeners appreciate Lenten rose for its early flowers, beginning in February or March and lasting for 2 to 3 months. Flowers come in a range of colors from whites, yellows, and greens to pinks, plums, purples, blue-blacks and even spotted shades. Flower forms are single, semi-double, or double, are up to 3 inches in diameter (13, 17), and are generally nodding (6). It grows in alkaline, semi-shade conditions in its native habitat (13) and is adaptable to USDA hardiness zones 4 to 9 (15) and partial to full shade. Lenten rose accepts a wide range of soil conditions, but dislikes poor drainage in the winter (13).

Lenten rose is propagated by division, in vitro propagation, or seed (14, 17). Division of rhizomes can be used when identical offspring are required, such as cultivars, but this process is time consuming and often results in a slow recovery time (13, 14, 17). In vitro propagation has the advantage of producing identical plants, thus allowing consumer selection of flower color prior to flowering. However, Lenten rose is difficult to produce in vitro (6) and better protocols are needed for a more efficient mass production system (18). Lenten rose is commonly propagated by seed when identical plants are not necessary or for the production of the named seed strains (13, 14).

Propagation by seed has the advantage of producing a substantial number of seedlings from one plant (13), but germination is slow in Lenten rose. Germination

requires many months and seeds often fail to germinate even under environmental conditions suitable for germination (14). Germination is commonly uneven forcing growers to transplant seedlings over several weeks or months until germination is concluded (16). The erratic and unpredictable nature of Lenten rose germination is undesirable to commercial growers who would desire higher and more synchronous germination.

Poor germination in Lenten rose is attributed to seed dormancy (6). This can be overcome by moist, warm stratification for 6 to 12 weeks followed by cold stratification (6, 9). Potassium nitrate and gibberellic acid soaks have been used to remove germination inhibitors in Christmas rose (*Helleborus niger* Linn.) but were ineffective (12). Once dormancy has been overcome, fresh hellebore seed will generally germinate at temperatures between 4C (40F) and 10C (50F) (9). This replicates the seeds natural temperature environment during dormancy release and germination (6).

Niimi et al. reported in a 2006 study on embryo development and seed germination of Christmas rose that there were few reports available on germination of *Helleborus* species. Observations of embryo development in this study indicated that Christmas rose has an underdeveloped, rudimentary embryo at seed dispersal and that dormancy release only occurred in seeds transferred from one temperature to another. Treatment at 25C for 8 weeks allowed the embryo to mature and chilling for at least 8 weeks at 4C was needed to break dormancy. The researchers concluded Christmas rose seeds have deep, simple morphophysiological dormancy, a combinational dormancy resulting from the immature embryo and a physiological dormancy (14).

Seed dormancy is described as a barrier to germination of an intact viable seed under favorable environmental conditions. Dormant, imbibed seeds are metabolically active and are capable of receiving external stimuli that can release dormancy. These stimuli include chemical or hormonal signals and environmental signals such as temperature, soil nitrate levels, moisture, or light (4). As an environmental signal, temperature has a strong effect on the dormancy status within seeds (5, 10, 11). Temperature controls dormancy cycling in the soil. Seeds have the ability to 'store' soil temperature information to monitor seasonal change for proper germination time (4, 10).

The majority of non-tropical imbibed seeds can be released from dormancy when stratified at 1-10C, but sometimes temperatures as high as 15C are effective. This is thought to be an adaptive mechanism within the seed to prevent loss of dormancy until after winter has passed. Chilling is a common practice in horticultural and forestry industries to break seed dormancy. Chilling treatments are not cumulative; periods of higher temperatures cancel chilling periods and can induce secondary dormancy (5).

Environmental factors also have an important role in the control of seed germination. Temperature impacts a seeds ability to germinate and the rate at which germination can occur. There is a defined minimum and maximum germination temperature for each species. The temperature at which a seed needs to germinate can be different, and must be studied separately, from the temperature needed to break dormancy (5). To understand how temperature affects dormancy and germination and how it is sensed within a seed, it is best to understand the type of seed dormancy that a seed possesses.

Based on the 2006 finding by Niimi et al., previous work by Lockhart on Christmas rose, and genus characteristics, it is most likely that Lenten rose has a deep, simple morphophysiological dormancy (6, 12, 14). Morphophysiological dormancy is a combinational dormancy (8). The morphological component is characteristic of seeds with embryos that are immature at dispersal (3). The embryos are not truly dormant, but require growth before germination can occur. Embryo growth occurs after the seeds have imbibed, but before germination begins (8). There are three types of morphological dormancy in herbaceous crops, rudimentary, linear, and undifferentiated, based on embryo type. Rudimentary embryos are minute embryos nested in substantial endosperm tissue (1) and have been reported in other members of the Ranunculaceae family (6, 12, 14).

The physiological dormancy component is attributed to restraints caused by the seed coverings, blocking the radicle from escaping the seed covering (8). Moist cold stratification relieves physiological dormancy in nature. Treatment of seeds with gibberellic acid, scarification, after-ripening in dry storage, cold or warm stratification, light, or darkness can be used to break dormancy, depending on the species (3, 8).

Physiological dormancy is divided into nondeep, intermediate, and deep levels, separated by the length of stratification time required to relieve dormancy. Short periods, days to a few months, are needed to break nondeep dormancy while intermediate dormancy requires at least 2 months of cold stratification and responds to external applications of GA as a substitute for chilling (8). Deep physiological dormancy requires several months of stratification and is divided into subtype a, requiring cold stratification

before germination can occur, and subtype b, requiring warm stratification before germination can occur (3). Seeds of *Helleborus* species most likely fall into subtype b.

Seeds with morphophysiological dormancy usually require a specific combination of warm and/or cold stratification to break dormancy (3). All conditions inhibiting germination must be removed in proper order to induce germination (8). There are eight known levels of morphophysiological dormancy (3), with two types of horticultural interest, simple and epicotyl (8). Simple morphophysiological dormancy is known to be present in Christmas rose (14). Warm conditions, 15C to 30C, followed by cold, 1 to 10C, are required for breaking simple, morphophysiological dormancy. Seeds have an immature, linear, embryo that develops during the warm temperature period. Once the embryo is of mature size, dormancy can be released during the chilling period (3, 8).

It is important to keep in mind when dealing with dormancy and germination that a seed is never just under the control of one factor in nature, but many factors concurrently (5). Predispersal environmental conditions, such as light quality, photoperiod, and temperature, and postdispersal conditions, such as temperature, light, moisture, and soil nutrients (7), can affect dormancy and germination. The conditions needed to remove dormancy can vary even within the same species (2). Dormancy loss and the initiation of germination is an ongoing, gradual process, especially in the case of temperature-dependent dormancy, where the temperature at which dormancy can occur expands as dormancy is released (3, 5).

The objectives of this experiment were to determine the number of weeks of warm followed cold stratification to increase germination rate, shorten time to germination, and increase synchronous germination in Lenten rose.

Materials and Methods

The experiment was conducted beginning in the winter of 2007 on the campus of Auburn University (Auburn, AL). Seeds of Lenten rose Hybrids and Red Hybrids (Jelitto Perennial Seed, Schwarmstedt, Germany) were received on February 12, 2007, and placed in dry, open paper bags at 4C (39F), until used in experiments.

Treatments consisted of warm stratification at 25C (77F) for 4, 6, 8, or 10 weeks followed by cold stratification at 4C (39F) for 4, 6, 8, or 10 weeks plus a control for Lenten rose Hybrids and Red Hybrids (Table 2.1). Each treatment and the control were replicated four times with 20 seeds per replication.

Germination containers were clear slim compact disc cases (14.2×12.5×0.5 cm) containing germination paper (Kimpak, Seedburo Equipment Company, Bozeman, MT) cut to 12×10 cm. Seeds were allowed to imbibe in aerated, distilled water for 8 h at room temperature on February 15, 2007 then surface sterilized in a 15% bleach (6% sodium hypochlorite) and distilled water solution for 3 min followed by three, 1 min rinses in distilled water. Seeds were placed on germination paper evenly moistened with distilled water, 20 seeds per case, cases sealed and placed in plastic racks. All treatments were sown on February 16, 2007. Treatments B through Q were placed in a Pro-Grow PC-70 germination chamber (Pro-Grow Supply Corp., Brookfield, WI) with a temperature set point of 25C (77F) in the Plant Sciences Center (Auburn, AL). The control, A, was

placed in a 10C (50F) cooler in Funchess Hall (Auburn, AL), until radicle emergence occurred. Treatments were watered with distilled water as needed to remain moist.

On February 26, 2007, heavy fungal infection was observed on all treatments in most replications. Symptoms consisted of white to light pink colored mycelia-like growth rapidly spreading outward from infected seeds. Severely infected seeds became soft and gelatinous and began to leak their contents. The decision was made to remove and surface sterilize seeds and containers, replace germination paper, and replace the distilled water with a 100 ppm solution of Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, CA) and distilled water to moisten germination paper. The Blocker 4F solution was also used to water treatments as needed until the completion of radicle emergence. Seeds were removed from treatment containers on March 1, 2007 and surface sterilized. Heavily infected seeds were removed from treatments reducing the number of seeds per replication to 16. Treatments B through Q were placed back in the germination chamber and monitored for additional infection.

On March 10, 2007, heavy infection was again observed in the Lenten rose Hybrids. The Lenten rose Red Hybrids remained free of infection. Throughout the next week, infection of the Lenten rose Hybrids seed progressed so rapidly that the majority of seed were destroyed and it was decided to restart this part of the experiment. A sample of infected treatments were kept and allowed to continue to progress for later identification of the fungal pathogen. The pathogen was diagnosed by the Auburn University Plant Diagnostic Lab as an unidentifiable water mold type, oospore producing fungal-like

organism, similar to *Pythium* spp., a common cause of seed rot. On March 16, 2007, seeds of Lenten rose Hybrids treatments were sown as they were on February 16, 2007 with the following changes. Only the Blocker 4F solution was used to moisten the germination paper and to keep treatments moist during the experiment.

Within weeks, all of the Lenten rose Hybrids were once again severely infected. It was decided at this point that the level of infection in this seed lot was too high to continue, so it was discontinued.

Radicle Emergence

After completion of their prescribed duration (Table 2.1) of warm stratification, Lenten rose Red Hybrids treatments B through Q were individually moved to a cooler set at 4C (39F) in the Paterson Greenhouse Complex (Auburn, AL). Following completion of their prescribed duration (Table 2.1) of cold stratification, treatments B through Q were individually moved to a cooler set at 10C (50F) in Funchess Hall (Auburn, AL), until radicle emergence occurred. Treatments were kept in darkness and exposed to light only when examined. Treatments were monitored approximately every 3 days to record the date of radicle emergence, defined as the day the radicle reached 3 mm in length. The range of radical emergence was determined from the start of the experiment on February 16, 2007. Radicle emergence and range were continuously recorded until this portion of the study was terminated on September 30, 2007.

Hypocotyl Emergence

Upon radicle emergence, individual seeds were removed from germination containers, planted directly into 10 cm (4 in) pots (Poppelman Plastics, Claremont, NC)

filled with growing medium (SunShine GBX General Purpose Professional Growing Mix, Sun Gro Horticulture, Bellevue, WA), and covered with 0.3 cm (0.125 in) of mix. Pots were placed back in the 10C (50F) cooler until hypocotyl emergence occurred. The media was watered as needed and misted once a week with a 100 ppm solution of Blocker 4F to inhibit fungal growth. Pots were examined for the emergence of seedlings approximately every 3 days to record the date of hypocotyl emergence, defined as the day when a visible shoot, at least 5mm, was present above the soil surface. The range of hypocotyl emergence was determined from the start of the experiment on February 16, 2007. Hypocotyl emergence and range were continuously recorded until this portion of the study was terminated on December 4, 2007.

Seedling Growth

Following hypocotyl emergence, individual seedlings were transferred to a climate controlled facility in the Patterson Greenhouse Complex (Auburn, AL). Seedlings were grown on tables receiving a 12 h daily photoperiod from 40 watt, cool-white fluorescent bulbs (762.8 lux intensity with 207.7 lux standard deviation) and watered as needed. Temperature was adjusted over a period of time from 10C (50F) to 22C (72F) to allow plants to acclimate from the climate controlled facility to existing outdoor daytime temperatures of approximately 22C (72F). Osmocote Classic 3-4 month release 14.25N-2.64P-10.56K (9-6-12, Scotts-Sierra Horticultural Products, Marysville, OH) was applied at the recommended rate on October 18, 2007 and as needed thereafter.

All plants were placed pot-to-pot on an outdoor nursery facility covered with approximately 50 % shade on October 18, 2007 and were watered by overhead irrigation

as needed. Substrate pH and electrical conductivity (EC) were monitored as recommended using the PourThru technique (19). Maintaining adequate fertility and appropriate pH were a significant problem January to March 2008. Additional fertilizer, ProCare Premium 7.5N-4.4P-8.8K (10-10-10, Gro Tec, Inc., Madison, GA), was applied at the recommended rate on January 18, 2008. In addition, plants were fertilized by hand as needed with TotalGro 15N-4.4P-8.8K (20-10-10, SDT Industries, Inc, Winnsboro, LA) applied at a rate of 150 ppm N. Citric acid at a pH of 3.9 from 50% citric acid (Seplex-L Organic Acid, Greencare Fertilizers, Inc., Kankakee, IL) was applied as needed to maintain soil pH at the recommended pH of 5.8-6.8 (16).

Root and crown rot disease were observed on February 18, 2008. A drench application was applied using Heritage (50% azoxystobin, Syngenta Crop Protection, Inc., Greensboro, NC) at 26.6 ml/3.79 l (0.9 oz/gal) of water and Truban 30 wettable powder (30% etridiazole, Scotts-Sierra Horticultural Products, Marysville, OH) at 204 g/3.79 l (0.45lbs/gal) of water through an injector was applied on February 19, 2008 and every three weeks thereafter.

The emergence of the first true leaves was recorded and the range of first true leaf emergence was determined from the start of the experiment on February 16, 2007. Plant height and average width (widest width + width 90°) / 2) were recorded approximately every three weeks beginning on February 26, 2008. The study was concluded on May 14, 2008.

The data were analyzed as a completely randomized design using SAS version 9.1.3 (SAS Institute, Cary, NC) with the warm and cold treatments in a factorial

treatment arrangement. The normality assumption for ANOVA was tested using the tests for normality statistics in PRO UNIVARIATE. Data were considered non-normal when the Shapiro-Wilk, the Kolmogorov-Smirnov, the Anderson-Darling, and the Cramér-von Mises tests were significant at $\alpha = 0.05$. The number of days to radicle emergence, plant height, and average plant width were analyzed with PROC GLM and the number of days to hypocotyl emergence and to first true leaf emergence were analyzed with PROC GENMOD using either the Poisson or negative binomial probability distribution depending on which distribution minimized the Pearson Chi-Square test for goodness of fit. Days to radicle emergence, hypocotyl emergence, and first true leaf were determined for each treatment and replication combination and subtracted to determine range. These data were analyzed using the same procedures as those above. The number of radicles emerged, the number of hypocotyls emerged, and the number of plants with true leaves out of the total number of seed sown per treatment combination was analyzed with PROC GENMOD using the binomial probability distribution. Plant height and average width were analyzed using PROC MIXED with the data collection period and warm and cold treatments in a factorial treatment arrangement. Single degree of freedom orthogonal contrasts were used to test linear and quadratic treatment trends at $\alpha = 0.05$. The control was not included in data analysis because there were an insufficient number of seeds that reached radicle emergence to complete the analysis.

Results and Discussion

There was an interaction of warm and cold duration for days to radicle emergence (Table 2.2). There were linear increases in the days to radicle emergence for both cold

and warm with increasing duration, 13-21 days increase with warm and 44-49 days increase with cold. Four weeks of warm followed by 4 weeks of cold resulted in the fewest days to radicle emergence, while 10 weeks of warm followed by 10 weeks of cold resulted in the greatest. There was a warm by cold duration interaction for radicle emergence range. Six weeks of warm resulted in a 22 day linear increase in the range of radicle emergence with increasing cold duration, but other warm durations were not different. Radicle emergence range at 4, 8, and 10 weeks cold resulted in a linear increase of 16-18 days, while 6 weeks cold resulted in a quadratic change of 18 days with the narrowest range at 4 weeks warm. The narrowest radicle emergence range was at 4 weeks warm and 10 weeks cold. However, this result may be misleading because only 17% of seed had radicles emerged.

Warm and cold main effects were significant for days to hypocotyl emergence (Table 2.2). There were linear increases of 33 days to hypocotyl emergence with increasing warm duration and 9 days with increasing cold duration. Therefore, increasing warm duration had a larger impact on days to hypocotyl emergence than increasing cold. The warm duration main effect was significant for hypocotyl emergence range with a quadratic change of 15 days with increasing warm. Like radicle emergence range, the narrowest range for hypocotyl emergence occurred after 4 weeks warm.

There was a warm by cold duration interaction for days to first true leaf emergence (Table 2.2). After 6 to 10 weeks warm, there were linear increases of 9 and 19 days, respectively, in the days to first true leaf emergence with increasing cold duration. Days to first true leaf emergence were not significant at 4 and 8 weeks of

warm. However, for all four durations of cold stratification, there were linear increases of 18, 42, 39, and 45 days, respectively, in the days to first true leaf emergence with increasing warm duration. Four weeks warm followed by 6 weeks cold resulted in the fewest days to first true leaf emergence, while 10 weeks warm followed by 10 weeks cold resulted in the greatest.

There was an interaction of warm and cold duration for the range of first true leaf emergence (Table 2.2). Range increased quadratically after 4 and 6 weeks of warm with increases of 11 and 33 days, respectively, with increasing cold duration. Conversely, after 8 and 10 weeks of warm, there were linear increases of 17 and 64 days, respectively, in the range of first true leaf emergence with increasing cold duration. With 4, 6, and 10 weeks of cold, there were increases of 71, 43, and 28 days, respectively, in the range of first true leaf emergence with increasing warm duration. Range of first true leaf emergence was not different at 8 weeks of cold stratification. The narrowest range of first true leaf emergence occurred at 4 weeks warm and 10 weeks cold. However, the result at 10 weeks cold may be suspect due to the low number of surviving plants.

The main effects were significant for warm duration in radicle and hypocotyl counts (Table 2.3). There was a linear increase of 36 seeds with radicle count and 31 seeds with hypocotyl count with increasing warm duration. Warm and cold duration main effects were significant for true leaf counts with linear increases of 26 seedlings with first true leaves with increasing warm duration and 15 seedlings with increasing cold duration.

There was an interaction of warm and cold duration for plant height (Table 2.4). After 4, 6, 8, and 10 weeks of warm, there were linear decreases of 1.1, 0.8, 0.5, and 2.8 cm, respectively, in plant height with increasing cold duration. Height decreased quadratically after 4, 6, and 8 weeks of cold with decreases of 1.3, 1.9, and 2.8 cm, respectively, with increasing warm duration. After 10 weeks of cold, there was a linear decrease of 2.6 cm in plant height with increasing warm duration. There was an overall trend toward taller plants with the shorter warm and cold durations.

Warm and cold duration main effects were significant for average plant width with linear decreases of 2.4 cm in average plant width with increasing warm duration and 1.3 cm with increasing cold duration (Table 2.4). Plant width followed a trend similar to plant height. Data collection period main effect was significant for both height and width. Both seedling height and width increased in a linear fashion by 5.9 cm between the first and last data collection periods.

It was a general pattern with this seed lot, although not always significant in the case of warm duration, that the fewest days to radicle, hypocotyl, and first true leaf emergence occurred with the shorter durations of warm and cold. The results for radicle, hypocotyl, and true leaf counts indicate that 10 weeks of warm duration produced the highest radicle, hypocotyl, and true leaf counts with no effect of cold duration on radicle and hypocotyl counts. The highest true leaf count for cold duration occurred at 4 weeks, indicating that 4 weeks cold duration is best.

The results for average width indicate that plant growth of Lenten rose decreased with increasing durations of cold and warm. While height decreased with increasing

warm durations, the best cold durations were found at 6 and 8 weeks. While these results indicate that shorter durations of warm and cold produced larger plants in this seed lot of Lenten rose, this is are potentially misleading. Actually an increase in growing time will increase plant size. Therefore, any method that decreases time to emergence and allows plants to begin growth earlier will benefit plant size.

The interactions of warm and cold duration reported in this study support previous research by Niimi et al. that members of the *Helleborus* genus have morphophysiological dormancy. The temperature range that is best for embryo development and release of dormancy differs at each embryo developmental stage (14). This is typical of other members of the Ranunculaceae family (1). However, 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 (14). The physiological component of dormancy determines the dormancy level based on cold stratification requirements and the response to external gibberellic acid (GA) applications (8). Based on the results of this study, this seed lot of Lenten rose exhibits nondeep, simple morphophysiological dormancy due to the fewest days to radicle and hypocotyl emergence and the highest first true leaf counts occurring after 4 weeks of cold stratification.

Nondeep, simple morphophysiological dormancy is typically reported in seeds that mature in late spring and germinate in fall. Physiological dormancy in these seeds is broken during high summer temperatures (2). Although this is not the case with Lenten rose, nondeep, simple morphophysiological dormancy has been reported in seeds of

Thalictrum mirabile Small, a member of the Ranunculaceae family that, like Lenten rose, requires cold stratification for the loss of physiological dormancy (20). These results are consistent with reports that Lenten rose is quicker to germinate than other acaulescent Hellebore species (6).

The overall results of this study indicate that there is not one clear warm and cold duration that provided the best emergence and count of radicle, hypocotyl, and first true leaves or the overall best seedling performance based on plant height and average width in this particular seed lot. Since only warm main effects were significant for radicle and hypocotyl count and the highest true leaf count was at 4 weeks cold duration, it is worth exploring if shorter periods of cold duration could be effective in germination of Lenten rose. It is up to the grower to select which characteristics are most important to their production system.

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Table 2.1. Warm followed by cold treatments of Lenten rose seeds.

Treatment	Warm weeks at 25C (77F)	Cold weeks at 4C (39F)
A	0	0
B	4	4
C	4	6
D	4	8
E	4	10
F	6	4
G	6	6
H	6	8
I	6	10
J	8	4
K	8	6
L	8	8
M	8	10
N	10	4
O	10	6
P	10	8
Q	10	10

Table 2.2. Effect of warm 25C (77F) followed by cold 4C (39F) stratification duration on days to and range of radicle, hypocotyl, and first true leaf emergence in Lenten rose seeds[z].

Cold (weeks)	Days to radicle emergence[y]					Radicle emergence range (days)				
	Warm (weeks)					Warm (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	

Days to hypocotyl emergence[w]				Hypocotyl emergence range (days)[v]	
Warm (weeks)		Cold (weeks)		Warm (weeks)	
4	152	4	170	4	13
6	165	6	172	6	35
8	175	8	173	8	29
10	185	10	179	10	28
Sign.	L***		L***		Q***

Cold (weeks)	Days to first true leaf emergence[u]					First true leaf emergence range (days)				
	Warm (weeks)					Warm (weeks)				
	4	6	8	10	Sign.	4	6	8	10	Sign.
4	238	235	252	256	L***	30	75	48	101	L***
6	220	237	259	262	L***	37	46	40	80	L***
8	234	250	258	273	L***	40	42	42	39	NS
10	230	244	258	275	L***	29	54	31	57	L***
Sign.	NS	L**	NS	L***		Q**	Q***	L***	L***	

[z]Control not included in analysis

[y]The warm by cold duration interaction was significant at $\alpha = 0.05$. Radicle emergence was when radicles were 3 mm long.

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

[w]Warm and cold duration main effects were significant at $\alpha = 0.05$. Hypocotyl emergence was when hypocotyls were 5 mm above the soil surface.

[v]Warm duration main effect was significant at $\alpha = 0.05$.

[u]The warm by cold duration interaction was significant at $\alpha = 0.05$. First true leaf emergence was when the first true leaves were visible.

Table 2.3. Effect of warm 25C (77F) followed by cold 4C (39F) stratification duration on radicle, hypocotyl, and first true leaf emergence counts in Lenten rose seeds[z].

Warm (weeks)	Radicle count[y]	Hypocotyl count
0	4	4
4	10	10
6	34	30
8	32	28
10	40	35
Sign.	L***	L***

True leaf count[x]

Warm		Cold (weeks)	
0	4	0	4
4	9	4	27
6	25	6	24
8	24	8	18
10	30	10	19
Sign.	L***		L***

[z]Four replications per treatment of 16 seeds per replication.
[y]Warm duration main effect was significant at $\propto$=0.05.
[x]Warm and cold duration main effects were significant at $\propto$=0.05.

Table 2.4. Effect of warm 25C (77F) followed by cold 4C (39F) seed stratification duration on plant height and width in Lenten rose[z].

Plant height (cm)[y]					
	Warm (weeks)				
Cold (weeks)	4	6	8	10	Sign.
4	9.5[x]	8.8	8.0	8.2	Q*
6	10.5	9.6	8.4	8.6	Q***
8	9.8	9.7	8.8	7.0	Q*
10	8.4	8.0	7.5	5.8	L***
Sign.[w]	L*	L***	L***	L***	

Plant width (cm)[v]			
Warm (weeks)		Cold (weeks)	
4	10.0	4	9.0
6	9.0	6	9.6
8	8.5	8	8.7
10	7.6	10	7.7
Sign.	L***		L***

Data collection period[u]		
Date	Plant height	Plant width
2/26/2008	5.0	5.6
3/18/2008	7.6	7.4
4/8/2008	10.3	10.4
5/14/2008	10.9	11.5
Sign.	L ***	L***

[z]The control was not included in the analysis due to low germination.
[y]The warm by cold duration interaction was significant at $\propto$=0.05.
[x]Least squares means.
[w]Linear (L) or quadratic at $\propto$=0.05(*), 0.01(**), or 0.001(***) using orthogonal contrasts.
[v]Plant width: (widest width + width 90˚)/2.
[u]Warm and cold duration main effects were significant at $\propto$=0.05.

CHAPTER III

SEED DENSITY EFFECTS ON SEED GERMINATION AND SEEDLING GROWTH OF LENTEN ROSE

Abstract

Seeds of Lenten rose (*Helleborus* × *hybridus*) face significant production difficulties due to poor germination. A possible method for enhancing germination in Lenten rose is seed conditioning by density sorting to remove immature, damaged, low vigor, and non-viable seeds. Studies have shown that seeds with larger size or mass exhibit better germination rates, faster germination, and larger seedlings than smaller or lighter seeds. The objective of this experiment was to determine if density sorting of Lenten rose seeds prior to sowing affects germination rate, time to germination, or range of germination. Treatments consisted of four seed density groups established using a density separator and an unsorted control. Density sorting had an effect on radicle, hypocotyl, and first true leaf counts the lowest density and the unsorted control, with the highest counts in the unsorted control and the lowest counts in the lowest density. Density sorting had little effect on germination rate, time, or range of germination. Seed in the control group had the highest radicle, hypocotyl, and first true leaf counts. The lowest density, was greatly impacted by the incidence of disease and it is recommended that this fraction of seed be removed by density separation before sowing.

Index words: Hellebore, *Helleborus* × *hybridus*, propagation, seed conditioning, seed dormancy, seed grading

Species used in this study: *Helleborus* × *hybridus* (Lenten rose)

Significance to Industry

Lenten rose is well suited to dry shade gardens in the southeastern United States but faces significant production difficulties due to poor germination. An increase in germination rate, decrease in time to germination, and more synchronous germination would result in more efficient propagation. Studies have shown that seeds with larger size or mass have better germination rates, faster germination, and larger seedlings than smaller or lighter seeds. Few differences in germination rate, time, and spread of germination were observed in this study. However, the incidence of diseased seed in this Lenten rose seed lot was greatly impacted by density sorting. The incidence of seed and seedling related infections could possibly be reduced by eliminating the lowest density seeds of Lenten rose.

Introduction

Lenten rose (*Helleborus* × *hybridus*) is a perennial member of the Ranunculaceae, or buttercup, family offering impressive winter to early-spring flowers in an assortment of colors. It is a popular ornamental, cut flower, and medicinal plant in Europe and is well suited to the southeastern United States (12, 15). Lenten rose is easy to cultivate, hardy, long-lived, tolerant of dry conditions, evergreen, deer resistant, and is considered to have the showiest flowers of the hellebore species (2, 13, 15). Lenten rose has enjoyed

increasing popularity in the United States since the 1980s (2). Gardeners are attracted to Lenten rose for its early flowering, beginning in February or March. Flowers last for 2 to 3 months and come in a multitude of colors from whites, yellows, and greens to pinks, plums, purples, blue-blacks and even spotted forms. Flowers appear as single, semi-double, or double forms, are up to 3 inches across (10, 15), and generally appear as nodding forms (2). Lenten rose accepts a wide range of soil conditions, but dislikes poor drainage in the winter. It grows in alkaline, semi-shade conditions in its native habitat (10) and is adaptable to USDA hardiness zones 4 to 9 (13) and partial to full shade. The plant grows 18-24 in (45-61 cm) in height and 24-30 in (61-76 in) in width and has pedately divided foliage with seven to eleven coarsely toothed, thick leaflets which add a nice texture to the garden (13, 15).

Lenten rose is difficult to propagate in both a nursery and laboratory setting (12, 15). When identical plants are necessary, division and in vitro propagation are the methods used for propagation. The division of rhizomes is time consuming and often results in a slow recovery time (10, 12, 15). Lenten rose is difficult to produce in a profitable quantity by in vitro propagation (2). Better protocols are needed for an efficient mass production system before in vitro propagation can become a reliable method of propagation (16). Lenten rose is commonly propagated by seed when identical plants are not necessary or for the production of the named seed strains (10, 12).

Propagation by seed has the advantage of producing a substantial number of seeds from one plant with 50 or more seeds produced by a single flower (10), but many months are needed for the seeds to germinate after they have been released from the mother plant

(12). A long germination period is undesirable to commercial growers who desire higher and more synchronous germination.

Lenten rose begins to germinate in December and continues to germinate throughout the spring (2) taking up to 18 months. Germination is uneven forcing growers to transplant seedlings over several weeks or months until germination has concluded (14). Hellebore seeds often fail to germinate even under environmental conditions suitable for germination (12). This is thought to be attributed to a combination of endogenous dormancies, both morphological and physiological (2). Morphological dormancy is attributed to a rudimentary embryo which must mature as the seed ripens (12). Physiological dormancy is believed to be caused by a chemical inhibitor which suppresses germination (2).

One possible method for increasing germination in Lenten rose is seed conditioning by density sorting. Conditioning improves the quality of the seed lot by removing immature or damaged seeds and those of low vigor or that are non-viable. Seed conditioning is used commercially and is well documented to improve uniformity and germination within a seed lot. Seed conditioning takes advantage of differences in seed size, weight, and density to separate seeds into higher quality lots (6).

Seed size is an essential factor to plant survival. The size of seeds determines the number the mother plant can produce, dispersal mechanisms, predation, germination, and long term seedling survival and performance (3, 7). Seed size, mass, density, and weight are used interchangeably in many references. Seed mass and weight are equal and are a function of seed density; however seed size relates to the volume of the seed. The

relationship between seed size, mass, and density is dependent on the structure of the seed coat, endosperm, and embryo, but can be used interchangeably for a given species if that species maintains a constant shape (3).

Seed mass varies tremendously within the plant kingdom. The variation is highest at the population level, among species in different habitats and at different stages of their life cycle. Variation continues to be high at the genus and family level and decreases at the species level, although variation at the species is often up to one order of magnitude (3).

The size of a seed is controlled by genetic and environmental factors. Genetic factors can include the pollinating plant, the mother plant, and the genetic makeup of the embryo. It appears that the mother plant has the most influence over genetic control of seed size, but little is known of the regulation of the genes involved (3). Seed size is a difficult trade off for the mother plant between producing many small seeds or fewer large seeds (9). Along with genetic factors, abiotic and biotic environmental conditions control seed size. In general, conditions which benefit the mother plant, such as proper water, nutrients, temperature, pH, and light, will increase seed size and weight (Castro).

Many studies have shown that seeds with larger size or mass will have better germination rates, faster germination, and larger seedlings than smaller or lighter seeds (3, 7, 9). A large size and mass could offer a seed many advantages over a smaller seed. These seeds have a larger embryo and/or endosperm providing them with the necessary reserves and greater defenses against environmental stress to increase their chances of fast embryo development and radicle elongation through the seed coat (3). Large seeds

are capable of emerging from greater depths after burial and of producing larger seedlings providing higher survival rates and competitive ability for water, light, and nutrients over smaller seeds (3, 9). Secondary compounds present in seed reserves and an increased resistance to environmental stress might also offer larger seeds better protection against pathogen infection than smaller seeds (4). Greater seed size and mass provide a seed with the most advantage when resources are scarce. Under adverse environmental conditions, the greater starting reserves give large seeds greater access to water, nutrients, and light than their smaller relatives. Variations in seed size and mass are less pronounced under agreeable environmental conditions (3).

However, Baskin and Baskin concluded that the effect of seed size on rate and percent germination varies by species based on their review of previous germination studies (1). In some studies, larger seeds have been shown to have slower germination rates, possibly due to their degree of dormancy or thicker seed coats which could inhibit imbibition and gas exchange (3, 11). It is believed that the combination of thicker seed coats and low ratio of surface to volume presents large seeds with a difficulty in obtaining sufficient water from the soil; however, nature has compensated for this by dispersing many large seeds in an undesiccated state (5). Larger seeds also have the disadvantage in nature of being more attractive to predators as a food source while they remain in the soil (7). It is interesting to note that small seeds in European flora have been associated with higher degrees of dormancy (9).

Although larger seeds generally have faster germination rates, their growth rates are slower across species. Small seeded species have the advantage in environmentally

favorable conditions of being able to quickly overtake large seeded, slow growing species (3, 17). Seedlings from larger seeds tend to store their carbon resources longer than smaller seeded seedlings, instead of using them to support respiration, resulting in a slower rate of growth in large seeded seedlings (9). The correlation between seed size and mass and seedling germination and growth is usually discontinued within a few weeks or months of emergence. Although this effect is short lived, it can still be a deciding factor between seedling survival and death (3). In the long term, large seed size has been attributed to greater plant height in a variety of temperate flora (8).

The density of Lenten rose seeds could have an effect on germination. Since it is known that one of the inhibitors of germination in Lenten rose is an immature embryo, sorting out lower density seeds could have the advantage of eliminating smaller embryos providing a higher percentage and more synchronous germination. Removing lower density seeds could also eliminate seeds less likely to survive adverse conditions, such as pathogen infections. Therefore, the objective of this experiment was to determine if density sorting of Lenten rose seeds prior to sowing affects germination rate, time to germination, or range of germination.

Materials and Methods

The experiment was conducted beginning in summer 2007 on the campus of Auburn University (Auburn, AL). Seeds of Lenten rose Red Hybrids (Jelitto Perennial Seed Schwarmstedt, Germany) were received on February 12, 2007, and placed in dry, open paper bags at 4C (39F), until used in experiments.

Treatments consisted of four seed density groups established using a density separator (Superior Fractioning Aspirator, Carter-Day Company, Minneapolis, MN). The aperture opening was adjusted until desirable density separation was achieved. Treatments one through three were collected from the first three bins and seed in the overflow bin was designated as the lowest density seed, density 4. An unsorted lot was also kept as a control. Seed weight of each density group was measured in 100 seed samples; density 1, 1596.2 mg/100 seeds, density 2, 1481.8 mg/100 seeds, density 3, 1346.5 mg/100 seeds, density 4, 1021.5 mg/100 seeds, and an unsorted control, 1448.6 mg/100 seeds. Each treatment and the control were replicated four times with 20 seeds per replication.

Germination containers were clear slim compact disc cases (14.2×12.5×0.5 cm) and germination paper (Kimpak, Seedburo Equipment Company, Bozeman, MT) cut to 12×10 cm. Seeds were allowed to imbibe in aerated, distilled water for 8 h at room temperature on August 7, 2007. The following day, seeds were surface sterilized in a 15% bleach solution (6% sodium hypochlorite) and distilled water for 3 min followed by three, 1 min rinses in distilled water. On August 8, 2007, treatments were placed on germination paper evenly moistened with a solution of 100 ppm Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, CA) and distilled water with 20 seeds per replication. Cases were sealed and placed on racks inside of plastic containers (42.4×27.9×27.4 cm) on benches in a climate controlled room at a constant 25C (77F) in the Plant Sciences Center (Auburn, AL). Container lids were kept partially opened to adjust relative humidity levels within the

containers. Treatments were kept adequately moist using a solution of 100 ppm Blocker 4F as needed until the completion of radicle emergence.

Radicle Emergence

After 8 wks of warm stratification at 25C (77F), all treatments were moved to a cooler set at 4C (39F) in the Paterson Greenhouse Complex (Auburn, AL). Following 4 wks of cold stratification in the 4C (39F) cooler, all treatments were moved to a cooler set at 10C (50F) in Funchess Hall (Auburn, AL), until radicle emergence occurred. Treatments were kept in darkness and exposed to light only when examined. Treatments were monitored approximately every 3 days to record the date of radicle emergence, which was defined as the day the radicle reached 3 mm in length. The number of seeds infected with fungus or fungal-like pathogens was recorded as this tended to be a problem in some treatments. Radicle emergence was continuously recorded until this portion of the study was terminated on March 10, 2008.

Hypocotyl Emergence

Upon radicle emergence, individual seeds were removed from germination containers, planted directly into 10 cm (4 in) pots (Poppelman Plastics, Claremont, NC) filled with potting medium (SunShine GBX General Purpose Professional Growing Mix, Sun Gro Horticulture, Bellevue, WA), and covered with 0.3 cm (0.125 in) of medium. Pots were placed back in the 10C (50F) cooler until hypocotyl emergence occurred. The medium was kept watered as needed and misted once a week with a 100 ppm solution of Blocker 4F to inhibit fungal growth. Pots were examined approximately every 3 days to record the date of hypocotyl emergence, defined as the day when a visible shoot was at

least 5mm above the medium surface. Hypocotyl emergence was continuously recorded until this portion of the study was terminated on March 31, 2008.

Seedling Growth

Following hypocotyl emergence, seedlings were transferred to an outdoor nursery facility covered with approximately 50 % shade in the Patterson Greenhouse Complex (Auburn, AL). Plants were placed pot-to-pot and watered by overhead irrigation as needed. Substrate pH and electrical conductivity (EC) were monitored as recommended using the PourThru technique (18).

Osmocote Classic 3-4 month release 14.25N-2.64P-10.56K (9-6-12, Scotts-Sierra Horticultural Products, Marysville, OH) was applied at the recommended rate three weeks after each plant was placed at the nursery facility. Citric acid at 3.9 pH from 50% citric acid (Seplex-L Organic Acid, Greencare Fertilizers, Inc., Kankakee, IL) was applied as needed to maintain soil pH at the recommended pH of 5.8-6.8 (14).

Root and crown rot disease were observed on February 18, 2008. A drench application of Heritage (50% azoxystobin, Syngenta Crop Protection, Inc., Greensboro, NC) at 26.6 ml/3.79 l (0.9 oz/gal), Truban 30 wettable powder (30% etridiazole, Scotts-Sierra Horticultural Products, Marysville, OH) at 204 g/3.79 l (0.45lbs/gal), and water through an injector was applied on February 19, 2008 and every 3 weeks thereafter.

The date of emergence of the first true leaves was recorded. Plant height and average plant width (widest width + width 90°) / 2) were recorded at study termination on May 14, 2008.

The data were analyzed as a completely randomized design using SAS version 9.1.3 (SAS Institute, Cary, NC) and PROC GLM. Tukey's mean separations was used to determine differences in density treatments at $\alpha = 0.05$.

Results and Discussion

There was a difference in response to seed density for radicle, hypocotyl, and first true leaf counts between density 4, the lowest density, and the unsorted control (Table 3.1). The highest radicle, hypocotyl, and first true leaf counts were in the unsorted control with the lowest counts found in density 4. However, radicle, hypocotyl, and first true leaf counts for densities 1, 2, and 3 were statistically similar to density 4 and the unsorted control. Days to radicle, hypocotyl, and first true leaf emergence and emergence range were not different.

During the study, it was observed that a large number of density 4 seeds became heavily infected with a fungal pathogen, which had been a problem in a previous study. Since larger seeds have the potential for greater resistance to pathogen infection than smaller seeds (4), it was determined that this effect should be studied. The pathogen was diagnosed by the Auburn University Plant Diagnostic Lab as an unidentifiable water mold type, oospore producing fungal-like organism, similar to *Pythium* spp., a common cause of seed rot. There was an effect of treatments on the incidence of diseased seed during the course of the study (Table 3.2). The highest incidence of diseased seed was found in density 4, while all other treatments were similar.

Density sorting of Lenten rose seed had little effect on germination rate, time, or range of germination. Surprisingly, unsorted seed had the highest radicle, hypocotyl, and

first true leaf counts. Although many studies have shown that larger seeds generally perform better (9), Baskin and Baskin have concluded that the effect of seed size on rate and percent germination varies by species (1) and other studies have concluded that larger seeds have slower germination rates (3, 11). It is possible that few differences in germination rate, time, and spread of germination were observed in this study because the seeds were not under conditions where resources were scarce. Under agreeable environmental conditions variations in seed size and mass are less pronounced (3).

The most important finding in this study was that the incidence of diseased seed in this seed lot was greatly impacted by density sorting. Secondary compounds in seed reserves and the increased resistance to environmental stress are thought to offer larger seeds better protection against pathogens (4). By eliminating the lowest density of Lenten rose seeds prior to sowing, a grower could reduce the incidence of seed and seedling related infections.

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Table 3.1. Effect of seed density on radicle, hypocotyl, and first true leaf emergence counts in Lenten rose.

Treatment[z]	Radicle count	Hypocotyl count	True leaf count
Density 1	4ab[y]	4ab	3ab
Density 2	4ab	4ab	4ab
Density 3	4ab	3ab	3ab
Density 4	2b	1b	1b
Unsorted	5a	4a	4a

[z]Density 1=1596.2 mg/100 seeds, density 2=1481.8 mg/100 seeds, density 3=1346.5 mg/100 seeds, density 4=1021.5 mg/100 seeds, and unsorted=1448.6 mg/100 seeds.
[y]Mean separation in columns using Tukey's studentized range test at $\propto$=0.05.

Table 3.2. Effect of seed density on disease incidence in Lenten rose.

Treatment[z]	Diseased seed count
Density 1	4b[y]
Density 2	4b
Density 3	6b
Density 4	14a
Unsorted	3b

[z]Density 1=1596.2 mg/100 seeds, density 2=1481.8 mg/100 seeds, density 3=1346.5 mg/100 seeds, density 4=1021.5 mg/100 seeds, and unsorted=1448.6 mg/100 seeds.
[y]Mean separation in columns using Tukey's studentized range test at $\propto$=0.05.

CHAPTER IV

GIBBERELLIC ACID EFFECTS ON SEED GERMINATION OF LENTEN ROSE

Abstract

Seed production of Lenten rose (*Helleborus* × *hybridus*) faces significant production difficulties due to poor germination. The seed have differentiated, but rudimentary embryos that are physiologically dormant at maturity. Temperatures of approximately 25C (77F) are required for embryo growth to occur followed by temperatures of approximately 4C (39F) to break physiological dormancy. Seeds of Lenten rose were treated with three GA_3 concentrations at 200 ppm, 400 ppm, or 800 ppm with three application times prior to warm stratification, between warm and cold stratification or after cold stratification and a control. Treatment of seeds with 200 ppm, 400 ppm, and 800 ppm GA_3 prior to warm stratification increased germination rates by 42% to 58%.

Index words: Hellebore, *Helleborus* × *hybridus*, gibberellins, growth hormone, growth regulator, morphophysiological dormancy, propagation, seed dormancy

Species used in this study: *Helleborus* × *hybridus* (Lenten rose)

Significance to Industry

Lenten rose is well suited to dry shade gardens in the southeastern United States but faces significant production difficulties due to poor germination. Higher and more synchronous seed germination would result in more efficient propagation. Treatment of Lenten rose seed with 200 ppm, 400 ppm, or 800 ppm GA_3 prior to warm stratification increased germination rates by 42% to 58%.

Introduction

Lenten rose (*Helleborus* × *hybridus*) is a perennial member of the Ranunculaceae, or buttercup, family with striking winter to early-spring flowers in an assortment of colors. It is a popular ornamental, cut flower, and medicinal plant in Europe and is well suited to the southeastern United States (13, 16). Lenten rose is easy to cultivate, hardy, long-lived, tolerant of dry conditions, evergreen, deer resistant, and is considered to have the showiest flowers of the hellebore species (3, 14, 16).

Gardeners are attracted to Lenten rose for its early flowers, beginning in February or March. Flowers last for 2 to 3 months and come in a multitude of colors including white, yellow, green, pink, plum, purples, blue-black and even spotted forms. Flowers can be single, semi-double, or double, are up to 3 inches across (12, 16), and are generally nodding (3). Lenten rose accepts a wide range of soil conditions, but dislikes poor drainage in the winter. It grows in alkaline, semi-shade conditions in its native habitat (12) and is adaptable to USDA hardiness zones 4 to 9 (14) and partial to full shade. The plant grows 18-24 in (45-61 cm) in height and 24-30 in (61-76 in) in width

and leaves are pedately divided with seven to eleven coarsely toothed, thick leaflets giving a nice texture to the woodland garden (14, 16).

Lenten rose is propagated by division, in vitro propagation, or seed (13, 16). Propagation by seed has the advantage of producing a substantial number of seedlings from one plant with 50 or more seeds produced by a single flower (12). However, many months are needed for seeds to germinate after they have been released from the mother plant and seeds often fail to germinate even under environmental conditions suitable for germination (13). Germination is often uneven forcing growers to transplant seedlings over several weeks or months until germination has concluded (15). The erratic and unpredictable nature of Lenten rose germination is undesirable for commercial growers who desire higher and more synchronous germination.

Poor germination in Lenten rose is thought to be attributed to a combination of morphological and physiological dormancies (3), better known as morphophysiological dormancy (13). Morphological dormancy is attributed to a rudimentary embryo that must mature as the seed ripens. This has been reported in others member of the Ranunculaceae family (3, 11, 13). The physiological component is believed to be caused by a chemical inhibitor which suppresses germination (3). Physiological inhibitors must be leached or neutralized from a fully developed embryo to allow germination to occur, typically by chilling, or cold stratification (3, 7, 11). Potassium nitrate and gibberellic acid seed soaks have been used to remove physiological dormancy in Christmas rose, *Helleborus niger*, but were ineffective (11).

Dormant, imbibed seeds are metabolically active and capable of receiving external stimuli that can release dormancy and initiate germination. These stimuli can include chemical and hormonal signals or environmental signals such as temperature, soil nitrate levels, or light (1). Abscisic acid (ABA), gibberellic acid (GA), ethylene, brassinosteroids, auxins, and cytokinins have a tremendous effect on plant development, even at low concentrations (10). ABA and GA have a considerable influence on seed maturation, dormancy, and germination and act antagonistically (4).

ABA is a principal controller of dormancy induction and maintenance (1, 10). There is overwhelming evidence to support ABA as an essential factor in the dormancy control of many seeds (1). Seeds deficient in ABA during development lack primary dormancy once dispersed. Conversely, an over expression of ABA synthesis genes during seed development enhances dormancy and hinders germination (4). Only ABA produced by the seed during development can provide a prolonged dormancy period (10).

ABA acts by preventing radicle protrusion. The effect is so strong that ABA can even inhibit protrusion late in the germination process, possibly preventing cell wall loosening processes from occurring (1). In nondormant *Brassica napus* embryos, complete germination was prevented when ABA application inhibited radicle protrusion preventing cell wall loosening from occurring (17).

GAs play an important role in dormancy release and the stimulation of seed germination (4, 10) by reducing many of the environmental requirements for germination and counteracting the inhibitory effects of ABA. It is most active after inhibition of

germination by ABA has been suppressed (1, 2) and accumulates at the highest concentrations just prior to radicle protrusion (10).

GAs act to regulate dormancy release and germination by an intricate interaction with ABA and environmental signals (10). An increase in GA and/or a reduction in ABA synthesis are necessary for dormancy release to occur, resulting in a shift to low ABA:GA ratios (4). Two types of dormancy are relieved by the functions of GA and the shift to low ABA:GA ratios (10). Embryo dormancy is attributed to a high ABA:GA ratio and an increase in GA sensitivity can increase the growth potential of the embryo and increase cell extension growth (4).

An increase in GA can also relieve dormancy imposed by the seed coverings (10). Dormancy imposed by the seed coverings is caused by the combination of low embryo growth potential and mechanical restraints imposed by the testa or endosperm tissue (7). This is different from hard seed coat induced physical dormancy (4). The testa is made up of mostly dead tissue and its characteristics are influenced during seed development by ABA. Rupture of the testa is assisted by enzymes released from the endosperm and/or radicle. ABA in some part inhibits endosperm weakening, while GA increases the embryo growth potential and assists in weakening micropylar endosperm, helping to alleviate seed covering imposed dormancy (1, 4, 10). Recent research has proposed that GA-induced cell-wall modifying proteins, such as endo-β-l,4-mannanases and endo-β-1,3-glucanases, cause endosperm weakening and seed covering imposed dormancy loss. β-1,3-glucanases regulate cell-to cell movement (1, 4, 8, 10) and are hypothesized to

cause endosperm weakening by breaking intercellular adhesion, causing cell separation (4).

The importance of GA in germination has been demonstrated by studying GA-deficient mutants of *Arabidopsis* and tomato; both required an external source of GA to conclude germination (1). Isolated embryos of GA-deficient mutants of tomato elongated without GA, demonstrating that GA is needed to overcome the mechanical restraints of the seed covering layers (10).

GA has been used effectively to substitute for cold stratification in members of the Ranunculaceae family such as *Thalictrum mirabile*, *Trollius ledebouri*, and *Eranthis hiemalis*. These species have morphophysiological dormancies similar to Lenten rose (5, 6, 18). Although GA has not been effective in other Hellebore species (11), there is the potential that it could be effective in Lenten rose and the role of GA in this species needs investigation. Therefore, the objectives of this experiment were to determine if the application of gibberellic acid (GA_3) would promote germination in Lenten rose seeds and to determine the best application time and concentration.

Materials and Methods

The experiment was conducted beginning in fall 2007 on the campus of Auburn University (Auburn, AL). Seeds of Lenten rose Red Hybrids (Jelitto Perennial Seed, Schwarmstedt, Germany) were received on February 12, 2007, and placed in dry, open paper bags at 4C (39F), until used in experiments.

Treatments consisted of 200 ppm, 400 ppm, or 800 ppm GA_3 (Gibberellic acid 90%, Acros Organics, Morris Plains, NJ) applied prior to warm stratification, between

warm and cold stratification, or after cold stratification plus a control receiving no GA_3 treatment (Table 4.1). Each treatment and the control were replicated four times with 20 seeds per replication.

Germination containers were clear slim compact disc cases (14.2×12.5×0.5 cm) containing germination paper (Kimpak, Seedburo Equipment Company, Bozeman, MT) cut to 12×10 cm. Seeds for treatments B, E, and H were allowed to imbibe in aerated, distilled water for 8 h at room temperature on November 18, 2007. The following day, they were surface sterilized in a 15% bleach (6% sodium hypochlorite) and distilled water solution for 3 min followed by three 1 min rinses in distilled water.

Seeds were placed for 24 h on germination paper to which 75ml of the appropriate concentration of GA_3 had been added. Cases were sealed and placed on benches in a climate controlled room in the Plant Sciences Center (Auburn, AL). The facility temperature was kept at a constant 25C (77F).

Seeds of all remaining treatments and control were allowed to imbibe in aerated, distilled water for 8 h at room temperature on November 19, 2007. The following day, they were surface sterilized as previously described. Treatments B, E, and H were removed from the GA_3 treated paper and blotted dry. Seeds of all treatments and control were placed on germination paper evenly moistened with a solution of 100 ppm Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, CA) and distilled water. Cases were sealed and placed on racks inside plastic containers (42.4×27.9×27.4 cm) on benches in the climate controlled room at the Plant Sciences Center. Container lids were kept partially opened to adjust for

appropriate humidity levels within the containers. Treatments were kept adequately moist using a solution of 100 ppm Blocker 4F as needed throughout the remainder of the experiment.

Radicle Germination

After 10 weeks of warm stratification at 25C (77F), treatments C, F, and I were removed from their germination containers, blotted dry, and placed for 24 h on GA_3 solution papers as previously described. Following the 24 h period, treatments C, F, and I were blotted dry and moved back to their germination containers and all treatments were moved to a cooler set at 4C (39F) in the Paterson Greenhouse Complex (Auburn, AL). Following 4 wks of cold stratification in the 4C (39F) cooler, treatments D, G, and J were removed from their germination containers, blotted dry, and placed for 24 h on GA_3 solution papers as previously described. Following the 24 h period, treatments D, G, and J were blotted dry and moved back to their germination containers and all treatments were moved to a cooler set at 10C (50F) in Funchess Hall (Auburn, AL) until radicle emergence occurred. Treatments were in darkness and exposed to light only when examined. Treatments were monitored approximately every 3 days to record the date of radicle emergence defined as the day the radicle reached 3 mm in length. Radicle emergence was continuously recorded until the study was terminated on May 9, 2008.

The data were analyzed as a completely randomized design using SAS version 9.1.3 (SAS Institute, Cary, NC) with GA_3 concentration and application time in a factorial treatment arrangement. The number of days to radicle emergence was analyzed with PROC GLM. The range in number of days to radicle emergence was determined for

each treatment and replication combination. These data were analyzed using the same procedures as above. The number of radicles emerged out of the total number of seed sown per treatment combination was analyzed with PROC GENMOD using the binomial probability distribution. Single degree of freedom group contrasts were used to test differences among selected treatment combinations at $\alpha = 0.05$.

Results and Discussion

GA_3 treatment at all concentrations applied pre warm resulted in 42% to 58% higher germination percentages when compared to post warm and post cold applications, and there was no difference in percent germination in post warm and post cold at any of the three concentrations (Table 4.1). There was also no difference between germination percentages among the three GA_3 concentrations when applied pre warm, post warm, or post cold. Germination percentages at all three GA_3 concentrations were higher than the control when applied pre warm and post warm, but not post cold

GA_3 applied at 200 ppm and 800 ppm resulted in differences in pre warm, post warm, and post cold for days to germination, but at 400 ppm GA_3, only the pre warm application was different from post warm and post cold applications (Table 4.1). There was no difference in germination percentages among the three GA_3 concentrations when applied pre warm. None of the three GA_3 concentrations decreased the number of days to germination when compared to the control. However, the fewest number of days to germination were found at 200 ppm, 400 ppm, and 800 ppm GA_3 when applied pre warm. There was no difference in the range of days to germination.

Although not a quantifiable measurement, it is interesting to note that within 24-48 hours of pre warm GA_3 treatment at all concentrations, a visually observable split in the seed coat was detected in most of the treated seeds. This supports the function of GA_3 in breaking physiological dormancy by weakening the mechanical resistance surrounding the endosperm and/or by increasing the growth potential of the embryo, thus expanding the overall size of the seed (10).

The release of physiological dormancy by the application of GA_3 in *Helleborus* prior to the release of morphological dormancy contradicts to previous research by Niimi, which concluded that morphological dormancy must first be broken by warm stratification followed by cold stratification to break physiological dormancy (13). It is thought that cold stratification releases bound GA or causes an initiation in its synthesis (10). However, there is evidence to support that ABA (GA inhibitors) could be leached out during imbition. ABA has been shown to leach from seeds of wild type tomato and lettuce into the medium upon imbition (9). Pre warm GA_3 applications could be more effective due to their proximity to ABA leaching prior to sowing.

This experiment demonstrates that GA_3 treatment can be used to increase germination percentage in this particular seed lot of Lenten rose and that of treatments used, 200 ppm was effective and economical. However, lower concentrations of GA_3 should be trialed as they might be just as effective as the 200 ppm. It might also be possible to use GA_3 in the imbibition solution for a greater effect. Greater uptake of GA has been reported in previously unimbibed seeds versus imbibed seeds and could allow for a reduction in concentration and thus expense of GA_3.

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Table 4.1. Concentration and application timing of GA_3 on Lenten rose seeds.

Treatment	GA_3 concentration (ppm)	GA_3 application time
A	0	control
B	200	prior to warm stratification
C	200	between warm and cold stratification
D	200	after cold stratification
E	400	prior to warm stratification
F	400	between warm and cold stratification
G	400	after cold stratification
H	800	prior to warm stratification
I	800	between warm and cold stratification
J	800	after cold stratification

Table 4.2. Effects of GA_3 concentration and application timing on germination of Lenten rose seeds.

GA_3 concentration (ppm)	Application time	Percent germination[z]	Days to germination[y]
–	control	20	136
200	pre warm	55	129
200	post warm	35	134
200	post cold	25	141
400	pre warm	65	131
400	post warm	30	137
400	post cold	25	140
800	pre warm	60	127
800	post warm	40	137
800	post cold	25	141
		Significance[x]	
GA_3 at 200 ppm applied pre warm vs. post warm and post cold		***	***
GA_3 at 200 ppm applied post warm vs. post cold		NS	**
GA_3 at 400 ppm applied pre warm vs. post warm and post cold		***	***
GA_3 at 400 ppm applied post warm vs. post cold		NS	NS
GA_3 at 800 ppm applied pre warm vs. post warm and post cold		***	***
GA_3 at 800 ppm applied post warm vs. post cold		NS	*
GA_3 at 400 ppm applied pre warm vs. 200 ppm and 800 ppm		NS	NS
GA_3 at 400 ppm applied post warm vs. 200 ppm and 800 ppm		NS	*
GA_3 at 400 ppm applied post cold vs. 200 ppm and 800 ppm		NS	*
Pre warm application of all GA_3 concentrations vs. control		***	NS
Post warm application of all GA_3 concentrations vs. control		*	NS
Post cold application of all GA_3 concentrations vs. control		NS	NS

[z]Based on mean of four replications and 20 seeds per replication.
[y]From the beginning of the experiment on November 20, 2007.
[x]Non-significant (NS) or significant at $\alpha = 0.05$(*), 0.01(**), or 0.001 (***) using orthogonal contrasts.

CHAPTER V

COLD DURATION EFFECTS ON SEED GERMINATION OF LENTEN ROSE

Abstract

Seed production of Lenten rose (*Helleborus* × *hybridus*) allows for lower propagation costs but significant difficulties are incurred due to poor germination. Seed have differentiated, but rudimentary embryos that are physiologically dormant at maturity. This is known as morphophysiological dormancy and requires a specific combination of warm and/or cold stratification to break dormancy. The objectives of this experiment were to determine the number of weeks of cold stratification required to increase germination rate, shorten time to germination, and increase synchronous germination in Lenten rose. Treatments consisted of five cold durations for 1, 2, 3, 4 or 5 weeks plus a control receiving no temperature treatment. The greatest percent radicle emergence and the lowest days to radicle emergence occurred in this seed lot after 1 week cold stratification. The shortest range of radicle emergence occurred after 3 weeks and the longest range after 0 weeks cold stratification.

Index words: *Helleborus* × *hybridus*, dormancy, stratification, dormancy, seed propagation, Hellebore, morphophysiological dormancy

Species used in this study: *Helleborus* × *hybridus* (Lenten rose)

Significance to Industry

Lenten rose is well suited to dry shade gardens in the southeastern United States but faces production difficulties due to complex dormancy and poor germination. An increase in germination rate, decrease in time to germination, and more synchronous germination would result in more efficient propagation. In this study, the greatest percent radicle emergence and the fewest days to radicle emergence occurred after 1 week cold stratification. There was a linear increase in the number of with increasing cold stratification, with the shortest days to emergence occurring after 1 week cold stratification. The shortest range of radicle emergence occurred after 3 weeks. Based on the seed lot studied, to achieve high germination rates in the shortest amount of time, it is recommended that seeds of Lenten rose undergo 1 week of cold stratification following 10 weeks of warm stratification.

Introduction

Lenten rose (*Helleborus* × *hybridus*) is a perennial member of the Ranunculaceae, or buttercup, family with attractive winter to early-spring flowers in a variety of colors. It is a popular ornamental, cut flower, and medicinal plant in Europe (14, 17). It is well suited to the southeastern United States and was named the 2005 Perennial Plant of the Year by the Perennial Plant Association (6, 15).

Lenten rose has many attractive features; it is easy to cultivate, hardy, long-lived, tolerant of dry conditions, evergreen, and deer resistant (6, 15, 17). Gardeners are most attracted to Lenten rose for its early flowers, beginning in February or March and lasting for 2 to 3 months. They come many shades of white, yellow, green, pink, and purple and generally appear as nodding forms (6). Flowers appear as single, semi-double, or double forms and are up to 3 inches across (13, 17). Lenten rose accepts a wide range of soil, is adaptable to USDA hardiness zones 4 to 9 (15), and prefers partial to full shade (13).

Lenten rose is propagated by division, in vitro propagation, or seed (14, 17). Division of rhizomes is time consuming and often results in a slow recovery time (13, 14, 17). In vitro propagation has the advantage of producing identical plants but has proven to be quite difficult to produce plants profitably (6) and better protocols are needed for an efficient mass production system (18). Lenten rose is commonly propagated by seed when identical plants are not necessary or for the production of the named seed strains (13, 14).

Propagation by seed has the advantage of producing a substantial number of seedlings from one plant (13), but germination is slow in Lenten rose. Germination requires many months and seeds often fail to germinate even under environmental conditions suitable for germination (14). Germination is commonly uneven thus forcing growers to transplant seedlings over several weeks or months until germination has concluded (16). The erratic and unpredictable nature of Lenten rose germination is undesirable to commercial growers who desire higher and more synchronous germination.

Poor germination in Lenten rose is attributed to its seed dormancy (6). This can be overcome in Lenten rose by moist, warm stratification at 60-80F for 6 to 12 weeks followed by cold stratification at 25-40F (6, 9). Once dormancy has been overcome, fresh hellebore seed will generally germinate at temperatures between 40F and 50F (9). This replicates the seeds natural temperature environment during dormancy release and germination (6).

Niimi et al. reported in a 2006 study on the embryo development and seed germination of Christmas rose that there were few reports available on germination of *Helleborus* species. Observation of embryo development in this study indicated that Christmas rose has an underdeveloped, rudimentary embryo at seed dispersal and that release of dormancy only occurred in seeds that were transferred from one temperature to another. Treatment at 25C for 8 weeks allowed the embryo to mature and chilling for at least 8 weeks at 4C was needed to break dormancy. The researchers concluded that seeds of Christmas rose have deep, simple morphophysiological dormancy, a combinational dormancy resulting from the immature embryo and a physiological dormancy (14).

Seed dormancy is described as a barrier to germination of an intact viable seed under favorable environmental conditions. As an environmental signal, temperature has an intense effect on the dormancy status within seeds and the initiation of germination (5, 10, 11).

The majority of non-tropical imbibed seeds can be released from dormancy when they are stratified at low temperatures from 1-10C, but sometimes temperatures as high as 15C can release dormancy. This is thought to be an adaptive mechanism within the seed

to prevent loss of dormancy until after winter has passed. Chilling is a common practice in horticultural and forestry industries to break seed dormancy. Chilling treatments are not cumulative; periods of higher temperatures cancel chilling periods and can induce secondary dormancy (5).

Temperature has a substantial impact on a seeds ability to germinate and the rate at which germination can occur. There is a defined minimum and maximum germination temperature for each species. The temperature at which a seed needs to germinate can be different, and must be studied separately, from the temperature needed to break dormancy (5). To understand how temperature affects dormancy and germination and how it is sensed within a seed, it is best to understand the type of seed dormancy that a seed possesses.

Based on the 2006 finding by Niimi et al., previous work by Lockhart on Christmas rose, and genus characteristics, it is most likely that Lenten rose has a deep, simple morphophysiological dormancy (6, 12, 14). Morphophysiological dormancy is a combinational dormancy (8). The morphological component is characteristic of seeds with embryos that are immature at dispersal (3) and require growth before germination can occur. Embryo growth occurs after the seeds have imbibed, but before germination begins (8).

The physiological dormancy component is attributed to the restraints caused by the seed coverings, blocking the radicle from protruding the seed covering (8). Moist cold stratification relieves physiological dormancy in nature. Treatment of seeds with

gibberellic acid, scarification, after-ripening in dry storage, cold or warm stratification, and light or darkness can be used to break dormancy, depending on the species (3, 8).

Physiological dormancy is divided into nondeep, intermediate, and deep levels, separated by the length of stratification time required to relieve dormancy. Short periods, days to a few months, are needed to break nondeep dormancy while intermediate dormancy requires at least 2 months of cold stratification and responds to external applications of GA as a substitute for chilling (8). Deep physiological dormancy requires several months of stratification and is divided into subtype a, requiring cold stratification before germination can occur, and subtype b, requiring warm stratification before germination can occur (3). Seeds of *Helleborus* species most likely fall into subtype b.

Seeds with morphophysiological dormancy usually require a specific combination of warm and/or cold stratification to break dormancy (3). All conditions inhibiting germination must be removed in proper order to induce germination (8). Simple morphophysiological dormancy is known to be present in Christmas rose (14). Warm conditions, 15C to 30C, followed by cold, 1 to 10C, are required for breaking simple, morphophysiological dormancy. Seeds have an immature, linear, embryo which develops in the warm temperature period. Once the embryo is of mature size, dormancy can be released during the chilling period (3, 8).

It is important to keep in mind when dealing with dormancy and germination that a seed is never just under the control of one factor in nature, but many factors concurrently (5). Predispersal environmental conditions, such as light quality, photoperiod, and temperature, and postdispersal conditions, such as temperature, light,

moisture, and soil nutrients (7), can all affect dormancy and germination. The conditions needed to remove dormancy can vary greatly even within the same species (2). Dormancy loss and the initiation of germination is an ongoing, gradual process, especially in the case of temperature-dependent dormancy, where the temperature at which dormancy can occur expands as dormancy is released (3, 5).

The results of Chapter II were inconclusive for the effect of cold temperature duration on germination rate, time to germination, and range of germination. The results indicated that a short cold duration might be desirable in producing the highest true leaf counts in Lenten rose; however, the shortest cold duration studied was 4 weeks. The number of weeks of cold required to break dormancy and initiate germination in Lenten rose should be further investigated using shorter durations of cold than were used in Chapter II. Therefore, this experiment was conducted to further investigate the weeks of cold stratification needed to increase germination rate, shorten time to germination, and increase synchronous seed germination in Lenten rose.

Materials and Methods

The experiment was conducted beginning in fall 2007 on the campus of Auburn University (Auburn, AL). Seeds of Lenten rose Red Hybrids (Jelitto Perennial Seed, Schwarmstedt, Germany) were received on February 12, 2007 and placed in dry, open paper bags at 4C (39F), until used in experiments.

Treatments consisted of 1, 2, 3, 4, or 5 weeks of cold stratification plus a control. Each treatment and the control were replicated four times with 20 seeds per replication.

Germination containers were clear slim compact disc cases (14.2×12.5×0.5 cm) containing germination paper (Kimpak, Seedburo Equipment Company, Bozeman, MT) cut to 12×10 cm. Seeds were allowed to imbibe in aerated, distilled water for 8 h at room temperature on November 19, 2007. The following day, they were surface sterilized in a 15% bleach (6% sodium hypochlorite) and distilled water solution for 3 min followed by three 1 min rinses in distilled water. On November 20, 2007, treatments were placed on germination paper evenly moistened with a solution of 100 ppm Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, CA) and distilled water, 20 seeds per replication. Cases were sealed and placed on racks inside plastic containers (42.4×27.9×27.4 cm) on benches in a climate controlled room at the Plant Sciences Center (Auburn, AL). The facility temperature was kept at a constant 25C (77F). Container lids were kept partially opened to adjust for appropriate humidity levels within the containers. Treatments were kept moist using a solution of 100 ppm Blocker 4F as needed throughout the remainder of the experiment.

Radicle Emergence

After 10 wks of warm stratification at 25C (77F), treatments 1 through 5 were moved to a cooler set at 4C (39F) at the Paterson Greenhouse Complex (Auburn, AL). The control, was moved directly to a cooler set at 10C (50F) in Funchess Hall (Auburn, AL). After completion of the respective cold conditioning period, treatments were moved to the 10C (50F) cooler in Funchess Hall until germination occurred. Treatments were in darkness and exposed to light only when examined. Treatments were monitored approximately every 3 days to record the date of radicle emergence defined as the day the

radicle reached 3 mm in length. Radicle emergence was continuously recorded until the study was terminated on May 9, 2008.

The data were analyzed as a completely randomized design using SAS version 9.1.3 (SAS Institute, Cary, NC). The number of days to radicle emergence was analyzed with PROC GLM. The range in number of days to radicle emergence was determined for each treatment and replication combination. This data was analyzed using the same procedures as above. The number of radicles emerged out of the total number of seed 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 $\alpha = 0.05$.

Results and Discussion

There was a cubic effect of cold stratification duration on percent radicle emergence with the highest emergence occurring after 1 week cold stratification (Table 5.1). There was a linear increase in the number of days to radicle emergence with increasing cold stratification, with the shortest days to emergence occurring after 1 week cold stratification. Concurrently, there was a linear decrease in radicle emergence range with increasing cold stratification with the shortest range occurring after 3 weeks and the longest range after 0 weeks cold stratification.

The study indicates that after 10 weeks of warm stratification very little cold stratification is needed for the germination of Lenten rose, only 1 week. Although treatments with longer periods of cold stratification had a shorter range of radicle emergence, this can be explained by the small germination percentages. In the longer

stratification periods, seeds began to germinate in the treatment then quickly stopped within 1 to 2 weeks, but in the more effective stratification periods, seeds were able to continue germinating to achieve a higher overall percent germination. It is left to the grower to decide what feature is most desirable, a higher percent and shorter time to radicle emergence or more synchronous germination.

The requirement of cold stratification following a warm period reported in this study supports previous research by Niimi et al. that members of the *Helleborus* genus have morphophysiological dormancy (14). This is typical of other members of the Ranunculaceae family (1). 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 this seed lot of Lenten rose has a level of dormancy as extreme as the Christmas rose, which has a deep, simple morphological dormancy (14). The physiological component of dormancy determines the dormancy level based on cold stratification requirements and the response to external gibberellic acid (GA) applications (8). Based on the results of this study, this seed lot of Lenten rose has nondeep, simple morphophysiological dormancy due to the highest percent radicle emergence and the shortest days to radicle emergence occurring after 1 week of cold stratification.

Nondeep, simple morphophysiological dormancy is typically reported in seeds that mature in late spring and germinate in fall. Physiological dormancy in these seeds is broken during high summer temperatures (2). Although this is not the case with Lenten rose, nondeep, simple morphophysiological dormancy has been reported in seeds of *Thalictrum mirabile*, a member of the Ranunculaceae family that, like Lenten rose,

requires cold stratification for the loss of physiological dormancy (19). These results are consistent with reports that Lenten rose is quicker to germinate than other acaulescent Hellebore species (6).

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Table 5.1. Effect of cold 4C (39F) stratification duration on Lenten rose seeds.

Treatments	Percent radicle emergence[z]	Days to radicle emergence[y]	Radicle emergence 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.[x]	C**	L***	L***

[z]Based on mean of four replications and 20 seed per replication.
[y]From the beginning of the experiment on November 20, 2007.
[x]Non-significant (NS), linear (L), or cubic (C) at $\propto$=0.05(*), 0.01(**), or 0.001(***) using orthogonal contrasts.

CHAPTER VI

FINAL DISCUSSION

The purpose of these studies was to develop a method to increase germination percentage, shorten germination time, provide more synchronous germination, and result in more efficient seed propagation techniques for Lenten rose. Chapter two investigated the number of weeks of warm followed by cold stratification needed for germination. Seeds received warm durations for 4, 6, 8, or 10 weeks followed by four cold durations for 4, 6, 8, or 10 weeks plus a control.

There was an interaction between warm and cold duration for days to radicle emergence and radicle emergence range. Four weeks warm followed by 4 weeks cold resulted in the fewest days to radicle emergence, while 10 weeks of warm followed by 10 weeks of cold resulted in the greatest days to radicle emergence. The narrowest radicle emergence range was after 4 weeks of warm and 10 weeks of cold. Warm and cold main effects were significant for days to hypocotyl emergence. There were linear increases in days to hypocotyl emergence with increasing warm duration and cold durations, with increasing warm durations having a larger impact on time to emergence than increasing cold. The warm duration main effect was significant for hypocotyl emergence range with the narrowest range occurring after 4 weeks warm. There was an interaction of warm and cold duration for days to and range of first true leaf emergence. Four weeks of warm

followed by 6 weeks of cold resulted in the fewest days to first true leaf emergence, while 10 weeks of warm followed by 10 weeks of cold resulted in the greatest. The narrowest range of first true leaf emergence occurred after 4 weeks of warm followed by 10 weeks of cold.

The main effects were significant for warm duration in radicle and hypocotyl counts with counts increasing as warm duration increased. Warm and cold duration main effects were significant for first true leaf counts with linear increases in seedling counts as warm and cold durations increased. There was an interaction between warm and cold duration for plant height with an overall trend toward taller plants with the shorter warm and cold durations. Warm and cold duration main effects were significant for average plant width with linear decreases as warm and cold durations increased. Data collection period main effect was significant for both height and width. Both seedling height and width increased in a linear fashion between the first and last data collection periods.

Results of Chapter two indicate that there was a general pattern in this seed lot, although not always significant in the case of warm duration, that the shortest days to radicle, hypocotyl, and first true leaf emergence occurred with the shorter durations of warm and cold. The results for radicle, hypocotyl, and true leaf counts indicate that 10 weeks of warm duration produced the highest radicle, hypocotyl, and true leaf counts with no effect of cold duration on radicle and hypocotyl counts. The highest true leaf counts for cold duration were reported after 4 weeks, indicating that 4 weeks of cold duration is best.

The results for average width indicate that plant growth of Lenten rose decreased with increasing durations of cold and warm. While height decreased with increasing warm durations, the best cold durations were found after 6 and 8 weeks. While these results indicate that shorter durations of warm and cold produced larger plants for this seed lot of Lenten rose, this is are potentially misleading. Actually an increase in growing time will increase plant size. Therefore, any method that decreases time to emergence and allows plants to begin growth earlier will benefit plant size.

Chapter three investigated the effect of density sorting on seed germination and seedling growth in Lenten rose. Treatments consisted of four seed density groups and an unsorted control. The highest radicle, hypocotyl, and first true leaf counts were found in the unsorted control, while the lowest were found in density 4, the lowest density. However, radicle, hypocotyl, and first true leaf counts for densities 1, 2, and 3 were statistically similar to density 4 and the unsorted control. During the study, it was observed that a large number of density 4 seeds became heavily infected with a fungal pathogen with the highest incidence of diseased seed found in density 4, while all other treatments were similar.

Density sorting of Lenten rose seed had little effect on germination rate, time, or range of germination. Unsorted seed had the highest radicle, hypocotyl, and first true leaf counts. Although many studies have shown that larger seeds generally perform better (6), it is possible that few differences in germination rate, time, and spread of germination were observed in this study because the seeds were not under conditions where resources were scarce. Under agreeable environmental conditions, variations in seed size and mass

are less pronounced (2). The most important finding in chapter three was that the incidence of diseased seed in this seed lot was greatly impacted by density sorting. Secondary compounds in seed reserves and the increased resistance to environmental stress are thought to offer larger seeds better protection against pathogens (4). By eliminating the lowest density of Lenten rose seeds prior to sowing, a grower could reduce the incidence of seed and seedling related infections.

In Chapter four, seeds of Lenten rose were treated with three GA_3 concentrations at 200 ppm, 400 ppm, or 800 ppm with three application times prior to warm stratification, between warm and cold stratification or after cold stratification, and a control. Treatment of seeds with 200 ppm, 400 ppm, and 800 ppm GA_3 prior to warm stratification increased germination rates by 42% to 58%.

Although not a quantifiable measurement, it is interesting to note that within 24-48 hours of pre warm GA_3 treatment at all concentrations, a split in the seed coat was observed in most of the treated seeds. This supports the function of GA_3 in breaking physiological dormancy by weakening the mechanical resistance surrounding the endosperm and/or by increasing the growth potential of the embryo, thus expanding the overall size of the seed (5).

The results of Chapter two indicated that a short cold duration might be desirable in producing the highest true leaf counts in Lenten rose; however, the shortest cold duration studied was 4 weeks. Therefore, Chapter five was conducted to further investigate the appropriate weeks of cold stratification for germination of Lenten rose seed. Treatments consisted of five cold durations for 1, 2, 3, 4 or 5 weeks plus a control.

The highest percent radicle emergence and the lowest days to radicle emergence occurred after 1 week of cold stratification. The shortest range of radicle emergence occurred after 3 weeks and the longest range after 0 weeks of cold stratification. Although treatments with longer periods of cold stratification had a shorter range of radicle emergence, this can be explained by the small germination percentages. In the longer stratification periods, seeds began to germinate during treatment then quickly stopped within 1 to 2 weeks, but in the more effective stratification periods, seeds were able to continue germinating to achieve a higher overall percent germination.

The study indicates that after 10 weeks of warm stratification very little cold stratification is needed for the germination of Lenten rose, only 1 week. The interactions of warm and cold duration reported in Chapters two and five and effect of GA_3 in breaking physiological dormancy reported in Chapter four support previous research by Niimi et al. (7) that members of the *Helleborus* genus have morphophysiological dormancy. The temperature range that is best for embryo development and release of dormancy differs at each embryo developmental stage. This is typical of other members of the Ranunculaceae family (1). However, 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 (7). The physiological component of dormancy determines the dormancy level based on cold stratification requirements and the response to external gibberellic acid (GA) applications (3). Based on the results of these studies with regard to temperature requirements and the ability of GA_3 to promote germination, this seed lot of Lenten rose has a nondeep, simple morphophysiological dormancy.

The results with this seed lot of Lenten rose indicates that, for more efficient seed propagation, low density Lenten rose seed should be removed prior to sowing to reduce disease incidence, seeds should be pre-treated with 200 ppm GA_3 after imbibtion, and warm stratified at 25C (77F) for 10 weeks followed by 1 week of cold stratification at 4C (39F). Further studies should include a combination of these treatments, possible cold elimination studies, and trials to determine if lower rates of GA_3 can be used, possibly in the imbibition water.

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