THESIS

OVERCOMING SEED DORMANCY IN *GLAUCIUM SPP.* (*PAPAVERACEAE*)

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KAREN CHRISTINA ELSNER ENTITLED, "OVERCOMING SEED DORMANCY IN *GLAUCIUM SPP.* (*PAPAVERACEAE*)" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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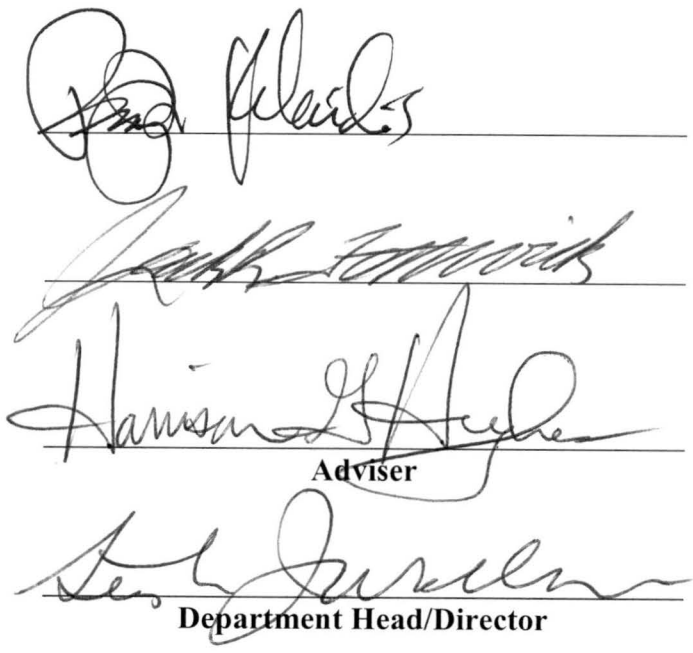

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ABSTRACT OF THESIS

OVERCOMING SEED DORMANCY IN *GLAUCIUM SPP.* (*PAPAVERACEAE*)

The Horned Poppy, *Glaucium sp.*, is a potential candidate for introduction as an herbaceous perennial to Colorado. However, its introduction has been hindered by poor germination, less than 10% in informal studies across the state. Nine seed lots of *Glaucium sp.*, representing four different species and a hybrid harvested from four different years, were collected at Denver Botanic Gardens and used in research to develop a protocol for seed treatment to improve germination.

Four different trials were conducted to evaluate germination. The first trial evaluated stratification temperatures and germination with and without light on two collection years of *G. flavum*. The greatest germination was 69.5% for the seeds collected in 2005 that were stratified for 45 days at 7°C and germinated with light. The seeds harvested in 2003 had 53% germination with 7°C stratification for 45 days and germinated without light.

The second trial evaluated scarification treatments, hot water, concentrated sulfuric acid (2 levels) and nicks in the seed coat, on two collection years of *G. flavum* and *G. acutidentatum*. 2005 *G. flavum* had the greatest germination at 57% with a 30-minute sulfuric acid scarification. 2003 *G. flavum* had 20% germination with the 60-minute acid scarification. *G. acutidentatum* seeds from 2003 and 2005 germinated with acid treatments but not as well as *G. flavum*. 2003 *G. acutidentatum* had 6% germination with 60-minute acid scarification and 2005 *G. acutidentatum* had 1.5% germination with 30-minute acid scarification.

Stratification and scarification were evaluated together in the third trial. Additional seed lots; 2003 *G. grandiflorum*, 2003 G. corniculatum and 1999 *G. corniculatum x flavum* were included. Although each seed lot had varying responses to the treatments, 30-minute acid scarification combined with 30- or 45-day stratification at 8°C were the optimal pretreatments. The germination percentages for these treatments ranged from 60 to 92%.

The final trial compared gibberellic acid to stratification to determine if GA could substitute for cold stratification. Hydrogen peroxide was also evaluated for comparison purposes. For all the seed lots, 30-minute acid scarification with 400 ppm or 500 ppm GA_3 generated the greatest germination. The percentages for these treatments ranged from 58 to 95%. Gibberellic acid proved to be a substitute for cold stratification; however, seeds germinated slower than cold stratification. Germination with GA took pproximately 10 days while stratified seed germinated in five days. *G. grandiflorum* and *G. acutidentatum* can be considered to have intermediate complex morphophysiological dormancy with a hard seed coat, while *G. corniculatum* appears to have only a hard seed coat.

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Chapter I

Introduction

The Horned Poppy is a potential candidate for introduction to Colorado as a new herbaceous perennial. The various Horned Poppy species originate from Europe and the Middle East, specifically from the Mediterranean Basin to the arid Caucasus Mountains in the countries of Georgia and Azerbaijan. These areas generally have hot, dry summers and wet winters. There are four species of the Horned Poppy genera, *Glaucium*, that are at the Denver Botanic Gardens, *G. flavum, G. corniculatum, G. grandiflorum* and *G. acutidentatum*. The blue-green ruffled leaves and yellow or orange flowers, which bloom all summer, could add interesting texture and color to Colorado home-gardener's palette.

Colorado's climate and soil can make it difficult for many ornamental plants to grow here. Therefore, nurseries are always interested in new plants that will survive well here but also satisfy the customer's desire for new beautiful plants. Colorado's average annual rainfall is only 17 inches a year statewide. However, it is usually less along the Front Range because storms that move from the Pacific Ocean usually lose their moisture in the mountains and storms from the Gulf of Mexico usually lose their moisture across the plains part of the state (Doesken, N. J. et al, 2003). Winter conditions also contribute to dehydration problems for perennials. Large diurnal temperature swings from day to night cause the soil to expand when it is warm and contract when it freezes. This causes the root system of dormant perennials to be exposed to drying conditions.

Irrigation is often used by homeowners to supplement the scarce precipitation for their gardens. Home landscapes can use as much as 50% of the water for a household

(Feucht and Wilson, 2007). If homeowners used more xerophytic plants, they could reduce their overall water usage significantly.

Colorado soils also present many problems. The heavy clayey texture, alkalinity and high salinity of the soil can impact a plant's health and vigor. Clay textured soils have small pore spaces that limit the amount of oxygen available to roots. When it rains, the small pores hold water tightly, reducing the oxygen level even more. Colorado soils have high levels of lime or calcium carbonate, $CaCO_3$, as well. The high levels of lime increase the pH of soils to levels around 7.2 to 7.5 (Havelin et al, 2005), which reduces the availability of nutrients, like iron and zinc, to plants. Colorado's semiarid climate causes rapid evaporation of water from soil surfaces leaving behind salts and increasing the salinity of soil, especially at the root zone. Saline soils limit plant growth by causing imbalances in the plant's water levels and ions (Havelin et al, 2005). Thus it is important to select plants that are salt tolerant and can withstand many of the problems inherent in Colorado.

There is a limited selection of plants that are xerophytic, cold hardy, and halophytic as well as aesthetically pleasing. Therefore, the Denver Botanic Gardens, Colorado State University and leaders of Colorado's landscape and nursery industry joined together to create a program for introducing plants called, "Plant Select." Since 1997, "Plant Select" chooses a few plants each year, either natives or introductions, to highlight and market for their ability to grow well in Colorado's climate. *Glaucium grandiflorum* and *G. acutidentatum* is currently being evaluated due to their drought and salt tolerance. However, the introduction of *G. grandiflorum* and *G. acutidentatum* into Colorado has been slow because their seeds have not germinated well in nursery

greenhouses. Growers have seen poor germination rates of single digit percentages when seeds stored at room temperature are then grown in greenhouses without seed coat treatments or stratification. Therefore, this research will evaluate the influence of cold stratification and/or scarification on seed germination. A recommended protocol for increased germination will be developed for the nursery industry.

Chapter II

Literature Review

2.1 Background of *Glaucium* species

Commonly referred to as Horned Poppies, *Glaucium* species are members of the Poppy family, *Papaveraceae*. *Glaucium* species originate from the Mediterranean and Middle East with some species having a wider distribution than others, Figure 2.1.a. *Glaucium* species have many similar characteristics and can be difficult to distinguish from each other. They all have blue-green foliage that is deeply pinnatified to pinnatisect and typically grow 30-50 cm long. The leaves have varying degrees of texture from glaucous to villous. All leaves are lyrate to sublyrate shaped and have a rosette growth habit. They have solitary blooms on flower stalks that grow above the foliage. All species have four petals in their corolla and their pistil is completely surrounded by stamens. They all develop long horned-shaped seed siliquiforms with the stigma remaining to cap off the top of the fruit. This study focuses primarily on *G. flavum*, *G. grandiflorum*, *G. acutidentatum* and *G. corniculatum*.

Figure 2.1 A map of the Mediterranean and Middle East which illustrates the region in which *Glaucium* is found.

Figure 2.2 A picture of *Glaucium acutidentatum* prior to bloom taken at Denver Botanic Gardens.

Figure 2.3 A close-up picture of *Glaucium flavum* illustrating the deeply pinnatifid leaf that was grown at CSU.

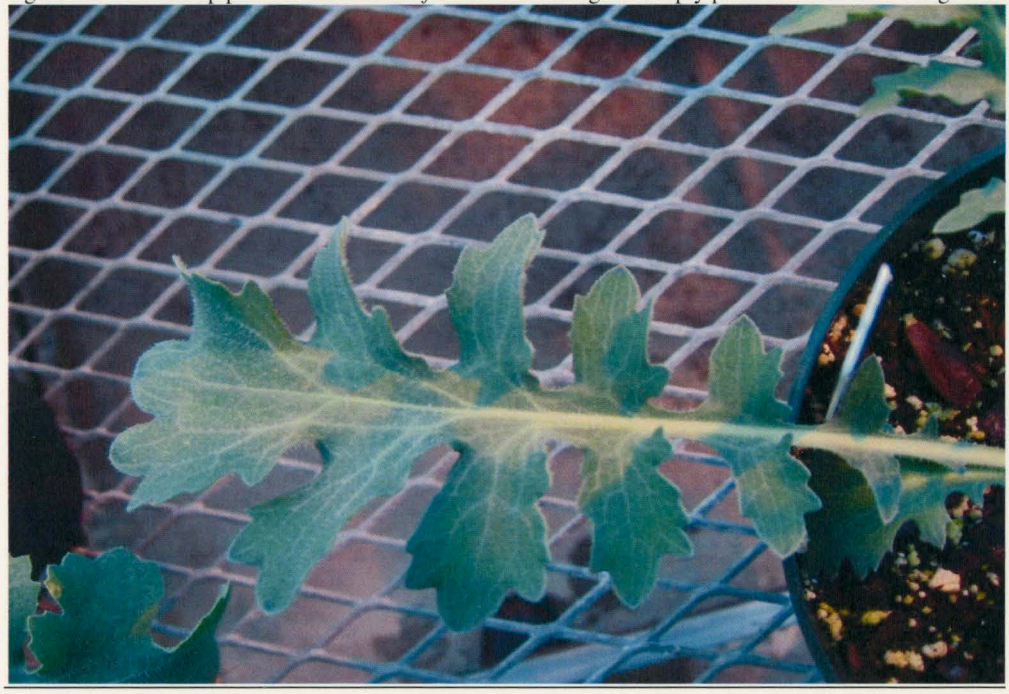


Figure 2.4 A picture which illustrates the developing silique of *G. grandiflorum.*

Figure 2.5 Pictures that demonstrate the flower for *G. grandiflorum* (a) and for *G. flavum* (b).

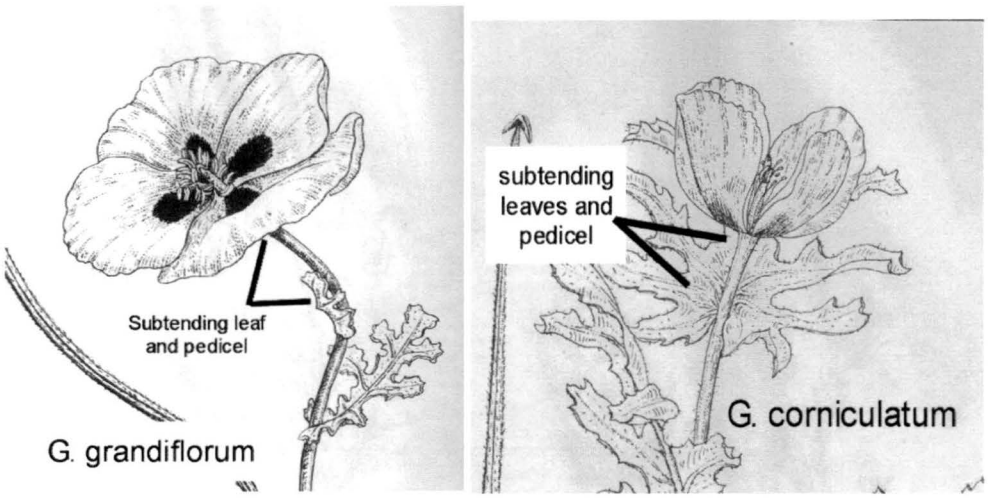
G. flavum Crantz has one of the widest distributions in the genus. It is found from the coasts of Britain and the Atlantic Islands to the coasts of the Mediterranean Basin and the Black Sea (Grey-Wilson, 2000). It grows predominantly on sandy beaches, which is why it is commonly known as the Sea Horned Poppy (Eisikowitch, 1979). This seems to indicate that *G. flavum* is salt tolerant since it grows on the seaside. According to the Flora of Turkey, *G. flavum* can be distinguished from other species by several

characteristics. The sepals have crisp, pilose hairs on the surface and the petals can be solid yellow, red or reddish mauve. *G. flavum* is most often recognized for the yellow petals and is commonly referred to as the Yellow Horned Poppy. The ovary is densely papillose to tuberculate, basically a bumpy surface. The siliquae will retain the papillose to tuberculate texture. In Turkey, *G. flavum* normally flowers from May through the summer and even though it is most often found at sea level, it does grow into river valleys as well.

G. grandiflorum Boiss & É. Huet is native to Turkey in the southern part of the Caucasus Mountains but it is also found in Syria, Iran and the Sinai (Grey-Wilson, 2000). Turkey is situated between the Mediterranean Sea and the Black Sea, where the Mediterranean coasts can receive 580 mm per year (approximately 23 in. per year) of precipitation, while the Black Sea coasts receive 1300 mm/yr (approximately 51 in. per year). However, there are mountain ranges in the country that introduce large climate changes with harsh winters and drier conditions with 400 mm/yr (~16 in./yr) of precipitation (Guide to Turkey, 2006). *G. grandiflorum* has features that distinguish it from the other species of *Glaucium*. It typically grows one main flower stem while other species have mulitiple flower stalks growing from the base of the rosette (Davis, P.H. ed, 1965). The sepals have short, stiff hairs making the surface hirsute. The petals are dark orange to crimson red with a black spot at the base of the petal. The pedicle of the flower exceeds the subtending leaf, which is different than most of the other *Glaucium* species. There are two varieties of *G. grandiflorum*: var. *grandiflorum* and var. *torquatum*. *G. grandiflorum* var. *torquatum* has red petals with a black blotch and can be found in

calcareous hillsides . *G. grandiflorum* var. *grandiflorum* is found in fields, banks and rocky slopes.

Figure 2.6 An illustration of G. *grandiflorum* pedicel as compared to the *G. corniculatum* pedicel. Drawings from Flora Palaestina, 1966.



G. acutidentatum Hausskn. & Bornm. is endemic to Turkey where it is found on dry hillslopes and rocky places (Grey-Wilson, 2000). *G. acutidentatum* is the most glabrous species with smooth sepals and ovaries. Although the ovary is smooth, the resulting siliquae is subtorulose. The petals are a solid orange-buff color. *G. acutidentatum* is found at elevations of 950-1400 m on dry hills (Davis Ed, 1965).

G. corniculatum (L.) J.H. Rudolph is native to the Mediterranean basin, Atlantic islands, Caucasus mountains, Bulgaria, Romania, northern Iraq and northwestern Iran (Grey-Wilson, 2000; Davis, 1965). *G. corniculatum* also has some unique characteristics. Its leaves have a soft, villous texture. Its sepals are scabrous to hirsute. There is some conflicting information about *G. corniculatum*'s corolla. According to the Flora of Turkey, the petals are yellow, orange or red with no mention of a basal spot.

However, other sources such as Grey-Wilson's book Poppies and the American Horticultural Society's A-Z Encyclopedia of Garden Plants, indicate that the petals are orange to red with a black basal spot.

Information about pollination and seed distribution is rather limited and focused primarily on *G. flavum*. It was first studied in 1963, in Britain, and was determined to be insect pollinated (Scott, 1963). The most frequent insect visitors were flies and bees. Scott performed artificial cross-pollination and enclosed some flower buds in muslin bags and found seed set with both cross-pollination and self-pollination. However, since the insect visits were common, it was hypothesized that cross-pollination is more common. Scott did not find any evidence of apomixes, cleistogamy or vivipary. Another study of *G. flavum* in Israel confirmed that varieties there were also insect pollinated and were visited by bees and flies (Eisikowitch, 1979). The study from Israel confirmed that *G. flavum* was self-compatible and there was no evidence of apomixes. The dry, dehiscent fruit develops an average of 282 seeds per silique (Scott, 1963). Considering the average fruit production is 17 pods per plant, each plant on average produces approximately 4,800 seeds a season. The seeds are dispersed when the wind shakes the dry fruit open. The seeds typically stay dormant until the spring when they germinate during periods of significant precipitation.

2.2 Seed Dormancy

Seeds are plant embryos in protective coverings held in a suspended state of development. This suspended state of development is a plant's way to wait for favorable conditions to germinate which would include adequate water, oxygen availability, ideal

temperature and light. When a viable seed does not germinate under ideal conditions it is most likely dormant. The purpose of dormancy is to ensure that germination only occurs when the environmental conditions are conductive to successful plant growth. The benefit of dormancy is that it can distribute germination across time and space ensuring longevity and species survival (Bewley and Black, 1994). Variations in seed size and seed coat thickness allow dormancy to break at different times spreading germination across different weeks, months or even years. A seed's dependency on environmental cues like a cold winter period helps ensure that the seed will not germinate right before a period of harsh weather. Finally, seeds are easily moved around by environmental events like wind, water flow or animals, so they can spread to new areas; when a seed is dormant it has more time to be transported. Also, light dependency prevents a seed from germinating under the leaf canopy of other plants until it has moved to a more open location (Bewley and Black, 1994). Dormant seeds need certain environmental factors or metabolic changes to happen before they can germinate.

Different types of dormancy have been categorized most clearly by Nikolaeva who divided it into 2 main types. One type is endogenous dormancy where the embryo is inhibited to germinate. The other type is exogeneous dormancy. This occurs when any other structure within or surrounding the embryo prevents germination; these structures normally could be the endosperm, the testa (seed coat), or the pericarp. Seeds can have combined exogenous and endogenous dormancy as well.

According to Nikolaeva's classification strategy, there are three types of exogenous dormancy: physical, mechanical and chemical. Physical dormancy is where a covering, most often the seed coat, but it could also be the pericarp, impedes water

absorption. The impermeability of the testa protects the embryo during times of scarce water availability when the seed would imbibe the available water but then not have enough to complete germination. Most seeds with physical (hard seed coats) dormancy, can spread their germination across different years but have very specific seasons, usually during the seasons of heavy precipitation, in which they germinate (Baskin and Baskin, 1998). This indicates that environmental conditions break down the seed coat. This can happen at different rates for different seed; following this, the seed will readily imbibe when given adequate moisture.

Mechanical dormancy is when the pericarp or testa simply does not give the embryo any space to elongate and germinate. However, according to Baskin and Baskin, most species with this stony endocarp will either be impermeable to water (physical dormancy), or the seed will have a type of endogenous dormancy. Once that dormancy is broken, the seedling will have enough vigor to push through the endocarp.

Finally, there is chemical dormancy, which is associated with high levels of germination inhibitors. Köckemann, in 1934, discovered that tomato seeds failed to germinate inside the fruit because of the presence of inhibitors in the juice and not due to lack of oxygen or osmotic potential. Cocklebur, *Avena fatua*, was discovered to have inhibitiors in its seed coats as well (Black, 1959). The most well-known germination inhibitor is abscisic acid or ABA, which was identified in the mid-1960's. The auxin indolebutyric acid, IBA, is also known to inhibit germination. Seeds can also have both physical and chemical dormancy. The seed coat or pericarp may not only inhibit water uptake but also block the exit of inhibitors (Bewley and Black, 1994). The testa or pericarp thus plays a key role in dormancy.

There are many different types of endogenous or embryo dormancy. They are due to highly decreased activity of the embryo or due to the embryo not being fully developed or a combination of both situations. Physiological dormancy is the decreased activity of the embryo. Physiological dormancy (PD) is difficult to differentiate from physical dormancy because the testa or pericarp still plays a role inhibiting germination. When PD embryos are excised, they grow normally, which most times indicate physical dormancy. However, when they are germinated immediately after harvest (before the seed coat can harden) the seeds do not germinate, indicating that another dormancy mechanism prevails. When they are given dormancy breaking treatments they germinate readily. Therefore, it is hypothesized that physiological dormancy is an interaction between the embryo and testa. Originally, it was theorized that the testa contained phenolics that react with oxygen before oxygen reached the embryo and the lack of oxygen prevented germination (Come and Tissaoui, 1972; Coumans et al., 1976). However, more recent research has shown that oxygen levels were not a large factor in dormancy. Jacobsen *et al* studied *Apium graveolins* (celery) and suggested that the embryo produces a chemical signal, gibberellic acid, that stimulates hydrolase production in the endosperm. The hydrolase then breaks down the endosperm for embryo use and germination follows. Nikolaeva classified three levels of physiological dormancy: nondeep, intermediate and deep. These indicate the strength of the dormancy inducing mechanism as well as how GA can break dormancy; it is effective in nondeep and intermediate but not for deep PD.

Morphological dormancy occurs when the embryo is underdeveloped. It is most prevalent in families with rudimentary or linear seeds (Baskin and Baskin, 1998). Also,

morphological dormancy (MD) is more common in large seeds where the embryo is less than 1% of volume. The seed cannot germinate until embryo development is complete. This does not happen until seeds separate from the parent plant (Nikolaeva, 1969). MD is most common in plant species from the tropics; however, it is also found in temperate regions. Typically, an underdeveloped embryo is associated with the presence of inhibitors.

Seeds can also have both physiological and morphological dormancy, termed morphophysiological dormancy (MPD). This type of dormancy is prevalent in many families, including *Papaveraceae*. Nikolaeva identified seven types of MPD and Baskin and Baskin added an eighth type. Nondeep and intermediate simple MPD is typical of winter annuals where the seeds mature in late spring and need the warm temperatures of summer to break the PD. This is followed by embryo development and it germinates in autumn. Deep simple MPD is more common for seeds that mature in summer but do not germinate until spring. Two types of physiological dormancy are involved in deep simple MPD and usually require different treatments to break both types of PD (usually a warm stratification and cold stratification). Epicotyl MPD refers to the observation that the radicle emerges in autumn yet the shoot does not emerge until the following spring. Barton, in 1933, showed that *Paeonia suffruticosa* seeds exhibit this behavior and that the epicotyl remains dormant even when the radicle is not. Double MPD refers to seeds that take 2 years to germinate. Seeds disperse in autumn and radicles emerge in the first spring. During the summer a corm-like structure forms from the hypocotyls with leaf development the following spring. Finally, there is complex MPD with three levels: nondeep, intermediate and deep. Nondeep MPD was identified by Baskin and Baskin in

1991 after investigating the germination of lilies in the *Erythronium* genus. These seeds mature in summer, disperse in autumn and germinate in spring. If the seeds did not separate from the maternal plant until winter, then they did not germinate the next spring but the following year in spring. This is strong evidence that the seeds need to imbibe water when temperatures are warm to finish embryo development. They then require cold temperatures to break the physiological dormancy. Nikolaeva classified many seeds from the *Trollius* genus (common name, Globeflower) with intermediate complex MPD. For these seeds, maturation and dispersal happen through the summer and autumn, but germination only occurs in the spring. Cold temperatures break both the morphological and physiological dormancies and laboratory tests showed that gibberellic acid could substitute for the cold temperatures. Deep complex MPD is similar to intermediate complex MPD with the exception that GA did not substitute for cold temperatures.

Although each dormancy type can occur in any region, some types are more prevalent in some geographical locations than others. Considering the origins of the different *Glaucium* species, woodlands, montanes and beaches were looked at more closely. In sclerophyllous woodlands within the Mediterranean Basin, herbaceous perennials will most likely have morphological, morphophysiological and physical dormancy (Baskin and Baskin, 1998). California's vegetation is also considered asclerophyllous woodland because of its thick dense, drought-resistant foliage. *Papaver californicum* has MPD because it has underdeveloped embryos and freshly matured seeds do not germinate. Hebaceous perennials from deciduous forests, like the Caucasus Mountains, exhibit morphological, physiological, morphophysiological dormancy or no dormancy at all (Baskin and Baskin, 1998). Rock outcrops, dunes and river banks in the midst of the

forests usually account for special circumstances of plants with different types of dormancy. Mountain regions have hebaceous species that are usually nondormant or have physiological dormancy (Baskin and Baskin, 1998). However, these species are not well researched and it is presumed that physical dormancy occurs too since many families with physical dormancy populate montane regions. Finally, beaches and foredunes are typical areas for halophytes. Unfortunately, only 7% of these species have known germination data for them. The most common were nondormant or morphophysiological dormancy with *G. flavum* being reported for MPD. Since the only dormancy listed for *Papaveraceae* is MPD and it was the most common among all regions of *Glaucium*, it is expected that MPD will be the predominant dormancy to overcome.

2.3 Dormancy Breaking Strategies

The reason for understanding the various types of dormancy is to understand how best to overcome that dormancy. Physical dormancy is probably the most involved because there are many different strategies for softening or breaking the seed coat. Chemical dormancy is broken by using germination promoters like Gibberellic acid, GA, to counteract the inhibitors. The different endogenous dormancy types usually require either warm or cold stratification periods or a combination of both. Nondeep physiological dormancy is common for freshly collected seeds and it is lost over time during dry storage (Baskin and Baskin, 1998). Intermediate and deep PD need cold stratification. Nondeep MPD requires only warm stratification, while other types of MPD need combinations of warm and cold stratification. The interaction of light can

make it more challenging to find the best strategy to break dormancy. A closer look at each technique explains how they overcome each type of dormancy.

Warm Stratification

Warm stratification is a period of time after dry storage where the seeds are in moist conditions (media or substrate) at temperatures between 20°C and 35°C. Warm stratification is the only way to break morphological dormancy and nondeep simple MPD (Baskin and Baskin, 1998). It is also used in combination with cold stratification to break other types of MPD. Warm stratification is a simulation for summer temperatures for the seeds. It gives the underdeveloped embryo the environmental conditions it needs to complete development and prepare to germinate.

Cold Stratification

Cold stratification is a pre-chilling treatment before seeds are germinated. It simulates winter temperatures; therefore, seeds are placed in a moist media or substrate and kept in temperatures from 0°C to 10°C (Baskin and Baskin, 1998). Moist chilling induces changes in hormonal levels by decreasing abscisic acid and raising cytokinin and gibberellic acid levels (Hartmann and Kester, 2002). It is also theorized that cold stratification weakens the seed coverings and increases enzyme activity (Hartmann and Kester, 2002). Cold stratification is used to break physiological and morphophysiological dormancy (Baskin and Baskin, 1998). It is often the only way to break deep PD and deep MPD dormancy. When breaking MPD, cold stratification is used in combination with warm stratification for all the simple types.

Gibberellic Acid

Gibberellic acid, GA, is a hormone that has shown a strong ability to break many types of dormancy. Cold stratification raises levels of a GA producing mechanism and then when seeds are transferred to warm temperatures for germination, GA levels increase (Jones and Stoddart, 1977). Since cold stratification is a proven method to break dormancy and it improves a seed's ability to produce GA, then exogenously applied GA should also stimulate germination. In cases of nondeep or intermediate PD or MPD, GA has been shown to effectively substitute for a cold stratification period. However, deep PD and MPD cannot be broken with GA, indicating that cold stratification does more to stimulate embryos than just GA. Gibberellic acid can substitute for dry storage in simple PD. This is due to the concept that during dry storage the embryo is releasing GA to stimulate the endosperm to breakdown in preparation for germination. Exogenously applied GA simply speeds up the process. Based on studies on cereal grains, it is suggested that in all seeds whose germination is stimulated by GA that the breakdown of starch and other substrates is a result of GA induced enzyme action as the first step (Jones and Stoddart, 1977).

Cytokinins

Cytokinins are a group of plant hormones that stimulate cell division and are key hormones in plant growth. Cytokinins also have been shown to play a role in breaking dormancy. In 1971, A.A. Khan proposed a model for the hormonal control of seed germination. Gibberellins play the primary role in promoting germination while cytokinins play a secondary role in overcoming the inhibitors. This model was developed

from research on weed and crop seeds where they observed that ABA inhibited germination and application of GA alone either did not overcome the ABA or only partially overcame it. When they combined cytokinin, specifically kinetin, with GA and applied it to seeds inhibited by ABA, the two hormones together completely reversed inhibition and the seeds germinated. Brown and Van Staden, in 1975, demonstrated that exogenous applications of cytokinins promoted germination as well as GA treatment in *Leucadendron daphnoides*. They demonstrated that cytokinins may play a primary role in breaking dormancy. Their work also showed that exogenously applied cytokinins can substitute for cold stratification. Studies comparing the effect of cytokinins on breaking dormancy in different temperatures and light conditions showed an interaction among the three. In lettuce seed germination, exposure to red light improved the effect of cytokinin (Reynolds and Thompson, 1973).

Potassium Nitrate (KNO_3)

While GA and cytokinins are plant hormones, potassium nitrate is an inorganic chemical that is used to overcome dormancy. Concentrations of 0.1 to 0.2% KNO_3 solutions are used to promote germination, primarily in light-sensitive seeds (Copeland and McDonald, 2001). The exact mechanism as to how KNO_3 promotes germination is still undetermined; however, research has identified a few possibilities. Copeland (2001) has identified research trials in recent years that have led to a better understanding of the role of KNO_3. He cited work by Adkins, et al in 1984, which demonstrated that KNO_3 affects the respiratory system in seeds. Copeland also cited that in 1986, Hilton and Thomas demonstrated that KNO_3 stimulated oxygen uptake. How the interaction of KNO_3 and oxygen affects germination is still undetermined. The latest research by

Hilhorst in 1990, demonstrated that KNO_3 works with phytochrome to stimulate germination.

Light

Some seeds require light to germinate while some seeds need an absence of light. Many times light interacts with chemical treatments to break dormancy. The influence of light on germination and other biological processes is due to the photoreceptor phytochrome. Phytochrome is a blue pigment protein that influences many biological processes, including dormancy, by its ability to absorb red light and far red light (Taiz and Zeigler, 2002). When a plant is grown in the dark, the form of phytochrome the plant naturally synthesizes is P_r. The P_r form absorbs red light (650-680 nm) and converts it to the P_{fr} form. P_{fr} can convert back to P_r when exposed to far red light (710-740 nm) or when maintained in dark for a sufficient length of time. In seeds, phytochrome is located primarily in the embryo axis and in very low concentrations in the cotyledons (Bewley and Black, 1994). Phytochrome in its P_{fr} form, activates the transcription for the gene that increases the level of GA, which then promotes germination (Taiz and Zeigler, 2002). Light requiring seeds contain limited amounts of P_{fr} and need light exposure to convert P_r to P_{fr} (Vidaver, 1977). Germination can be inhibited by light in some seeds. Some of seeds are desert plants and survival could depend on being buried at depths where there is adequate moisture (Hartmann and Kester, 2002).

Mechanical scarification

Scarification is the process of making breaks in the seed coat to allow for imbibition and thus overcome physical dormancy. Mechanical scarification uses abrasive

methods to soften, scratch or cut into the seed coat. If the seed only has physical dormancy then the only reason for the dormancy is that the seed coat is impermeable and thus does not allow water into the seed. Scratching the seed coat will either thin it sufficiently to make the testa permeable or make holes into the surface and allow water through. One method of mechanical scarification is to use a razor blade and cut a slit into the seed coat. Needles are also used to prick a hole in the coat (Baskin and Baskin,1998). Another method, which is more practical for large amounts of seed, is to blow seeds across sand paper under pressurized air. The coarseness of the sand paper and the intensity of the pressure are variables that need to be determined for each seed lot.

Hot Water Scarification

Immersing seeds in hot water is a technique for softening the testa and breaking physical dormancy. The boiling water causes the palisade layer in the seed coat to separate from the mesophyll and create cracks in the seed coat (Baskin and Baskin, 1998). It may also leach out inhibitors in the testa, especially salts. Seeds can be placed in a bag or strainer and dipped into water; duration in the water will be a variable to test for each species (Baskin and Baskin, 1998). Another way to perform hot water scarification is to bring water up to a temperature between 77°C and 100°C, drop the seeds in and immediately remove the water/seeds from the heat source (Hartmann, et al. 2002). The seeds remain in the water for 12 to 24 hours and imbibe.

Acid Scarification

Acid scarification is another seed coat softening strategy to break physical dormancy. The acid eats holes into the seed coat as well as destroys the naturally

plugged opening in the strophiole. Concentrated sulfuric acid is the most commonly used acid. It is poured over the seeds; the amount of time seeds stay in the sulfuric acid is a species-dependent variable. The seeds need to be washed for 10 minutes afterwards or rinsed in water with a small amount of baking soda dissolved in it (Hartmann et al, 2002).

Hydrogen Peroxide

Hydrogen peroxide is a pretreatment that has been shown to improve germination in some species. Like KNO_3, the mechanism of how it works is undetermined and many theories have been developed. In 1959, Ching studied the effect of H_2O_2 on the germination of *Psuedotsuga menziesii*. The results demonstrated that H_2O_2 increased the germination rate in the seeds which correlated with an increase in respiration rates. They determined that hydrogen peroxide increased the rate of conversion of fats in the seed and thereby increased the rate of synthesis of cellular components for germination. Chien and Lin, in 1994, determined from their research that H_2O_2 is helpful in cracking the hard seeds, allowing them to interact with water. In 2001, Ogawa and Iwabuchi demonstrated that hydrogen peroxide promotes germination by decomposing inhibitors. The increase in respiration rate indicates hydrogen peroxide would break physiological dormancy, while cracking the seed coat would break physical dormancy and the decomposition of inhibitors would break chemical dormancy.

2.4 Previous Research on *Glaucium sp.* Germination

In 1963, G.A. M. Scott reported his research on *G. flavum* in the Journal of Ecology. He studied the habitat and geological distribution of the species across all of Europe and determined that it grows mostly in warm climates without preference for

differences for specific rainfall regimes although it grows in areas with approximately 20-in to 60-in (508 to 1524 mm) of precipitation annually. He found a striking similarity among the locations of *G. flavum* in that the areas all have high pH soils from 7.5 to 8.4 with the mean being 8.1. Scott reported observations of *G. flavum* on the Southern coasts of England. He noted that the plants began flowering in mid-May and continued to as late as October although they were mostly finished by August. He observed that they were insect pollinated and performed experiments on cross- vs. self-pollination where he determined both were possible. He noted that the first fruits developed and matured in July yet did not dehisce until late autumn and early winter and that the seeds did not germinate until March or April.

Scott also performed seed germination trials as part of his research with *G. flavum*. He compared unripened and ripened seed. He found that unripen seed germinated quite readily while ripe seed did not. He determined that the testa hardens as it ripens and prevents imbibition. He then trialed many techniques to overcome seed coat dormancy such as cutting, cracking, sulfuric acid, hot water, auxins, cold, heat, leaching and shaking seeds with sand and water. The only effective method was shaking with 'Analar' sand and water in a shaker for 8-9 hours. This resulted in 50% germ. Older seed had better germination and he hypothesized that seed coats were worn down over time.

In 1976, Formanowicz and Koziowski studied the influence of stratification on germination of *G. flavum*. They determined that the optimal conditions was stratification for 30 days at -2°C to 2°C with germination at 20°C to 30°C in either light or dark. They also found that seeds did not germinate after harvest because of a requirement for an

after-ripening period. This apparently contradicts Scott's findings; however, the difference in seed behavior could have been due to the different environments in which the seeds were produced. They also performed trials with KNO_3 and determined that it did not aid germination.

In 1984, Mermerska performed seed germination trials on *G. flavum.* He compared different germination temperatures, stratification and other dormancy breaking strategies. Mermerska achieved 73-82% germination at temperatures between 4 and 15°C. However, gamma irradiation, KNO_3, germisane, and gibberellin did not improve germination.

In 1988, Thanos, e al., investigated the influence of light, especially red and far red light, combined with stratification and various temperatures on germination of *G. flavum*. They found that in the absence of any light or pretreatment, the best germination (greater than 80%) occurred below 20°C; at 20°C and above the germination rate dropped below 25%. A pretreatment of imbibing seeds in a mannitol solution inhibited germination especially as the concentration increased. For germination temperatures at 20 and 25°C, a 15-minute pulse of R light administered at 3 different 4-hour intervals after imbibition, improved germination (80% and 35% respectively); however, a 20-day stratification at 3°C increased germination at those temperatures to greater than 80% for both temperatures. When stratification was combined with R illumination, it produced germination rates around 90% for both 20°C and 25°C. They also tested imbibition at 25°C in varying amounts of light and then germinated seeds in complete darkness at 15°C. They found that even 1 hour of light inhibited germination. They also planted seeds in pots of sand media at varying depths from 0 to 6 cm. The best germination and

seedling emergence occurred at the planting depth of 0.5 cm; it singularly stood out from all the other depths including surface planting. Throughout the experiments, the researchers noticed a prolonged imbibition period, which they hypothesize is the seeds way of ensuring that it germinates during a period of sustained rainfall instead of responding to a brief period of rain. The light interaction indicates to the authors a surface-avoiding mechanism. When seeds are at the soil surface, complete daylight inhibits germination; however, a shallow burial will block enough light to encourage germination.

In 1997, Walmsley and Davy studied *G. flavum* as one of six species for germination for vegetative restoration of a shingle beach habitat in Sizewell, Suffolk, UK. Seed was collected during the growing season of 1986 and again in 1992 after the construction of a power plant. Viability of the 7-year old seed (collected in 1986) was compared to the 1-year old seed by TZ tests. Germination pretreatments included a control with no stratification and stratification of varying weeks (2-8 weeks) at two different temperatures, 25°C and 2°C. Germination was tested at 25/15°C, 20/10°C and 15/5°C in total darkness and 12 hours of daylight. Viability of *G. flavum* was 100% in the 1-year old seed and over 90% in the 7-year old seed. *G. flavum* had no germination without stratification, and less than 15% germ with 25°C stratification. However, stratification at 2°C resulted in high germination (over 90%) for the 1-year old seed in all treatments. The 7-year old seed had lower germination (between 48% and 64%) in the warmer germination temperature of 25/15°C, but had approximately 90% germination in the 15/5°C conditions for both light and dark. The improved germination at cooler

temperatures corresponds to the normal temperatures during March and April when *G. flavum* naturally germinates.

The researchers also compared germination with different levels of saline salt to mimic the sea water normally found on coasts. Germination in *G. flavum* dropped dramatically at 20% salinity. The authors indicate that *G. flavum* could be influenced by a salinity-enforced dormancy which would prevent it from germinating in winter when sea water inundates the beaches and force the seeds to wait until spring when rainwater would leach the salts from the sand and seeds. Unripened seed was also tested. Walmsley and Davy found that unripe seeds germinated readily and determined that *G. flavum* seeds have a hard seed coat dormancy as noted by Scott in 1963. Seed age increased the seeds sensitivity to supraoptimal temperatures. Therefore, they concluded that reduced germination in older seeds is not necessarily due to lower viability but because of growing sensitivity to inappropriate conditions such that the optimal environmental conditions has a more narrow range; this is likely a symptom of decreased vigor.

Overall, previous research has clearly indicated a need to stratify *G. flavum* seeds and a consensus that the seeds have seed coat dormancy (hard seed). However, a combination of stratification with a seed coat dormancy breaking strategy has not been tested. Previous research has also verified that *G. flavum* germinates better in cooler temperatures and that light can interact with germination at warmer temperatures (breaking a surface-germination avoidance mechanism) but light impedes germination at cooler temperatures. There is contradictory evidence relative to the ability to germinate unripen seeds. This may be an environmental effect since the research that supports

germination of unripe seed were both from England and the research that supports an inability to germinate unripe seed is from Poland. There is no reported seed germination trials on other species of *Glaucium*, so it is still to be determined if other *Glaucium* species will germinate under similar conditions.

Chapter III

Materials and Methods

3.1 Seed Acquisition

Seeds were acquired from Denver Botanic Garden's collection of *Glaucium* plants including: *G. flavum* collected in 2003 and 2005, *G. acutidentatum* collected in 2003 and 2005, *G. grandiflorum* collected in 2003, *G. corniculutatum* collected in 2003 and 2004 and *G. corniculatum x flavum* collected in 1999. All five lines are grown in the Rock Alpine Garden with an area of 43,560 ft^2 or approximately 4046.9 m^2. *Glaucium* species are generally considered cross-pollinators. Therefore, since the plants were grown in close proximity to each other, the seeds obtained most likely were not pure species. Impurity was confirmed when the germinated seeds were grown to point of flowering and the characteristics varied from species type of the lines among the progeny. Species type characteristics were based on the descriptions from Flora of Turkey and the data is presented in Appendix A.

Seed germination behavior is highly influenced by the maternal plant. Maternal plants influence seed germination by chemicals passed to the developing seeds or by the influence of the seed coat (Baskin and Baskin, 1998). The testa is derived from the integuments of the ovule (Hartmann, et al., 1997); therefore, the testa's genetic makeup is completely maternal in origin. Differences in germination behavior among the species were expected to be detectable.

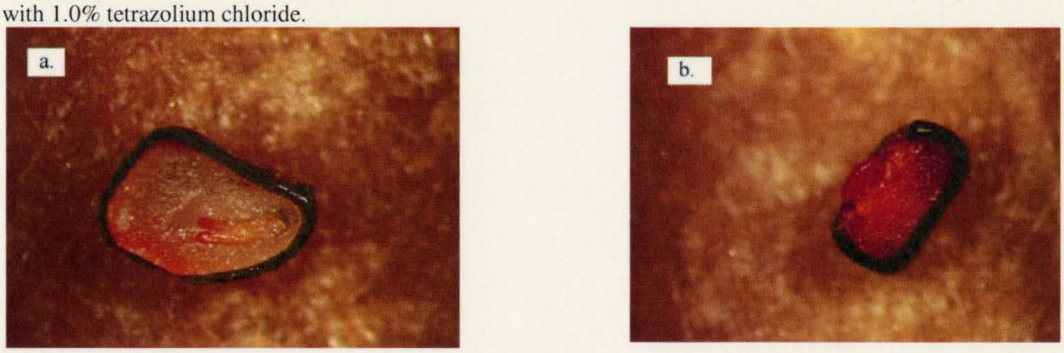
3.2 Viability Testing

A seed is considered viable if the embryo is alive. The viability of the seeds was tested with a 1.0% concentration of 2,3,5-triphenyltetrazolium chloride (TZ). The testing method was in accordance with the standards set by the Association of Official Seed Analysts (AOSA) for seeds of the *Papaveraceae*. Two replications of 100 seeds from each seed lot were set on moist blotter paper to soften overnight. Off-center, longitudinal cuts were then made into the seeds and they were placed in dishes of 1.0% TZ. The dishes were set in a dark store room at 19°C overnight. Viability was determined based on diagrams and descriptions of *Papaver sp.* seed staining in the AOSA handbook. Viability tests were performed on the 2005 and 2003 *G. flavum* seeds prior to the stratification trial and the combined stratification/scarification trial. 2003 and 2005 *G. acutidentatum* seeds were tested prior to the scarification trial and the combined stratification/scarification trial. The rest of the seeds were tested before the combined stratification/scarification trial.

Table 3.1 Relative viability (in percent) was determined using a 1.0% tetrazolium stain at specific times prior to germination trials.

Seeds	5/19/2006	11/1/2006	2/7/2007
G. flavum, 2003	65.0		
G. flavum, 2005	85.5		86.0
G. acutidentatum, 2003		74.5	87.0
G. acutidentatum, 2005		89.5	92.0
G. grandiflorum, 2003			86.0
G. corniculatum, 2003			93.0
G. corniculatum, 2004			97.0
G. corniculatum x flavum, 1999			92.0

Figures 3.1 Pictures that illustrate the differences between (a) nonviable and (b) viable *Glaucium sp.* seeds stained with 1.0% tetrazolium chloride.



3.3 Imbibition Testing

The purpose of the imbibition test was to determine if the seeds had impermeable seed coats. 50 seeds were weighed and placed between two moist blotter papers in Petri dishes. They were weighed at 4 hour intervals for 12 hours and reweighed according to the procedure from Baskins and Baskins (1998). Since the imbibition test demonstrated the seeds were not imbibing water, it was assumed that the seed coat was not permeable.

Table 3.2 Weight, in grams, of 50 *Glaucium flavum* seeds from two collection years during 4-hr intervals of imbibition test.

	Initial Wt.(g)	**Wt.(g) at 4 hrs.**	**Wt.(g) at 8 hrs.**	**Wt.(g) at 12 hrs.**	**% Wt gain**
2003 G. flavum	0.045	0.051	0.053	0.053	17
2005 G. flavum	0.040	0.045	0.045	0.045	14

3.4 Germination

Germination tests were all conducted in accordance with testing standards specified by AOSA for *Papaver sp.* seeds since they did not have procedures outlined for *Glaucium sp.* One exception to their procedure was that KNO_3 was omitted since it

would interact with the other dormancy-breaking methods being evaluated. 50 seeds were used in each replication and 4 replications for each treatment were used. The seeds were placed on moistened blotters inside Petri dishes. The dishes were wrapped with parafilm to seal them shut. They were then placed in a germinator at the Colorado State Seed Laboratory. The germinator was set at 15°C and held that temperature at +/- 0.6°C. The germinator's light source was 6 cool, white fluorescent bulbs, which emits approximately 10.25 µmol/s m^2 of light. When the light treatment was used, the germinator was set to give 8 hours of light from 9AM to 5PM. For the dark treatments, the four replications of each treatment were wrapped in aluminum foil. For every trial, the observation window for germination was 28 days before termination of the experiment. Protrusion of the radicle was the criteria for successful germination.

Figure 3.2 An illustration of radicle protrusion in germination of *Glaucium sp.*

3.5 Stratification Trial

The first trial compared 2003 *G. flavum* and 2005 *G. flavum* germination. The purpose was to verify if seeds grown here in Colorado behaved similarly to the germination behavior reported in the European studies. Stratification at 7°C +/- 1°C in a refrigerator for 45 days was compared to non-stratified seeds directly germinated from dry storage at room temperature. Four different germination conditions: 4°C/dark, 4°C/light, 15°C/dark and 15°C/light. The germinator at the Colorado State Seed Lab was used for the 15°C conditions and a walk-in cooler was used for the 4°C conditions. The cooler held temperatures between 3°C and 6°C. Four fluorescent bulbs was the light source in the cooler and they emitted 6.47 μmol/s m^2 of light.

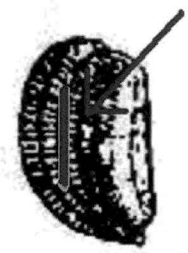
After non-stratified seeds in the 4°C conditions (both light and dark) did not germinate, they were moved to the 15°C germinator to evaluate if they would still germinate. The seeds that were stratified at 7°C and tested in the 4°C did not germinate either, but they were discarded after growing mold. The seeds that were moved to the 15°C germinator germinated and the data was added to the analysis. This introduced two aspects of confoundedness. First, the duration of the different temperatures of stratification was different; the stratification at 7°C lasted 45 days while the stratification at 4°C was 28 days (the observation period for germination). Second, the stratification at7°C was in darkness and the treatments with light were only exposed to light during germination. However, the treatments with light and stratification at 4°C included light during the stratification and the germination period because the stratification was originally a germination treatment. Although the data was confounded, it was still believed to be useful.

Therefore, the analysis changed from a 2x2x2x2 factorial, comparing seed age, stratification, light and germination temperature to a 2x3x2 factorial comparing seed age (2 levels), stratification (3 levels – none, 7°C and 4°C) and light (2 levels). Statistical analysis was done using Statistical Analysis System software (SAS Inst. Inc, with the general linear models. Analysis of variance was performed and the F-values were considered significant at p-values of 0.05 or less.

3.6 Scarification Trial

Since the imbibition test showed impermeability, a scarification trial was conducted. 2003 *G.flavum*, 2005 *G. flavum*, 2003 *G.acutidentatum* and 2005 *G. acutidentatum* seeds were used in order to compare differences between age of seed and species. Five scarification treatments were compared: hot water, cuts into the testa, 30-minute soak in sulfuric acid, 60-minute soak in sulfuric acid and no scarification. For the hot water scarification, water was brought to boiling and the heat source was turned off. The seeds were added and the beakers were removed from the heat source. After 24 hours the seeds were removed from the beakers and put into Petri dishes. Cuts into the testa were performed by hand with a razor blade using a dissecting scope. Cuts were off center to avoid the embryo in case the cut was deeper than the seed coat.

Figure 3.3 A diagram that illustrates the area of cut into the *Glaucium sp.* seed coat.



For acid scarification, seeds were placed in small glass jars (6 cm tall and 5 cm in diameter) and sulfuric acid was poured into the jar to the point that the seeds were completely submerged in acid. They remained in the acid for 30 minutes +/-3 minutes or 60 minutes +/- 3 minutes. All seeds were germinated in Petri dishes with moistened blotter paper, wrapped in foil at 15°C in the Colorado State Seed Lab germinator. Statistical analysis was done using Statistical Analysis System software (SAS Inst. Inc) with the general linear models. Analysis of variance was performed and the F-values were considered significant at p-values of 0.05 or less. Differences were compared with the arcsin transformation because of low variation at low germination rates and higher variation at higher germination rates. The means were compared from the original data.

3.7 Stratification and Scarification Combined Trial

The combined trial compared scarification, stratification and combined scarification/stratification on all the collected seed species. It was a 3 x 3 x 7 factorial design, comparing 3 levels of scarification, 3 levels of stratification and 7 different seed lots. The three levels of scarification were 30 minutes of sulfuric acid, 60 minutes of sulfuric acid and no scarification. The three levels of stratification were 30 days of stratification, 45 days of stratification (both at 8°C) and no stratification. The seed lots were 2005 *G. flavum*, 2003 *G. acutidentatum*, 2005 *G. acutidentatum*, 2003 *G. grandiflorum*, 2003 *G. corniculatum*, 2004 *G. corniculatum* and 1999 *G. corniculatum x flavum*. Seeds were stratified in a walk-in cooler set at 47°F (8°C). All Petri plates with seeds were wrapped in foil so light was not a concern. Scarification treatments were 30-

minute concentrated sulfuric acid soak, 60-minute concentrated sulfuric acid soak or no scarification.

All seeds were wrapped in foil and germinated at 15°C, in the germinator at the Colorado State Seed Lab. Statistical analysis was done using Statistical Analysis System software (SAS Inst. Inc) with the general linear models. Analysis of variance was performed and the F-values were considered significant at p-values of 0.05 or less.

During the trial, mold growth affected the results of many of the stratification treatments. Mold grew within just a few days after being moved from the cooler to the germinator. It occurred in the 2004 *G. corniculatum* and with the 30-minute acid and 60-minute acid treatments. It killed the seeds in the first week of the trial. Some replications of *G. grandiflorum* were also killed by the mold, but enough survived to run an analysis. A second repeat trial was performed with additional efforts towards cleaning to prevent or slow down the mold growth. Alcohol was used to wipe down all surfaces and equipment that came in contact with the seeds. Unfortunately, the efforts made to make the set up procedure more sterile to prevent mold growth dramatically decreased germination in all treatments. Therefore, those results were not included in the statistical analysis.

3.8 Gibberellic Acid and Hydrogen Peroxide Trial

The final trial compared exogenously applied Gibberellic acid, cold stratification and hydrogen peroxide. The design was a 6 x 9 factorial design, comparing 6 seed lots and 9 different treatments. The 9 treatments were: 24-hr soak in 3% hydrogen peroxide,

48-hour soak in 3% hydrogen peroxide, 30-minute acid scarification, 30-day stratification, 30-minute acid scarification with 30-day stratification, 30-minute acid scarification combined with 400 ppm GA, 30-minute acid scarification combined with 500 ppm GA, 30-minute acid scarification combined with 1000 ppm GA and a control. For the hydrogen peroxide treatments, seeds were placed in glass jars (6 cm tall and 5 cm in diameter) and 3% H_2O_2 was poured over them to fill the jar 1/3 of the way up and submerge the seeds. They were kept at room temperature in cabinets to prevent light interaction. Stratification was at 8°C in the same cooler as the combined trial. Acid scarification was 30-minute soaks in concentrated sulfuric acid. Powdered GA_3 was mixed with nanopure water to make concentrations of 400 ppm, 500 ppm and 1000 ppm. These were poured into the Petri dishes to moisten the blotter paper. The same seeds used in the combined trial were used again for this trial with the exception of 2005 *G. flavum* because of insufficient seed. Analysis of variance was performed and the F-values were considered significant at p-values of 0.05 or less. Differences were compared with the arcsin transformation because of low variation at low germination rates and higher variation at higher germination rates. Student-Nueman-Keul (SNK) means separation test was used to compare the treatments.

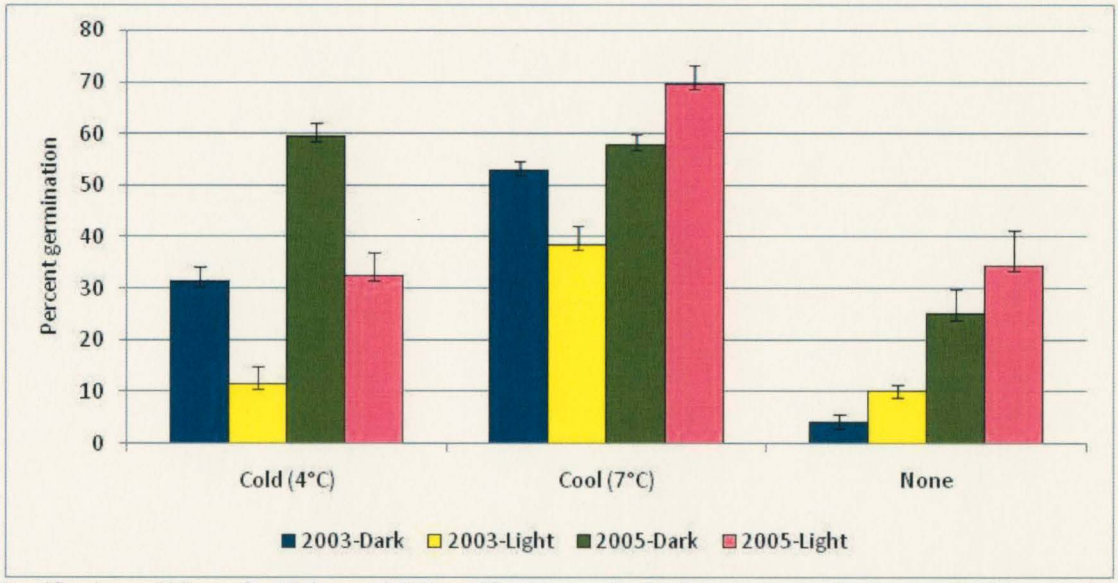
Chapter IV

Results and Discussion

4.1 Stratification Trial

Papaveraceae seeds are classified with morphophysiological dormancy (Baskin and Baskin, 1998). Therefore, *Glaucium flavum* was expected to require cold stratification. Stratified seeds (harvested in 2003 and 2005) had greater germination than those not stratified (Figure 4.1 and Table 4.1). Germination varied when the two different temperatures of stratification were compared.

Figure 4.1 Average percent germination and standard error of *Glaucium flavum* seeds (harvested in 2003 and 2005) germinated at 15°C with two stratification treatments and with or without light.



Stratification at 7°C was for 45 days and 4°C stratification was for 28 days. Light germination was for 8 hours a day provided by 6 fluorescent bulbs emitting 10 µmol/s m^2 of light and the dark treatment was achieved by wrapping the Petri dishes with foil. Four replications of 50 seeds were used for each treatment.

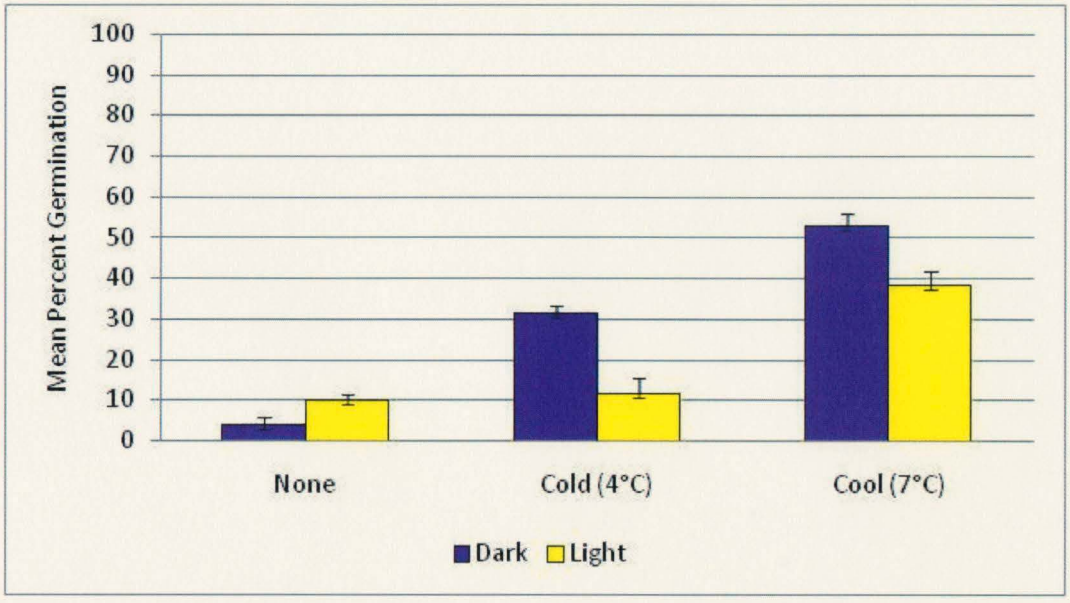
Table 4.1 Average percent germination and standard error of *Glaucium flavum* seeds (harvested in 2003 and 2005) germinated at 15°C with different stratification treatments and with or without light.

Year	Strat[a]	Light[b]	mean of % germ[c]	Standard Error
2003	Cold (4°C)	Dark	31.5	2.8723
2003	Cold (4°C)	Light	11.5	3.304
2003	Cool (7°C)	Dark	53	1.7321
2003	Cool (7°C)	Light	38.5	3.7749
2003	None	Dark	4	1.633
2003	None	Light	10	1.4142
2005	Cold (4°C)	Dark	59.5	2.63
2005	Cold (4°C)	Light	32.5	4.5735
2005	Cool (7°C)	Dark	58	1.8257
2005	Cool (7°C)	Light	69.5	3.8622
2005	None	Dark	25	4.9329
2005	None	Light	34.5	6.8007

a. 7°C stratification was for 45 days while 4°C stratification was for 28 days.
b. Dark – Petri dishes were wrapped in foil, Light was 8 hours a day provided by 6 fluorescent bulbs emitting 10 µmol/s m^2 of light.
c. Four replications of 50 seeds were used for each treatment.

The greatest germination for the 2003 seeds was 53% with 7°C stratification followed by germination in the dark (Table 4.1). The 7°C stratification had greater germination than the 4°C stratification with or without light (Figure 4.2). The stratification treatments were consistent with greater germination without light than with light. The non-stratified seeds had greater germination with light. The improvement in germination may be due to the light activating phytochrome which is theorized to activate GA synthesis (Taiz and Zeigler, 2002).

Figure 4.2 Average percent germination of *G. flavum* seed (collected in 2003) at 15°C with stratification treatments and with or without light.

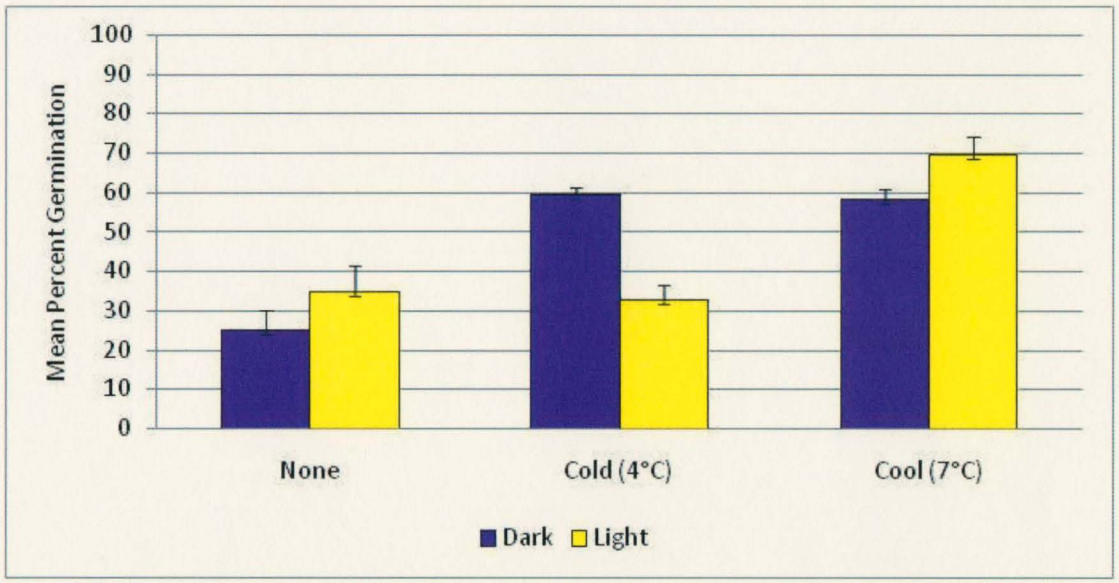


Stratification at 7°C was for 45 days and 4°C stratification was for 28 days. Light germination was for 8 hours a day provided by 6 fluorescent bulbs emitting 10 µmol/s m^2 of light and the dark treatment was achieved by wrapping the Petri dishes with foil. Four replications of 50 seeds were used for each treatment.

The most favorable conditions for the 2005 seeds were 7°C stratification and germination in light with 69.5% germination (Table 4.1). There was no significant difference in germination between the two temperatures of stratification in dark conditions; however, light improved germination for the non-stratified seeds and cool stratified seeds. The improved germination with light and stratification can be explained by Hilhorst and Karssen's theory of phytochrome receptors. Hilhorst and Karssen (1992) proposed a model where cold stratification promotes the synthesis of phytochrome receptors. When the receptor, X_A, binds with P_{fr} it creates phytochrome-receptor complex, X_P, which actives germination. However, in the 4°C stratification the effectiveness of light dropped (Figure 4.3). The 4°C was originally a germination treatment; therefore, the seeds were not only germinated in light when transferred to 15°C but also stratified in light. According to Steadman (2004), light during stratification can

cause phytochrome to inhibit germination. She expanded on Hilhorst and Karssen's theory of the phytochrome receptors and proposed that the phytochrome-receptor complex, X_P, has a limited time before it degrades. Therefore, light during stratification builds up X_p too soon and it likely degrades by the time the seeds are moved to warmer temperatures for germination.

Figure 4.3 Average percent germination of *G. flavum* seed (collected in 2005) at 15°C with stratification treatments and with or without light.



8°C stratification was for 45 days, 4°C stratification was for 28 days. Dark germination: Petri dishes were wrapped in foil. Light germination was for 8 hours a day provided by 6 fluorescent bulbs emitting 10 µmol/s m^2 of light. Four replications of 50 seeds were used for each treatment.

When the seeds from the two different collection dates are compared, there are three prominent interactions. First, there was an age effect with the older seeds displaying reduced germination, which could be due to loss of vigor or lower viability since 2003 seeds were only 65% viable and the 2005 seeds were 85.5%. Second, at the 7°C stratification, the 2003 seeds germinated better in darkness, while the 2005 seeds germinated better with 8 hours at light. According to Vidaver (1977), seed sensitivity to light is due to four factors: conditions applied during germination, post-harvest treatment,

growth conditions of the parent plant and parental genetic make-up. Therefore, although the variability in light response could be due to the hybridization among species resulting in genetic combinations, it could also be due to an age effect or growth conditions of the parent plant. Finally, for both ages of seed, the 4°C stratification treatment had a more dramatic difference in the dark and light germination than other treatments (Figure 4.1). This is likely due to the difference in stratification periods. The 4°C stratification was for 28 days while the 7°C stratification was 45 days. The shorter period may have contributed to the different reaction to light as well as the difference in temperature and light duration.

When average-days-to-germinate was statistically analyzed, seeds with germination below 30% were removed from the analysis due to likelihood that it would give an imprecise approximation. Since the 2003 non-stratified seeds did not germinate well, all non-stratified seeds were removed from the analysis. For both years of seed, germination took about 1 week; however, it took longer for seeds to germinate in light that were stratified at 4°C (Table 4.2). Most likely, light is slowing down or interfering with the enzymatic activities that occur during stratification. For both years of seed, there was also a significant difference between stratification treatments in light but not in the dark. This difference could be due to the different temperatures, but they also are likely due to the interaction with light.

Table 4.2 Average days-to-germinate and standard error of *Glaucium flavum* seeds (harvested in 2003 and 2005) germinated at 15°C with different stratification treatments and with or without light.

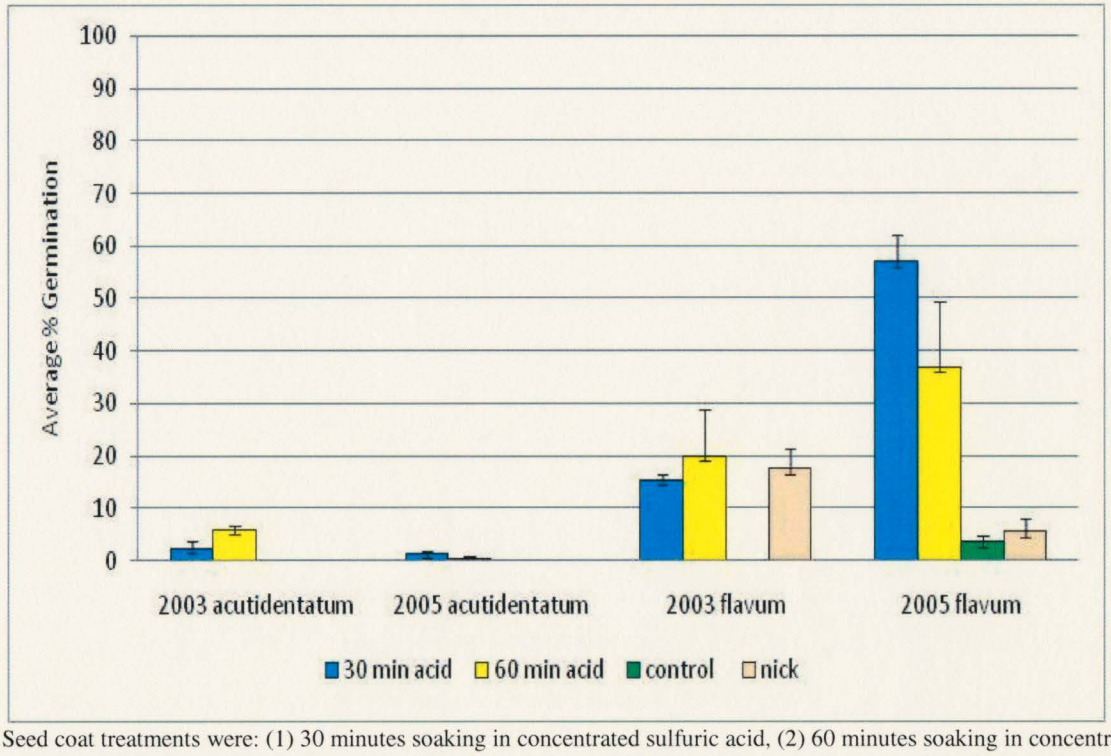
Year	Strat[a]	Light[b]	Days[c]	Standard Error
2003	4°C	Dark	6.3	0.225
2003	4°C	Light	7.9	0.278
2003	8°C	Dark	7.0	0.293
2003	8°C	Light	6.6	0.227
2005	4°C	Dark	6.0	0.209
2005	4°C	Light	10.2	0.248
2005	7°C	Dark	6.1	0.221
2005	7°C	Light	4.7	0.170

a. 7°C stratification was for 45 days while 4°C stratification was for 28 days.
b. Dark – Petri dishes were wrapped in foil, Light was 8 hours a day provided by 6 fluorescent bulbs emitting 10 µmol/s m^2 of light.
c. Four replications of 50 seeds were used for each treatment.

4.2 Scarification Trial

The scarification trial did not result in higher germination rates; however, it did indicate physical dormancy. There was no germination for the hot water treatment it was dropped from analysis. There was a major difference between species; *G. flavum* germinated more readily than *G. acutidentatum* (Figure 4.4). Despite the difference between species, acid scarification yielded the best germination. 2005 *G. flavum* had 57% germination with 30-minute acid scarification and 37% germination with 60-minute acid scarification. The control and wounding (nicks) were only 3.5% and 5.5% respectively (Table 4.3). 2003 *G. flavum* was the only seed lot that responded to nicks equally as well as the acid scarification (Figure 4.3). There was no statistically significant difference among three scarification treatments (30-minute acid, 60-minute acid and nicks) for the 2003 *G. flavum*. Since *G. flavum* from both collection years responded to scarification, a hard seed coat is likely a contributing factor to its dormancy.

Figure 4.4 Average percent germination at 15°C in darkness from two collection years, 2003 and 2005, of *G. acutidentatum* and *G. flavum* seed with four seed coat treatments.



Seed coat treatments were: (1) 30 minutes soaking in concentrated sulfuric acid, (2) 60 minutes soaking in concentrated sulfuric acid, (3) hand nicks by cutting into the testa with a razor blade and (4) control – no conditioning. Average germination was taken from four replications, 50 seeds each replication.

Table 4.3 Average percent germination at 15°C in darkness from two collection years, 2003 and 2005, of *G. acutidentatum* and *G. flavum* with four seed coat treatments.

Year	Species	Treatment	Mean Germination %	Standard Error
2003	*acutidentatum*	30 min acid	2.5	1.26
2003	*acutidentatum*	60 min acid	6	0.50
2003	*acutidentatum*	control	0	0.96
2003	*acutidentatum*	nick	0	5.20
2005	*acutidentatum*	30 min acid	1.5	0.82
2005	*acutidentatum*	60 min acid	0.5	0.50
2005	*acutidentatum*	control	0	9.02
2005	*acutidentatum*	nick	0	12.40
2003	*flavum*	30 min acid	15.5	0.00
2003	*flavum*	60 min acid	20	0.00
2003	*flavum*	control	0	0.00
2003	*flavum*	nick	17.5	1.26
2005	*flavum*	30 min acid	57	0.00
2005	*flavum*	60 min acid	37	0.00
2005	*flavum*	control	3.5	3.86
2005	*flavum*	nick	5.5	2.50

Seed coat treatments were: (1) 30 minutes soaking in concentrated sulfuric acid, (2) 60 minutes soaking in concentrated sulfuric acid, (3) hand nicks by cutting into the testa with a razor blade and (4) control – no conditioning. Average germination was taken from four replications, 50 seeds each replication.

G. acutidentatum had minimal germination in this trial. The 2003 seeds had 6% germination with 60-minute acid scarification and 2.5% with 30 minutes (Table 4.3). The 2005 seeds had 1.5% germination with 30-minute acid scarification and 0.5% germination with 60-minute acid. Neither 2003 nor 2005 *G. acutidentatum* seeds germinated with nicking or the control treatment. The poor germination indicates that the predominant dormancy for *G. acutidentatum* is likely endogenous dormancy.

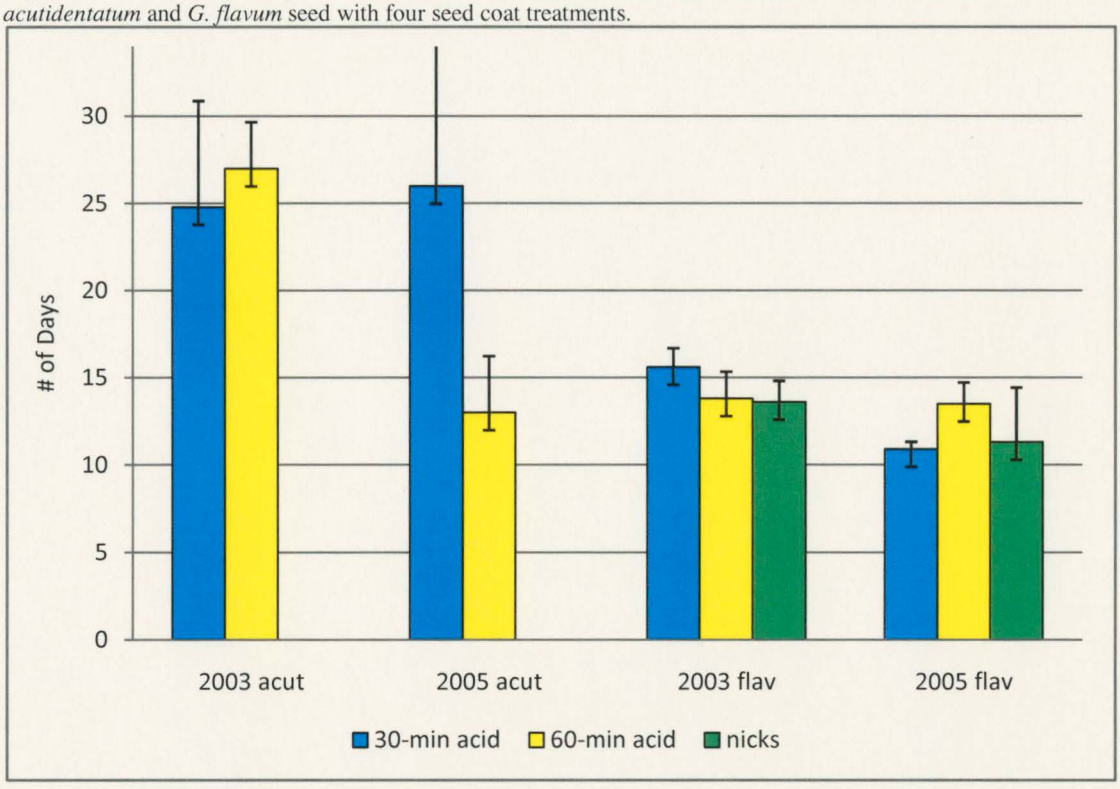
The statistical analysis for the average days to germinate was weighted by the number of germinated seeds. The analysis of differences indicated that the main influence on the length of time it takes to germinate is the species. The *G. acutidentatum* seeds from both years took significantly longer to germinate (25 – 27 days) than the *G. flavum* seeds (approximately 2 weeks); although the 60-minute acid treatment on the 2005 *G. acutidentatum* was similar to *G. flavum* (Table 4.4). The extended length of time for *G. acutidentatum* is also likely an indication of endogenous dormancy.

Table 4.4 Average days-to-germinate in darkness at 15°C from two collection years, 2003 and 2005, of *G. acutidentatum* and *G. flavum* with four seed coat treatments.

Seed Lot	treatment	average days	std. error
2003 acut	30-min acid	24.8	6.087
2005 acut	30-min acid	26	8.088
2003 flav	30-min acid	15.6	1.101
2005 flav	30-min acid	10.9	0.431
2003 acut	60-min acid	27	2.679
2005 acut	60-min acid	13	3.250
2003 flav	60-min acid	13.8	1.555
2005 flav	60-min acid	13.5	1.234
2003 acut	nicks	0	0.000
2005 acut	nicks	0	0.000
2003 flav	nicks	13.6	1.231
2005 flav	nicks	11.3	3.134

Seed coat treatments were: (1) 30 minutes soaking in concentrated sulfuric acid, (2) 60 minutes soaking in concentrated sulfuric acid, (3) hand nicks by cutting into the testa with a razor blade and (4) control – no conditioning. Average germination was taken from four replications, 50 seeds each replication.

Figure 4.5 Average days-to-germinate at 15°C in darkness from two collection years, 2003 and 2005, of *G. acutidentatum* and *G. flavum* seed with four seed coat treatments.



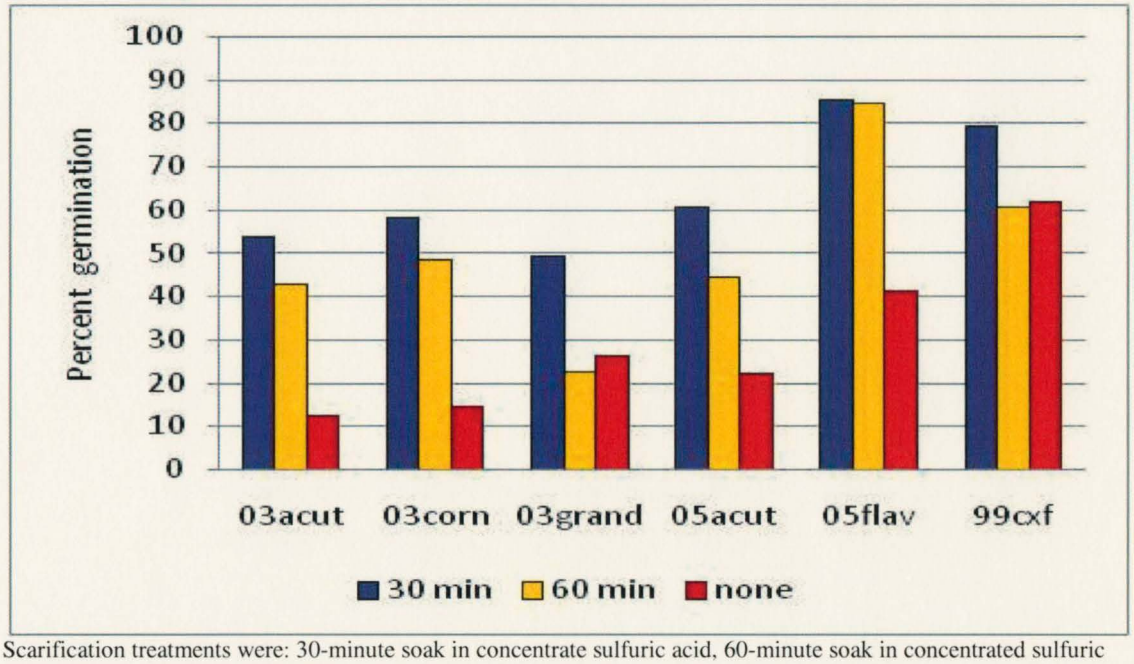
Seed coat treatments were: (1) 30 minutes soaking in concentrated sulfuric acid, (2) 60 minutes soaking in concentrated sulfuric acid, (3) hand nicks by cutting into the testa with a razor blade and (4) control – no conditioning. Average germination was taken from four replications, 50 seeds each replication.

4.3 Stratification and Scarification Combined Trial

When scarification and stratification were both used, germination increased in most of the seed lots used in the trial. However, the different seed lots varied in their germination response with treatment. Statistical analysis using ANOVA indicated that the main effect of the acid treatments was the most significant factor to consider; its F-value was four times greater than the main effects of the seed lot and stratification (Appendix B.5). The 30-minute acid scarification was significantly different than the 60-minute acid scarification and the control for all but one seed lot; 2005 *G. flavum* had a similar response to both acid scarifications (Figure 4.6). The difference in germination between

the control and the scarified seeds indicated that the seed coat imposes dormancy on the seeds.

Figure 4.6 Percent seed germination at 15°C in darkness averaged across the stratification treatments for multiple seed lots of *Glaucium* species.

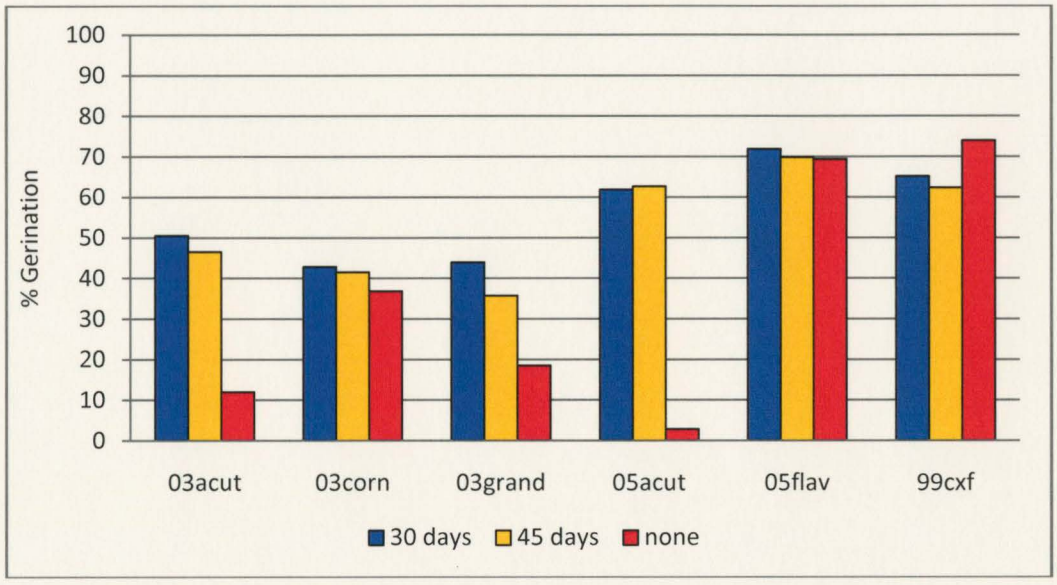


Scarification treatments were: 30-minute soak in concentrate sulfuric acid, 60-minute soak in concentrated sulfuric acid, or no acid soak. Each bar represents the average for 12 replications for the scarification and seed lot; 4 replications with no stratification, 4 replications with 30-day stratification at 8°C and 4 replications with 45-day stratification at 8°C.

Stratification also had a significant effect on germination; although, it is not as clearly indicated as the effect of scarification because of interactions with the different seed lots. There was no significant difference between the 30-day and 45-day stratification, indicating that the shorter stratification period would be effective in breaking dormancy (Figure 4.7). Non-stratified treatments had poor germination for *G. grandiflorum* and both years of *G. acutidentatum*; however, the rest of the seed lots seem to germinate equally as well without stratification than with stratification. Since these results were averaged over the acid scarification treatments to evaluate the main effect of stratification, it is difficult to determine why these seed lots did not need stratification.

Therefore, a closer look at each seed lot and the interactions between acid scarification and stratification is necessary.

Figure 4.7 Percent seed germination at 15°C in darkness averaged across the scarification treatments for multiple seed lots of *Glaucium* species.

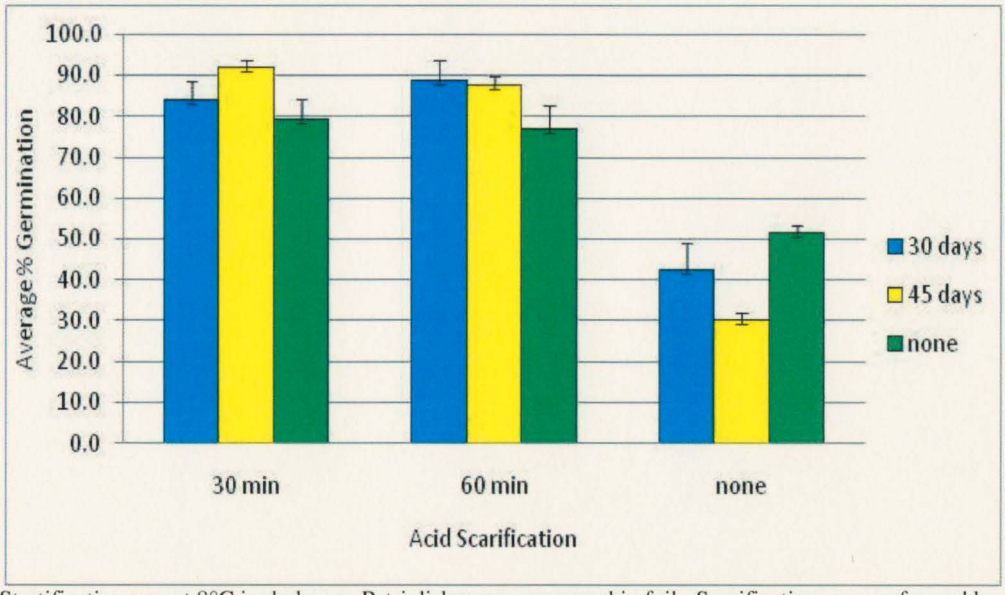


Stratification treatments were: 30 days at 8°C, 45 days at 8°C, or no stratification. Each bar represents the average for 12 replications for the stratification and seed lot; 4 replications with no acid scarification, 4 replications with 30-min soak in concentrated sulfuric acid and 4 replications with 60-min soak in concentrated sulfuric acid.

2005 *G. flavum*

2005 *G. flavum* demonstrated high germination between 77 and 92% for both the 30-minute acid and the 60-minute acid scarification for all three stratification periods. There was no significant difference among them (Figure 4.8 and Table 4.5). Germination decreased by half without acid scarification; however, the double-control seeds (no acid, no stratification) had greater germination than the non-scarified seeds with stratification. This is curious since the stratification trial indicated that stratification was needed for higher germination rates.

Figure 4.8 Average percent germination of *G. flavum* seed (collected in 2005) at 15°C in darkness for nine combinations of stratification and acid scarification.



Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Table 4.5 Average percent germination and standard error of *Glaucium flavum* seed (collected in 2005) germinated in darkness at 15°C with nine different combinations of stratification and acid scarification.

stratification period	acid scarification	Average percent germination	standard error
30 days	30 min	84	4.546
30 days	60 min	89	4.726
30 days	none	42.5	6.397
45 days	30 min	92	1.826
45 days	60 min	87.5	2.217
45 days	none	30	2.160
none	30 min	79.5	4.924
none	60 min	77	5.686
none	none	51.5	1.893

Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Comparing 2005 *G. flavum* across all three trials, it is evident that it displayed variable behavior. In the stratification trial, the control had 25% germination (Table 4.1). However, in the scarification trial, the control was 3.5% (Table 4.3) and 51.5% in the combined trial (Table 4.5). The controls from all three experiments had no pretreatment

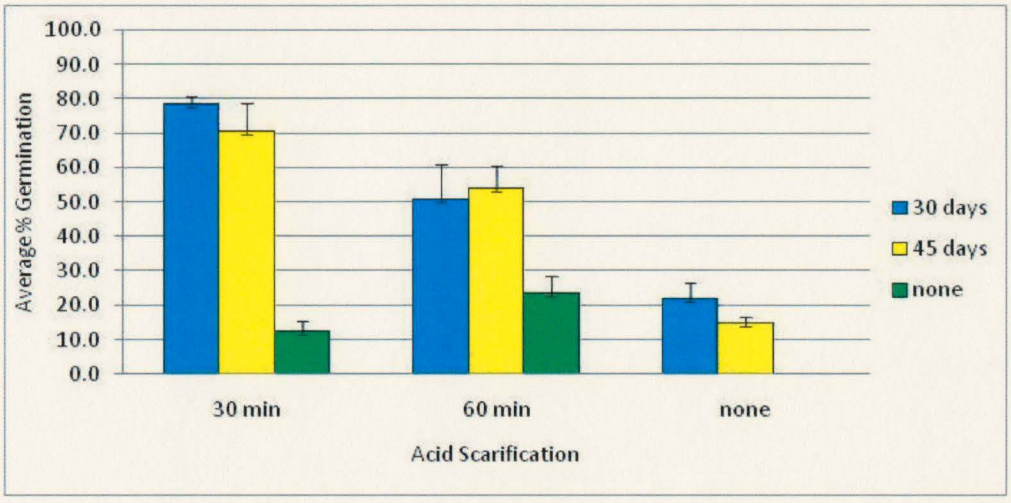
and were germinated at 15°C in the dark. Comparing similar stratification treatments; in the stratification trial, the 8°C, 45-day stratification yielded 58% germination (Table 4.1) while the same conditions (wrapped in foil for complete darkness and same germination temperature at 15°C) in the combination trial yielded 30% germination (Table 4.5). In the scarification trial, the germination was 57% for 30-minute acid and 37% for 60-minute acid (Table 4.3), while in the combination trial; it was 79.5% germination for 30-minute acid (no stratification) and 77% for 60-minute acid scarification without stratification (Table 4.5). In both experiments, germination was at 15°C and dishes were wrapped in foil for complete darkness. This great variation may be attributed to the fact that the seeds are most likely progeny of cross-pollination and with differing genetic traits controlling dormancy. Since, the scarification treatments in the combination trial demonstrate good germination (greater than 50%) the hard seed dormancy is considered likely.

2003 *G. acutidentatum*

2003 *G. acutidentatum* seeds clearly required acid scarification and stratification (Figure 4.9). The optimal conditions for greatest germination, 78.5%, were 30 minutes of acid scarification with 30 days of stratification (Table 4.6). There was no significant difference between 30-day and 45-day stratification for each acid treatment. This indicates that the shorter period is equally as effective. However, there was a significant difference between 30-minute acid and 60-minute acid for each stratification treatment (Figure 4.9), suggesting that the seed coat may not have been thick enough to withstand the long acid treatment. The endosperm and embryo may have been affected by the 60-minute acid soak. Since *G. acutidentatum* requires both scarification and stratification to

effectively overcome dormancy, it likely has a combination of physical and embryo dormancy.

Figure 4.9 Average percent germination of *G. acutidentatum* seed (collected in 2003) at 15°C in darkness for nine combinations of stratification and acid scarification.



Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Table 4.6 Average percent germination and standard error of *G. acutidentatum* seed (collected in 2003) germinated in darkness at 15°C with nine combinations of stratification and acid scarification.

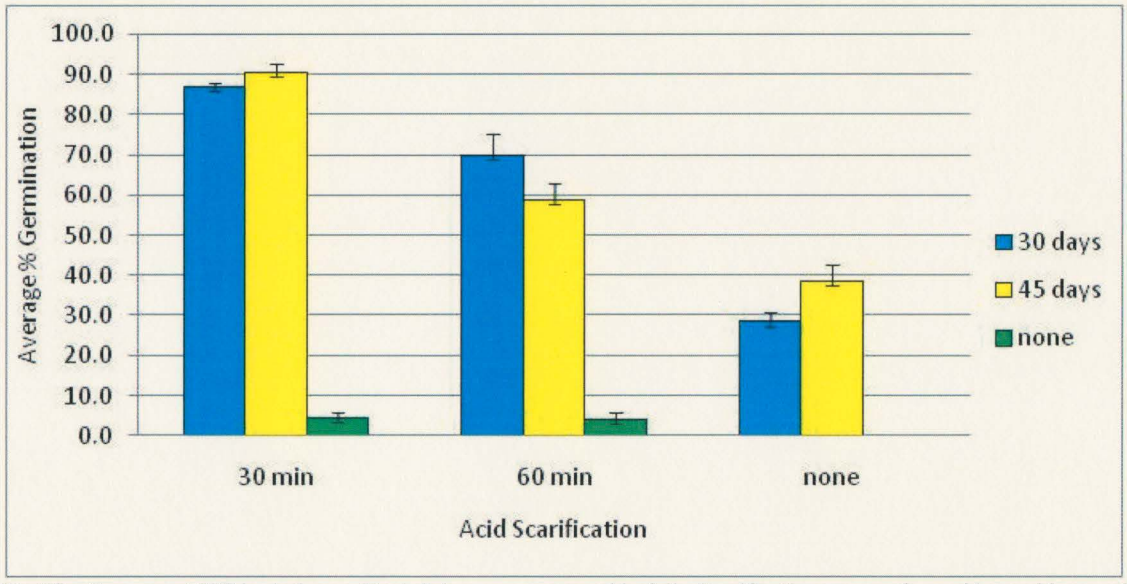
stratification period	**acid scarification**	**Average percent germination**	**standard error**
30 days	30 min	78.5	2.2174
30 days	60 min	51	9.8826
30 days	none	22	4.3205
45 days	30 min	70.5	8.1394
45 days	60 min	54	6.6833
45 days	none	15	1.7321
none	30 min	12.5	3.0957
none	60 min	23.5	4.9244
none	none	0	0

Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

2005 *G. acutidentatum*

A dramatic improvement in germination in 2005 *G. acutidentatum* was observed when acid scarification was combined with stratification. There was no germination in the double control (no acid scarification and no stratification) and minimal germination without stratification (Figure 4.10). A 30-minute scarification yielded 87% germination with a 30-day stratification and 90.5% with a 45-day stratification (Table 4.7). This was a significantly greater germination than with 60-minute scarification (70% with 30-day stratification and 59% with 45-day stratification). The longer scarification period likely caused damage to the embryo while in the acid. There was no significant difference in stratification periods, indicating that the shorter period was equally effective. The success of the combined acid scarification and stratification treatment indicates the seeds have both exogenous and endogenous dormancy.

Figure 4.10 Average percent germination of *G. acutidentatum* seed (collected in 2005) at 15°C in darkness for nine combinations of stratification and acid scarification.



Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

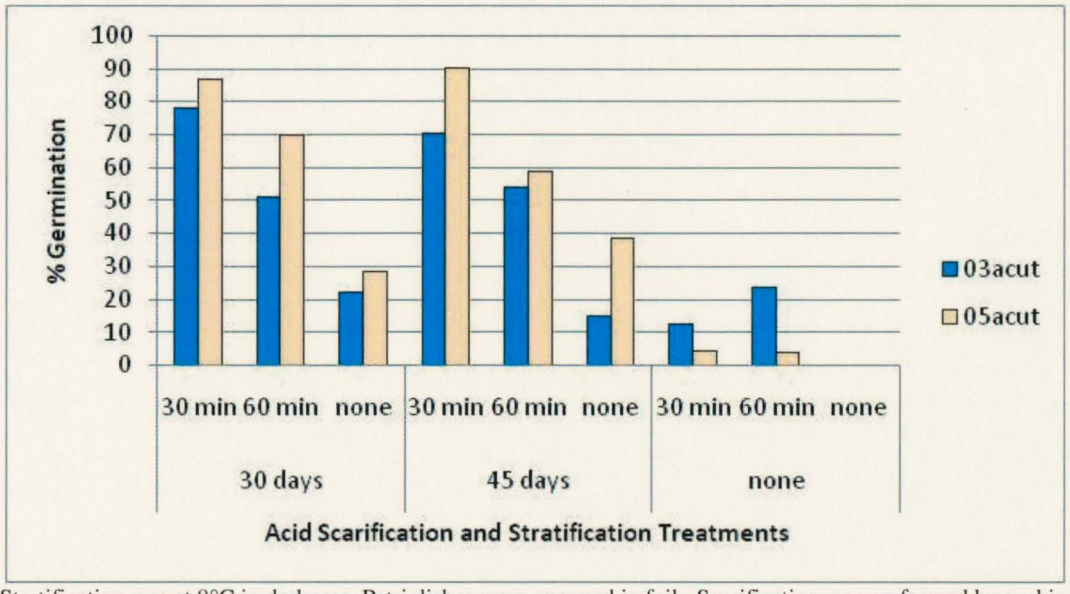
Table 4.7 Average percent germination and standard error of *G. acutidentatum* seed (collected in 2005) germinated in darkness at 15°C with nine combinations of stratification and acid scarification.

stratification period	acid scarification	Average percent germination	standard error
30 days	30 min	87	1
30 days	60 min	70	5.2281
30 days	none	28.5	2.2174
45 days	30 min	90.5	2.2174
45 days	60 min	59	3.873
45 days	none	38.5	4.3493
none	30 min	4.5	1.5
none	60 min	4	1.8257
none	none	0	0

Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

The two different years of *G. acutidentatum* responded similarly to the treatments in the trial. The 2003 seed had slightly lower germination than the 2005 in most treatments except for the two acid treatments without stratification (Figure 4.11). It is expected that older seed will have lower germination than younger seed due to a decrease in vigor followed by a decrease in viability (Walmsley and Davy, 1997). The seed likely has a combination of physical and endogenous dormancy. The endogenous dormancy is most likely morphophysiological as it is common in the *Papaveraceae*.

Figure 4.11 Average percent germination of *G. acutidentatum* seed (collected in 2003 and 2005) at 15°C in darkness for nine combinations of stratification and acid scarification.

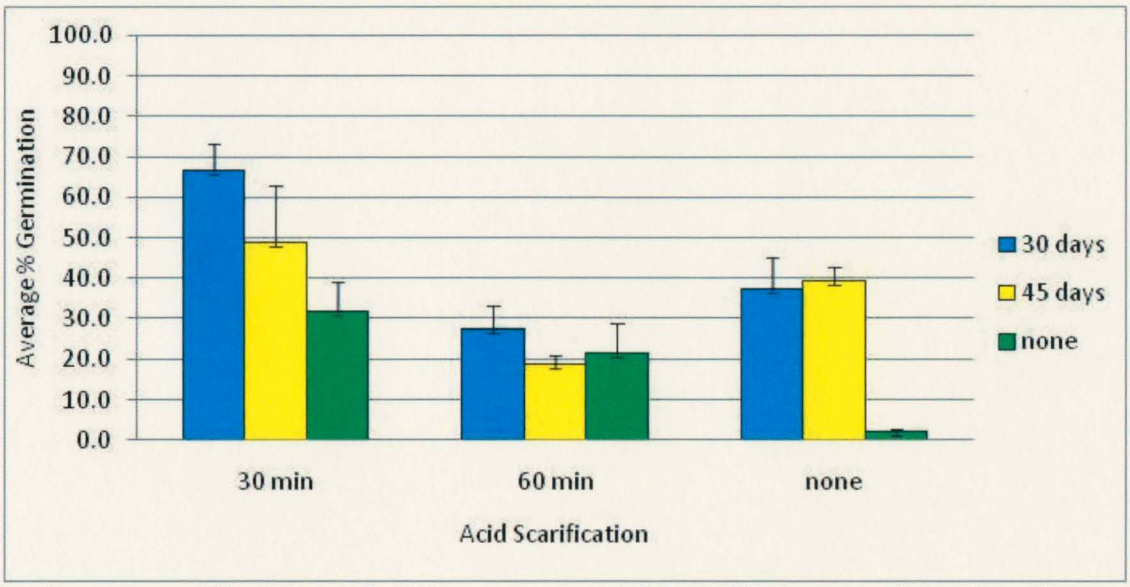


Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

2003 *G. grandiflorum*

The optimal treatment for 2003 *G. grandiflorum* was 30-minute acid scarification with 30-day stratification (Figure 4.12). All the other treatments yielded germination rates below 50% (Table 4.8). The unique behavior of this seed lot was that non-scarified seeds that were stratified had greater germination than the 60-minute scarified seeds. Since *G. grandiflorum* had mold growth with the acid/stratification treatments, it is likely that the acid was causing significant damage to the seeds. It is possible that the seed coat of *G. grandiflorum* is too thin for a long period of acid scarification. However, since it responded well to 30-minute scarification and 30-day stratification, it is still likely that *G. grandiflorum* has both hard seed and embryo dormancy.

Figure 4.12 Average percent germination of *G. grandiflorum* seed (collected in 2003) at 15°C in darkness for nine combinations of stratification and acid scarification.



Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Table 4.8 Average percent germination and standard error of *G. grandiflorum* seed (collected in 2003) germinated in darkness at 15°C with nine combinations of stratification and acid scarification.

stratification period	acid scarification	Average percent germination	standard error
30 days	30 min	66.67	6.566
30 days	60 min	27.5	5.737
30 days	none	37.5	7.632
45 days	30 min	49	13.820
45 days	60 min	18.67	2.404
45 days	none	39.5	3.403
none	30 min	32	7.257
none	60 min	21.5	7.544
none	none	2	0.817

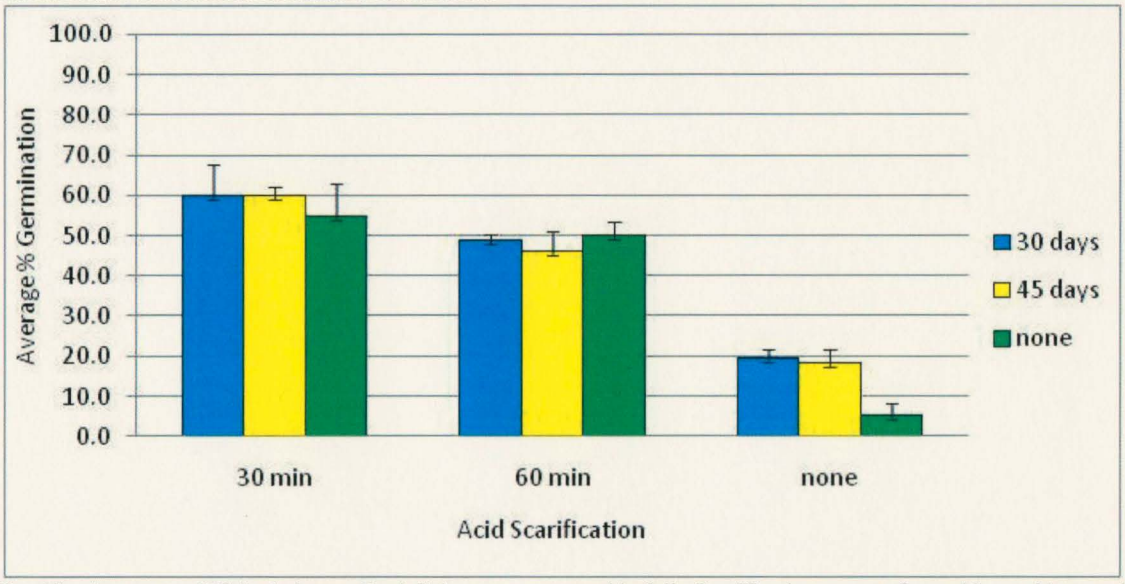
Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

2003 *G. corniculatum*

2003 *G. corniculatum* responded primarily to the acid scarification treatment (Figure 4.13). There was no significant difference among the stratification periods,

including no stratification, for both acid treatments. There was a statistically significant difference between the 30-minute and 60-minute acid scarification for the 45-day stratification; however, there was no difference among acid treatments for the other stratification treatments. Therefore, it is likely that *G. corniculatum* may only have a physical dormancy. Since it originates from the Mediterranean area where physical dormancy is common, it is possible that these seeds may not have morphophysiological dormancy.

Figure 4.13 Average percent germination of *G. corniculatum* seed (collected in 2003) at 15°C in darkness for nine combinations of stratification and acid scarification.



Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Table 4.9 Average percent germination and standard error of *G. corniculatum* seed (collected in 2003) germinated in darkness at 15°C with nine different combinations of stratification and acid scarification.

stratification period	acid scarification	Average percent germination	standard error
30 days	30 min	60	7.874
30 days	60 min	49	1.291
30 days	none	19.5	2.2174
45 days	30 min	60	2.1602
45 days	60 min	46	5.099
45 days	none	18.5	3.4034
none	30 min	55	7.9373
none	60 min	50	3.559
none	none	5.5	2.9861

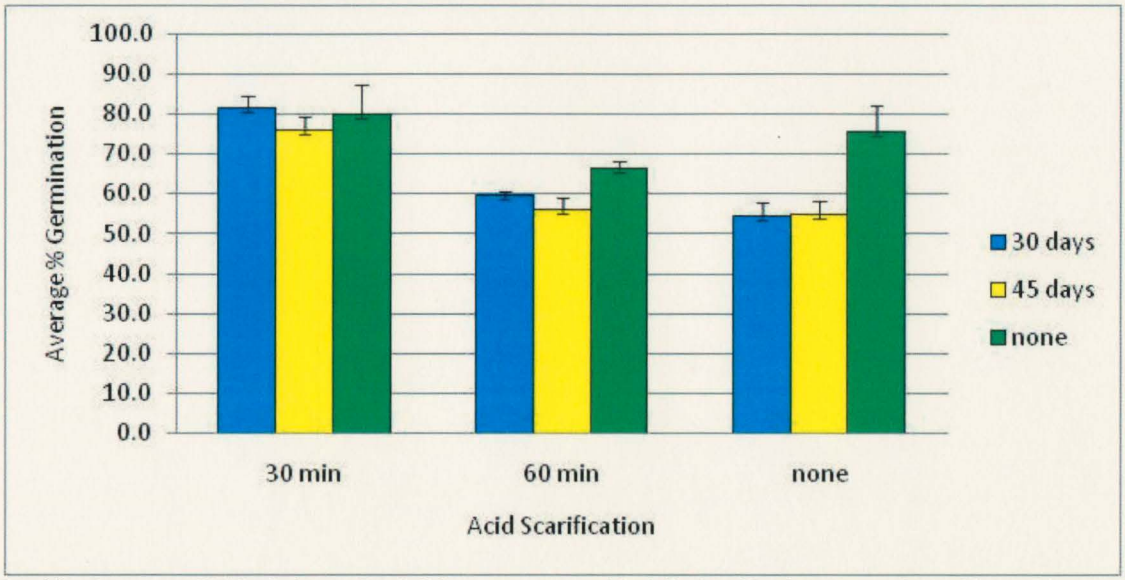
Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

1999 *G. corniculatum x flavum*

The hybrid, *G. corniculatum x flavum*, germinated in similar fashion to both its parent plants; they responded to the acid scarification but not to stratification. Comparing the different stratification periods for 30-minute acid treatment, there is no significant difference among the stratification periods (Figure 4.14). There is also no significant difference between stratification periods for the 60-minute acid scarification. There was a significant difference between the acid scarification treatments with a 30-minute scarification yielding between 76 and 82% germination, while the 60-minute scarification and no scarification yielded between 54 and 67% germination, excluding the double control (Table 4.10). The double control had germination comparable to the 30-minute acid scarification at 76% (Table 4.10). Since germination was over 50% for all treatments it suggests that the hybrid seeds may be nondormant. However, since the 30-

minute acid scarification improved germination rates, the hybrid seed may still have hard seed.

Figure 4.14 Average percent germination of *G. corniculatum x flavum* seed (collected in 1999) at 15°C in darkness for nine combinations of stratification and acid scarification.



Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Table 4.10 Average percent germination and standard error of *G. corniculatum x flavum* seed (collected in 1999) germinated in darkness at 15°C with nine combinations of stratification and acid scarification.

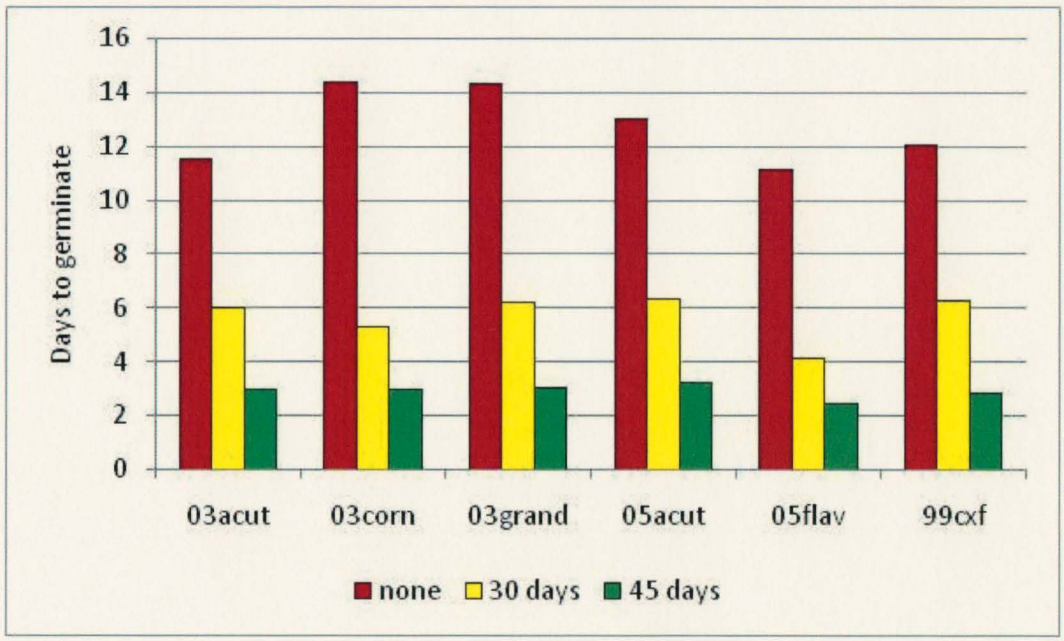
stratification period	acid scarification	average percent germination	standard error
30 days	30 min	81.5	3.2016
30 days	60 min	59.5	1.2583
30 days	none	54.5	3.304
45 days	30 min	76	3.559
45 days	60 min	56	3.1623
45 days	none	55	3.3166
none	30 min	80	7.1674
none	60 min	66.5	1.893
none	none	75.5	6.5511

Stratification was at 8°C in darkness, Petri dishes were wrapped in foil. Scarification was performed by soaking seeds in concentrated sulfuric acid.

Average Days to Germinate

The analysis for days to germinate did not include the double control treatment of no acid scarification and no stratification because of the low germination for many of the seed lots. The average days to germinate for the combination trial had fairly clear results; stratification was the main factor that influenced how long it took for seeds to germinate (Figure 4.15). Non-stratified seeds took twice as long to germinate as stratified seeds. The 30-day stratification took longer to germinate than the 45-day stratification (Figure 4.15).

Figure 4.15 Average days-to-germinate at 15°C in darkness averaged across the acid scarification treatments for multiple seed lots of *Glaucium* species.

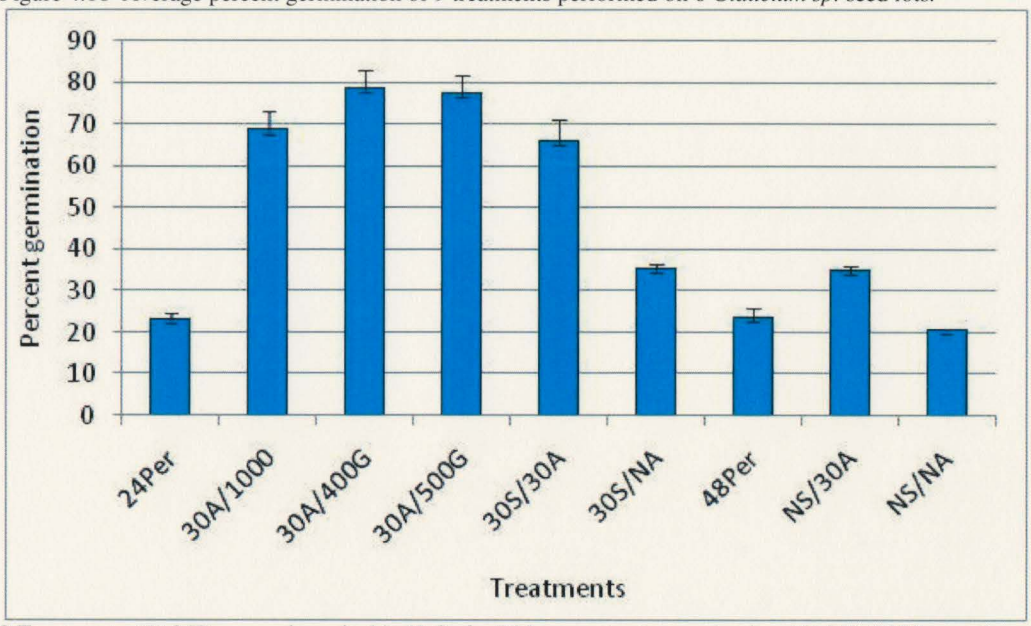


Scarification treatments were: 30-minute soak in concentrate sulfuric acid, 60-minute soak in concentrated sulfuric acid, or no acid soak. Each bar represents the average for 12 replications for the scarification and seed lot; 4 replications with no stratification, 4 replications with 30-day stratification at 8°C and 4 replications with 45-day stratification at 8°C.

4.4 Gibberellic Acid (GA) and Hydrogen Peroxide (HP) Trial

The GA and HP trial compared nine treatments with 6 seed lots. The entire experimented was repeated. The ANOVA (Appendix B.7) indicated that the main effect of seed lots and treatments were the most significant factors and that there were no significant differences between experiments. SNK (Student Neuman Keul) means separation test indicated that there was no significant difference between the treatment using 400ppm GA_3 and 500ppm GA_3 (Appendix B.8). Looking first at the germination of each treatment averaged over the different seed lots and experiments, there were some treatments that promoted germination better overall than others; 30-minute acid scarification with exogenously applied GA_3 at either 400ppm or 500ppm promoted the greatest germination overall (Figure 4.16).

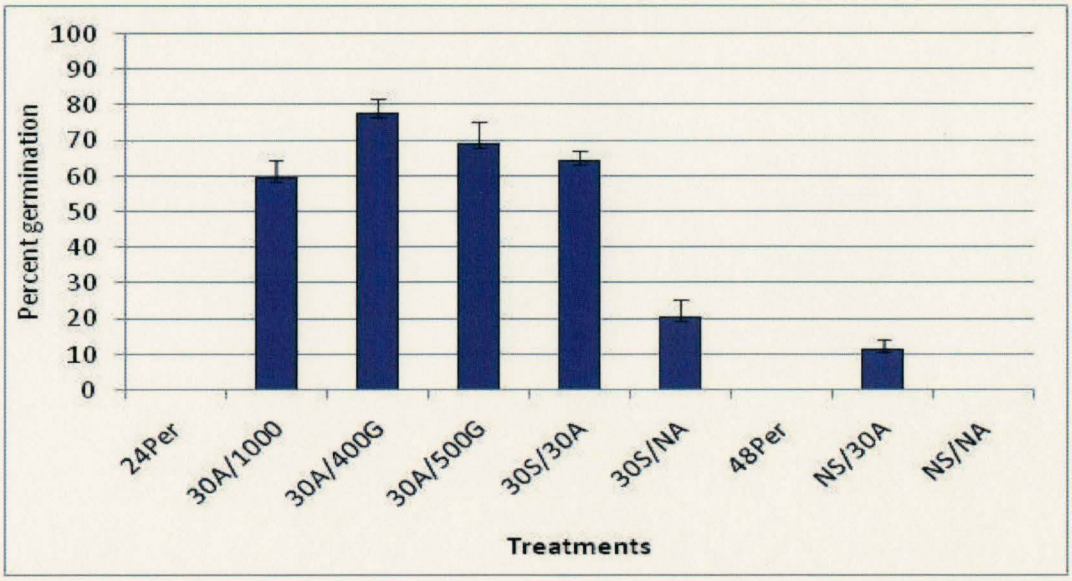
Figure 4.16 Average percent germination of 9 treatments performed on 6 *Glaucium sp.* seed lots.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification. 6 Seed Lots: 1999 *G. corniculatum x flavum*, 2003 *G. grandiflorum*, *2003 G. acutidentatum*, 2003 *G. corniculatum*, 2004 *G. corniculatum* and 2005 *G. acutidentatum*.All seeds germinated at 15°C with dishes wrapped in foil. All treatments were 4 replications of 50 seeds.

However, each seed lot responded uniquely to the varying treatments. The 2003 *G. acutidentatum* had no germination in the control and peroxide treatments (Figure 4.17). It responded favorably to the 30 min acid scarification/30-day stratification and to all GA treatments. However, the 400 ppm GA treatment was the most effective, yielding 77% germination, which was significantly greater than the other treatments. Since germination does not need warm stratification and gibberellic acid substitutes for cold stratification, the 2003 *G. acutidentatum* seeds apparently have an intermediate complex morphophysiological dormancy combined with physical dormancy.

Figure 4.17 Average percent germination of *G. acutidentatum* seed (collected in 2003) with 9 preconditioning treatments.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30-minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification. All seeds germinated at 15°C with dishes wrapped in foil. All treatments were 4 replications of 50 seeds

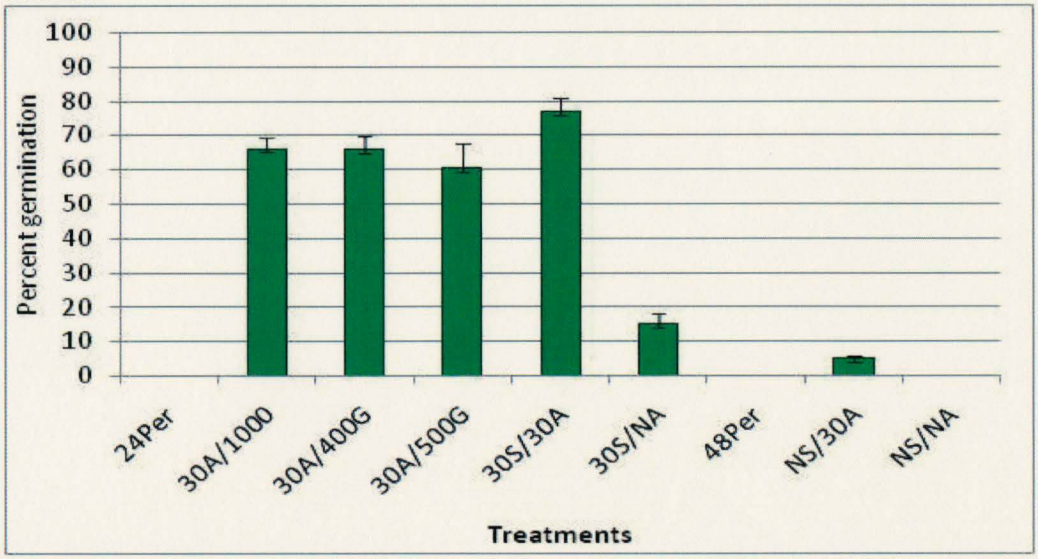
Table 4.11 Average percent germination and standard error of *G. acutidentatum* seed (collected in 2003) germinated in darkness at 15°C with 9 different preconditioning treatments.

Treatment	Experiment	average percent germination	Standard Error
24Per	1	0	0
24Per	2	0	0
30A/1000	1	41	8.544
30A/1000	2	78	1.4142
30A/400G	1	78.5	6.702
30A/400G	2	77	1.7321
30A/500G	1	59	9.1104
30A/500G	2	79.2	3.5926
30S/30A	1	69.5	3.304
30S/30A	2	59.5	1.7078
30S/NA	1	27.5	6.2383
30S/NA	2	13.5	3.8622
48Per	1	0	0
48Per	2	0	0
NS/30A	1	15.675	3.4362
NS/30A	2	7.5	1.7078
NS/NA	1	0	0
NS/NA	2	0	0

9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30-minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30-minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

Seeds of 2005 *G. acutidentatum* responded well to the giberellic acid, although the highest germination occurred with the 30-minute acid scarification and 30-day stratification (Figure 4.18). There was no germination in the control or hydrogen peroxide treatments. 2005 *G. acutidentatum* seeds responded to the treatments similarly to the 2003 *G. acutidentatum*. Although the greatest germination occurred with the scarification/stratification, since the gibberellic acid treatments had greater than 50% germination (Table 4.12), it is still possible to conclude that 2005 *G. acutidentatum* has intermediate complex MPD combined with physical dormancy. This is similar to the findings for 2003 *G. acutidentatum*.

Figure 4.18 Average percent germination of *G. acutidentatum* seed (collected in 2005) with 9 preconditioning treatments.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification. All seeds germinated at 15°C with dishes wrapped in foil. All treatments were 4 replications of 50 seeds.

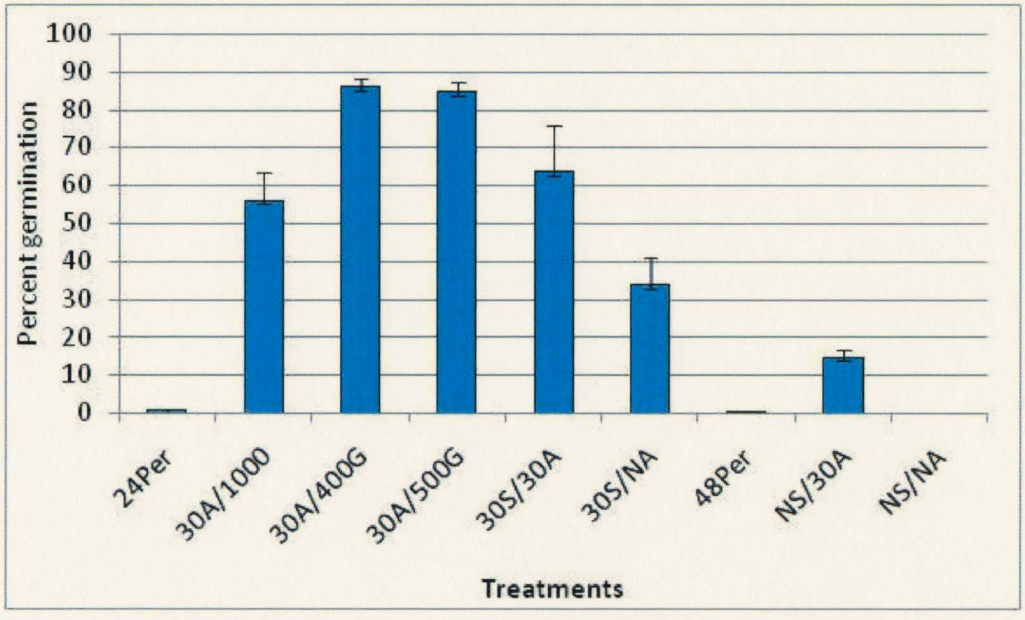
Table 4.12 Average percent germination and standard error of *G. acutidentatum* seed (collected in 2005) germinated at 15°C in darkness with 9 different preconditioning treatments.

Treatment	Experiment	average percent germination	Standard Error
24Per	1	0.0	0.000
24Per	2	0.0	0.000
30A/1000	1	68.8	4.912
30A/1000	2	63.5	1.500
30A/400G	1	58.0	6.976
30A/400G	2	73.5	0.957
30A/500G	1	53.2	11.937
30A/500G	2	67.7	1.827
30S/30A	1	85.0	0.577
30S/30A	2	68.5	7.500
30S/NA	1	14.0	2.000
30S/NA	2	16.0	3.559
48Per	1	0.0	0.000
48Per	2	0.0	0.000
NS/30A	1	2.5	1.258
NS/30A	2	7.2	0.644
NS/NA	1	0.0	0.000
NS/NA	2	0.0	0.000

9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30-minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

2003 *G. grandiflorum* had greater germination with the gibberellic acid treatments than with stratification (Figure 4.19). It had no germination without acid scarification and stratification. It showed minimal response to hydrogen peroxide. Its germination doubled with scarification and stratification (30S/30A) as compared to stratification only (30S/NA). This indicates that *G. grandiflorum* has physical dormancy. Since it responded to the GA, it can be concluded that *G. grandiflorum* has combined physical dormancy with intermediate complex MPD.

Figure 4.19 Average percent germination and standard error of *G. grandiflorum* seed (collected in 2003) with 9 preconditioning treatments and germinated in darkness at 15°C.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

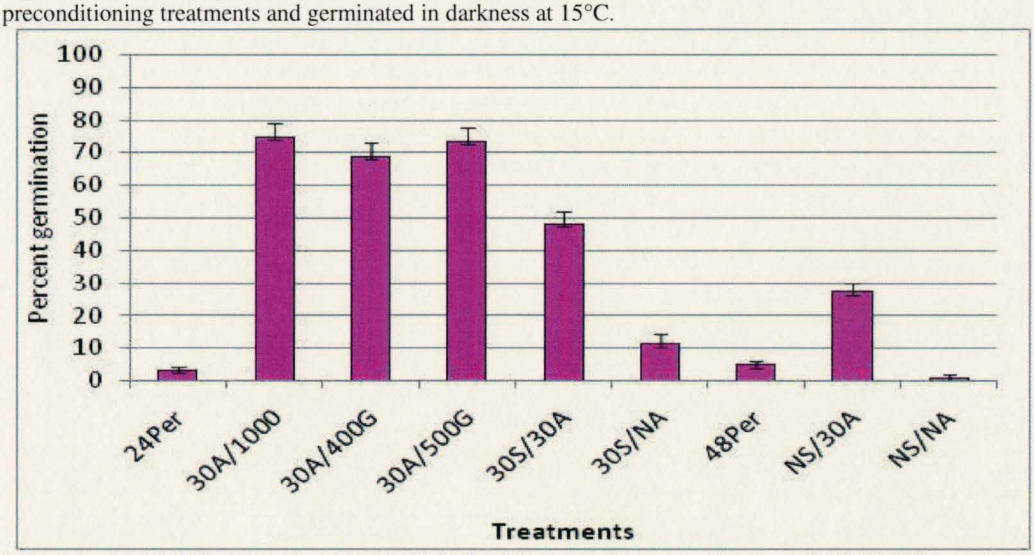
Table 4.13 Average percent germination and standard error of *G. grandiflorum* seed (collected in 2003) germinated in darkness at 15°C with 9 preconditioning treatments.

Treatment	Experiment	average percent germination	Standard Error
24Per	1	1.5	0.9574
24Per	2	0	0
30A/1000	1	32.275	12.0045
30A/1000	2	79.75	2.45
30A/400G	1	90.9	3.2057
30A/400G	2	80.8	1.2
30A/500G	1	92.5	2.0616
30A/500G	2	77	3
30S/30A	1	79.85	3.4654
30S/30A	2	47	21
30S/NA	1	30.5	7.6757
30S/NA	2	37	7
48Per	1	0.5	0.5
48Per	2	0	0
NS/30A	1	29.5	3.4034
NS/30A	2	0	0
NS/NA	1	0	0
NS/NA	2	0	0

9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

Seeds of 2003 *G. corniculatum* responded favorably to the GA treatments; germination for all three concentrations was higher than the germination for other treatments (Figure 4.20). Germination ranged from 69% to 79% for the GA treatments and there was no significant difference among them (Table 4.14). This would indicate that 2003 *G. corniculatum* exhibits intermediate complex MPD combined with physical dormancy since it exhibits high germination when scarified and in the presence of exogenously applied gibberellic acid.

Figure 4.20 Average percent germination and standard error of *G. corniculatum* seed (collected in 2003) with 9 preconditioning treatments and germinated in darkness at 15°C.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

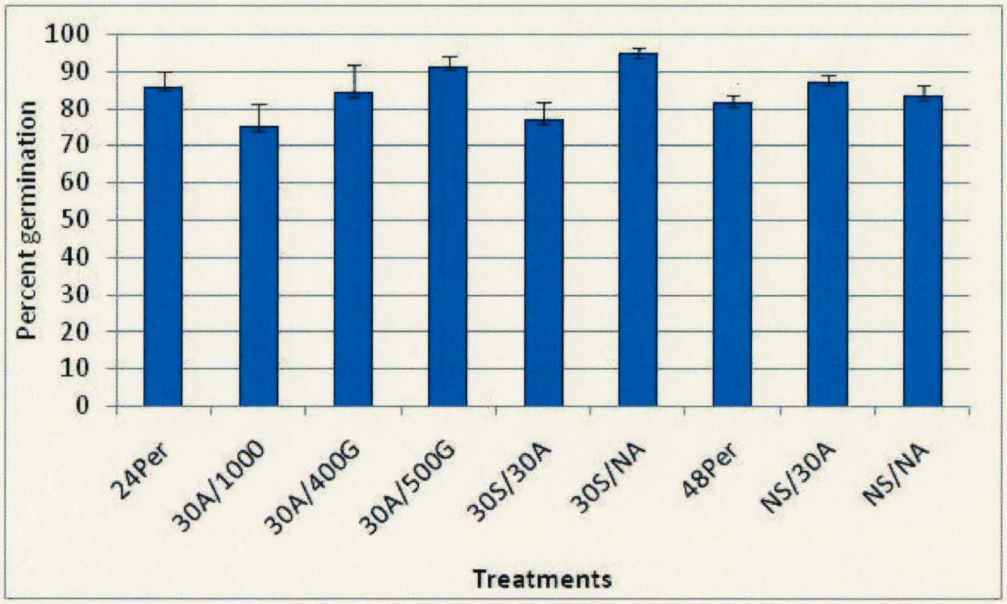
Table 4.14 Average percent germination and standard error of *G. corniculatum* seed (collected in 2003) germinated in darkness at 15°C with 9 preconditioning treatments.

Treatment	Experiment	average percent germination	Standard Error
24Per	1	5	1.291
24Per	2	1.5	0.5
30A/1000	1	79.95	2.2526
30A/1000	2	69.5	6.1305
30A/400G	1	68	4.1633
30A/400G	2	69.775	4.105
30A/500G	1	75.275	4.8531
30A/500G	2	71.5	3.4034
30S/30A	1	45	3.4157
30S/30A	2	51.425	3.6328
30S/NA	1	14.575	3.5493
30S/NA	2	8	2.8284
48Per	1	3.5	1.7078
48Per	2	6	0.8165
NS/30A	1	17.1	1.7916
NS/30A	2	37.5	3.594
NS/NA	1	0.5	0.5
NS/NA	2	1.525	0.9621

9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

The 2004 *G. corniculatum* seed germination was very different from the other seed lots. It germinated well for all treatments (Figure 4.21). The lowest germination was 67.5% with 30-minute acid scarification and 1000ppm of GA (Table 4.15). The 30-minute acid scarification combined with 30-day stratification also had a statistically significant reduced germination rate (73-80%) as compared to other treatments with percentages greater than 80% (Figure 4.21). This data indicates that 2004 *G. corniculatum* is non-dormant. This germination behavior could indicate why *G. corniculatum* is considered a weed in some states because it seemed to germinate without scarification or stratification.

Figure 4.21 Average percent germination and standard error of *G. corniculatum* seed (collected in 2004) with 9 different preconditioning treatments and germinated in darkness at 15°C.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

Table 4.15 Average percent germination and standard error of *G. corniculatum* seed (collected in 2004) germinated in darkness at 15°C with 9 preconditioning treatments.

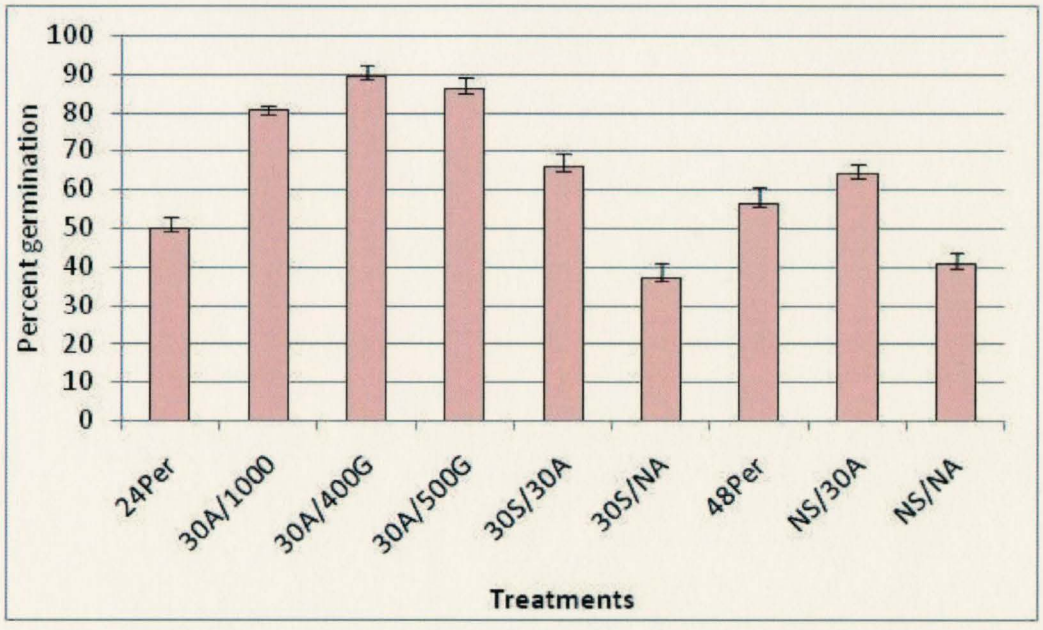
Treatment	Experiment	average percent germination	Standard Error
24Per	1	82	5.7155
24Per	2	89.5	2.63
30A/1000	1	82.7	7.9424
30A/1000	2	67.5	4.113
30A/400G	1	89.5	1.7078
30A/400G	2	79	13.6748
30A/500G	1	94.5	1.7078
30A/500G	2	88	4.3205
30S/30A	1	80.5	3.5
30S/30A	2	73	6.455
30S/NA	1	94.5	1.7078
30S/NA	2	95	1.9149
48Per	1	77.65	3.4141
48Per	2	85	1.291
NS/30A	1	91.5	1.7078
NS/30A	2	82.875	1.983
NS/NA	1	92	4.2426
NS/NA	2	74.5	2.0616

9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

The 1999 *G. corniculatum x flavum* had the highest germination with acid scarification and exogeneously applied GA at concentrations of 400 ppm or 500ppm (Figure 4.22). All three gibberellic treatments had significantly greater germination than the 30-minute acid scarification combined with 30-day stratification treatment (Table 4.16). Acid scarification alone had a similar germination rate to acid scarification combined with stratification in the 60-70% range (Table 4.16). This indicates that the hybrid seed may only have a physical dormancy; however, there must be some endogenous dormancy being overcome for the GA to increase germination to the 80-90% range. Looking back at the germination behavior of *G. flavum* in the combined

scarification/stratification trial and the *G. corniculatum* from this trial, the parents of this hybrid exhibit a combination of intermediate complex MPD, physical dormancy only and no dormancy. Therefore, it would seem reasonable to expect varying dormancy behavior from the seed derived from hybridized plants.

Figure 4.22 Average percent germination and standard error of *G. corniculatum x flavum* seed (collected in 1999) with 9 preconditioning treatments and germinated in darkness at 15°C.



9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30 minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

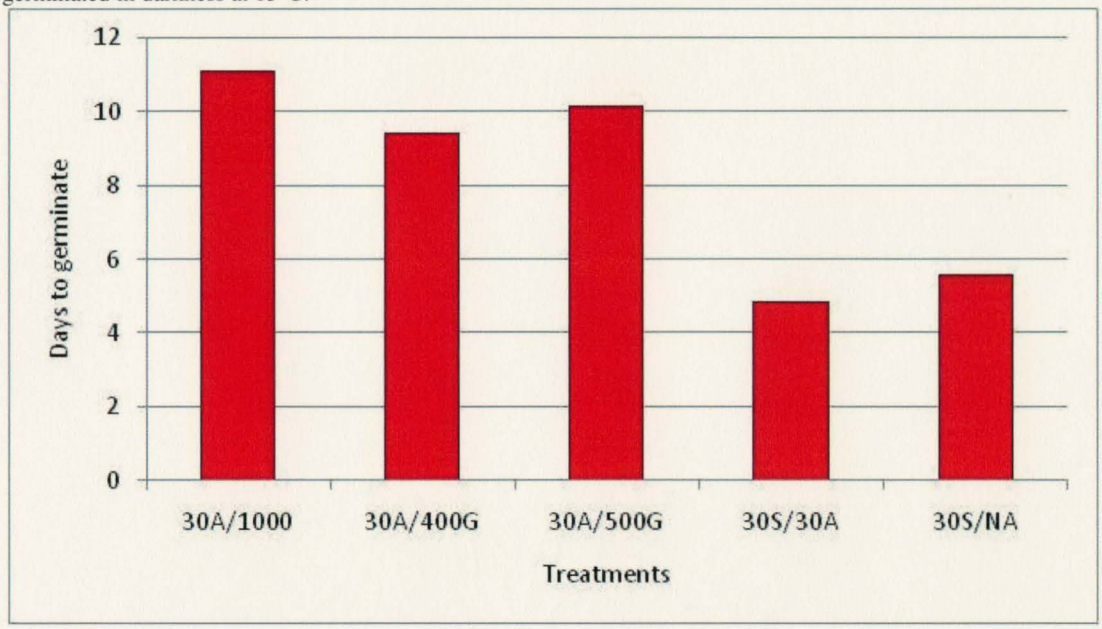
Table 4.16 Average percent germination and standard error of *G. corniculatum x flavum* seed (collected in 1999) germinated in darkness at 15°C with 9 preconditioning treatments.

Treatment	Experiment	average percent germination	Standard Error
24Per	1	47	3.6968
24Per	2	53	2.3805
30A/1000	1	70.3	1.3159
30A/1000	2	90.5	1.5
30A/400G	1	87.925	4.0954
30A/400G	2	91	1.291
30A/500G	1	88.3	1.3
30A/500G	2	84	4.3205
30S/30A	1	60	3.559
30S/30A	2	71.4	3.9783
30S/NA	1	37.5	4.5
30S/NA	2	37	3
48Per	1	46	4.899
48Per	2	67	3
NS/30A	1	62.5	3.5
NS/30A	2	65.5	1.893
NS/NA	1	36.125	4.4365
NS/NA	2	45.375	1.789

9 Treatments: (1) 24Per – seeds soaked in H_2O_2 for 24 hours prior to germination, (2) 30A/1000 – seeds scarified for 30 minutes in concentrated sulfuric acid then 1000ppm GA_3 exogenously applied, (3) 30A/400G - 30 minutes acid scarification then 400ppm GA_3 exogenously applied, (4) 30A/500G – 30-minute acid scarification then 500ppm GA_3 exogenously applied, (5) 30S/30A –30-minute acid scarification then stratified for 30 days at 8°C, (6) 30S/NA – seeds were stratified for 30 days at 8°C, (7) 48Per – seeds soaked in H_2O_2 for 48 hours prior to germination, (8) NS/30A – seeds scarified for 30 minutes in concentrated sulfuric acid and (9) NS/NA – no stratification or acid scarification.

The difference between the average days to germinate of the treatments was analyzed without the treatments: no scarification/no stratification, 24-hr peroxide, 48-hr peroxide and the 30-minute acid scarification because their germination was less than 30% for many of the seed lots. Overall, 30-minute scarification with 30-day stratification had the shortest germination time with an average of 5.8 days (Figure 4.23). The ANOVA analysis indicated that the combination treatment was the most significant factor with an F-value 5 times greater than the other F-values.

Figure 4.23 Average days-to-germinate of 6 seed lots of *Glaucium sp.* with 5 dormancy-breaking treatments and germinated in darkness at 15°C.



All seeds germinated at 15°C in darkness (Petri dishes wrapped in foil). 4 replications of 50 seeds used for each seed lot in each treatment. Seed lots: 2003 G. acutidentatum, 2005 G. acutidentatum, 2003 G. corniculatum, 2004 G. corniculatum, 2003 G. grandiflorum and 1999 G. corniculatum x flavum. Treatments: 30A/1000 – 30 minute acid scarification in concentrated sulfuric acid with exogenous application of GA_3 at 1000 ppm, 30A/400G - 30-minute acid scarification with exogenous application of GA_3 at 400 ppm, 300A/500G - 30-minute acid scarification with exogenous application of GA_3 at 500 ppm, 30S/30A – 30-minute scarification with 30 days of stratification at 8°C and 30S/NA – 30 days of stratification without acid scarification.

Chapter V

Conclusion

The purpose of this research was to establish seed propagation protocols for *Glaucium* species, particularly *G. grandiflorum* and *G. acutidentatum. G. corniculatum* is classified as a weed by the Southern Weed Science Society (1998) and *G. flavum* is a prohibited weed in the state of Massachusetts; therefore, more investigation in methods to sterilize or control these two species is recommended before introduction. Although there was some variation among and within the species, overall, 30-minute acid scarification with 30-day stratification is recommended; the stratification may be replaced with 400 ppm exogenously applied GA_3.

Glaucium sp. demonstrated morphophysiological dormancy that is characteristic of *Papaveraceae.* However, they also had a hard seed coat as well. The seeds germinated well with cold stratification only which indicates complex MPD. They also germinated well with exogenously applied gibberellic acid and this typically indicates that the level of complex MPD is intermediate. Germination was improved with acid scarification and this is an indication of physical dormancy.

G. flavum and *G. corniculatum* demonstrated high variability in response to stratification compared to no stratification. They may not have MPD; but they could be nondormant or have a hard seed coat. The lack of embryo dormancy can be a contributing factor to their weediness. The loss of MPD could be an adaptation to climate conditions here in the United States.

Chapter IV

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Appendix A

Characterization of *Glaucium* species

Davis and Cullen assembled thorough descriptions of different *Glaucium* species in Flora of Turkey (1965). The characteristics that were easily identified were used to characterize the plants grown from selected germinated seeds of this research. The plants were characterized to ascertain genetic variability in the seeds.

Table A.1 Distinguishing characteristics of four different *Glaucium* species referenced from Flora of Turkey, 1965.

	Stems	**Leaves**	**Sepals**	**Petals**	**Peduncle**
G. flavum	multiple		crisply pilose	yellow reddish reddish-mauve	
G. corniculatum	multiple	villous	scabrous to hirsute	yellow orange red	shorter than subtending leaves
G. grandiflorum	one main		scabrous to hirsute	dark orange to crimson with black basal spot	exceeds subtending leaves
G. acutidentatum	multiple		glabrous	concolorous, orange-buff	

Figure A.1 Pictures of *Glaucium sp.* leaves that illustrate leaf texture surfaces; (a) slightly hirsute, (b) hirsute and (c) tomentose.

Figure A.2 A picture of Glaucium sp. sepals that illustrate the hirsute surface.

Figure A.3 A picture that illustrates differences among plants grown from *G. grandiflorum* seed (collected in 2003).

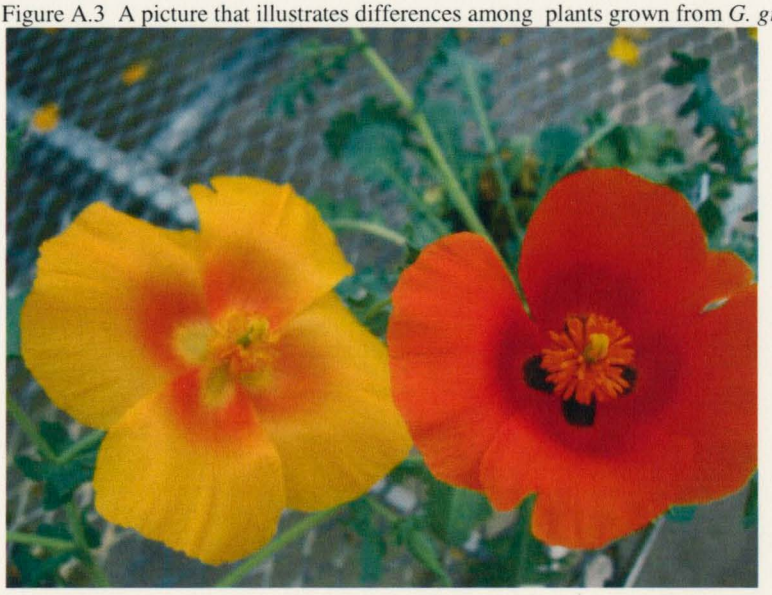

Table A.2 Characteristics of *G. grandiflorum* plants grown from selected seeds from the research trials.

Number	**Stems**	**Leaves**	**Sepals**	**Petals**	**Peduncle**
Reference	**1 main**		**scabrous to hirsute**	**dark orange to crimson with black basal spot**	**exceeds subtending leaf**
03 grand	multiple	sparsely hirsute	hirsute	light orange to dark, with faint basal spot	exceeds subtending leaf
03 grand	multiple	slightly hirsute	hirsute	light orange to dark with faint basal spot	equal length as leaves
03 grand	1 main	sparsely hirsute	hirsute	light orange to dark, with black basal spot	does not exceed subtending leaf
03 grand	multiple	hirsute	hirsute	light orange to dark, with fuzzy basal spot	does not exceed subtending leaf
03 grand	multiple	slightly hirsute	hirsute	light orange to dark, with fuzzy basal spot	does not exceed subtending leaf
03 grand	multiple	hirsute	slightly hirsute	light orange with black basal spot	exceeds subtending leaf
03 grand	multiple	hirsute	hirsute	medium-dark orange with faint basal spot	exceeds subtending leaf
03 grand	multiple	hirsute	slightly hirsute	light to medium orange with solid basal spot	some exceed, some do not

Number of data points is limited because few bloomed due to problems growing out the plants to the point of blooming in the greenhouse.

Table A.3 Characteristics of *G. acutidentatum* plants grown from selected seeds from the research trials.

	Stems	**Leaves**	**Sepals**	**Petals**	**Peduncle**
Reference	**multi-stemmed**		**glabrous**	**concolorous, orange-buff**	
03 acut	multi-stemmed	hirsute	slightly hirsute	light-medium orange, black basal spot	does not exceed subtending leaves
03 acut	multi-stemmed	hirsute	glabrous	medium to dark orange, black basal spot	does not exceed subtending leaves
05 acut	multi-stemmed	tomentose	hirsute	light-medium orange, black basal spot	exceeds subtending leaves
05 acut	multi-stemmed	hirsute	hirsute	deep orange with yellow basal spot	exceeds subtending leaves
05 acut	multi-stemmed	hirsute	hirsute	deep orange with black basal spot	equal length to subtending leaves

Number of data points is limited because few bloomed due to problems growing out the plants to the point of blooming in the greenhouse.

Table A.4 Characteristics of *G. corniculatum* plants grown from selected seeds from the research trials.

Number	Stems	Leaves	Sepals	Petals	Peduncle
Reference	**multi-stemmed**	**villous**	**scabrous to hirsute**	**yellow, orange or red**	**shorter than subtending leaf**
03 corn	one main stem	hirsute	slightly hirsute	medium to dark orange with faint basal spot	does not exceed subtending leaf
03 corn	one main stem	hirsute	slightly hirsute	light orange	exceeds subtending leaf
03 corn	one main stem	slightly hirsute	very slightly hirsute	light to medium orange with black basal spot	exceeds subtending leaf
03 corn	multi-stemmed	very slightly hirsute	slightly hirsute	dark yellow	exceeds subtending leaf
03 corn	multi-stemmed	hirsute	hirsute	light to medium orange with black basal spot	does not exceed subtending leaf
03 corn	multi-stemmed	slightly hirsute	glabrous	medium orange with black basal spot	does not exceed subtending leaf
03 corn	multi-stemmed	hirsute	hirsute	yellow	exceeds subtending leaf
03 corn	multi-stemmed	hirsute	hirsute	medium to dark orange with faint basal spot	does not exceed subtending leaf
03 corn	multi-stemmed	hirsute	hirsute	light orange to dark orange with faint yellow basal spot	equal length to subtending leaf
04 corn	one main stem	tomentose	hirsute	orange with yellow basal spot	does not exceed subtending leaf
04 corn	one main stem	tomentose	hirsute	orange with yellow basal spot	does not exceed subtending leaf

Number of data points is limited because few bloomed due to problems growing out the plants to the point of blooming in the greenhouse.

Appendix B

SAS Anova Tables

Table B.1 SAS ANOVA table of percent germination of the stratification trial evaluating germination of *G. flavum* seed harvested in 2003 and 2005, with three levels of stratification (4°C, 7°C, none) and with or without light.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
year	1	5676.75	5676.75	107.96	<.0001
strat	2	10669.5	5334.75	101.45	<.0001
year*strat	2	90.5	45.25	0.86	0.432
light	1	396.75	396.75	7.55	0.009
year*light	1	168.75	168.75	3.21	0.082
strat*light	2	2061.5	1030.75	19.6	<.0001
year*strat*light	2	568.5	284.25	5.41	0.009

Table B.2 SAS ANOVA table of average days-to-germinate for the stratification trial evaluating germination of *G. flavum* seed harvested in 2003 and 2005, with three levels of stratification (4°C, 7°C, none) and with or without light.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
year	1	5.93	5.93	1.29	0.2682
strat	1	310.59	310.59	67.2	<.0001
year*strat	1	196.03	196.03	42.41	<.0001
lgt	1	131.30	131.30	28.41	<.0001
year*lgt	1	17.65	17.65	3.82	0.0624
strat*lgt	1	478.18	478.18	103.45	<.0001
year*strat*lgt	1	113.45	113.45	24.55	<.0001

Table B.3 SAS ANOVA table of transformed data (arcsin transformation) for percent germination of the scarification trial evaluating germination of *G. flavum* and *G.acutidentatum* seed harvested in 2003 and 2005, with four levels of scarification: control, nicks, 30-minute acid scarification (concentrated sulfuric acid) and 60-minute acid scarification.

Source	DF	Type III SS	Mean Square	F Value	Pr>F
year	1	0.03	0.03	2.59	0.1144
species	1	1.70	1.70	138.42	<.0001
year*species	1	0.17	0.17	14.01	0.0005
treat	3	1.19	0.39	32.3	<.0001
year*treat	3	0.22	0.07	6.15	0.0013
species*treat	3	0.39	0.13	10.8	<.0001
year*species*treat	3	0.30	0.10	8.27	0.0002

Table B.4 SAS ANOVA table of average days-to-germinate for the scarification trial evaluating germination of *G. flavum* and *G.acutidentatum* seed harvested in 2003 and 2005, with four levels of scarification: control, nicks, 30-minute acid scarification (concentrated sulfuric acid) and 60-minute acid scarification.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
year	1	188.17	188.17	3.02	0.0951
species	1	818.82	818.82	13.14	0.0014
year*species	1	38.32	38.32	0.61	0.4406
trt	2	81.25	40.62	0.65	0.53
year*trt	2	74.23	37.11	0.6	0.5592
species*trt	1	81.71	81.71	1.31	0.2635
year*species*trt	1	218.06	218.06	3.5	0.0736

Table B.5 SAS ANOVA table for percent germination of the scarification/stratification trial evaluating germination of *G.acutidentatum* (harvested in 2003 and 2005), *G. grandiflorum* and *G. corniuculatum* (harvested in 2003), *G. flavum* (harvested in 2005) and *G. corniculatum x flavum* (harvested in 1999) seed, with three levels of scarification: control, 30-minute acid scarification (concentrated sulfuric acid) and 60-minute acid scarification and three levels of stratification at 8°C (30 days, 45 days, none).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
seed	5	47084.10	9416.82	97.96	<.0001
strat	2	17463.67	8731.83	90.83	<.0001
seed*strat	10	26607.77	2660.77	27.68	<.0001
acid	2	43441.41	21720.70	225.94	<.0001
seed*acid	10	11386.63	1138.66	11.84	<.0001
strat*acid	4	3478.95	869.73	9.05	<.0001
seed*strat*acid	20	7808.71	390.43	4.06	<.0001

Table B.6 SAS ANOVA table for average days-to-germinate of the scarification/stratification trial evaluating germination of *G.acutidentatum* (harvested in 2003 and 2005), *G. grandiflorum* and *G. corniuculatum* (harvested in 2003), *G. flavum* (harvested in 2005) and *G. corniculatum x flavum* (harvested in 1999) seed, with three levels of scarification: control, 30-minute acid scarification (concentrated sulfuric acid) and 60-minute acid scarification and three levels of stratification at 8°C (30 days, 45 days, none).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
seed	5	1271.64	254.32	15.99	<.0001
strat	2	29105.48	14552.74	914.89	<.0001
seed*strat	10	1033.13	103.31	6.5	<.0001
acid	2	468.32	234.16	14.72	<.0001
seed*acid	10	438.81	43.88	2.76	0.0039
strat*acid	3	208.56	69.52	4.37	0.0056
seed*strat*acid	15	362.26	24.15	1.52	0.1063

Table B.7 SAS ANOVA table for percent germination of the GA/hydrogen peroxide trial evaluating germination of *G.acutidentatum* (harvested in 2003 and 2005), *G. grandiflorum* and *G. corniuculatum* (harvested in 2003), *G. flavum* (harvested in 2005) and *G. corniculatum x flavum* (harvested in 1999) seed, with nine treatments.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
seed	5	163060.90	32612.18	480.48	<.0001
trt	8	213508.48	26688.56	393.21	<.0001
seed*trt	40	82363.72	2059.09	30.34	<.0001
exp	1	28.60	28.60	0.42	0.5167
seed*exp	5	1940.00	388.00	5.72	<.0001
trt*exp	8	2861.13	357.64	5.27	<.0001
seed*trt*exp	40	13246.40	331.16	4.88	<.0001

Treatments: 30A/1000 – 30-minute acid scarification in concentrated sulfuric acid with exogenous application of GA_3 at 1000 ppm, 30A/400G - 30-minute acid scarification with exogenous application of GA_3 at 400 ppm, 300A/500G - 30-minute acid scarification with exogenous application of GA_3 at 500 ppm, 30S/30A – 30-minute scarification with 30 days of stratification at 8°C and 30S/NA – 30 days of stratification without acid scarification.

Table B.8 SAS SNK table for percent germination of the GA/hydrogen peroxide trial evaluating germination of *G.acutidentatum* (harvested in 2003 and 2005), *G. grandiflorum* and *G. corniuculatum* (harvested in 2003), *G. flavum* (harvested in 2005) and *G. corniculatum x flavum* (harvested in 1999) seed, with nine treatments.

SNK	Mean	N	trt
A	78.6	46	30A/400G
A	77.5	46	30A/500G
B	68.2	46	30A/1000
B	66.7	46	30S/30A
C	36.5	46	NS/30A
C	35.4	46	30S/NA
D	24.8	46	48Per
D	24.3	46	24Per
D	21.7	46	NS/NA

Treatments: 30A/1000 – 30-minute acid scarification in concentrated sulfuric acid with exogenous application of GA_3 at 1000 ppm, 30A/400G - 30-minute acid scarification with exogenous application of GA_3 at 400 ppm, 300A/500G - 30-minute acid scarification with exogenous application of GA_3 at 500 ppm, 30S/30A – 30-minute scarification with 30 days of stratification at 8°C and 30S/NA – 30 days of stratification without acid scarification.

Table B.9 SAS Anova table for average days-to-germinate of the GA/hydrogen peroxide trial evaluating germination of *G.acutidentatum* (harvested in 2003 and 2005), *G. grandiflorum* and *G. corniuculatum* (harvested in 2003), *G. flavum* (harvested in 2005) and *G. corniculatum x flavum* (harvested in 1999) seed, with nine treatments.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
seed	5	8823.00	1764.60	104.96	<.0001
trt	4	36785.41	9196.35	547.01	<.0001
seed*trt	20	5724.79	286.23	17.03	<.0001
exp	1	190.53	190.53	11.33	0.0009
seed*exp	5	512.45	102.49	6.1	<.0001
trt*exp	4	2316.39	579.09	34.45	<.0001
seed*trt*exp	20	1098.90	54.94	3.27	<.0001

Treatments: 30A/1000 – 30-minute acid scarification in concentrated sulfuric acid with exogenous application of GA_3 at 1000 ppm, 30A/400G - 30-minute acid scarification with exogenous application of GA_3 at 400 ppm, 300A/500G - 30-minute acid scarification with exogenous application of GA_3 at 500 ppm, 30S/30A – 30-minute scarification with 30 days of stratification at 8°C and 30S/NA – 30 days of stratification without acid scarification.

Appendix C

Seed Testing Rules by Association of Official Seed Analysts

C.1 Tetrazolium test procedure for viability

FAMILY: PAPAVERACEAE

Genera: *Argemone, Eschscholzia, Hunnemannia, Papaver*

1. PRECONDITIONING:

METHOD	TIME (hrs)	TEMP (°C)
1. soak in beaker of water	overnight	20-25
2. imbibe on moist blotters, filter paper, or paper towels	overnight	20-25

Morphology

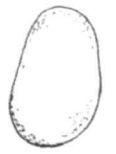

Fig 1 External

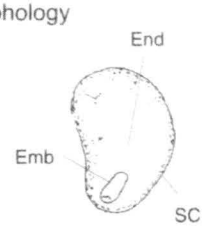

Fig 2 Embryo

Notes:

2. PREPARATION & STAINING:

METHOD	TZ Conc(%)	TIME (hrs)	TEMP (°C)
1. pierce with needle through endosperm	1.0	overnight	30-35
2. cut longitudinally, leaving seed intact at distal end	1.0	6-18	30-35

Notes: With *Eschscholzia and Papaver* there is a line on the seed that, if located, can be used as a guideline for cutting.

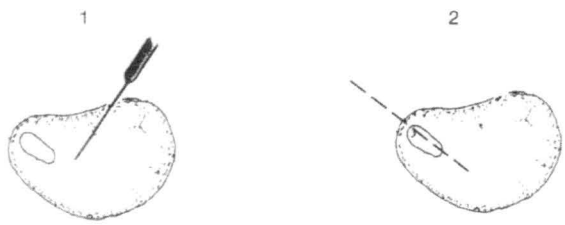

Fig 3 Preparation method

FAMILY: PAPAVERACEAE

Post Staining Notes: Embryo may have to be gently teased (pulled) out to evaluate.

3. EVALUATION:

VIABLE (NORMAL STAINING)

- entire embryo evenly stained
- endosperm may stain
- orange coloration inside seed coat, on periphery of endosperm, acceptable

NON-VIABLE (ABNORMAL OR NO STAINING)

- any essential part of the embryo unstained
- orange coloration penetrating into endosperm
- no embryo or only partial embryo development

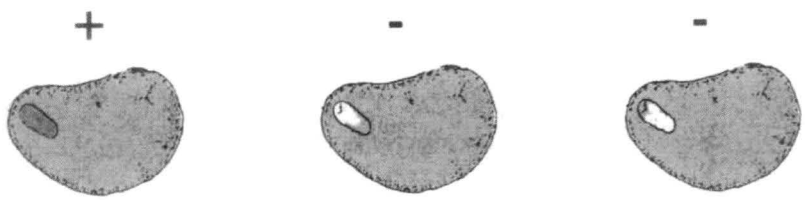

Fig 4 Seed stain evaluation

REFERENCES: 1, 3, 7

C.2 Germination test procedures, in the absence of procedures for *Glaucium sp.*, *Papaver glaucum* was used for this trial.

Table 3. Methods of testing for laboratory germination (cont.).

Kind of Seed	Substrata	Tempera-ture °C	First count days	Final count days	Additional Directions See Sec. 4.2 and 4.9
Oryzopsis miliacea smilograss	see *Piptatherum miliaceum*				
Panicum antidotale blue panicgrass	P, TS	20-30	7	28	Light.
Panicum maximum guineagrass	see *Megathyrsus maximus*				
Panicum maximum green panicgrass	see *Megathyrsus maximus*				
Panicum maximum var. *trichoglume* green panicgrass	see *Megathyrsus maximus*				
Panicum miliaceum subsp. *miliaceum* proso millet	B, T	20-30	3	7	
Panicum virgatum switchgrass	P, TS	15-30	7	14	Light; KNO_3. Fresh and dormant: Prechill at 5 °C for 2 weeks. Ungerminated seeds: see sec. 4.2 e and 4.9 k.
Papaver glaucum tulip poppy	TB, P	15	6[a]	14[b]	KNO_3.
Papaver nudicaule Iceland poppy	TB, P	15	6[a]	14[b]	KNO_3.
Papaver orientale Oriental poppy	TB, P	20-30	6[a]	12[b]	Light and KNO_3.
Papaver rhoeas corn poppy, shirley poppy	TB, P	15	none[a]	8[b]	
Pascopyrum smithii western wheatgrass	B, P, T	15-30	7	28	Dark. Fresh and dormant: KNO_3 or soil. Ungerminated seeds: see sec. 4.2 e and 4.9 k.
Paspalum dilatatum dallisgrass	P	20-35	7	21	Light; KNO_3.
Paspalum notatum bahiagrass 'Pensacola'	P, S	20-35	7	28	Light; see sec. 4.8 b.
All other cvs.	P	30-35	3	21	Light; remove enclosing structures; see sec. 4.8 b Fresh and dormant: Scratch caryopses; KNO_3.
Paspalum urvillei vaseygrass	P	20-35	7	21	Light. Fresh and dormant: KNO_3.
Pastinaca sativa parsnip	T, B, TS	20-30	6	28	
Pelargonium spp. geranium	B, T, TB	20	7	28[b]	Hard seed: see sec. 4.2 d and 4.9 k(6).
Alternate method (for clipped and scarified seeds)	B, T, TB	20	7	14[b]	Hard seed: see sec. 4.2 d and 4.9 k(6).
Pennisetum americanum pearl millet	see *Pennisetum glaucum*				
Pennisetum glaucum pearl millet	B, T	20-30	3	7	
Pennisetum purpureum napiergrass	B, T	20-30	3	10	
Penstemon barbatus, P. grandiflorus, P. laevigatus, P. hirsutus penstemon	P	15	8[a]	18[b]	Light.

4.9 Explanation of Table 3

Table 3 contains specific germination requirements for the kinds of seeds listed in column 1. If the genus and species is listed, that listing shall be used. If the species is not listed, use the spp. listing for that genus. Some explanations of these tables and additional germination requirements and conditions are as follows:

a. **Substrata.** — Symbols for substrata in column 2, Table 3 are: B = between blotters: TB = top of blotters; T = paper toweling, used either as folded towel tests or as rolled towel tests in horizontal or vertical position; S = sand; TS = top of sand; P = covered petri dishes with (a) two layers of blotters, or (b) three thicknesses of filter paper, or (c) top of sand; PT = substrata listed for P with the following substrata also allowed: sponge rok, vermiculite, terralite, or a mixture of 50% sand and vermiculite, sand and perlite, etc.; C = creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed that are pressed for about one-half their thickness into the paper wadding; RB = blotters and raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds; TC = on top of creped cellulose paper without a blotter. PP = pleated filter paper; TCS = on top of creped cellulose paper without a blotter and covered with ½ to 3/4 inch layer of sand.

   Since it is generally difficult to obtain consistent supplies of soil, it is not to be used as primary testing substrate. However, it may be necessary to use it when seedlings show phytotoxic symptoms or if evaluation of seedlings is in doubt. Soil is commonly used for comparative or investigative purposes. Refer to section 4.5b(1).

   If there is a question as to whether a paper substratum is toxic to developing seedlings, check tests should be made on Whatman's No. 2 filter paper or its equivalent. Seeds of celery, celeriac, chicory, dandelion, timothy, and bermudagrass are particularly sensitive to toxic substrata. If root injury is evident on substratum moistened with potassium nitrate, retests should be made on substratum moistened with water.

b. **Moisture.** — The directions and suggestions given under the heading "Moisture and Aeration" in section 4.3 should be observed. In Table 3 the term "moisture on dry side" means that the moistened substratum should be pressed against a dry absorbent surface such as a dry paper towel or blotter to remove excess moisture.

c. **Temperature.** — Single numerals in Table 3 indicate constant temperatures. Two numerals separated by a dash indicate an alternation of temperature, the test to be held at the first temperature for approximately 16 hours and at the second temperature for approximately 8 hours per day. A sharp alternation of temperature, such as obtained by hand transfer, may be beneficial in breaking dormancy. If the tests are not subjected to alternating temperatures over weekends and holidays, they are to be held at the lower temperature during this time. Variation from the temperature specified in the rules should not be more than $\pm 1^{\circ}C$ due to the apparatus. In the case of species of *Trifolium, Medicago* and *Vicia faba,* the temperature should not exceed $20^{\circ}C$.