DORMANCY IN PRE-VARIETY GERMPLASM OF NATIVE *COREOPSIS* SPECIES

By

DZINGAI RUKUNI

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To my wife Miriam and my daughters Shingirai Tadziripa and Tanyaradzwa Nyasha who gave me all the support that I needed to accomplish this research and dissertation. This is a special dedication to my son Dzingai Junior who arrived on October 28th 2008. Also to my mother and late father who inspired me to achieve all that I have to this day.

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By

Dzingai Rukuni

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Cochair: Jeffrey G. Norcini
Cochair: Daniel J. Cantliffe
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Coreopsis is Florida's state native wildflower, and the Florida Department of Transportation adopted it for roadside revegetation. However, *C. floridana* and *C. lanceolata* seeds have dormancy that can impede economic establishment of sustainable populations. *Coreopsis* seeds (achenes) consist of a pericarp, testa and endosperm surrounding a dicotyledonous embryo. In *C. lanceolata,* lateral endosperm was a single cell-layer, but 1 to 3 cell-layers at the micropylar end, while in *C. floridana* a single cell-layer persisted throughout. Endosperm ploidy was 1.5 times that of embryo, consistent with angiosperms. Endosperm removal alleviated dormancy in dark-imbibed *C. floridana* seeds, while in both species, excision of the same tissue permitted germination at supraoptimal temperatures. Optimum germination temperatures in C*oreopsis* were 15 and 20^{o}C, and germination declined above 25^{o}C. In *C. lanceolata,* endo-β-mannanase (EBM) activity was detected at 90 hours imbibition but *C. floridana* seeds did not exhibit enzyme activity anytime during germination. There was an association between EBM activity and endosperm rupture. Abscisic acid, tetcyclacis or supraoptimal temperatures inhibited EBM activity and germination.

In 4-week-old *C. floridana* seeds, pericarp, testa and endosperm imposed dormancy in dark but all naked embryos germinated. Exogenous gibberellic acid (GA) overcame dormancy in dark. Cold (5^{o}C) stratification partially overcame dormancy in dark-imbibed *C. floridana* seeds, but potassium nitrate was not effective. Four-week-old *C. lanceolata* seeds required 150 days dry afterripening to overcome dormancy; endosperm enforced dormancy for 90 days, while naked embryos were non-dormant. Potassium nitrate, light and GA did not alleviate dormancy in *C. lanceolata*, and cold stratification reduced germination.

Osmopriming *C. floridana* seeds in PEG or PEG+GA improved germination rate and uniformity at 30^{o}C but these treatments were less effective than priming in PEG+6-benzyladenine (BA), which led to 100% germination at 20 or 30^{o}C in dark. When solid matrix priming (SMP) was used, BA gave similar results as in osmopriming, but GA effectiveness improved. In *C. lanceolata*, osmopriming in PEG+BA increased germination at supraoptimal temperatures.

Dormancy alleviation in *Coreopsis* was modulated by embryo envelopes. There is great potential for using SMP to enhance germination and improve field establishment in Florida's native *Coreopsis* sowing programs.

CHAPTER 1
INTRODUCTION

1.1 Overview

The genus *Coreopsis* is Florida's state wildflower. The Florida Department of Transportation (FDOT) deems use of this native wildflower appropriate and consistent with roadside ecosystem management goals (FDOT, 2008). Roads impact the ecosystem in several ways such as altered vegetation patterns, hydrology, biodiversity and habitat, decrease water quality via runoff, and affect a larger area than they occupy (Forman and Alexander, 1998; Trombulak and Frissel, 2000; Gelbard and Belnap, 2003; Hansen and Clevenger, 2005; Flory and Clay, 2006; Watts *et al.*, 2007). Planting and restoration of locally adapted (ecotypes) native wildflowers besides their aesthetic value, has the potential for lowering roadside maintenance costs by reducing mowing and re-seeding frequency (McCully, 1987; Bryant and Harper-Lore, 1997; Dana *et al.*, 1996; Norcini and Aldrich, 1998; Norcini *et al.*, 2001a, 2001b; FDOT, 2008). For example, use of native wildflowers in Texas roadside plantings increased aesthetic value, reduced maintenance costs, enhanced wildlife habitat and biodiversity, augmented soil erosion control and suppressed noxious weeds (Bryant and Harper-Lore, 1997). Other states using local ecotypes for revegetation programs include, Idaho, Michigan, Ohio and Wisconsin.

Native wildflower populations established with locally or regionally adapted ecotype germplasm are likely to be self-sustaining with proper management (Norcini and Aldrich, 1998; Norcini *et al.*, 2001b; FDOT, 2008), because the environment favors flowering, seed production (Norcini *et al.*, 2001b; Norcini and Aldrich, 1998) and the possible establishment of a persistent seed bank (Kabat *et al.*, 2007; Frances, 2008), coupled with opportune conditions for subsequent seed germination and recruitment (Frances, 2008).

Because of adaptive attributes and their aesthetic value, Florida ecotypes are especially valued for ecological restoration, roadside revegetation, mine site reclamation, and enhancement of natural areas. Seeds of Florida ecotypes of native *Coreopsis* species are costly ($110-$220/kg) because of limited supply (Norcini and Aldrich, 2008), and are well adapted, low maintenance plants (Norcini and Aldrich, 1998; Norcini *et al.*, 2001b; Kabat *et al.* 2007; Norcini, 2008). However, native Florida ecotypes of *Coreopsis* can have germination problems that hinder establishment of sustainable populations. Additionally, some species require light for germination.

1.2 Taxonomy and Distribution

Coreopsis is a genus of about 132 species and is distributed throughout the Americas, the near Pacific and Africa. Formerly known as *Calliopsis, Coreopsis* belongs to the family *Asteraceae* (*Compositae*), the daisy or aster family. The aster family has some of the most important agricultural and horticultural species such as lettuce (*Lactuca sativa*) and sunflower (*Helianthus annuus*). *Coreopsis* species in the current study belong to the Division *Magnoliophyta;* Class, *Magnoliopsida;* Order, *Asterales;* Family, *Asteraceae;* Tribe, *Coreopsideae;* Genus, *Coreopsis*: *C. basalis, C. floridana, C. lanceolata, C. leavenworthii* and *C. pubescens* (USDA-NRCS, 2007; Crawford and Mort, 2005). According to Crawford and Mort (2005) the species *basalis, lanceolata* and *pubescens* belong to the section *Coreopsis*, *leavenworthii* to section *Calliopsis,* and *floridana* to section *Eublepharis.* Important botanical and reproductive information on the study species was documented by Smith (1976) and is summarized in Table 1-1. Further hybridization work in *Coreopsis* is reported by Smith (1983) and Smith and Deng (2008), and Czarnecki *et al.* (2008) presents information on genetic variation in *C. leavenworthii*. *Coreopsis* species have sporophytic self-incompatibility (Ferrer

and Good-Avila, 2007) to maintain genetic variability, and cross-pollination is necessary for seed production.

The genus *Coreopsis* is commonly referred to as tickseed because the flat, small, indehiscent fruit (achene) is oval to round, and has two short spines or awns at one end that give it a bug-like appearance. The achene (hereafter referred to as seed) is a cypsela because it develops from an inferior ovary (Esau, 1977). Tickseed flowers generally have eight showy ray flowers (petals) that usually have toothed ends. There are 14 tickseed species in Florida; *C. floridana* is endemic while *C. leavenworthii* is nearly so (USDA NRCS, 2007; Wunderlin and Hansen 2008).

1.3 Problem Statement and Research Objectives

Poor seed germination due to dormancy is an obstacle to propagating and establishing non-domesticated species. Successive sowing cycles of seeds without applying germination improving treatments leads to reduced dormancy in cultivated populations of *Helianthus annuus* (Gandhi *et al.*, 2005) and *Echinaceae purpurea* (Qu *et al.*, 2005) due to sustained passive selection against dormancy. In *C. leavenworthii* and *C. lanceolata* prevariety germplasm, seeds harvested in different seasons had variable germination levels (Norcini *et al.*, 2004, Kabat, 2004; Norcini *et al.* 2006, Kabat *et al.*, 2007). Using higher seeding rates to compensate for low germination can be expensive because of high seed prices (Kabat *et al.* 2007; Norcini 2008; FDOT, 2008) which range from $110 and $220 per kilogram (Norcini and Aldrich, 2008).

Dry afterripening increases germination of *C. lanceolata* and *C. leavenworthii* (Norcini *et al.* 2004, 2006; Norcini and Aldrich 2007a). However, afterripening under cold moist conditions induces dormancy in *C. lanceolata* (Banovetz and Scheiner (1994a, 1994b). Gibberellic acid stimulates germination in non-afterripened *C. basalis* seeds (Carpenter and Ostmark, 1992). However, there is a lack of information on germination behavior in native *Coreopsis* seeds.

Optimizing germination continues to present a challenge to both seed producers and end users of pre-variety *Coreopsis* germplasm, and techniques to improve germination are presently being explored.

The objective of this study was to investigate methods of alleviating dormancy, and enhance germination to attain acceptable field establishment under a wide variety of environmental conditions, and promote efficient and cost effective native *Coreopsis* sowing programs in roadside revegetation and ecological restoration projects. Several approaches were used to accomplish the objective, and these were: 1) studying *Coreopsis* seed anatomy, and examining effects of embryo envelopes on dormancy regulation, and assessing the relationship between endo-β-mannanase (EBM) enzyme activity in endosperms and need for during germination; 2) appraisal of temperature and light effects on germination, and GA to promote germination; 3) evaluation of influence of dry afterripening on germination; and 4) exploration of seed priming techniques to improve germination.

Table 1-1. Distribution and characteristics of *Coreopsis* species used in the study.

Species	Distribution	Main blooming time	Chromosome number	Habitat	Hybridizations
C. basalis (A. Diert.) Blake. Goldenmane tickseed	It was apparently introduced into the USA, but is now widespread on the coastal plain from Florida to North Carolina. Southern states; North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Arkansas, Louisiana and Texas.	April-July	n=13	low sandy areas	Successful with: *C. wrightii* only when it was the male parent (pollen donor) Failed with: *C. grandiflora*, var. *grandiflora* and *saxicola*, *C. auriculata, C. rosea* and *C. tinctoria*.:
C. floridana E.B. Smith. Florida tickseed	Endemic to Florida. Thought to be hybrid of *C. linifolia* and *C. gladiata* (or possibly *C. falcata*). Distribution –Florida	mid October-December	n=ca.78 Mid October-December Individuals blooming late in the season (December-March) are nearly scapose. This probably represents a high polyploidy (duodecaploid) derived from hybridization.	low sandy bogs, ditches, pine barrens, everglades	Successful with: none Failed with: *C. linifolia* (n=13 and n=26) and *C. gladiata*
C. lanceolata L. Lanceleaf tickseed. Varieties: *glabella* and *villosa* Michx.	Connecticut, New Jersey, Delaware, Pennsylvania, Maryland, West Virginia, New York, North Carolina, South Carolina, Georgia, Florida, Alabama, Tennessee, Kent, Mississippi, Louisiana, Texas, Arkansas, Oklahoma, Missouri, Kansas, Illinois, Indiana, Iowa, Michigan, Wisconsin.	mid March-May, in the south to June-July in the north.	n=13 (+0-2B). Exhibits variability and has intergrading phases. The most prevalent variation is a subglabrous and pubescent phase. Four phases have been drawn up: typical, entire-leaved, robust and dwarf hairy. All intergrade and it does not appear useful to segregate any of them. In Florida, all "dwarf" glabrous or *villosa.*	prairies, glades, sandy slopes and roadsides.	Successful with: *C. auriculata, C. grandiflora* var. *grandiflora, harveyana* and *saxicola; C. pubescens* var. *pubescens.* Failed with: *C. intermedia, C. nuecensoides, C. basalis, C. rosea, C. gladiata, C. tinctoria* var. *tinctoria* and *C. palmata.*

Table 1-1. Continued

Species	Distribution	Main Blooming time	Chromosome number	Habitat	Hybridizations
C. leavenworthii Torr. & Gray. Leavenworth's tickseed. Varieties: *garberi* Gray, *curtissii* Sherff and *lewtonii* (Small) Sherff.	Nearly endemic to Florida. Alabama - 2 counties.	April-October (sporadically all year in southern Florida)	n=12	low wet sandy flatwoods and savannas.	Successful with: *C. tinctoria* var. *tinctoria* and Failed with: *C. basalis, C. wrightii, C. nuecensoides, C. linifolia (n=26), C. rosea, C. gladiata,* and *C.grandiflora* var. *longipes.*
C. pubescens Ell. Star tickseed. Varieties: *debilis, pubescens* and *robusta*	Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Tennessee, Kent, Mississippi, Louisiana, Arkansas, Oklahoma, Missouri, Illinois.	June-August.	n=13 (+0-2B).	alluvial banks, pan woods, sandy fields.	Successful with: *C. pubescens var pubescens X var debilis, C. auriculata, C. intermedia, C. grandiflora* var. *saxicola* and *C. lanceolata.* Failed with: *C. basalis, C. nuecensoides, C. palmata,* and *C. tripteris.*

Based on information from Smith (1976), Wunderlin (2008) and Norcini (Pers. Comm.).

CHAPTER 2
LITERATURE REVIEW

In seed biology, there are four chronological developmental programs, namely, morphogenesis, maturation, dormancy and germination, in that order (Bewley and Black, 1994). Integration of the phases is less strong than previously thought since some stages can be bypassed without detriment to the seed (Golovina *et al.,* 2001; Gutierrez *et al.*, 2007). A brown seeded tomato mutant does not exit the developmental program and germinates after physiological maturity without entering dormancy or quiescence (Downie *et al.*, 2004); vivipary also occurs in corn kernels (White *et al.*, 2000). Many dormancy features are determined by genetics and mediated by the environment, and the balance between the phytohormones abscisic acid (ABA) and GA plays a crucial role in seed dormancy (White *et al.*, 2000; Finch-Savage and Leubner-Metzger, 2006; Leubner-Metzger, 2003; Groot and Karsen, 1992).

2.1 Seed Dormancy in *Coreopsis* Species

Coreopsis lanceolata seed dormancy is influenced by afterripening (Banovetz and Scheiner, 1994a, 1994b), and the phytohormones GA_3 and ethylene govern germination in *Coreopsis basalis* seeds collected from natural stands (Carpenter and Ostmark, 1992). In *C. leavenworthii* seeds, warm stratification alleviated dormancy (Kabat, 2004; Kabat *et al.*, 2007), while in *C. lanceolata* seeds dry afterripening overcame dormancy (Norcini *et al.,* 2004, 2006; Norcini and Aldrich, 2007a). Fresh *C. basalis, C. floridana* and *C. leavenworthii* seeds require slight afterripening (Norcini and Aldrich, 2007a). *Coreopsis leavenworthii* and *C. lanceolata* prevariety seeds harvested in different seasons had variable dormancy levels (Norcini *et al.*, 2004, 2006; Kabat, 2004; Kabat *et al.*, 2007). Cold stratification induces dormancy in *C. lanceolata* seeds (Banovetz and Scheiner (1994a, 1994b).

2.2 Seed Morphology and Anatomy

The typical fruit of *Asteraceae*, the achene or cypsela (hereafter referred to as seed), is comprised of a dicotyledonous embryo surrounded by a thin endosperm, testa, and pericarp (Borthwick and Robbins, 1928; Esau, 1977; Puttock, 1994; Sancho *et al.,* 2006). Seeds of *Asteraceae* are classified as cypselas because they develop from an inferior ovary (Esau, 1977). Like other angiosperms, the diploid pericarp and testa are of maternal origin. The triploid endosperm has one set of genes from the paternal parent and two sets from maternal parent, and the diploid bi-parental embryo is derived equally from both male and female parental gametes (Friedman 1998; Herr 1999; Floyd and Friedman 2001; Friedman and Williams 2003). In lettuce (*Lactuca sativa*), the embryo is enveloped by a two-cell layer of endosperm which could be three or more cell layers thick at the micropylar end, a testa and pericarp (Borthwick and Robbins, 1928; Jones, 1974; Psaras *et al.* 1981; Nijsse *et al.* 1998). In seeds of *Arabidopsis thaliana* and *Lepidium sativum* (both *Brassicaceae*), endosperm is single cell-layered (Muller *et al.*, 2006). Pericarp and testa protect the dispersal unit while endosperm nourishes the embryo during seed development and germination (Floyd and Friedman 2000; Friedman and Floyd, 2001; Williams and Friedman 2002; Feurtado *et al.*, 2004). Costa *et al.* (2004) reported that endosperm contains proteins that repress precocious germination and promote desiccation tolerance.

The developmental origin of the endosperm from a second fertilization event was independently discovered by Nawaschin of Russia in 1898 and Guignard of France in 1899 (Friedman, 1997; Friedman and Floyd, 2001). Before then, the endosperm had been regarded as a fission product of polar nuclei of the female gametophyte (embryo sac). There are three types of endosperm development, *viz*: cellular, helobial and nuclear (syncytial) of which the last is the most common in angiosperms (Carmichael and Friedman, 1995; 1996; Friedman 1998; 1992a; 1992b; 1990a; 1990b; Friedman, 1994; 1995; 1998; Friedman and Carmichael, 1996; Floyd *et*

al., 1999; Floyd and Friedman 2000; Friedman and Floyd, 2001; Williams and Friedman 2002; Costa *et al.*, 2004). The endosperm could be regarded as an organism homologous (but not in function) to the embryo rather than as a tissue because it is genetically identical to embryo except for gene dosage (Friedman, 1998; 1995; Friedman, 1998; 1991; 1990a; 1990b). The three defining characteristics of an endosperm are altruism, determinate growth and programmed death (Friedman, 1998; 1995).

2.3 Germination

Germination is the sum of all the processes that occur in a seed and results in exit from dormancy or quiescence to produce a seedling. This occurs when sufficient moisture is provided under optimal temperatures, adequate oxygen levels and may sometimes require exacting light quality and/or quantity (Baskin *et al.*, 2003; Bewley and Black, 1994; Finch-Savage and Leubner-Metzger, 2006; Tamura *et al.*, 2006; Bradford *et al.*, 2007). Water uptake in seeds occurs in a triphasic pattern, starting with an initial stage of rapid water uptake, followed by a lag phase, and finally, a period of resumed water uptake as a result of embryonic axis growth (Bewley and Black, 1994). Failure of seeds to enter the third stage of mass increase is a sign of dormancy (Loercher, 1974; Bewley and Black, 1994; Baskin and Baskin, 1998; Jayasuriya *et al.*, 2007).

2.3.1 Temperature and Light

Temperature influences germination via its inherent control of respiration and metabolic rates in seeds (Bewley and Black, 1994; Baskin and Baskin, 1998). Germination and respiration rates vary with temperature and water potential (Dahal *et al.*, 1996; Marshall and Squire, 1996; Steinmaus *et al.*, 2000; Cheng and Bradford, 1999; Grundy, 2002; Larsen *et al.*, 2004). Seeds have an optimum, maximum and minimum temperature at which germination occurs, and non-dormant seeds germinate in a wide temperature range (Bewley and Black, 1994; Baskin and

Baskin, 1998; Pritchard *et al.*, 1999; Kabat *et al.*, 2007). Fluctuating temperatures promote germination in *Cynara cardunculus* and *Sisymbrium altissimum* seeds under high osmotic potential (Huarte and Benech-Arnold, 2005), and alternating temperatures in the natural habitat means that seeds germinate under wider environmental conditions (del Monte and Tarquis, 1997; Chachalis and Reddy, 2000; Steinmaus *et al.*, 2000; Steadman, 2004; Baskin *et al.*, 2006; Tarasoff *et al.,* 2007; Merritt *et al.*, 2007; Leon *et al.*, 2006; 2007; Cristaudo *et al.,* 2007; Merritt *et al.*, 2007). Temperature and light interaction cause variable germination responses (Taylorson and Hendricks, 1972; Steadman, 2004). For example, light responses vary with temperature in germinating *Abutilon theophrasti* and *Setaria faberi* seeds (Leon and Owen, 2003).

Light is a critical environmental factor regulating germination, and acts through the phytochrome system (Hendricks *et al.*, 1968; Wooley and Stoller, 1978; van der Woude and Toole, 1980; Ensminger and Ikuma, 1988; Ensminger and Ikuma, 1988; Derkx *et al.*, 1994; Poppe and Schafer, 1997; Casal *et al.*, 1997; 1998; Toyomasu *et al.*, 1998; Milberg *et al.,* 2000; Yamaguchi and Kamiya, 2002; Oh *et al.*, 2006; Appenroth *et al.*, 2006; Donohue *et al.,* 2007; 2008). Phytochrome photoreceptors are located in the embryonic axis (Bewley and Black, 1994). The phytochrome system is widely believed to control germination through two forms of the inter-convertible phytochrome pigment that exists as P_{fr} or $P_{r,}$ with P_{fr} being the biologically active form (Bewley and Black, 1994; Baskin and Baskin, 1998). Exposure of seeds to red light (660 nm) converts the pigment to the P_{fr} form and irradiation with far-red light (730 nm) causes it to revert (Poppe and Schafer, 1997; Casal *et al.*, 1997; 1998).

Three types of phytochrome responses operate in seeds: Low Fluence Response (LFR), Very Low Fluence Response (VLFR) and High Irradiance Response (HIR). Very low proportions of P_{fr} are sufficient to induce VLFR under low fluence red light, whereas higher

levels are required to induce LFR. Under far-red light, VLFR would occur but not LFR. The HIR occurs under continuous far-red light. The LFR response is under control of the more stable phytochrome B while the labile phytochrome A mediates VLFR and HIR. Reciprocity experiments are utilized to dissect these light responses since LFR and VLFR exhibit reciprocity but HIR does not (Yanovsky *et al.*, 1997; Casal *et al.*, 1997; 1998). The best-characterized light response is controlled by phytochrome B, and lettuce seeds are a good example for LFR (Grubisic *et al.,* 1985; Shinomura *et al.,* 1994; Yanovsky *et al.*, 1997; Leon and Owen, 2003; Yamauchi *et al.*, 2004; Appenroth *et al.*, 2006;).

In six annual *Asteraceae* weeds (*Bidens pilosa, Galinsoga parviflora, Guizotia scabra, Parthenium hysterophorus, Tagetes minuta* and *Verbesina encelioides*), germination was promoted by light (Karlson *et al.*, 2008). Lettuce seeds responded to light quality at 4 to 32% seed moisture content (Vertucci *et al.,* 1987; Lewak and Khan, 1977). In lettuce, far-red irradiance can not reverse the effects of red light exposure when applied 14 hours after red light induction of germination (Loercher, 1974). The light requirement in some lettuce seeds could be substituted for by cold stratification (Lewak and Khan, 1977; van der Woude and Toole, 1980). Light and cold stratification promoted germination in *Amaranthus tuberculatus* (Leon and Owen, 2003; Leon *et al.*, 2006; 2007). Nitric oxide was found to promote light-mediated events in germinating light-requiring lettuce seeds (Beligni and Lamattina, 2000).

Germination is less dependent on light in larger seeds (Milberg *et al.,* 2000; Schutz *et al.,* 2002; Jankowska-Blaszczuk and Daws, 2007). Small seeds of *C. lanceolata* germinated frequently at shallow depths and large ones at deeper positions (Banovetz and Scheiner, 1994b). In *Tragopogon pratensis* (*Asteraceae*) seeds, dormancy is controlled by seed size (van Molken *et al.*, 2005). In buried weed seeds with VLFR, sensitivity to light is high, and 70 to 400% more

seeds germinated when tillage was carried out in daylight than at night (Scopel *et al.*, 1994). Many seeds such as those of *Polygonum aviculare* lose the light-requirement for germination as dormancy wanes (Batlla and Benech-Arnold, 2005).

In many species, activation of phytochrome is linked to subsequent *de novo* GA production (Grubisic *et al.,* 1985; Yanovsky *et al.*, 1997; Toyomasu *et al.*, 1998; Kucera *et al.,* 2005; Oh *et al.*, 2006; Yamauchi *et al.*, 2007; Donohue *et al.*, 2007; 2008), and enhanced sensitivity of embryos to synthesized GA (Hilhorst and Karsen, 1988; Yamaguchi and Kamiya, 2002).

2.3.2 Gibberellic Acid

Gibberellic acid promotes germination (Hole *et al.*, 1989; Yamaguchi *et al.*, 1998; Debeaujon and Koornneef, 2000; Olszewski *et al.,* 2002; Feurtado *et al.*, 2004; Ali-Rachedi *et al.*, 2004; Kucera *et al.,* 2005; Finch-Savage and Leubner-Metzger, 2006; Muller *et al.*, 2006; Yamauchi *et al.*, 2007). Exogenous application of GA induces germination in many seeds by substituting for the light requirement (Braun and Khan, 1975; Derkx *et al.*, 1994; Yoshioka *et al.,* 1998; Yamaguchi *et al.*, 1998; Yamaguchi and Kamiya, 2002; Hedden, 2002; Yamauchi *et al.*, 2007). Gibberellins stimulate embryo growth potential by promoting cell elongation and division of embryo hypocotyls (Taiz and Zieger, 2002), and at times, induces *de novo* biosynthesis of hydrolases that digest and weaken endosperm (Wu *et al.*, 2001; Yamaguchi and Kamiya, 2002; Kucera *et al.,* 2005; Oh *et al.,* 2006).

The importance of GA in germination is demonstrated in *Arabidopsis thaliana* and tomato GA-deficient mutants that require exogenous GA for germination (Leon-Kloosterziel *et al.,* 1996; Debeaujon and Koorneef, 2000; Olszewski *et al.,* 2002; Hedden, 2002; Mo and Bewley, 2003). Gibberellin requirement for germination is determined by testa integrity in

Arabidopsis testa mutants (Debeaujon and Koornneef, 2000). Ethylene action substitutes for GA requirement in *Arabidopsis* seed germination (Leon-Kloosterziel *et al.,* 1996).

2.3.3 Ethylene

The role of ethylene in germination is widely acknowledged (Wang and Ecker, 2002; Kepczynski and Kepczynka, 1997). Ethylene promotes germination in lettuce seeds by stimulating radial cell expansion in the embryonic hypocotyl (Abeles, 1986). The phytohormone promotes germination in seeds of lettuce (Nascimento *et al.,* 2000; 2001), peanut (*Arachis hypogeae*) (Ketring and Morgan, 1972), *Avena fatua* (Cranston *et al.*, 1996), barley (Locke *et al.,* 2000), tomato (Siriwitayawan *et al.*, 2003), beet (*Beta vulgaris*) (Hermann *et al.*, 2007), *Lannea microcarpa* (Neya *et al.*, 2008) and in *Stylosanthes humilis* (Pinheiro *et al.*, 2008). Ethylene also overcomes thermoinhibition in lettuce seeds (Abeles, 1986; Nascimento *et al.,* 2000; 2001). Seeds of some species do not respond to ethylene, or promotive effects are minimal (Kucera *et al.,* 2005).

2.3.4 Cytokinins

Cytokinins can either inhibit or promote germination, and response is dependent on environmental conditions such as temperature (Hutchinson and Kieber, 2002). Cytokinins promote germination through regulation of cell division (Khan, 1971; Braun and Khan, 1975; Hutchinson and Kieber, 2002). They also alleviate thermoinhibition in lettuce seeds (Abeles, 1986; Kucera *et al.,* 2005).

2.4 Dormancy

Seed dormancy is an innate property that prevents seeds from germinating under any environmental conditions that are otherwise favorable for germination in a particular species (Baskin and Baskin, 1998; 2004; Baskin *et al.*, 2003; Bewley and Black, 1994; Finch-Savage and Leubner-Metzger, 2006). Primary dormancy is acquired at seed maturation (Bewley and Black,

1994; Baskin and Baskin, 1998; Mollard *et al.*, 2007). Dormancy is a quantitative character though its inheritance is not well understood (Foley and Fennimore, 1998). Finch-Savage and Leubner-Metzger (2006) proposed a broad classification of five seed dormancy classes: physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy and combinational dormancy. Non-deep physiological dormancy is the most common and occurs in both gymnosperms and angiosperms (Baskin and Baskin, 1998; 2004).

In the natural environment, seed dormancy plays an important role by stretching the "germination window" by several seasons or years, an important survival strategy (Paterson *et al.*, 1976; Cavieres and Arroyo, 2000; Foley, 2001; Traba *et al.,* 2004; Masin *et al.*, 2006; Karlsson *et al.*, 2006). Based on 2-year burial results, the longevity of *C. lanceolata* seeds were 2, 6 and 13 years for small, medium and large seeds respectively (Banovetz and Scheiner, 1994a). Seed age-related changes in dormancy levels in a soil seed bank is adaptive rather than spontaneous, and maintains genetic diversity (Valleriani and Tielborger, 2006).

Growth and maturation environment of maternal plants influence dormancy in *Arabidopsis thaliana* (Blodner *et al.*, 2007; Donohue *et al.,* 2007; 2008), *Sicyos deppei* (Orozco-Segovia *et al.*, 2000), and *Avena fatua* and *A. barbata* (Paterson *et al.,* 1976). In a particular species, there are variations in seed dormancy among populations, maternal parents and years of seed production (Andersson and Milberg, 1998; Pritchard *et al.*, 1999; El-Keblawy and Al-Ansari, 2000). The shift in seed population sensitivity to environmental cues together with individual seed variation in sensitivity accounts for the wide array of dormancy phenotypes (Silverton, 1999; Mo and Bewley, 2003; Bradford, 2005).

2.4.1 Abscisic Acid (ABA)

Abscisic acid is a putative dormancy enhancer (Schopfer and Plachy, 1984; Hole *et al.*, 1989; Page-Degivry and Garello, 1992; Leon-Kloosterziel *et al.,* 1996; Leung and Giraudat,

1998; Toorop *et al.*, 2000; Feurtado *et al.*, 2004; Benech-Arnold *et al.,* 1999, 2006; Ali-Rachedi *et al.*, 2004; Kucera *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006; Muller *et al.*, 2006; Leon *et al.*, 2006; 2007; Toh *et al.*, 2008). In dormant imbibed seeds, ABA is produced in the embryo and regulates germination by arresting embryo growth (Taiz and Zeiger, 2002). The nature of ABA receptors and its signal transduction are still not well understood (Leung and Giraudat, 1998). Abscisic acid is synthesized in plants in response to abiotic stress such as drought, and this phytohormone plays a major role during seed development, maturation and dormancy (White *et al.*, 2000; Suzuki *et al.*, 2000; Frey *et al.*, 2004).

Abscisic acid is a sesquiterpenoid synthesized from xanthophylls (Nambara and Marion-Poll, 2003; Taylor *et al.,* 2000). Carotenoids are the main precursors of ABA and inhibition of their biosynthesis would prevent ABA biosynthesis. Studies in which carotenoid biosynthesis inhibitors are used to study ABA effects in seed germination could however be misleading, because carotenoids are channeled to many other physiological processes (Yoshioka *et al.*, 1998). Abscisic acid in seeds is inactivated by metabolism into phaseic and dihydrophaseic acids (Jacobsen *et al.*, 2002; Feurtado *et al.*, 2004).

Abscisic acid is required during late seed maturation to induce dormancy (Hole *et al.*, 1989), and in *Arabidopsis thaliana*, this is controlled by four master regulator genes, *viz*, *ABSCISIC ACID INSENSITIVE 3 (ABI3), FUSCA3 (FUS3), LEAFY COTYLEDON 1 (LEC1)* and *LEC2* (Gutierrez *et al.*, 2007). Production of ABA during seed development is of dual origin, arising from developing embryo and maternal tissues, but only embryonic ABA induces lasting dormancy (Hole *et al.*, 1989; Page-Degivry and Garello, 1992; Groot and Karssen, 1992; Nambara; Suzuki *et al.*, 2000 and Marion-Poll, 2003; Kucera *et al.,* 2005). Developing seeds of *Cucumis sativus* were more sensitive to ABA than mature seeds (Amritphale *et al.*, 2005). This

disparity in embryo ABA sensitivity prevents vivipary. Pre-harvest sprouting in *Sorghum bicolor* was caused by low embryonic sensitivity to ABA (Steinbach *et al.*, 1997). However, Berry and Black (1992) proposed that the osmotic environment within the tomato fruit contributed more to preventing precocious germination of developing seeds than did endogenous seed ABA. Embryo axes excised from seeds after radicle protrusion were insensitive to ABA in *Aesculus hippocastanum* (Gumulevskaya and Azarkovich, 2004).

In ABA-deficient tomato seeds, exogenous ABA restricted embryo growth potential (Groot and Karsen, 1992). Embryonic ABA in *Arabidopsis* seeds influences dormancy (Debeaujon and Koornneef, 2000; Debeaujon *et al.,* 2000). Plant mutants impaired in ABA biosynthesis and/or sensitivity produce viviparous seeds that germinate on the mother plant. Such mutants have been isolated in tomato, *Zea mays*, *Sorghum bicolor*, *Triticum aestivum* and *Arabidopsis thaliana*; and these genotypes are invaluable in the study of ABA and its role in seed dormancy (Leon-Kloosterziel *et al.*, 1996; Steinbach *et al.*, 1997; Kawakami *et al.*, 1997; Donohue, 2005; Gualano *et al.*, 2007; Gianinetti and Vernieri, 2007).

Seed sensitivity to ABA is associated with physiological status (Ni and Bradford, 1992; 1993). Lettuce seeds imbibed in conditions favoring germination had rapid decline in endogenous ABA content (Braun and Khan, 1975). Dry dormant seeds of the Cvi ecotype of *Arabidopsis thaliana* were found to contain higher amounts of ABA than dry, non-dormant seeds, and when imbibed, ABA content declined in both dormant and non-dormant seeds until 3 days after sowing when ABA content increased and remained high only in dormant seeds (Ali-Rachedi *et al.*, 2004). The mechanisms blocking germination when exogenous ABA is used were found to be different from those that blocked germination in dormant *Arabidopsis thaliana* seeds (Chibani *et al.*, 2006).

Abscisic acid breakdown permits seeds to exit dormancy (Jacobsen *et al.*, 2002; Feurtado *et al.*, 2004). Fluridone, an ABA biosynthesis inhibitor overcame dormancy in lettuce seeds (Yoshioka *et al.,* 1998; Gonai *et al.*, 2004), and in a light-requiring lettuce cultivar, the ABA-inhibitor norflurazone promoted germination in dark-imbibed seeds (Roth-Bejerano *et al.,* 1999). The capacity of embryos to metabolize (de-activate) ABA and low ABA sensitivity are two factors associated with dormancy termination in *Chamaecyparis nootkatensis* seeds (Schmitz *et al.,* 2000, 2002), and similar results were found with *Pinus monticola* (Feurtado *et al.*, 2004) and in barley (Jacobsen *et al.*, 2002). This corroborates that ABA is involved in dormancy imposition (Gonai *et al.*, 2004; Tamura *et al.*, 2006; Toh *et al.*, 2008).

Abscisic acid inhibits endosperm rupture in *Lepidium sativum, Arabidopsis thaliana* and *Nicotiana tabacum* seeds (Leubner-Metzger, 2002; Muller *et al.,* 2006), and inhibits water uptake in *Brassica napus* seeds by preventing cell wall loosening (Schopfer and Plachy, 1985, 1984). Abscisic acid action is associated with arrested embryo growth as confirmed by studies in which micropylar endosperm is removed (Finch-Savage and Leubner-Metzger, 2006).

2.4.2 Afterripening

Afterripening is a natural process that occurs in dry seeds under a set of environmental conditions. This process allows them to germinate after a certain period from physiological maturity, and may take a few weeks to years depending on species, storage relative humidity and temperature, and oxygen levels (Widrlechner, 2006). Afterripening occurs below a certain seed moisture content and is delayed when seeds are too dry; it is accelerated by high temperatures and elevated oxygen levels (Bewley and Black, 1994). It is a common property of many dicot and monocot seeds (Baskin and Baskin, 1976; Fennimore and Foley, 1998; Steadman *et al.,* 2003; Merritt *et al.*, 2007; Tarasoff *et al.*, 2007; Cristaudo *et al.*, 2007), but is a phenomenon whose physiology is not well understood (Wettlaufer and Leopold, 1991; Berma-Lugo and

Leopold 1992; Bewley and Black, 1994; Baskin and Baskin 1998). The process is associated with non-enzymatic biochemical reactions between carbohydrates (sugars) and proteins (amino acids) called Maillard and Amadori reactions (Wettlaufer and Leopold, 1991; Berma-Lugo and Leopold 1992; El-Keblawy and Al-Ansari, 2000; Murthy and Sun, 2000; Murthy *et al.*, 2003). These reactions occur best at warm temperatures. Changes associated with afterripening are linked to seed aging, implying that these two are part of a continuum, with full afterripening being the peak of seed vigor after which seed deterioration occurs (Foley, 2001).

Dry afterripening is not an abrupt change from dormancy to full germinability, rather seeds become more responsive to a wider range of conditions favoring germination and less responsive to those which do not (Bewley and Black, 1994; Baskin and Baskin, 1998; Foley, 2001). The equilibrium seed moisture content, which is influenced by seed storage temperature and relative humidity, is an important factor in afterripening (Baskin and Baskin, 1979; Leubner-Metzger, 2005; Paterson *et al.,* 1976; Fennimore and Foley, 1998; Steadman *et al.,* 2003). Afterripening can occur at temperatures as low as -20^{o}C (Baskin *et al.*, 2006). In *Asteraceae,* dormancy is overcome by afterripening (Leubner-Metzger, 2003).

Possible mechanisms for dormancy alleviation through afterripening are postulated to act via the capacity of seeds to degrade ABA and synthesize GA during imbibition (Ramagosa *et al.*, 2001). Afterripened barley seeds have increased ABA degradation during imbibition, compared to non-afterripened ones (Jacobsen *et al.*, 2002; Chono *et al.*, 2006). Afterripening involves both a decline in ABA levels and sensitivity (Steinbach *et al.*, 1997; Ramagosa *et al.,* 2001). Response of *Arabidopsis* seeds to dormancy breaking treatments such as cold stratification, nitrate and light were dependent on afterripening (Leubner-Metzger, 2002; Ali-Rachedi *et al.*, 2004; Finch-Savage *et al.*, 2007). Afterripening is not only important in making decisions on

sowing certain seed lots, but is a critical factor in weed management in cropping systems (Foley, 2001).

2.4.3 Coat Imposed Dormancy

The two most common ways by which primary dormancy is conferred are coat-imposed dormancy conferred by restrictive embryo coverings, and embryo dormancy in which dormancy is regulated by the embryo's physiological status (Foley and Fennimore, 1998; Li *et al.,* 1999; Ren and Kermode, 1999; Baskin *et al.*, 2003; Leubner-Metzger, 2003; 2005; Baskin *et al.*, 2006; Chen *et al.*, 2007). Seed coats may influence dormancy through interference with water uptake and gaseous exchange, leaching endogenous inhibitors to embryo, or mechanical restriction of embryo growth (Shull 1913; Borthwick and Robbins 1928; Jones 1974; Koller *et al.*, 1963; Watkins and Cantliffe, 1983; Abeles, 1986; Soltani, 2003; Leubner-Mezger, 2003; Benech-Arnold *et al.,* 1999, 2006; Chen *et al.*, 2007; Bradford *et al.* 2007). In lettuce, presence of endosperm regulated dormancy (Leubner-Metzger, 2003). In *Arabidopsis thaliana,* testa removal or mutational defects in testa structure, led to reduced dormancy (Debeaujon and Koorneef, 2000).

2.5 Overcoming Dormancy

2.5.1 Nitrates

Compounds that donate the nitric oxide (NO) molecule stimulate germination via the nitric oxide pathway (Jovanovic *et al.,* 2005). Cyanide, nitric oxide, sodium nitroprusside, nitrates and nitrites have dormancy breaking properties in seeds of *Arabidopsis* (Alboresi *et al.,* 2005; Bethke *et al.,* 2006a, 2007) and *Zoysia japonica* (Xu *et al.*, 2005), because they donate nitric oxide, a signaling molecule that plays an important role in seed dormancy loss (Bethke *et al.,* 2006a, 2006b). Potassium nitrate is recommended for dormancy alleviation in many species (ISTA, 1985; AOSA, 1998).

2.5.2 Moist Cold and Warm Stratification

Cold (0-10^{o}C) or warm (>15^{o}C) stratification are pre-sowing treatments in which seeds are imbibed for various durations, normally in dark, before they are exposed to requisite germination conditions; stratification requirements vary by species. Cold stratification or moist chilling alleviates dormancy in many species (ISTA, 1985; del Monte and Tarquis, 1997; Pritchard *et al.*, 1999; Yang *et al.*, 1999; AOSA, 1998; Cavieres and Arroyo, 2000; Soltani, 2003). Cold stratification reduces embryo sensitivity to ABA (Jacobsen *et al.*, 2002; Feurtado *et al.*, 2004; Chono *et al.*, 2006) and promotes ABA catabolism (Sondheimer *et al.*, 1968), thereby breaking dormancy. Another mechanism by which cold stratification breaks dormancy is by enhancing embryo sensitivity to GA (Leon *et al.*, 2006, 2007). Moist chilling replaces the light requirement in some species (Noronha *et al.,* 1997; Leon *et al.,* 2006, 2007). In the *Asteraceae* species *Guizotia scabra*, *Parthenium hysterophorus* and *Verbesina encelioides*, cold stratification overcomes dormancy, whereas warm stratification relieves dormancy in *Bidens pilosa* and *Galinsoga parviflora* (Karlson *et al.*, 2008), and in *C. leavenworthii* (Kabat *et al*., 2007).

Twelve weeks of warm stratification followed by 20 weeks cold stratification alleviated dormancy in *Empetrum hermaphroditum* seeds (Baskin *et al.*, 2002). Lettuce seeds imbibed at low temperatures germinate better when transferred to supraoptimal temperatures compared to those initially imbibed at warmer temperatures (Nascimento, 2003).

2.5.3 Seed Priming

Priming is a pre-sowing imbibition treatment that improves seed germination and field establishment through advancing the biological pre-germination processes, but does not permit radicle protrusion or loss of desiccation tolerance (Cantliffe, 1981). Seeds are primed in various liquids (osmopriming or osmoconditioning) or in hydrated inert solid-matrix materials (solid

matrix priming [SMP] or matriconditioning) (Cantliffe, 2003). Various specific terms are used to describe priming, for example, priming seeds in salt solutions is called halopriming. When priming in water, it is called hydropriming or drum priming, using heat/cold, thermopriming, and seed hydration with biological agents, biopriming (Ashraf and Foolad, 2005).

Seed priming can also be used to improve germination under stress conditions such as high soil temperatures which inhibit germination, as for example in lettuce seeds (Guedes and Cantliffe, 1980; Cantliffe *et al.*, 1984; Cantliffe, 1991; Sung *et al.*, 1998; Nascimento and Cantliffe, 1998; Nascimento *et al*, 2000, 2001; Nascimento, 2003).

2.5.4 Other Dormancy Breaking Substances

Chemicals with phytohormone-like action such as N6-benzyladenine (BA) and fusicoccin overcome dormancy in lettuce seeds by promoting embryonic hypocotyl elongation (Abeles, 1986). Recently discovered brassinosteroids relieve seed dormancy (Bishop and Koncz, 2002; Kucera *et al.*, 2005). Indole-3-acetic acid (IAA), a naturally occurring auxin overcomes dormancy in many species (Kepinski and Leyser, 2002; Kucera *et al.,* 2005). Of late, polypeptide hormones were discovered in plants and could be involved in dormancy alleviation (Ryan *et al.,* 2002).

Organic chemicals like ethanol, methanol, butanol, propanol, iso-propanol, pentanol, aldehydes, nitriles and ketones can break dormancy in red rice (Cohn *et al.,* 1989; Cohn, 2002). Seed anatomy influences permeability of alcohols through the pericarp, testa, endosperm and into embryo. However, these chemicals may not directly break dormancy. For example, in red rice, alcohols are converted to carboxylic acids which then overcome dormancy (Taylorson, 1988; Abeles, 1986; Taylorson and Hendricks, 1980; Priestly and Leopold, 1980; Reynolds, 1977, 1987).

Non-organic chemicals like selenium compounds break dormancy in *Stylosanthes humilis* seeds (Pinheiro *et al.*, 2008). Sodium hypochlorite overcomes dormancy in lettuce through endosperm weakening (Drew and Brocklehurst, 1984). Various salts alleviated seed dormancy in *Haloxylon ammodendron* (Tobe *et al.*, 2004), and potassium hydroxide reduced dormancy in *Zoysia japonica* (Xu *et al.*, 2005). Seeds of parasitic species such as *Orobanche Cumana* and *Striga hermonthica* exit dormancy when they get exposed to root exudates from their hosts (Matusova *et al.*, 2004).

CHAPTER 3
ACHENE MORPHOLOGY AND ANATOMY AFFECT DORMANCY IN *COREOPSIS* (*ASTERACEAE*) SPECIES

3.1 Introduction

Seed dormancy impedes propagation and establishment of many non-domesticated native species such as *Coreopsis* (Norcini and Aldrich, 2007a; Kabat *et al.,* 2007). The state of Florida is encouraging planting of native wildflowers in roadside revegetation projects (FDOT, 2008), but germination can be poor due to dormancy (Norcini and Aldrich, 1998; Norcini *et al.*, 2001b, 2004, 2006). In native *C. leavenworthii* and *C. lanceolata*, harvest season of prevariety germplasm influenced the proportion of dormant seeds (Norcini *et al.* 2004, 2006; Kabat *et al.*, 2007). Prevariety germplasm of native *Coreopsis* seeds are in high demand because they are well adapted, low maintenance plants (Norcini and Aldrich, 1998; Kabat *et al.* 2007; Norcini, 2008), with potential to be self-sustaining given proper management (Norcini and Aldrich, 1998; Norcini *et al.*, 2001b; FDOT, 2008), making them highly suitable for roadside revegetation. Increasing seeding rates to compensate for low germination can be expensive because seeds cost between $110 and $220 per kilogram (Norcini and Aldrich, 2008), and supply is limited. Information on dormancy and germination physiology in *C. floridana* and *C. lanceolata* is limited.

In *Coreopsis* species, seed anatomy is not well documented. Information on morphology and anatomy of seeds is important to understanding their biology (Bewley and Black, 1994). Many species have coat-imposed dormancy (Groot and Karsen, 1992; Welbaum *et al.,* 1998; Sung *et al.,* 1998; Debeaujon and Koornneef, 2000; Toorop *et al.,* 2000; Yamaguchi and Kamiya, 2002; Leubner-Metzger *et al.,* 2003; Kucera *et al.,* 2005; Muller *et al.,* 2006). In *Asteraceae*, of which *Coreopsis* belongs, the typical achene or cypsela (seed) develops from an inferior ovary (Esau, 1977), and is comprised of a dicotyledonous embryo enclosed in an

endosperm, testa, and pericarp (Sancho *et al.,* 2006; Puttock, 1994; Borthwick and Robbins, 1928). In angiosperms, pericarp and testa are diploid and of maternal origin, and the embryo and endosperm are derived from both male and female parent gametes but the former is diploid, while the later is triploid (Friedman, 1998; Herr, 1999; Floyd and Friedman, 2001; Friedman and Williams, 2003). In lettuce (*Lactuca sativa*) the embryo is enveloped by a two cell-layer of endosperm which could be three or more cell-layers thick at the micropylar end (Borthwick and Robbins, 1928; Jones, 1974; Psaras *et al.,* 1981; Nijsse *et al.,* 1998).

The selective permeability of endosperm, testa and pericarp controls dormancy and germination by regulating diffusion of solutes (Shull, 1913; Borthwick and Robbins, 1928; Jones, 1974) and oxygen (Bradford *et al.,* 2007) to the embryo. Some seeds have high concentrations of abscisic acid (ABA) in embryo envelopes, and leaching of ABA to the embryo during imbibition maintained dormancy (Chen *et al.,* 2007). Pericarp and testa protect the dispersal unit while endosperm is important to embryo growth during seed development, and also germination in endospermic seeds. In lettuce, the endosperm regulates dormancy by physically restricting embryo growth (Leubner-Metzger, 2003).

Gibberellic acid (GA) is implicated in induction of embryo growth potential and initiation of hydrolases that weaken structures surrounding the embryo (Groot and Karsen, 1992; Welbaum *et al.,* 1998; Toorop *et al.,* 2000; Morohashi and Matsushima, 2000; Yamaguchi and Kamiya, 2002; Leubner-Metzger *et al.,* 2003; Kucera *et al.,* 2005; Muller *et al.,* 2006; Chen *et al.,* 2007). Gibberellins stimulated germination in non-afterripened *C. basalis* seeds (Carpenter and Ostmark, 1992). Removal of seed tissues enveloping the embryo in many species had similar effects as GA (Leon-Kloosterziel *et al.,* 1996; Debeaujon and Koorneef, 2000; Leubner-Mezger, 2003). Light is important in dormancy regulation and is presumed to initiate *de novo*

GA biosynthesis (Hilhorst and Karsen, 1988; Shinomura *et al.,* 1994; Poppe and Schafer, 1997; Milberg *et al.,* 2000; Yamaguchi and Kamiya, 2002; Yamaguchi *et al.,* 2004; Leon and Owen, 2003; Leon and Knapp, 2004). The color or transmittance of the embryo envelopes determined the quality and quantity of light reaching the embryo (Bewley and Black, 1994; Xu *et al.,* 2005). Light and/or GA are important in alleviating coat-imposed dormancy in several species (Watkins and Cantliffe, 1983; Groot and Karsen, 1992; Leon-Kloosterziel *et al.,* 1996; Sung *et al.,* 1998; Debeaujon and Koornneef, 2000; Leubner-Metzger *et al.,* 2003; Kucera *et al.,* 2005; Muller *et al.,* 2006).

Endo-β-mannanase (EBM) activity is associated (through endosperm digestion) with germination of several species including lettuce (Dutta *et al.,* 1997; Nonogaki and Morohashi, 1999) and tomato (Nonogaki and Morohashi, 1996; 1999; Nonogaki *et al.*, 1998; Toorop *et al.,* 2000; Wu *et al.*, 2001; Mo and Bewley, 2003; Downie *et al.,* 1999; 2004). Activity of this enzyme varies in individual tomato seeds in a population, and single-seed assay techniques are appropriate to measure activity levels (Still *et al.*, 1997; Still and Bradford, 1997; Mo and Bewley, 2003). Lettuce seed germination at supraoptimal temperatures in some cultivars is linked to greater EBM activity (Abeles, 1986; Dutta *et al.,* 1997; Nascimento *et al.,* 2000; 2001). Thermoinhibition in imbibed lettuce (Yoshioka *et al.,* 1998; Gonai *et al.*, 2004) and *Arabidopsis thaliana* (Tamura *et al.*, 2006; Toh *et al.*, 2008) seeds is associated with endosperm resistance to rupture and elevated *de novo* ABA biosynthesis. Abscisic acid biosynthesis inhibitors permitted germination at elevated temperatures in lettuce (Gonai *et al.*, 2004) and *A. thaliana* (Yoshioka *et al.,* 1998; Tamura *et al.*, 2006; Toh *et al.*, 2008) seeds. There is strong evidence of an association between EBM hydrolase activity in endosperm and dormancy termination.

Dormancy in *Coreopsis* continues to present a challenge to revegetation efforts, and dormancy regulation needs to be understood in order to develop techniques to alleviate it. The overall objectives were to 1) identify seed tissues (components), 2) test whether these tissues could be excised to diminish or terminate dormancy, and 3) establish whether EBM activity was involved in release from dormancy. Morphology and anatomy of *C. floridana* and *C. lanceolata* seeds were examined, and their potential roles in dormancy investigated in light or dark. Various tissues surrounding the embryo were excised, and seeds or embryos were imbibed at various temperatures in light or dark. The EBM activity in endosperms of imbibed seeds was also quantified to determine if this enzyme might be linked to dormancy. To address this, a single-seed assay technique was used to measure enzyme activity in *C. floridana* and *C. lanceolata* seeds, and to ascertain effects of ABA (putative dormancy enhancer), tetcyclacis (GA biosynthesis inhibitor) and high temperature (30^{o}C) on enzyme activity.

3.2 Materials and Methods

3.2.1 Plant Material

3.2.1.1 Anatomy and accompanying germination studies

Seeds of *C. floridana* E.B. Smith (Ex. Polk County, FL) and *C. lanceolata* L. (composite population, Ex. Leon, Wakulla, Gadsden and Jefferson Counties, FL) were harvested in 2005 from cultivated populations in Florida (Norcini and Aldrich, 2007a).

3.2.1.2 Endo-β-mannanase analysis and related germination studies

Coreopsis floridana and *C. lanceolata* seed lots used in anatomical studies (above) were sown to raise seedlings in a growth chamber in mid August 2006. These were transplanted at the end of October 2006 into 11 liter pots containing pine bark and drip fertigated in a high roof, plastic, passively ventilated greenhouse under natural photoperiods. Fertigation was set to supply 2.5 liters/plant/day with a nutrient solution containing (ppm): nitrogen (140), phosphorus

(50), potassium (200), calcium (120), magnesium (50), sulfur (65), iron (2.8), boron (0.7), copper (0.2), manganese (0.8), zinc (0.2) and molybdenum (0.05). The EC of the solution was 1.8 dSm^{-1} and pH was 5.5. Bumble bees (*Bombus impatiens*; Kopert Biological Systems, Romulus, MI) were used to ensure cross pollination since *Coreopsis* is self-incompatible (Ferrer and Good-Avila, 2007). Seeds of *C. lanceolata* were collected in May 2007 and those of *C. floridana* in November 2007. Seeds were bulked over 4 weeks in each species to provide sufficient quantities. *Coreopsis floridana* seeds were dried to 8.7% seed moisture content and *C. lanceolata* 8.1%, sealed in moisture-proof plastic bags, and stored at 10°C and 50% relative humidity in dark.

3.2.2 Microscopy

3.2.2.1 External seed morphology

A LEICA MZFLIII Stereomicroscope (Leica Microsystems Inc., Bannockburn, IL) and a Kodak MDS290 camera (Eastman Kodak Co., Rochester, NY) were used to record seed images.

3.2.2.2 Scanning electron microscope (SEM)

Seed anatomy and morphology were studied using SEM. Seeds were examined whole, or cut longitudinally or transversely to view internal anatomy. Specimens from the two *Coreopsis* species were sputter coated with platinum and examined using a HITACHI S-4000 FE-Scanning Electron Microscope (Hitachi High Technologies America Inc., Pleasanton, CA).

3.2.2.3 Light microscopy

Individual seeds of each species were bisected transversely or longitudinally and fixed overnight in a solution of 500 mM phosphate buffer (pH 7.0), 20 mM EDTA, 20 mM $MgSO_4$, 0.5% paraformaldehyde and 1.5% (v:v) glutaraldehyde. A light vacuum was applied until the seeds sunk to the bottom of the tubes. Samples were washed twice in phosphate buffered saline (PBS) to remove the fixative. The specimens were post-fixed overnight in 2% OsO_4 (osmium) in

100 mM phosphate buffer (pH 7). They were washed twice in PBS to remove the osmium, dehydrated in a graded ethanol series starting from 10% to 100%. The ethanol was replaced with increasing concentrations of Spurrs resin (Electron Microscopy Sciences, Ft. Washington, PA). To speed up infiltration of the resin, samples were heated for 3 minutes in a bio-microwave (PELCO BioWave 34700 + Coldspot, Ted Pella Inc., Redding, CA) at a power setting of 250 W, vacuum pressure 575 mm Hg, and a temperature of 37^{o}C in each of the Spurrs series from 10% to 90%. At 100% Spurrs, the microwave heating duration was increased to 4.5 minutes. The seed specimens were positioned lengthwise or cross-sectional in molds with 100% Spurrs resin and incubated at 65^{o}C until polymerization was complete.

Sections 1.0 μm thick were made using a Leica Reichert Ultracut R microtome (Leica AG, Reichert Division, Wien, Austria). Sections were mounted on glass slides and stained with 1% toluidine blue (T-blue) for 5 minutes on a warm hot-plate, and excess dye rinsed away using deionized water. The slides were mounted using Permount SP15-100, (Fisher Scientific, Atlanta, GA) and cover glasses. The stained sections were observed using a Zeiss Axioskop microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY).

3.2.2.4 Determination of seed tissue ploidy

Ploidy levels were determined to verify the identity of seed tissues. Seeds were fixed and embedded similarly to that for light microscopy. Sections (5 μm) were cut and stained with 0.1 μg/ml 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, St. Louis, MO) to stain deoxyribonucleic acid (DNA). Methylene blue (Polysciences Inc., Warrinton, PA) was used to quench auto-fluorescence from cell walls. Using a Zeiss Axioskop microscope, DNA fluorescence was quantified with a DAPI filter (excitation: 350/50 nm; emission LD 420). DAPI stained DNA fluorescence techniques were used to quantify relative ploidy levels in seed tissue nuclei (Williams and Friedman 2002; Friedman 1992b). Fluorescence measurements were made

in white pixels on 10 endosperm and 10 embryo nuclei per image (section). Twenty seed sections were evaluated for each species (*C. floridana* or *C. lanceolata*). Background fluorescence was quantified and adjustments made on the nuclei fluorescence readings. Mean fluorescence was calculated for each tissue type to obtain the ratio of average endosperm to embryo measurements.

3.2.3 Image Capture and Editing

Digital images were recorded with a Kodak MDS290 camera and stored in Tagged Image File Format (TIFF). Images were edited (adjusting color) and labeled using Adobe Photoshop version 7.0 (Adobe Systems Inc., San Jose, CA).

3.2.4 Germination Tests

Constant temperatures of 20 and 30^{o}C were provided in an Isotemp incubator model 304R (Fisher Scientific, Fair Lawn, NJ) in continuous cool white fluorescent light (~25 μmolm^{-2}s^{-1}) or dark. Darkness was achieved by covering Petri dishes with aluminum foil. Four replications of 25 seeds were placed on double blue blotter paper (Anchor Paper Company, St. Paul, MN) moistened with de-ionized water in 5 cm glass Petri dishes. Germination, defined as visible radicle protrusion of at least 2 mm, was recorded every 2 days up to 14 days. Interim germination counts for dark incubated seeds were made under a dim-green light (25W, A19, Specialty 90912, General Electric Company, Cleveland, OH) in a dark room.

3.2.5 Influence of Seed Envelopes on Germination

Experiments with *C. floridana* and *C. lanceolata* seeds harvested in 2005 were conducted in spring/summer 2006 (effects of embryo covers at 20^{o}C) and spring/summer 2007 (effects of embryo covers at various temperatures). Pericarp plus testa and endosperm of *Coreopsis floridana* and *C. lanceolata* seeds were removed under a dissecting microscope (Model, ASZ37L3, Bausch and Lomb, Rochester, NY). Ten to twenty embryos per replicate and three or

four replicates per treatment were tested for germination. Germination tests were conducted on a thermo-gradient table, Type db 5000 (Van dok and de Boer Machinefabriek BV, Enkhuizen, The Netherlands) at 10, 20 or 30^{o}C in continuous dark or light with intact seeds (S), intact seeds minus the pericarp and testa (S-P), and naked embryos (E). Germination test duration was 14 days. Intact seeds were used as controls.

3.2.6 Statistical Analysis

Digital images recorded in fluorescence microscopy were analyzed using NIH Image Version 1.61/ppc software (National Institute of Health, Bethesda, MD). Germination data for each species were analyzed separately. Data were adjusted for viability as determined by a TZ test (ISTA 2003), arcsine-square root transformed, if necessary, and analyzed by general linear model methods (SAS Version 9.1; SAS Institute, Cary, NC). Pearson product-moment correlation coefficients (r) were also computed in SAS Version 9.1. Treatment means were separated by least significant difference (LSD; α=0.05).

3.2.7 Endo-β-Mannanase Activity in *Coreopsis* Endosperms

Studies on EBM activity and germination were conducted after 6 months storage for *C. floridana* and 12 months for *C. lanceolata* seeds. Endo-β-mannanase activity during imbibition of *C. floridana* and C. *lanceolata* seeds was detected using a gel-diffusion assay (Bonina, 2005; Nascimento *et al.*, 2001; Downie *et al.*, 1994; Still *et al.,* 1997). Seeds were incubated at 20^{o}C in light as described for germination tests.

To prepare gel plates, 0.05% (w/v) galactomannan (Locust Bean Gum, Sigma Chemical Co., St. Louis, MO) was dissolved in incubation buffer (0.1 M citric acid, 0.2M sodium phosphate, pH 5.0) by stirring and heating for 30 minutes. The solution was then clarified by centrifugation at 15,000 g for 15 minutes at 4°C. Agar (Plant TC/Micropropagation grade, PhytoTechnology Laboratories, Shawnee Mission, KS) was dissolved at 0.7% (w/v) in the

clarified solution and stirred while heating to the boiling point. Thirty ml of solution was dispensed into 150x25 mm plastic Petri dishes (Falcon, Franklin Lakes, NJ). After solidification, 2-mm wells were made using a disposable plastic pipette, and excised gel removed by aspiration.

Endosperms were separated from the embryos under a dissecting microscope as described previously. In some experiments, endosperms were divided into lateral and micropylar sections. Each endosperm (whole, micropylar tip or lateral) was placed into an individual microtiter plate (Nalge Nunc, Naperville, IL) well containing 20 µL of sterile incubation buffer (0.1 M citric acid, 0.2 M sodium phosphate, pH 5.0) and incubated in the dark for 2 hours at 25°C. After incubation, 10 µL of buffer from each microtiter well was transferred to gel-diffusion plate wells. Endo-β-mannanase (Megazyme International Ireland Ltd., Wicklow, Ireland) was diluted to make a standard (control) EBM solution of 1 U (1 U = enzyme activity converting 1 µmol of galactomannan per minute under standard conditions). The standard (10 µL) was transferred into wells in the gel-diffusion plates alongside endosperm extracts. Petri dishes were sealed with Parafilm® "M" (Pechiney Plastic Packaging, Chicago, IL), covered with aluminum foil, and incubated at 25°C for 24 hours.

Gels were stained with 10 mL Congo Red (Sigma Chemical Co., St. Louis, MO) in distilled water (0.4% w/v) added to each plate. Plates were agitated on an orbital shaker for 20 minutes at 60 rpm during staining. The stain solution was decanted and the gel was gently rinsed with distilled water for 1 min after which 10 mL of citrate-phosphate buffer pH 7.0 was added and plates shaken again for 3 minutes on the orbital shaker at 60 rpm. The buffer was decanted and plates were scanned within 5-10 minutes using a Scan Jet 3c/T (Hewlett Packard, Palto Alto, CA). Scanned images were captured using WinRhizo™ (Regent Instruments Inc., Quebec, Canada) software. The diameter of the cleared areas on the plates was proportional to EBM

activity. Germination tests (as described earlier) were conducted and counts recorded daily up to 14 days.

3.2.8 Influence of ABA, Tetcyclacis and Supraoptimal Temperature (30°C) on Germination and EBM activity

Seeds were incubated at 20 or 30°C with 100 μM ABA or 100 μM tetcyclacis (a GA biosynthesis inhibitor) in light, as described in germination tests above. Light conditions were chosen because *C. floridana* and *C. lanceolata* seeds had highest germination in in light. Ten endosperms were excised as described previously after incubating seeds for 24 hours on each substrate, and endosperms were incubated for an additional 120 hours on respective media. Endosperms from control intact seeds were excised after 144 hours. In another control (incubated in de-ionized water), endosperms were excised after 24 hours but re-incubated in de-ionized water for a further 120 hours. Enzyme activity was evaluated using procedures described above. Accompanying germination tests were conducted on respective substrate and temperature combinations.

3.3 Results and Discussion

Seeds of *C. floridana* and *C. lanceolata* were dark brown to black with pale brown or beige wings (Figure 3-1). Only seeds of *C. lanceolata* had cavernous callus tissue (knob-like structures) at both longitudinal ends. Additionally, seeds of both species had hollow spikes on the pericarp (Rose, 1889; Tadesse *et al.,* 1995). Wings are a possible adaptation for dispersal in wind, whereas callus and spikes could facilitate water floatation during dispersal. Light microscopy and SEM images of *Coreopsis* seeds showed an outer woody pericarp (fruit wall), a testa (seed coat) and a single cell-layered endosperm that enveloped a dicotyledonous embryo (Figures 3-2 and 3-3). The inner part of the woody pericarp was composed of polygonal parenchyma cells. Measurements of imbibition rate and equilibrium moisture content

demonstrated that embryo envelops in both species were freely permeable to water. In *C. lanceolata* the radicle (micropylar) end of the endosperm consisted of one to three-cell layers, while in *C. floridana* the micropylar endosperm remained a single cell-layer throughout (Figure 3-3). In lettuce seeds the endosperm is composed of two cell layers but the micropylar end could have more than two cell-layers (Borthwick and Robbins, 1928; Psaras *et al.,* 1981; Bewley and Black, 1994; Nijsse *et al.,* 1998). Muller *et al.* (2006) reported a single cell-layer endosperm in *Lepidium sativum* and *Arabidopsis thaliana*; however, they found that in both species the micropylar end had one to two-cell layers.

The embryo had distinctive smaller epidermal cells and larger inner cells (Figure 3-2) resembling lettuce seeds (Borthwick and Robbins, 1928). Placement of the dicotyledonous spatulate embryo in both species was symmetrical and longitudinal. Seed morphology and anatomy of the two *Coreopsis* species was similar and typical of *Asteraceae* (Esau, 1977; Borthwick and Robbins, 1928; Psaras *et al.,* 1981; Nijsse *et al.,* 1998).

The mean ratio (± standard error) of endosperm to embryo DNA fluorescence (Figure 3-4) for *C. floridana* was 1.54 ± 0.03 and 1.52 ± 0.02 for *C. lanceolata*. The 3:2 DNA ratio indicated that the single-cell layer tissue enclosing the embryo was endosperm. Triploid endosperms and diploid embryos have been reported in many angiosperm seeds (Friedman and Williams, 2003; Williams and Friedman, 2002; Carmichael and Friedman, 1996, 1995; Friedman 1998, 1992a, 1992b, 1990a, 1990b; Floyd and Friedman, 2001, 2000; Floyd *et al.,* 1999).

In light at 20^{o}C, germination of *C. floridana* naked embryos, seeds without pericarp and testa, and intact seeds was 100%; however, in dark germination declined to 77% in naked embryos, 43% in seeds without pericarp and testa, and 15% in intact seeds (Figure 3-5). In *C. lanceolata,* germination was nearly 100% when naked embryos, seeds without pericarp and

intact seeds were incubated in light or dark. However, compared to intact seeds of *C. lanceolata* germination was rapid in naked embryos and seeds without pericarp and testa (Figure 3-5), indicating some level of dormancy imposition by the pericarp (Bewley and Black, 1994).

Tissues enveloping the embryo in both species imposed dormancy. In dark-imbibed *C. floridana* seeds, removal of pericarp plus testa and endosperm nearly alleviated dormancy. In light-requiring lettuce cultivars, removal of endosperm relieved dormancy in dark (Leubner, 2003; Gonai *et al.,* 2004). Debeaujon and Koornneef (2000) reported that testa-structural mutants of *Arabidopsis thaliana* had varying dormancy levels as dictated by testa integrity. Intact seeds of *C. floridana* had an obligate light requirement but not *C. lanceolata* (Milberg *et al.,* 2000; Yamaguchi *et al.,* 2004; Leon and Owen, 2003; Leon and Knapp, 2004).

In light, excised embryos of *C. floridana* germinated 100% in 4 days at 30^{o}C while at 20^{o}C it took 8 days, and more than 14 days at 10^{o}C. Naked embryos of *C. lanceolata* germinated 100% at 20^{o}C and 30^{o}C in about 4 days but 10 days at 10^{o}C. Greater germination rates by naked embryos indicated that pericarp, testa and endosperm had some restriction on germination of *C. floridana* and *C. lanceolata* seeds. In both species, intact seeds and those without pericarp did not attain 100% germination at 30^{o}C, because this was near the upper limit for germination. Pericarp restricts germination in thermoinhibited *Tagetes minuta* achenes (Taylor *et al.*, 2005). Lettuce (Cantliffe *et al.,* 1984; Yoshioka *et al.,* 1998; Nascimento *et al.,* 2000, 2001; Gonai *et al.,* 2004) and; *Arabidopsis* (Toh *et al.,* 2008) seeds have reduced germination at supraoptimal temperatures. When pericarp, testa and endosperm were removed, both *C. floridana* and *C. lanceolata* seeds germinated normally at elevated temperatures.

Endo-β-mannanase activity was assayed in imbibed *C. floridana* and *C. lanceolata* endosperms to determine if enzyme activity was linked to dormancy. *Coreopsis lanceolata*

seeds exhibited EBM activity from 90 hours of imbibition onwards; visible radicle protrusion was observed after 96 hours. Non-germinated *C. lanceolata* seeds had non-detectable EBM activity. *Coreopsis floridana* seeds did not exhibit EBM activity even in the post germination phase. *Coreopsis floridana* seeds did not exhibit EBM activity possibly because of the absence of galactomannans in the endosperm, which implies that a different enzyme system could be involved in endosperm digestion. In lettuce seeds, hydrolytic enzymes other than mannanase might be involved in endosperm weakening (Dutta *et al.*, 1994); cellulolytic, hemicellulolytic or pectolytic enzymes promoted germination in dormant dark-imbibed lettuce seeds (Ikuma and Thimann, 1963). Endosperm composition of both species is not known and no literature was available detailing the identity of deposits in these endosperms. Galactomannans constitute the bulk of deposits in lettuce endosperms (Dutta *et al.,* 1997; Nonogaki and Morohashi, 1999) and enzyme activity has been used to detect germination patterns in that species (Abeles, 1986; Dutta *et al.,* 1997; Nascimento *et al.,* 2000, 2001; Bonina, 2005). Endo-β-mannanase activity has also been reported in endosperms of germinating tomato seeds (Nonogaki and Morohashi, 1996; Nonogaki *et al.*, 1998; Mo and Bewley, 2003).

Endo-β-mannanase activity levels were significantly correlated with *C. lanceolata* seed germination ($r= 0.88$, $p = 0.0199$), incubation duration ($r=0.88$, $p = 0.0196$), and proportion of seeds with enzyme activity ($r=0.93$, $p = 0.0077$). Germination percentage was well correlated with proportion of seeds expressing EBM activity ($r=0.97$, $p = 0.0012$), and incubation duration ($r=0.99$, $p = 0.0001$). The proportion of seeds exhibiting EBM activity was highly correlated with incubation duration ($r = 0.99$, $p = 0.0002$) (Figure 3-7). Endo-β-mannanase activity increased as germination time progressed, together with the proportion of seeds expressing activity of this enzyme. The percentage of seeds exhibiting EBM activity reached 100% before

maximum germination was attained, implying that enzyme activity preceded germination (Figure 3-7). In seeds of lettuce (Abeles, 1986; Dutta *et al.,* 1997; Nascimento *et al.,* 2000, 2001; Bonina, 2005, 2007), tomato (Nonogaki and Morohashi, 1996, 1999; Still and Bradford, 1997; Nonogaki *et al.,* 1998), and in this case *C. lanceolata*, EBM activity is associated with germination.

Conditions unfavorable for germination inhibited EBM activity in *C. lanceolata* seeds (Halmer *et al.,* 1976; Toorop *et al.*, 2000). Germination percentage and EBM activity levels were highly correlated (0.87, $p = 0.0001$). When seeds were imbibed in ABA at optimal (20^oC) temperatures, germination percentage was 28% after 14 days, and only germinated seeds expressed normal levels of EBM activity (~0.45 U) while EBM activity levels were low (>0.10 U) in ungerminated seeds (Figure 3-8). At 30^oC (control) germination was 9% and non-germinated seeds exhibited low levels of enzyme activity. Seeds incubated in a combination of ABA and 30^oC had 4% germination and no enzyme activity was detected from ungerminated seeds (Figure 3-8). The correlation coefficient between the proportion of endosperms expressing EBM activity and enzyme activity levels was 0.98 ($p=0.0025$) (Figure 3-9). Failure of some *C. lanceolata* seeds to germinate on ABA substrate or at 30^oC was linked to low or no EBM activity. In lettuce, thermo-tolerant cultivars had greater EBM activity during germination at 35^oC compared to thermo-sensitive ones (Abeles, 1986; Dutta *et al.,* 1997; Nascimento *et al.,* 2000, 2001, 2005; Bonina, 2005, 2007). Thermoinhibition in lettuce was circumvented by removal of the endosperm (Cantliffe *et al.*, 1984; Sung *et al.*, 1998; Nascimento *et al*, 200, 2001, 2005). In *Arabidopsis thaliana* seeds, thermoinhibition was associated with endosperm resistance to rupture and elevated *de novo* ABA biosynthesis in embryos (Tamura *et al.*, 2006; Toh *et al.*, 2008), and fluridone (ABA biosynthesis inhibitor) relieved thermoinhibition

(Yoshioka *et al.,* 1998; Gonai *et al.,* 2004). In seeds such as those of *Datura ferox*, conditions that maintained dormancy inhibited EBM activity (Bewley, 1997). The mechanism of thermoinhibition at 30^{o}C and ABA-suppressed germination in *C. lanceolata* seeds was associated with low EBM activity in endosperms.

Seeds incubated in 100 μM tetcyclacis (GA biosynthesis inhibitor) failed to germinate and endosperms had no detectable EBM activity (data not shown). Gibberellin-deficient tomato mutants do not germinate or exhibit EBM activity without exogenous GA application (Mo and Bewley, 2003). When seeds were imbibed on de-ionized water for 24 hours and endosperm separated from embryo, EBM activity was detected in excised endosperms incubated for a further 120 hours on same substrate (Figure 3-9, End. Ctrl). In tomato seeds, EBM activity occurred only if endosperm was separated from embryo after at least 6 hours of initial imbibition, indicating that a signal was required from embryo (Mo and Bewley, 2003). Moreover in *Arabidopsis thaliana* and *Lepidium sativum* endosperm weakening processes, GA could substitute for this embryo signal (Muller *et al.*, 2006). Gibberellic acid induced enzymatic endosperm weakening in lettuce (Halmer *et al.*, 1976) and tomato (Groot *et al.*, 1988) seeds. It seems *de novo* GA biosynthesis may be required to induce EBM activity and germination in *C. lanceolata* seeds.

3.4 Conclusions

Coreopsis floridana and *C. lanceolata* seeds have an outer pericarp, a testa, endosperm and dicotyledonous embryo, and this is the first study to report the anatomy of *Coreopsis* seeds, paving way for further seed physiology research of this species. The pericarp, testa and endosperm are involved in imposing dormancy in *C. floridana* and *C. lanceolata*. Correlative and diagnostic evidence suggests that EBM activity is a prerequisite for germination in *C. lanceolata* seeds, but not in *C. floridana*. In *C. floridana*, a different enzyme system could be

involved in endosperm digestion during germination, but this was not explored further. This EBM activity in *C. lanceolata* seeds is reported here for the first time and may provide an answer to circumventing dormancy in non-afterripened *C. lanceolata* seeds. Seed enhancement technologies aimed at breaking dormancy and/or circumventing thermoinhibition for germination improvement and field uniformity should focus on techniques that weaken pericarp, testa and endosperm.

3.5 Summary

Morphology and anatomy of *C. floridana* and *C. lanceolata* achenes (seeds) were studied in order to ascertain possible tissue functions in dormancy modulation. Seeds were examined using light microscopy, scanning electron microscopy and fluorescence microscopy. Seeds of both species had a woody pericarp, testa and an endosperm surrounding a dicotyledonous embryo. The lateral endosperm in *C. lanceolata* was a single cell-layer but was one to three cell-layers thick at the micropylar end, while in *C. floridana* there was a single cell-layer throughout. Florescence microscopy confirmed endosperm ploidy was 1.5 times that of embryo, concurring with double fertilization outcomes in angiosperms. *Coreopsis floridana* seeds were dormant in dark; however, *C. lanceolata* seeds germinated in light and dark, but are normally dormant unless dry afterripened at 10^{o}C for 150 days. Dormancy in dark-imbibed *C. floridana* seeds at 20^{o}C was nearly alleviated by removal of pericarp, testa and endosperm; removal of the same tissues in light-imbibed seeds entirely overcame inhibition in both species at temperatures as high as 30^{o}C. In other *Asteraceae* lack of endo-β-mannanase (EBM) activity restricts germination. A single-seed assay technique was used to evaluate EBM activity in *C. floridana* and *C. lanceolata*. *Coreopsis floridana* seeds did not exhibit EBM activity, but in *C. lanceolata,* EBM activity occurred (at 90 hours) before radicle protrusion. There was a significant correlation (r = 0.97) between proportion of seeds exhibiting EBM activity and endosperm

rupture. Inhibited radicle protrusion in 100 μM abscisic acid substrate and/or at 30°C was associated (r = 0.87) with reduced EBM activity. Tetcyclacis (100 μM) prevented EBM activity and induced dormancy. Thus, dormancy in *C. floridana* is overcome by imbibing seeds in light while uniform and rapid germination of *C. lanceolata* is consistent with endosperm weakening. Potentially, this mechanism may be important to circumventing dormancy in non-afterripened seeds of *C. lanceolata*.

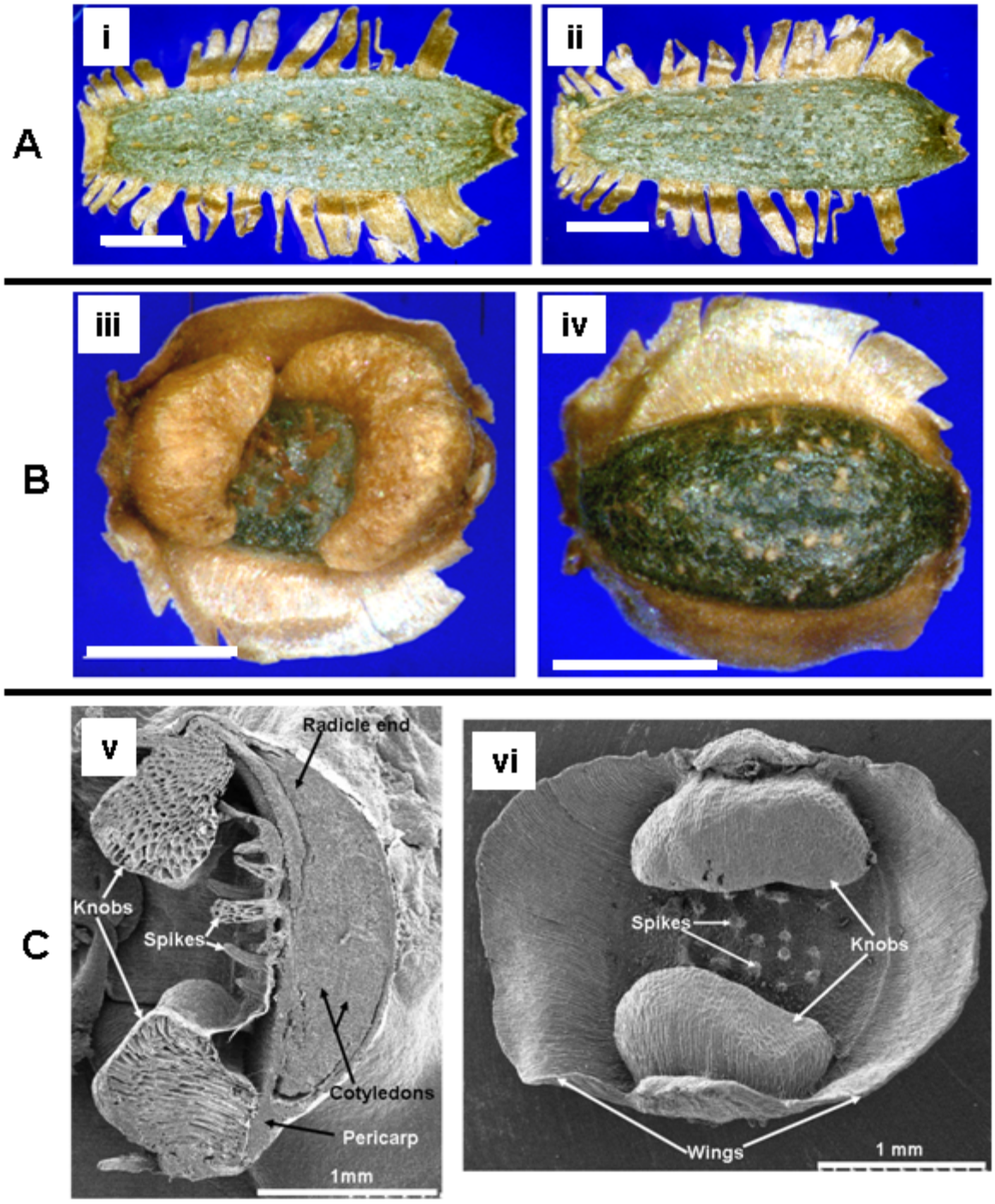


Figure 3-1. External appearance of *Coreopsis* seeds. A) *C. floridana* convex (i) and concave (ii) side. B) *C. lanceolata* convex (iii) and concave (iv) side. Bar scale = 1 mm. C) SEM longitudinal section of *C. lanceolata* seed (v), and whole seed (vi).

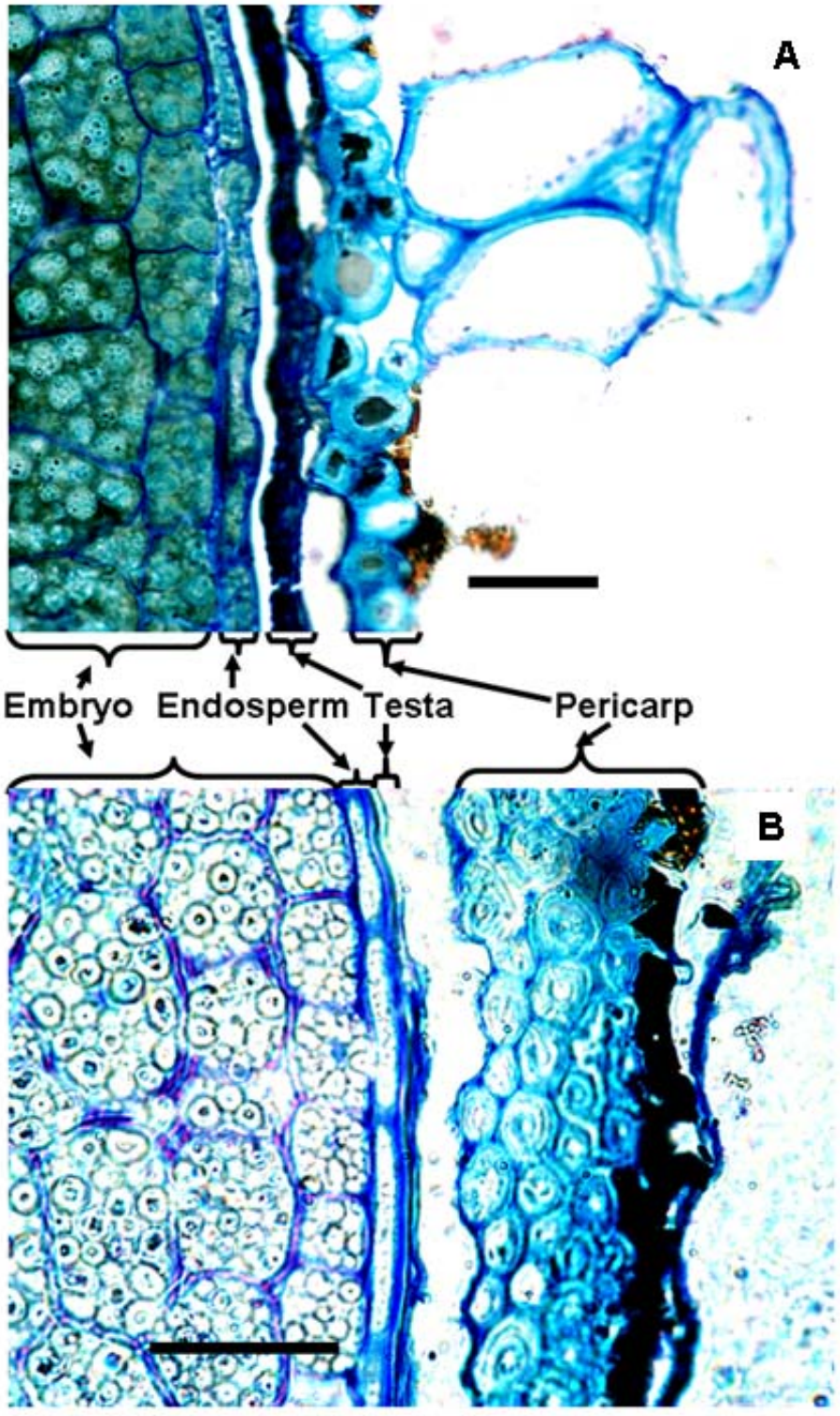


Figure 3-2. Light microscopy image of internal seed anatomy A) *C. floridana.* B) *C. lanceolata*. Bar scale = 25 μm.

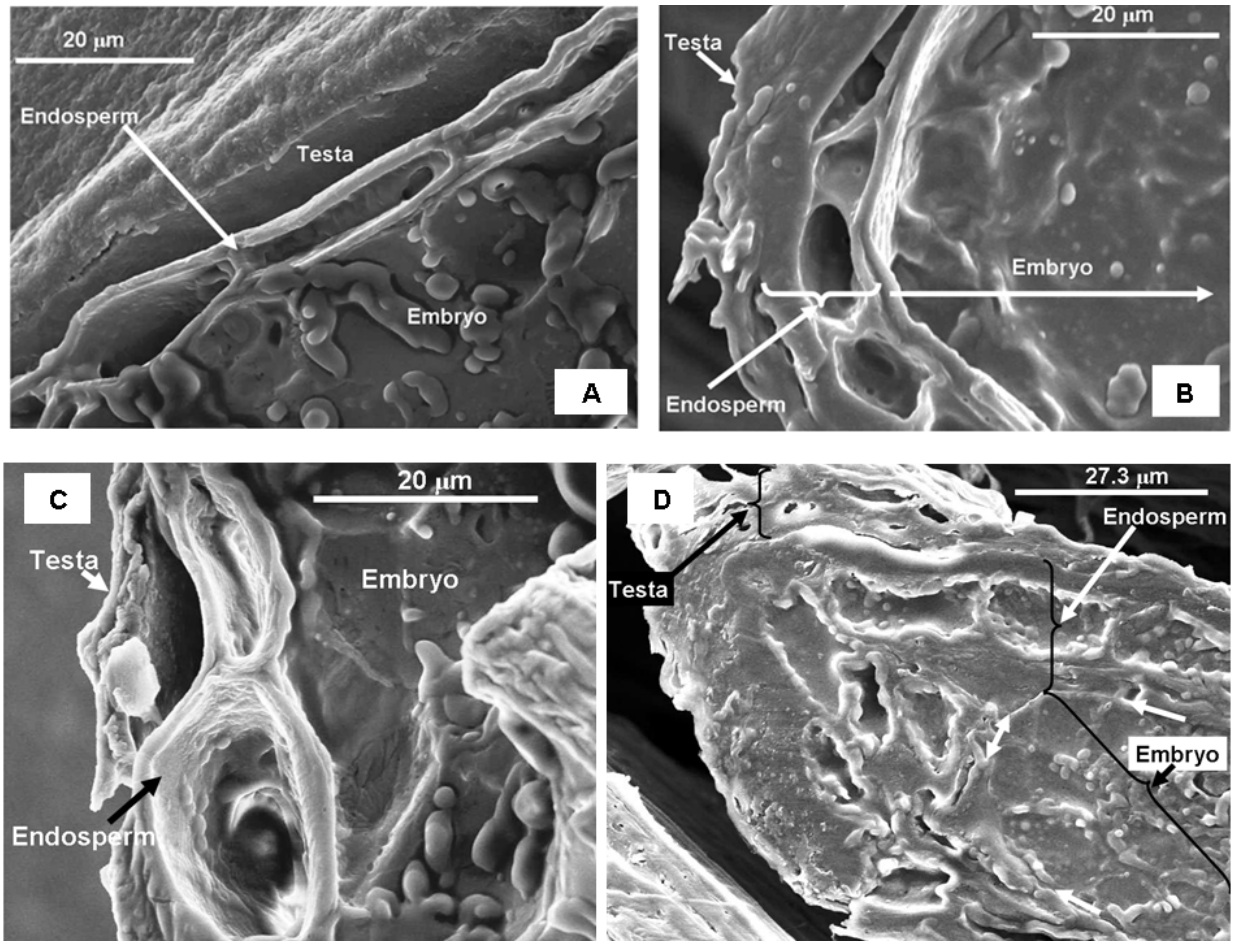


Figure 3-3. Lateral and micropylar endosperm of *Coreopsis* seeds A) *C. floridana* lateral endosperm is a single cell-layer. B) *C. lanceolata* lateral endosperm is also a single cell-layer. C) Micropylar endosperm of *C. floridana* seeds are single cell-layered. D) *C. lanceolata* seeds have micropylar endosperm with one or more cell-layers. Short white arrows on *C. lanceolata* image indicate the radicle/endosperm interface.

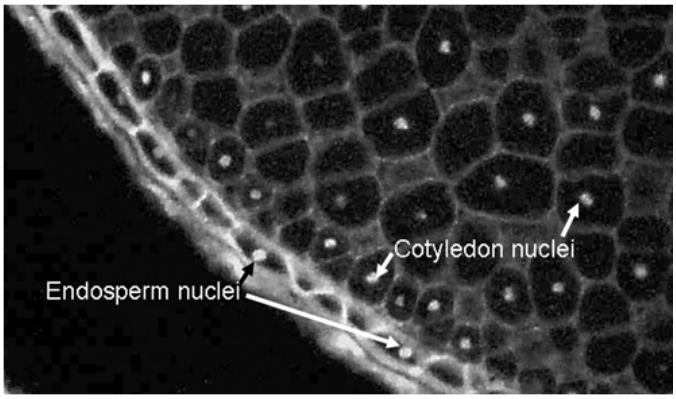


Figure 3-4. Photograph of the cotyledons (embryo) and the single cell-layer endosperm tissue showing DAPI stained fluorescent nuclei in *C. lanceolata* seeds.

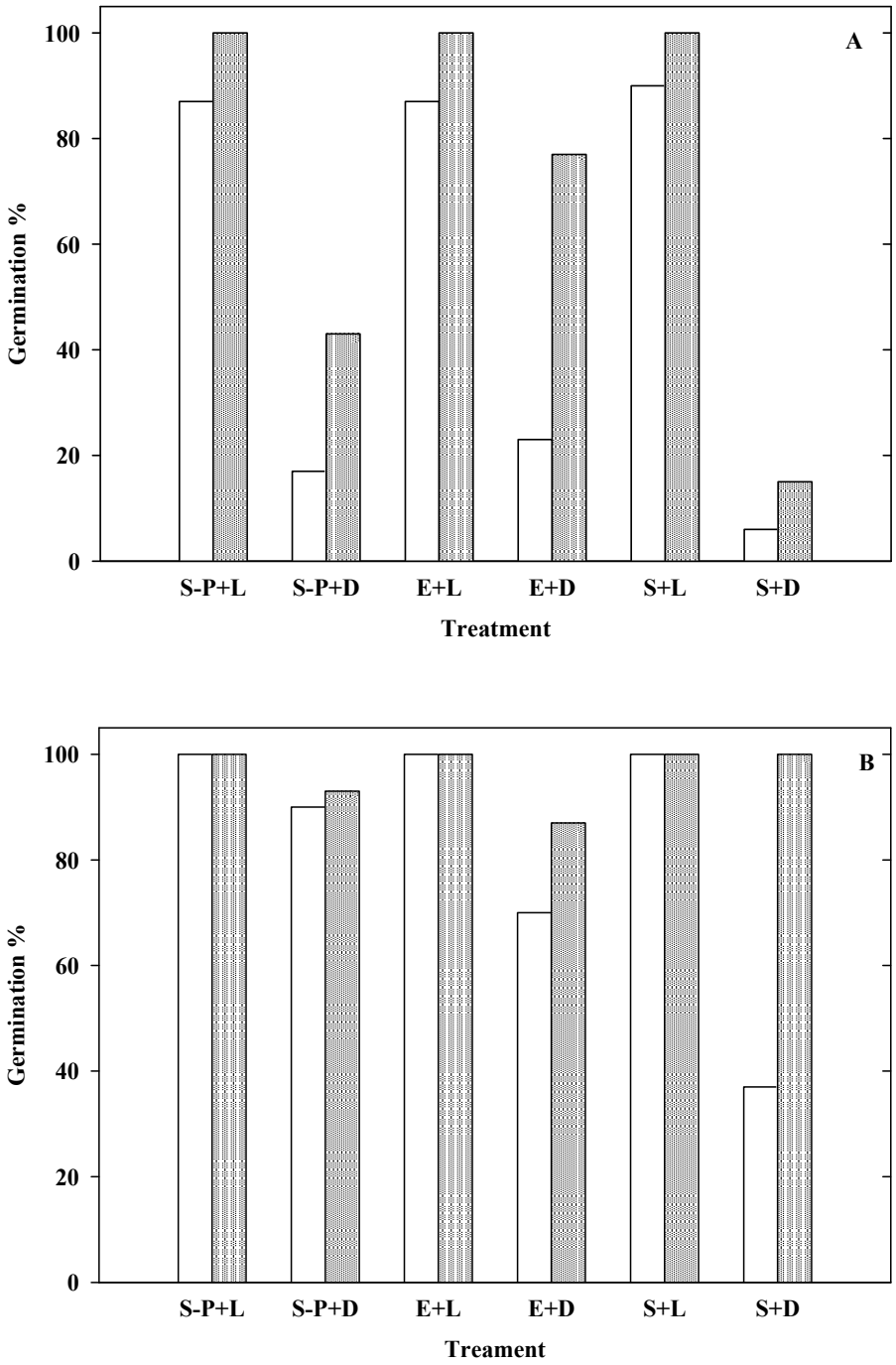


Figure 3-5. The influence of removal of pericarp and/or endosperm on germination in light or dark A) *C. floridana* seeds, at 7 days (white bars, LSD=18) and at 14 days (stipuled bars, LSD=11). B) *Coreopsis lanceolata* seeds at 7 days (white bars, LSD=33) and at 14 days (stipuled bars, LSD=16) in light or dark at 20^{o}C. S, Intact seed; P, pericarp; E, naked embryo; L, light and D, dark. Experiments were conducted in spring/summer 2006.

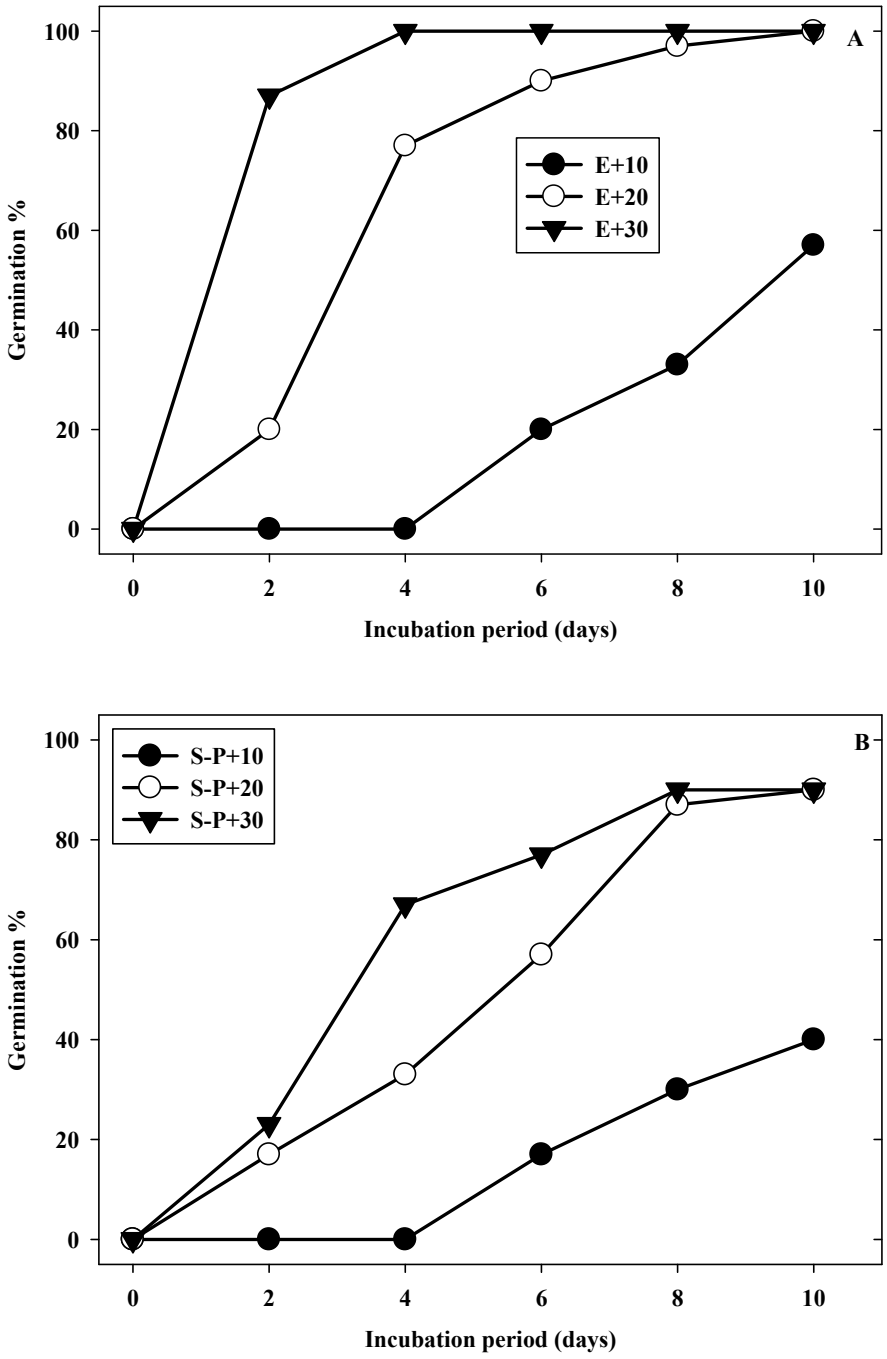
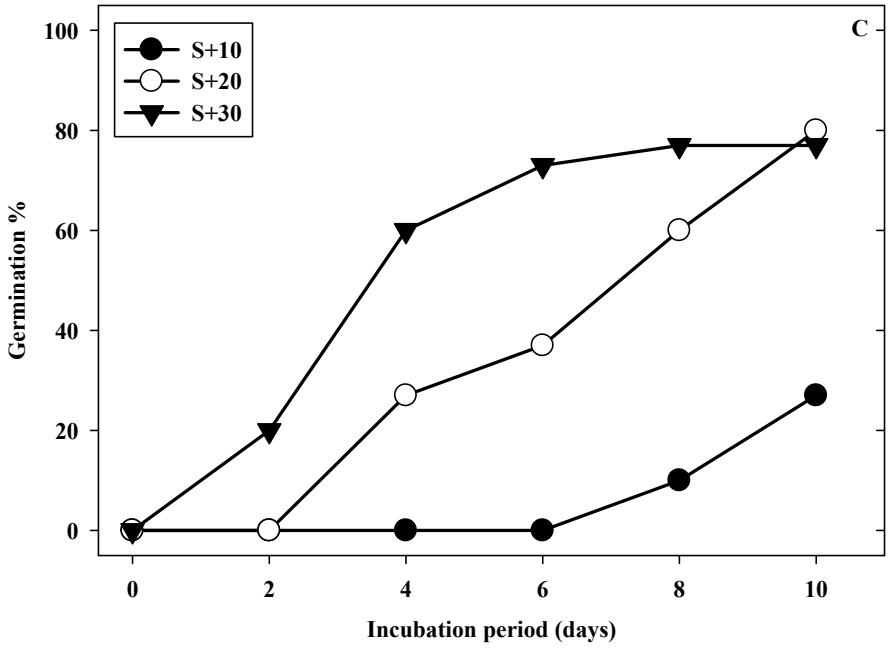
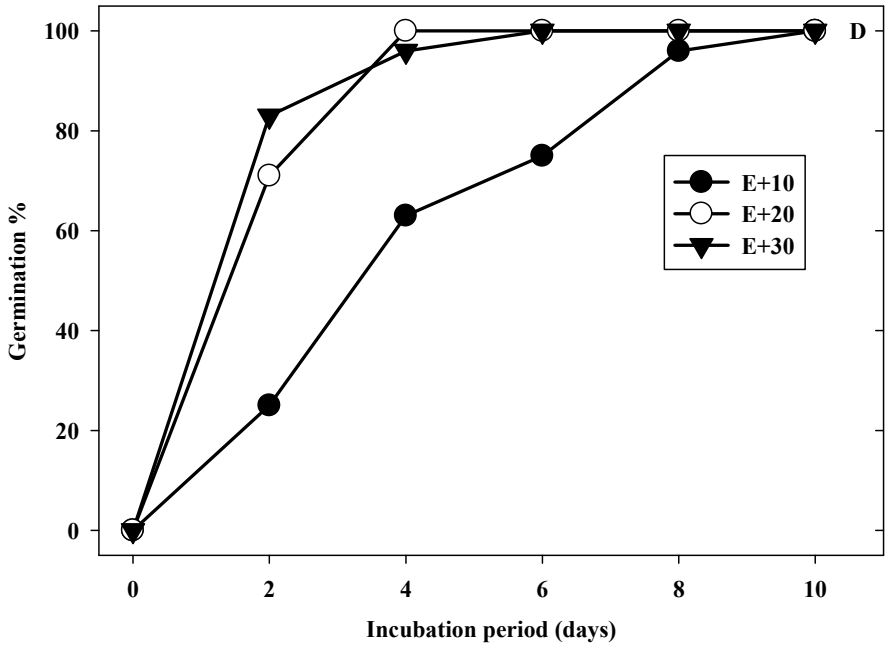


Figure 3-6. The influence of the removal of pericarp and testa or endosperm on germination in light at 10, 20 or 30°C. A), B) and C) *C. floridana*. D), E), and F) *C. lanceolata*. Seeds. LSDs = 13, 19, 16, 21 and 21 at 2, 4, 6, 8 and 10 days respectively for *C. floridana* (across all embryo envelope treatments). LSDs = 18, 12, 18, 14 and 18 at 2, 4, 6, 8 and 10 days respectively for *C. lanceolata* (across all embryo envelope treatments). S, Intact seed; P, pericarp; E, naked embryo. Experiments were conducted in spring/summer 2007.





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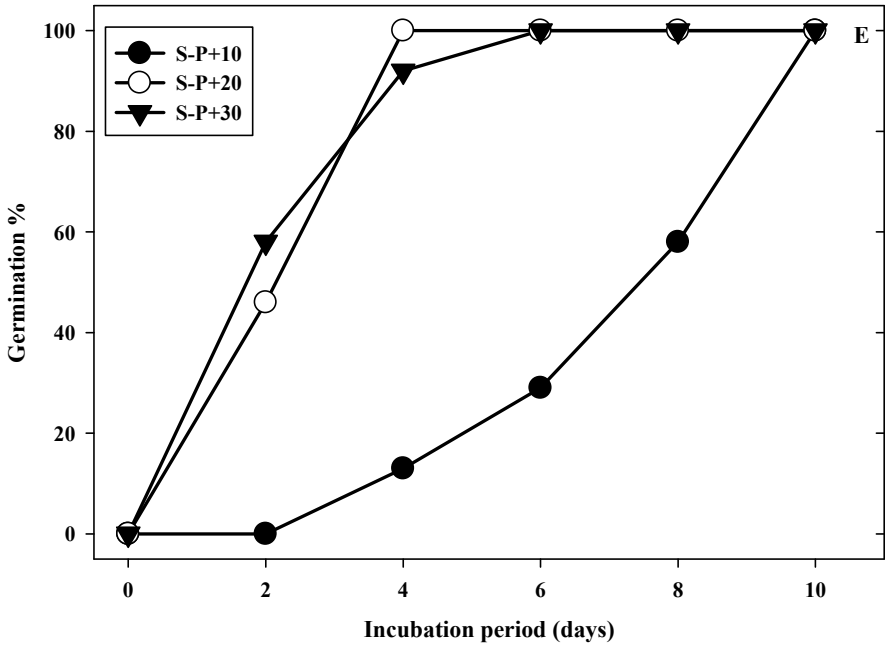

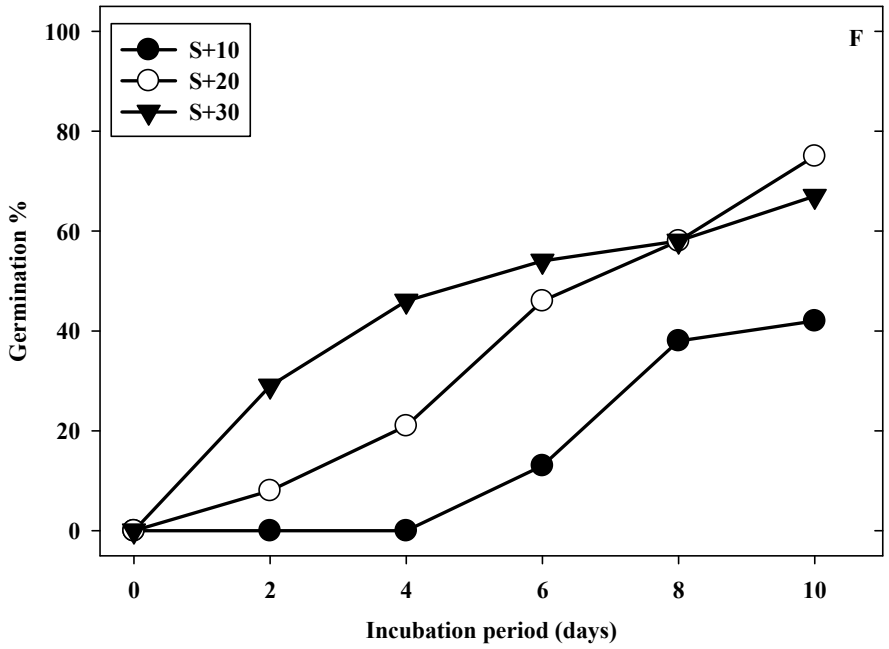

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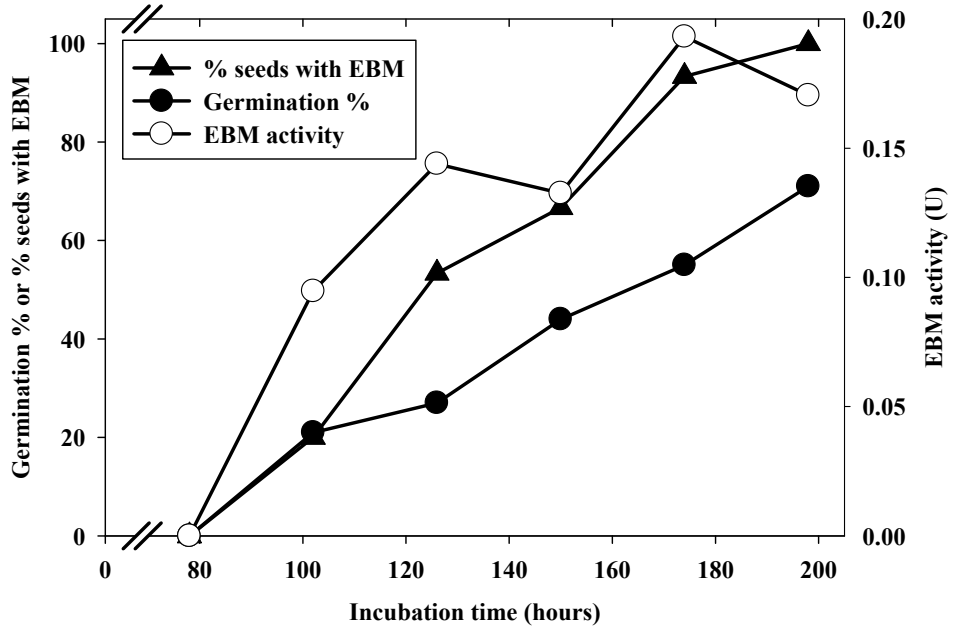


Figure 3-7. Relationship between germination percentage and percentage seeds with EBM activity (Y1 axis) and EBM activity (Y2 axis) in *C. lanceolata* seeds. EBM activity assessments were made every 6 hours and activity was first detected after 90 hours. Standard EBM activity was 1U, (1 U = EBM activity converting 1 μmol of galactomannan/minute under standard conditions).

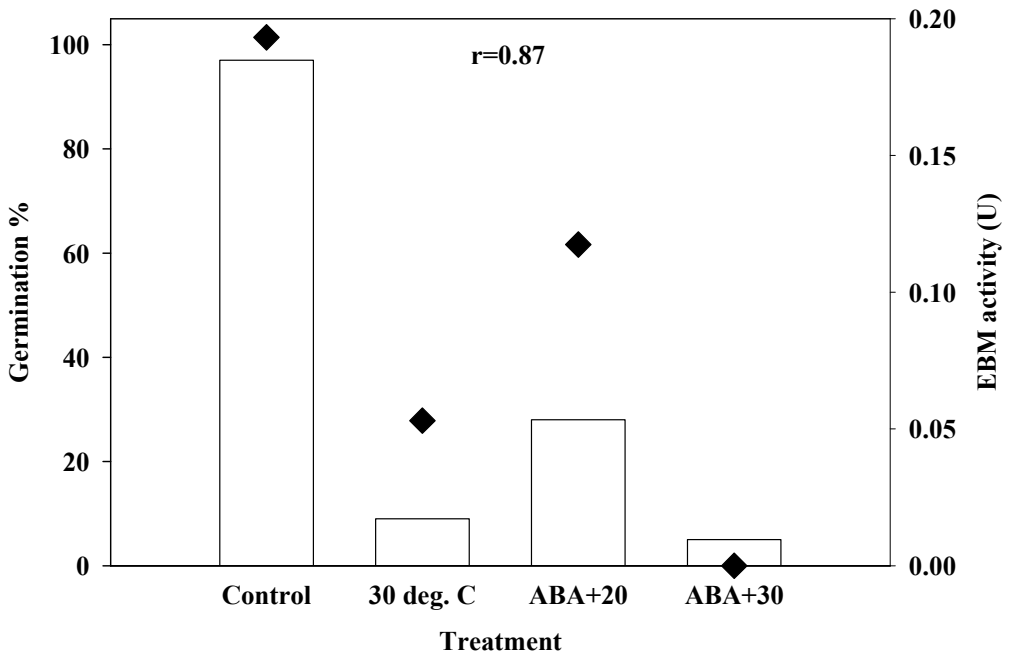


Figure 3-8. Correlation between *C. lanceolata* germination (white bars and Y1 axis), and EBM activity levels (diamond marks and Y2 axis). Enzyme activity was measured from endosperms of non-germinated seeds at the end of a 14-day incubation period under the respective treatments. Germinated seeds from non-control treatments had normal enzyme activity similar to control. Standard EBM activity was 1U, (1 U = EBM activity converting 1 μmol of galactomannan/minute under standard conditions).

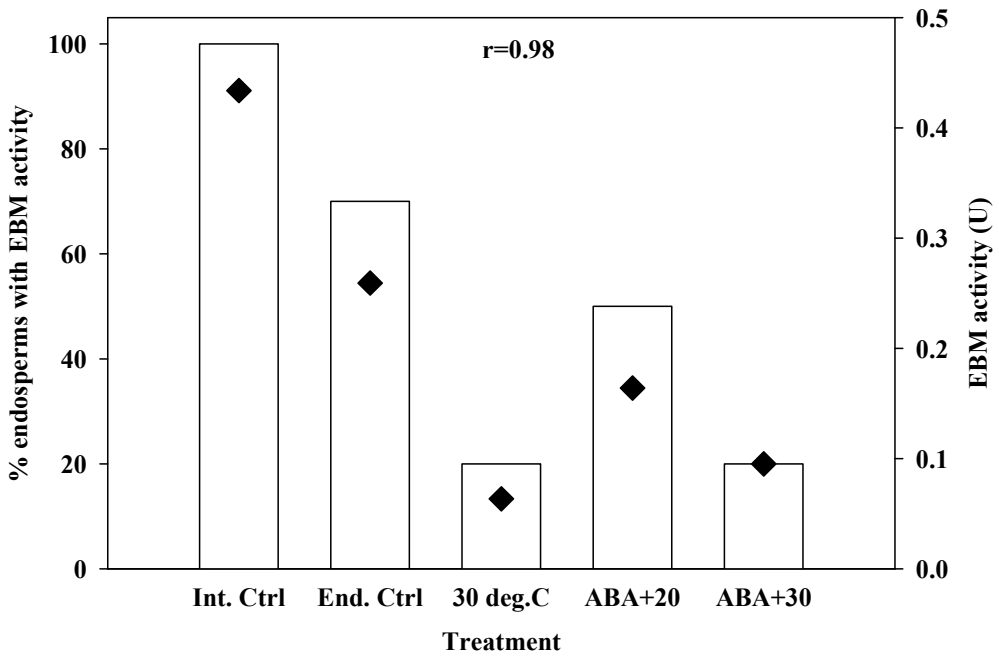


Figure 3-9. Percentage of endosperms exhibiting EBM activity, white bars and Y1 axis, and EBM activity levels, diamond marks and Y2 axis, in *C. lanceolata* endosperms. In 30 deg C, ABA+20 and ABA+30 treatments, endosperms were separated from embryos after 24 hours imbibition on respective media and were incubated for an additional 120 hours on same substrate. Intact control: endosperms were excised after 144 hours incubation at 20^{o}C in de-ionized water. End control: endosperms were excised after 24 hours at 20^{o}C in de-ionized water and re-incubated for another 120 hours in same substrate. Enzyme activity was measured after incubating endosperms for a total of 144 hours under each treatment. Standard EBM activity was 1U, (1 U = EBM activity converting 1 µmol of galactomannan/minute under standard conditions).

CHAPTER 4
OPTIMIZING GERMINATION IN *COREOPSIS* SEEDS

4.1 Introduction

Native *Coreopsis* wildflowers are useful in Florida ecosystem restoration and aesthetic plantings on roadsides and public parks. Widespread use can be hindered by low germination due to seed dormancy. In order to study dormancy in *Coreopsis*, various factors to alleviate dormancy need to be elucidated. Normally, germination occurs when seeds are provided with adequate moisture, optimal temperature, oxygen and sometimes light. Failure to germinate signifies dormancy (Bewley and Black, 1994; Baskin and Baskin, 1998, 2004). Various studies suggest that most seeds posses some level of dormancy at maturity (Finch-Savage and Leubner-Metzger, 2006; Steinbach *et al.,* 1995, 1997) and both dormancy release and subsequent germination depend on environmental cues (Baskin and Baskin, 1978, 2004).

The balance between germination and dormancy is determined by genetics, physiological status (Finch-Savage and Leubner-Metzger, 2006; Bradford *et al.*, 2007), and environmental factors including seed maturation environment (Sung *et al.*, 1998; Orozco-Segovia *et al.*, 2000; Blodner *et al.*, 2007). Norcini *et al.* (2004) reported that environmental conditions during seed maturity affects seed viability and dormancy in *Coreopsis lanceolata*. In some seeds, nitrates and cold stratification (moist chilling) break dormancy (ISTA, 1985; AOSA, 1998), while alternating temperatures overcome dormancy in other species (Leon and Knapp, 2004). Temperature controls germination in non-dormant seeds by its inherent influence on metabolic and physiological processes (Bewley and Black, 1994; Baskin and Baskin, 1998). Information on effects of environmental factors (moisture, temperature, oxygen, light) and seed properties (genotype, physiological and physical status) is important in dormancy studies because these factors regulate germination, and germination is a method of assessing dormancy.

There is strong evidence that ABA imposes and maintains dormancy (Finch-Savage and Leubner-Metzger, 2006). In many species the phytohormone ABA inhibits germination (Schopfer and Plachy, 1984; Leon-Kloosterziel *et al.,* 1996; Leung and Giraudat, 1998; Toorop *et al.*, 2000; Benech-Arnold *et al.,* 2006; Kucera *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006; Taylor *et al.*, 2000). In dormant seeds of barley (Jacobsen *et al.*, 2002; Chono *et al.*, 2006), and *Chamaecyparis nootkatensis* (Schmitz *et al.,* 2000, 2002), ABA is synthesized *de novo* during imbibition and accumulates due to the inability of the embryo to metabolize it. *Arabidopsis thaliana* ABA-mutants (insensitive or deficient) lack seed dormancy (Leon-Kloosterziel *et al.*, 1996). In species where coat-imposed dormancy is involved, there is an indirect interaction between ABA and GA in dormancy regulation (Jacobson *et al.*, 2002; Leubner-Metzger, 2003).

Gibberellic acid promotes germination (Debeaujon and Koornneef, 2000; Olszewski *et al.,* 2002; Yamaguchi and Kamiya, 2002; Kucera *et al.,* 2005) by stimulating cell elongation and division of embryo hypocotyls (Taiz and Zieger, 2002), and in some species, induces *de novo* biosynthesis of hydrolases that digest and weaken endosperm (Yamaguchi and Kamiya, 2002; Kucera *et al.,* 2005). Exogenous application of GA can substitute for light in positively photoblastic seeds (Braun and Khan, 1975; Yoshioka *et al.,* 1998; Yamaguchi and Kamiya, 2002).

The control of light response in germination is mediated by phytochrome photoreceptors (Loercher, 1974; Bewley and Black, 1994). Phytochrome controls germination through two inter-convertible protein conformations, termed P_{fr} or $P_{r,}$ with P_{fr} being the biologically active form. Exposing seeds to red light (660 nm) converts the pigment to the P_{fr} form and irradiation with far-red light (730 nm) causes it to revert (Poppe and Schafer, 1997; Casal *et al.*, 1997;

1998). There are three known types of phytochrome responses in seeds; Low Fluence Response (LFR), Very Low Fluence Response (VLFR) and High Irradiance Response (HIR) (Shinomura *et al.* 1994; Poppe and Schafer, 1997; Milberg *et al.,* 2000; Casal *et al.,* 1998; Yamaguchi and Kamiya, 2002; Leon and Owen, 2003; Yamauchi *et al.* 2004). Very low amounts of P_{fr} are sufficient to induce VLFR, whereas higher levels are required to induce LFR. The VLFR occurs under far-red light, but LFR does not. The HIR occurs under continuous high-irradiance far-red light (~715 nm). The LFR response is under control of the more stable phytochrome B while the labile phytochrome A mediates VLFR and HIR. Light is postulated to induce *de novo* GA biosynthesis in imbibed light-requiring seeds, and to enhance sensitivity of embryos to GAs (Shinomura *et al.,* 1994; Hilhorst and Karsen, 1988; Poppe and Schafer, 1997; Yamaguchi and Kamiya, 2002; Yamauchi *et al.*2004). Light is an important element in dormancy alleviation and germination in many seeds (Goggin *et al.*, 2008).

This study sought to identify important factors that might influence germination of *C. basalis, C. floridana, C. lanceolata, C. leavenworthii* and *C. pubescens* seeds. The effects of temperature, light, nitrate and cold stratification were examined. Additionally, effects of ABA, GA, and tetcyclacis (a GA biosynthesis inhibitor) were investigated to gain insight into possible roles of GA and ABA in germination of *Coreopsis* seeds.

4.2 Materials and Methods

4.2.1 Seed Material

Five *Coreopsis* species were studied: *C. basalis* (A. Dietr.) S.F. Blake, C. *floridana* E.B. Smith, *C. lanceolata* L., *C. leavenworthii* Torr. & A. Gray, and *C. pubescens* Elliot. *Coreopsis basalis, C. floridana, C. leavenworthii* and *C. lanceolata* (North Florida ecotype) were prevariety germplasm. *Coreopsis floridana* and *C. leavenworthii* are wetland species, with *C. floridana* being endemic to Florida and *C. leavenworthii* nearly so (USDA-NRCS, 2007). *Coreopsis*

basalis and *C. lanceolata* are upland species but *C. pubescens* is a facultative wetland species, and all three are more widely distributed than the wetland species (USDA-NRCS, 2007). Except for *C. basalis* which was harvested from a natural stand in northern Florida, seeds of *C. floridana, C. lanceolata* (North Florida ecotype - NF) and *C. leavenworthii* were harvested from cultivated populations in Florida (Norcini and Aldrich, 2007a). A North Carolina ecotype of *C. lanceolata* (NC) and a West Virginia ecotype of *C. pubescens* (WV) were purchased from Ernst Conservation Seeds (Meadville, PA). Two other accessions of *C. lanceolata*, a dwarf form (DW) and a typical form (Lance Leaf type - LL), were purchased from Applewood Seed Company (Arvada, CO).

4.2.2 Germination Tests

A thermo-gradient table, Type db 5000 (Van dok and de Boer Machinefabriek BV, Enkhuizen, The Netherlands) was used (minimizes non-treatment variation) to provide temperatures of 15, 20, 25 and 30^{o}C in continuous light or dark. When a single constant temperature was required, an Isotemp incubator model 304R (Fisher Scientific, Fair Lawn, NJ) was set at 20^{o}C in continuous light or dark.

Four replications of 25 seeds each were placed on double blue blotter paper (Anchor Paper Company, St. Paul, MN) moistened with de-ionized water in 5 cm glass Petri dishes. Dark was achieved by wrapping Petri dishes with aluminum foil and maintaining a dark environment in an incubator. Germination counts were made at 7 and 14 days. The 7-day counts for seeds germinated in dark were made under a dim-green light (25W, A19, Specialty 90912; General Electric Company, Cleveland, OH) in a dark room. Germination was defined as visible protrusion of radicle to at least 2 mm. A tetrazolium test (ISTA, 2003) was used to assess seed viability of non-germinated seeds.

4.2.3 Plant Growth Regulators

Germination tests were conducted at 20°C, except that the blotter papers were moistened with 0 to 100 µM of tetcyclacis (BASF, Florham Park, NJ), GA_3 (Fluka Chemie AG, Buchs, Switzerland), GA_{4+7} (Plant Protection Limited, Yalding, United Kingdom), or ABA (Sigma Chemical Company, St. Louis, MO). At intermediate counts, blotter papers were re-moistened with the appropriate concentration of plant growth regulator or tetcyclacis.

4.2.4 Cold Stratification

Seeds were imbibed in the dark at 5°C (Precision incubator model 816, Precision Scientific Group, Chicago, IL) for 7 or 14 days. After the prescribed period, four replicates (25 seeds each) of Petri dishes were transferred to an incubator at 20°C for germination tests in light or dark.

4.2.5 Statistical Analysis

Data were analyzed separately. Germination percentages were adjusted for percentage of viable seeds, arcsine-squre root transformed if necessary (to normalize data), and then analyzed; however, non-transformed means are presented. Analysis of variance was performed using PROC GLM (SAS Version 9.1; SAS Institute, Cary, NC). Treatment means were separated using the least significant difference (LSD) test at the 5% significance level.

4.3 Results

4.3.1 Temperature and Light

In all species, the greatest germination was attained at 15 and 20°C. Depending on species, germination improved in light, (Figure 4-1). In *C. lanceolata*, the NC ecotype germination was promoted by light compared to the NF ecotype. However, *C. floridana* seeds were dormant in the dark (Figure 4-1). When all 5 species were imbibed at 25 and 30°C

germination percentage fell and this effect was more pronounced in dark. However, germination was maximized if seeds were returned to optimal temperatures.

4.3.2 Gibberellic Acid, ABA and Tetcyclacis

Except *C. floridana* and *C. leavenworthii* where dark germination was promoted gibberellic acid did not affect germination of any species; interestingly, GA reduced germination in *C. leavenworthii* seeds imbibed in light. Abscisic acid reduced germination in all species, but the reduction was more drastic in dark (Figure 4-2).

When exogenous GA concentrations were increased in dark-imbibed *C. floridana* seeds, germination also increased; GA_3 and GA_{4+7} had similar efficacy in stimulating germination (Figure 4-4). In *C. lanceolata* (NF), neither GA_3 or GA_{4+7} affected germination. Germination in *C. floridana* and *C. lanceolata* (NF) seeds declined as concentrations of ABA or tetcyclacis increased (Figures 4-5 and 4-6).

4.3.3 Cold Stratification

Stratification at 5°C for 7 or 14 days did not affect germination of *C. basalis, C. lanceolata, C. pubescens* or *C. lanceolata* seeds in light, but there was a reduction in germination in dark (Figure 4-3). In *C. lanceolata*, dark germination reduction was pronounced in the NC ecotype compared to the NF ecotype. However, cold stratification of *C. floridana* and *C. leavenworthii* seeds promoted germination in dark, but in light *C. leavenworthii* seeds had reduced germination (Figure 4-3).

4.4 Discussion

Coreopsis seeds had greatest germination percentage at constant 15 or 20°C which concurs with other reports (ISTA, 1985; Carpenter and Ostmark, 1992; Banovetz and Scheiner, 1994; AOSA, 1998). Seeds that did not germinate at elevated temperatures (30°C) germinated normally when returned to optimal temperatures, which indicates that higher temperatures

inhibited germination but did not kill the seeds. *Coreopsis basalis, C. pubescens* and four accessions of *C. lanceolata* did not require light for germination but light was vital in *C. floridana* and *C. leavenworthii*. *Coreopsis floridana* seeds tolerated higher imbibitional temperatures in light compared to other *Coreopsis* species. Light requirement in *C. floridana* and *C. leavenworthii* seeds may be an ecological adaptation.

Gibberellic acid improved germination in dark-imbibed *C. floridana* and *C. leavenworthii* but not other species. Both GA_3 or GA_{4+7} had similar efficacy in promoting germination in *C. floridana*. In light-requiring lettuce seeds (Braun and Khan, 1975; Psaras *et al.*, 1981; Vertucci *et al.,* 1987; Bewley and Black, 1994; Nijsse *et al.*, 1998; Yoshioka *et al.,* 1998), in *Bidens pilosa* (Forsyth and Brown, 1982), and in other light-requiring seeds (Olszewski *et al.*, 2002; Yamaguchi and Kamiya, 2002) exogenous GA promoted germination. In endospermic seeds, such as those of *Coreopsis*, gibberellins may stimulate embryo growth and induce hydrolases that digest and weaken endosperm, allowing radicle protrusion (Yamaguchi and Kamiya, 2002; Kucera *et al.*, 2005).

Cold stratification promoted germination in dark-imbibed *C. floridana* and *C. leavenworthii* seeds but reduced germination in *C. basalis, C. lanceolata* and *C. pubescens* imbibed in dark. Cold stratification reduced seed germination in *C. lanceolata* (Banovetz and Scheiner, 1994), *Phacelia dubia* (Baskin and Baskin, 1978) and *Asparagus acutifolius* (Conversa and Elia, 2008). However, in light-requiring lettuce seeds, cold stratification improved germination (van der Woude and Toole, 1980; Psaras, 1981; Bewley and Black, 1994; Nijsse *et al.* 1998). Differences in cold stratification requirement between species might be an ecological adaptation.

Abscisic acid probably prevents the germination of *Coreopsis* seeds by antagonizing GA action, as has been shown for other species (Leubner-Metzger, 2003; Finch-Savage and Leubner-Metzger, 2006). Abscisic acid biosynthesis inhibitors alleviated dormancy in lettuce seeds (Roth-Bejerano *et al.*, 1999). Tetcyclacis inhibited germination by impairing *de novo* GA biosynthesis (Debeaujon and Koornneef, 2000). Data show that factors impeding GA activity in *Coreopsis* seeds inhibit germination (Figures 4-5 and 4-6).

This study identified factors influencing germination in *Coreopsis* species, and there is apparent inter-specific variation related to natural ecological habitats (Paterson *et al.*, 1976; Leon *et al.*, 2006). In addition to similar temperature optimum for germination (15 or 20^{o}C) in all *Coreopsis* species, light, GA and cold stratification promoted germination only in the wetland species *C. floridana* and *C. leavenworthii*. This information is important to *Coreopsis* seeds end users (for example, nursery personnel) because it enables them to optimize germination, or use these methods as pre-germination seed treatments to promote germination and improve subsequent seedling recovery.

4.5 Summary

This study sought to optimize factors influencing germination in *C. basalis, C. floridana, C. lanceolata, C. leavenworthii* and *C. pubescens* to facilitate dormancy studies. Germination effects of temperatures (15, 20, 25 and 30^{o}C), differing light regimes (continuous white light or dark), GA and two pre-germination cold stratification treatments (5^{o}C for 7 or 14 days) were examined. Additionally, effects of ABA and tetcyclacis were examined to evaluate germination response. Optimum germination temperature range in light or dark in all species was 15 to 20^{o}C. *Coreopsis basalis, C. pubescens* and four accessions of *C. lanceolata* did not require light for germination but for *C. floridana* and *C. leavenworthii,* light was essential. Cold stratification reduced total germination in *C. basalis, C. pubescens* and four accessions of *C. lanceolata*, but

improved germination in dark-imbibed *C. floridana* and *C. leavenworthii* seeds. Gibberellic acid (100 μM GA_3) did not promote germination in *C. basalis, C. pubescens* and *C. lanceolata* but improved germination in dark-imbibed *C. floridana* and *C. leavenworthii* seeds. Abscisic acid and tetcyclacis inhibited germination in all species. Factors important in germination of the wetland species *C. floridana* and *C. leavenworthii* were similar, and likewise in the upland species *C. basalis* and *C. lanceolata,* and the facultative species *C. pubescens*.

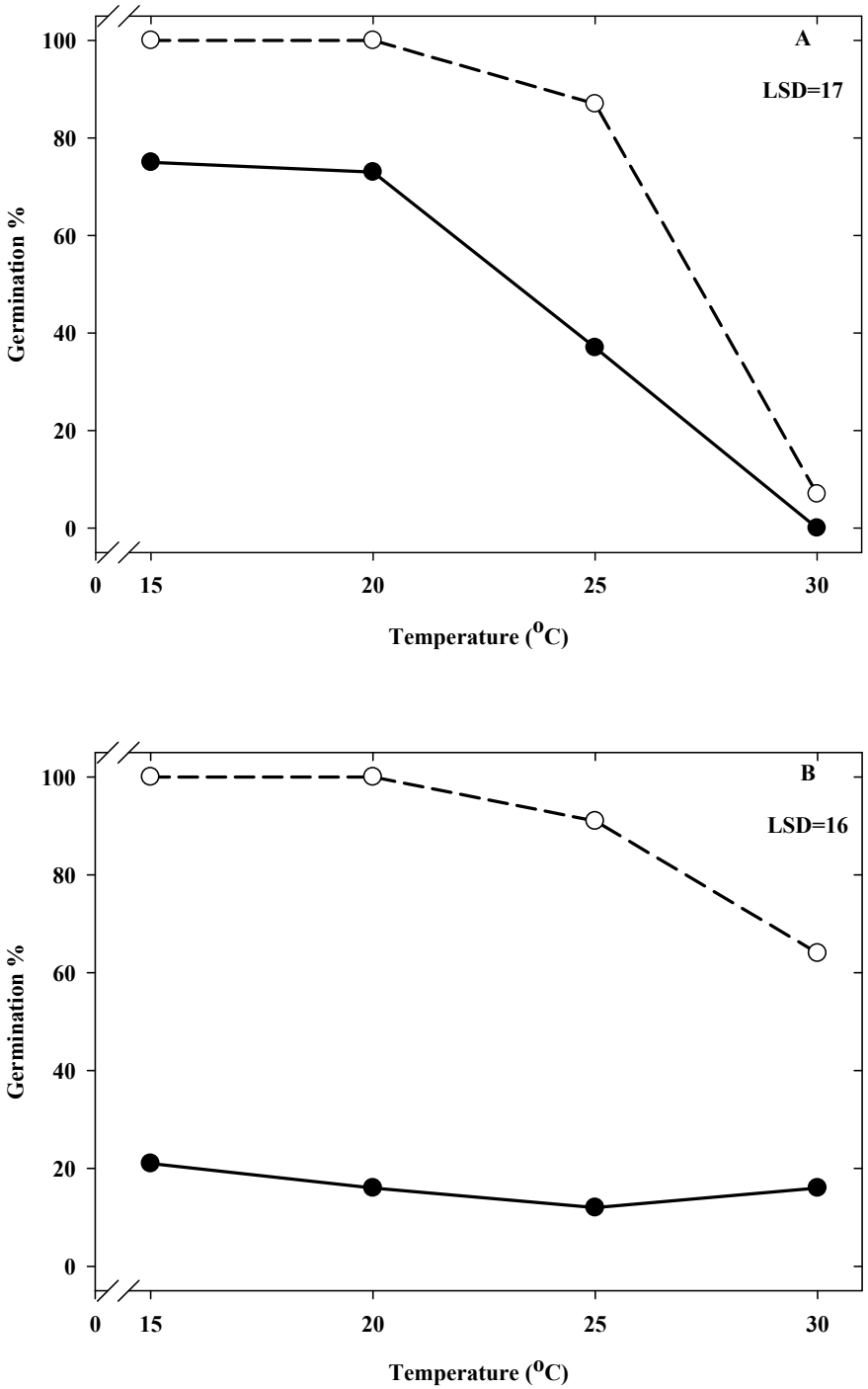
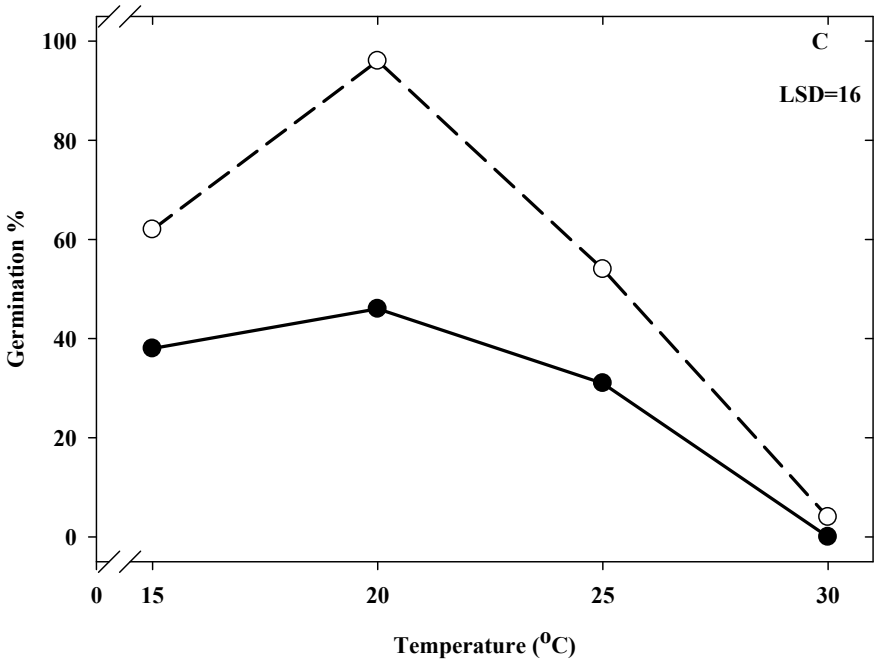
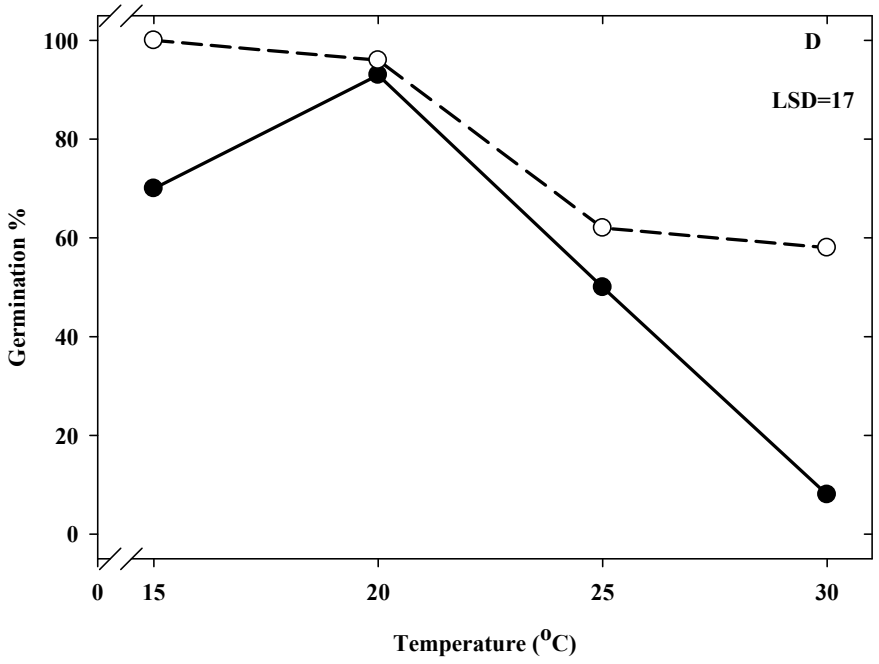


Figure 4-1. Effect of temperature on germination of *Coreopsis* seeds in light (broken lines) and dark (solid lines). A) C. *basalis.* B) *C. floridana*. C) *C. leavenworthii*. D) *C. pubescens*. E) *C. lanceolata*, North Florida ecotype. F) *C. lanceolata*, North Carolina ecotype. G) *C. lanceolata*, dwarf type. H) *C. lanceolata*, lanceleaf type.





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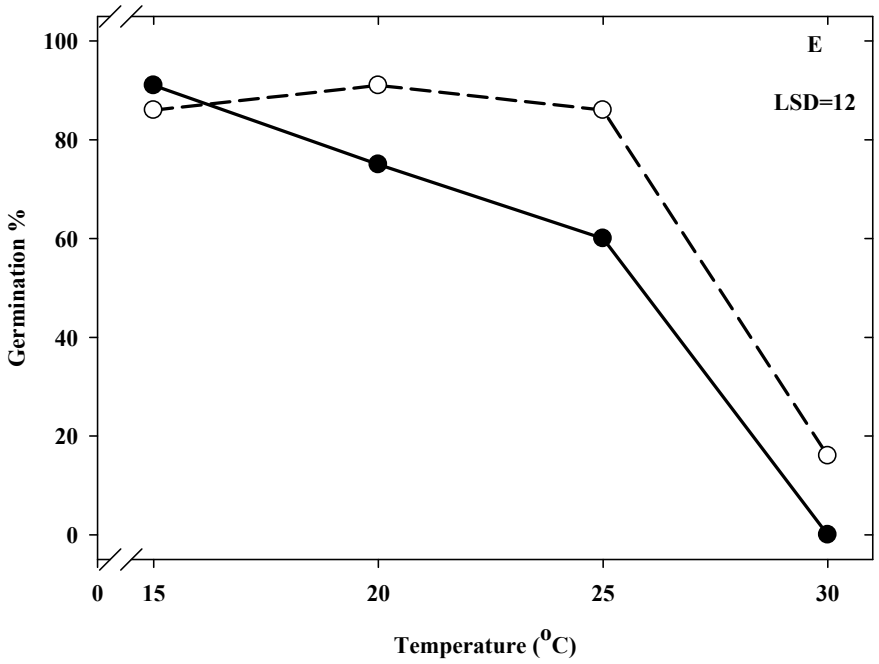

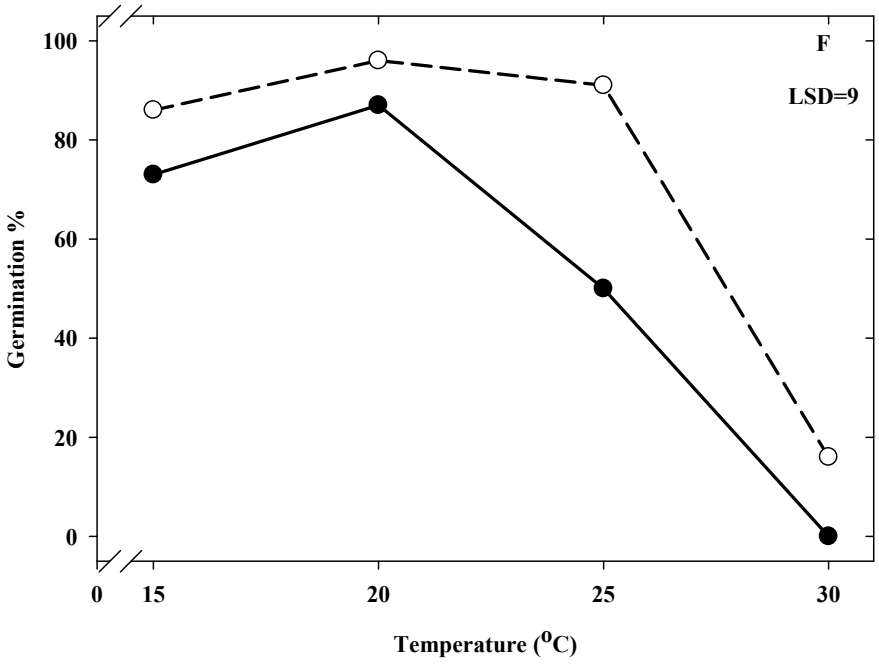

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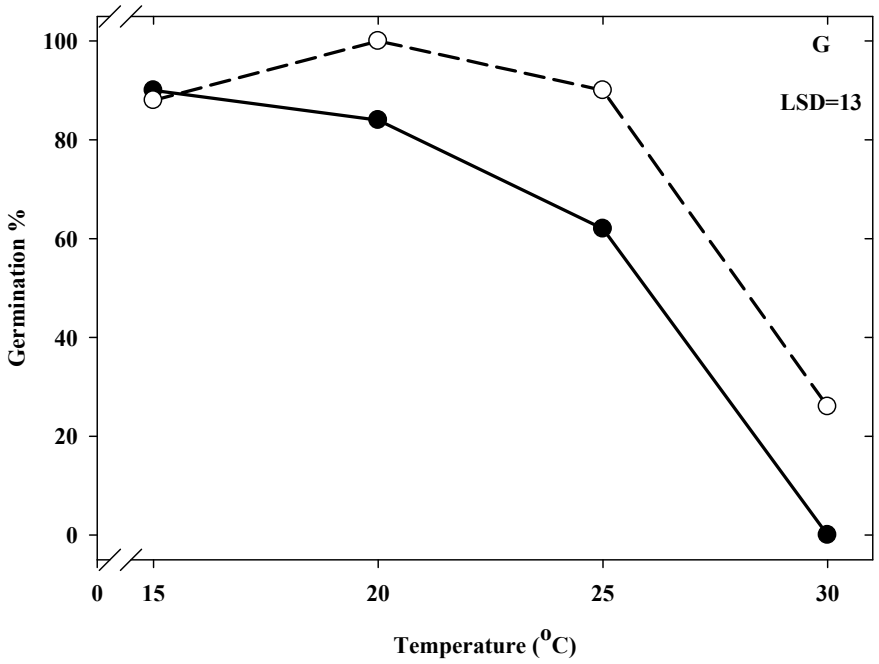

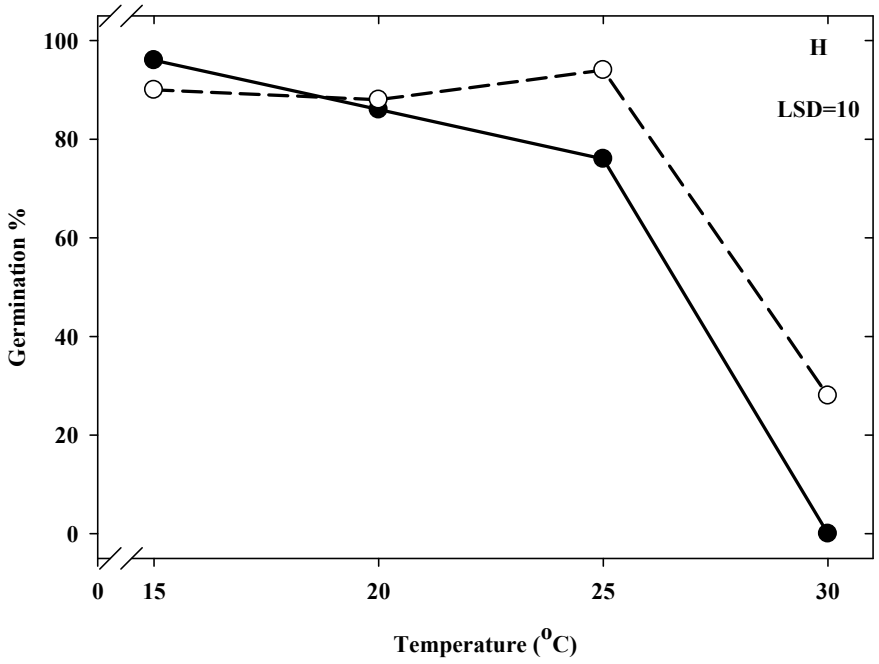

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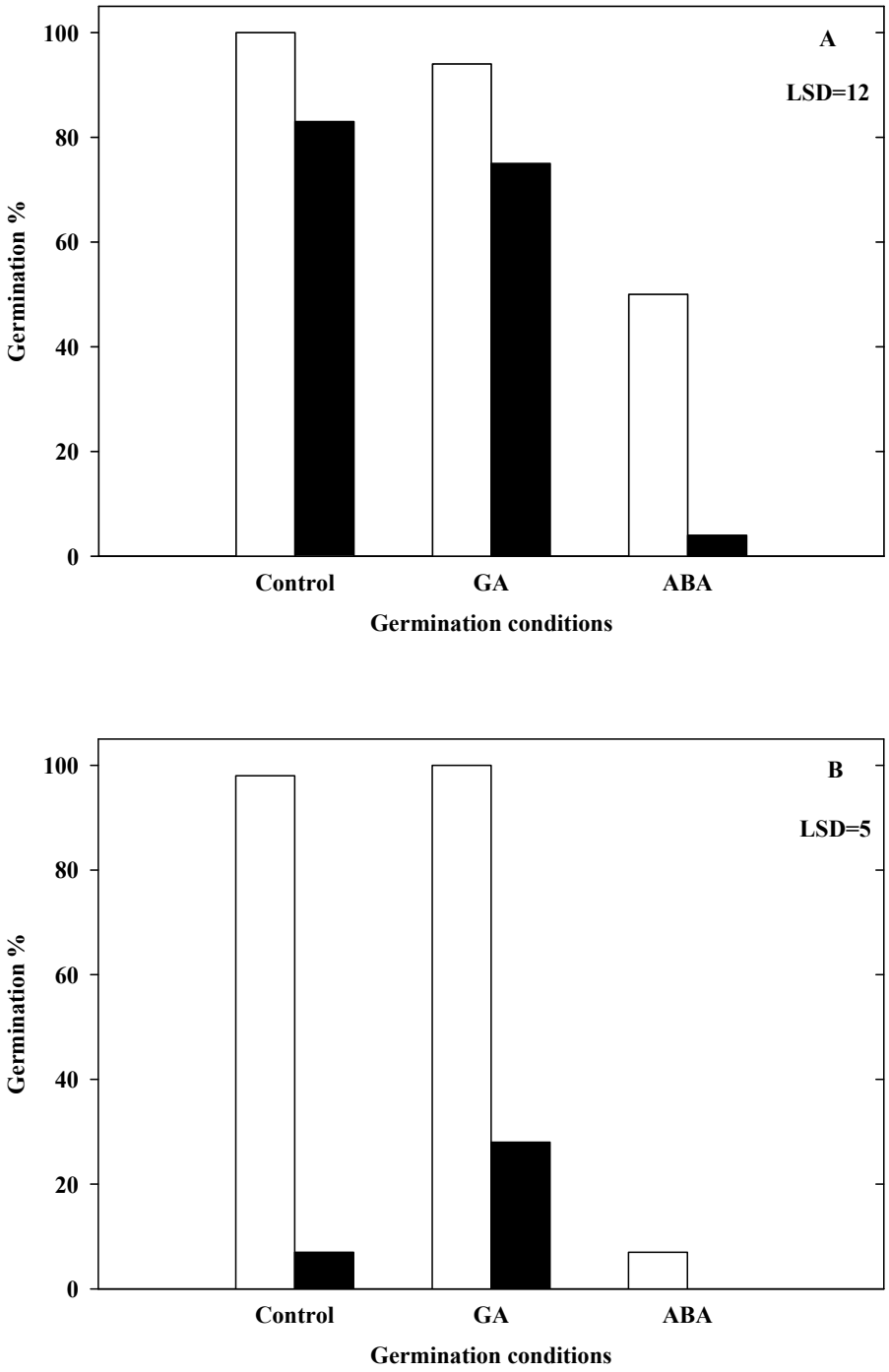
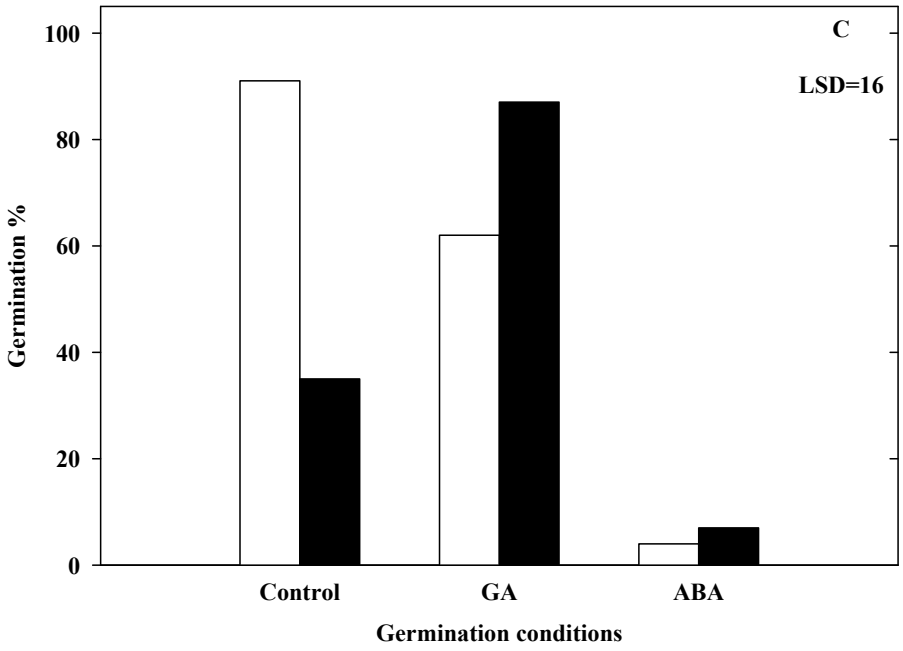
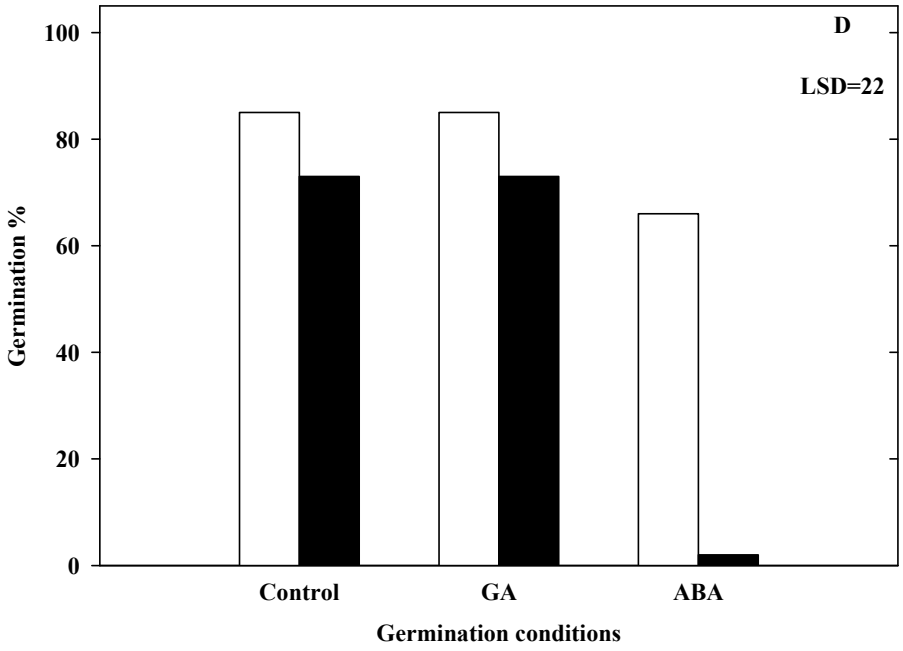


Figure 4-2. Influence of 100µM GA or ABA on germination of *Coreopsis* species at constant 20°C in light (white bars) and dark (black bars). A) C. *basalis.* B) *C. floridana*. C) *C. leavenworthii*. D) *C. pubescens*. E) *C. lanceolata*, North Florida ecotype. F) *C. lanceolata*, North Carolina ecotype. G) *C. lanceolata*, dwarf type. H) *C. lanceolata*, lanceleaf type.





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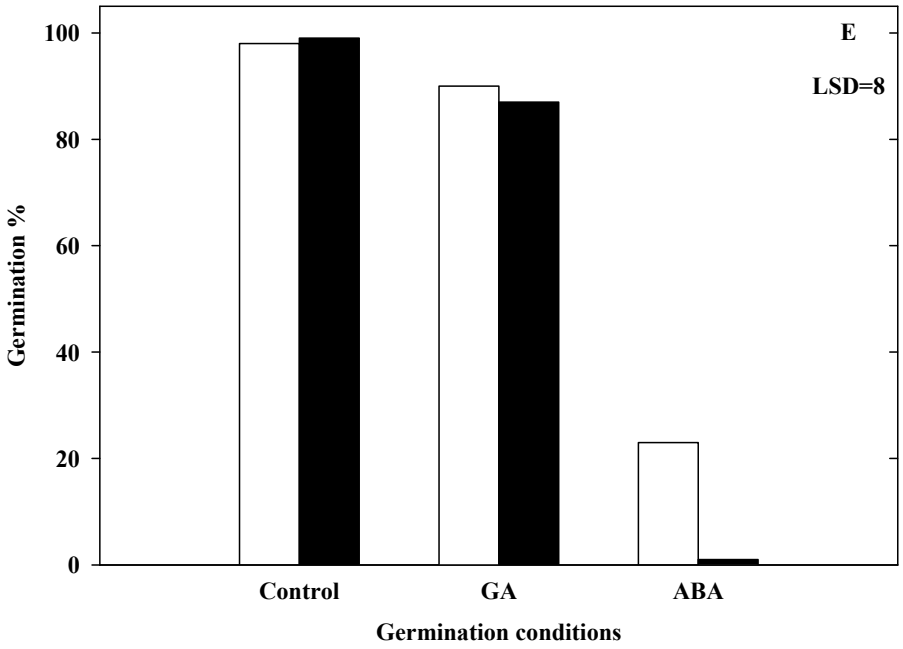

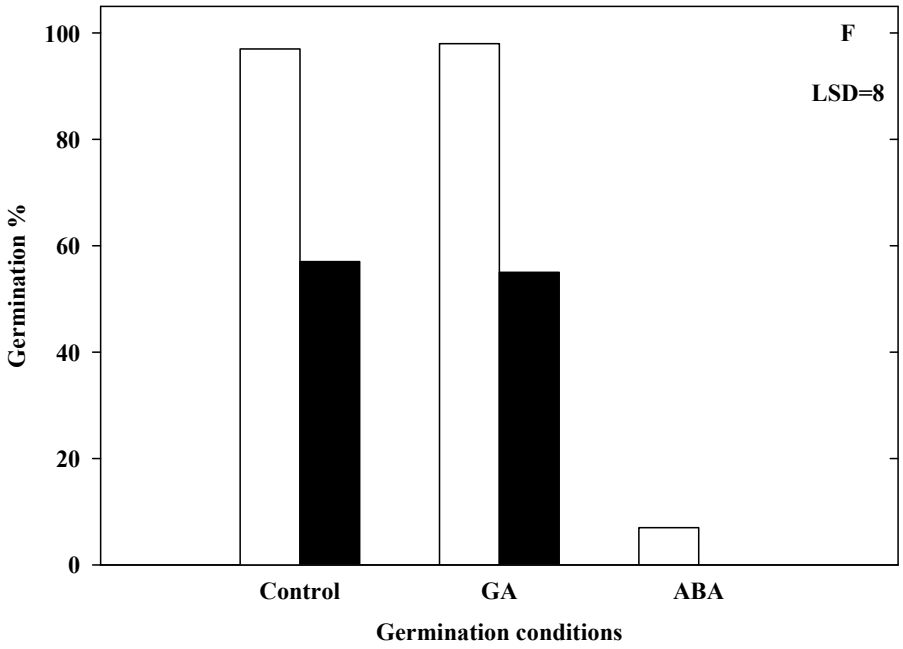

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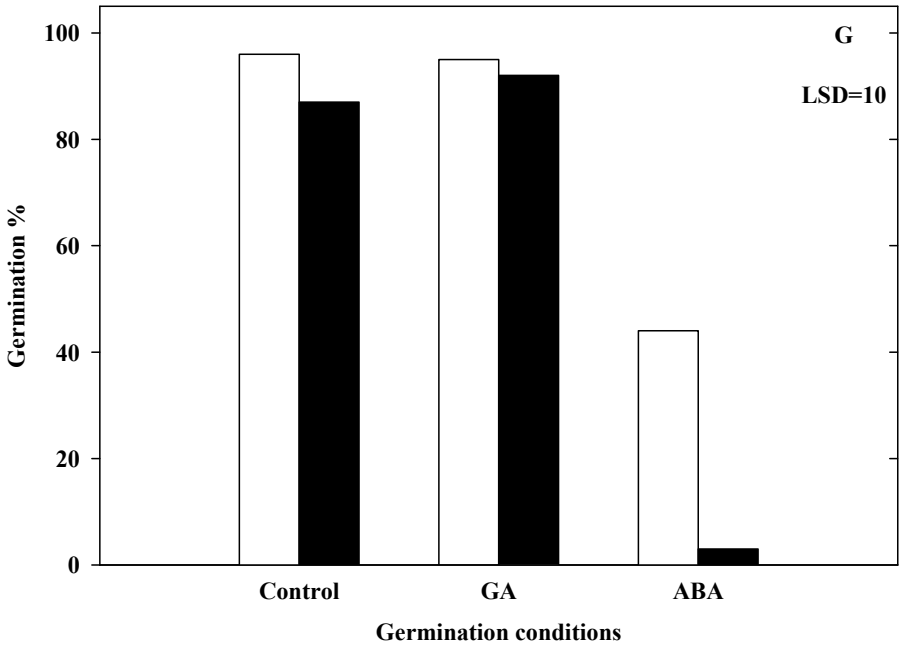

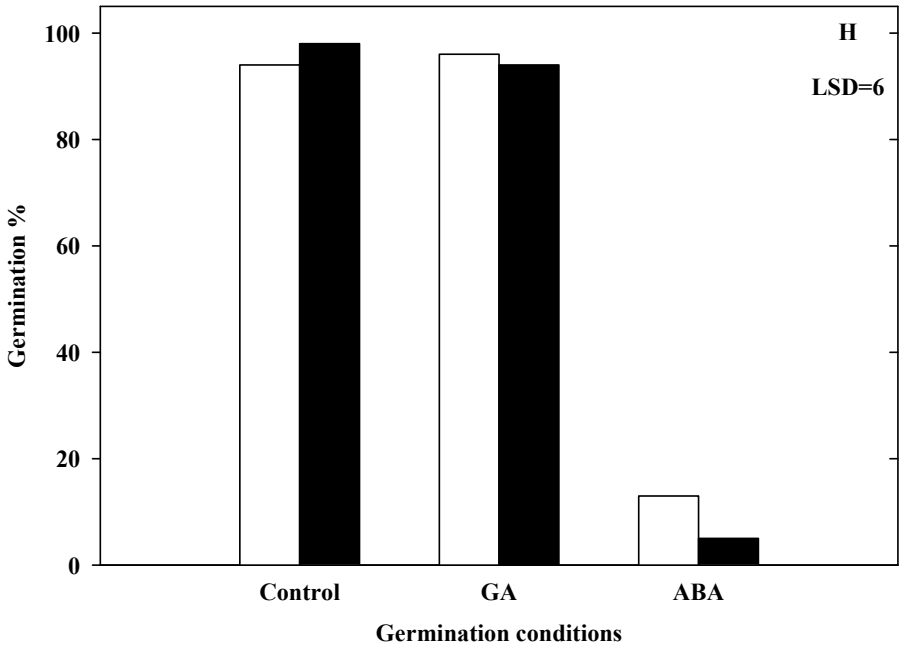

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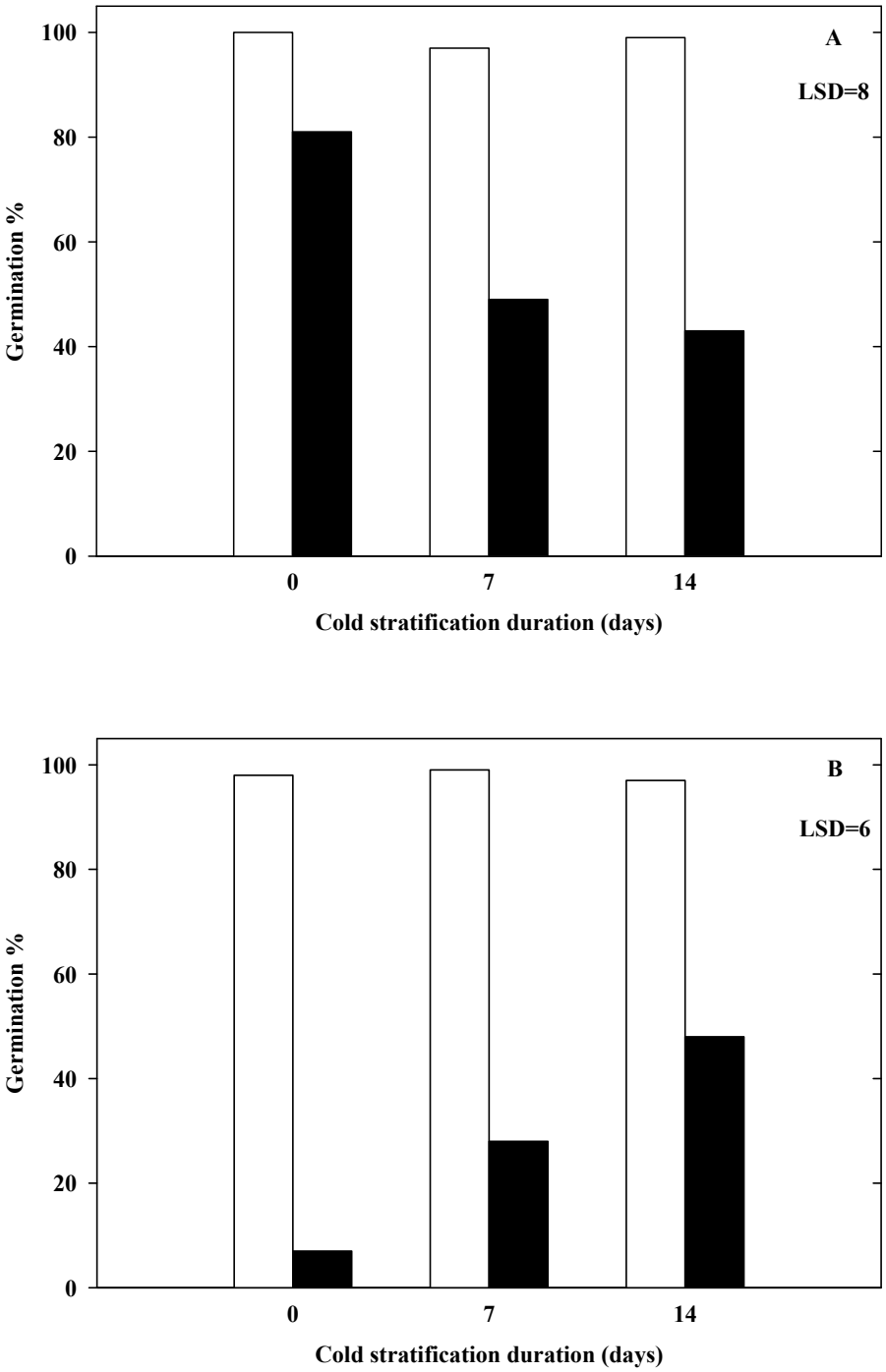
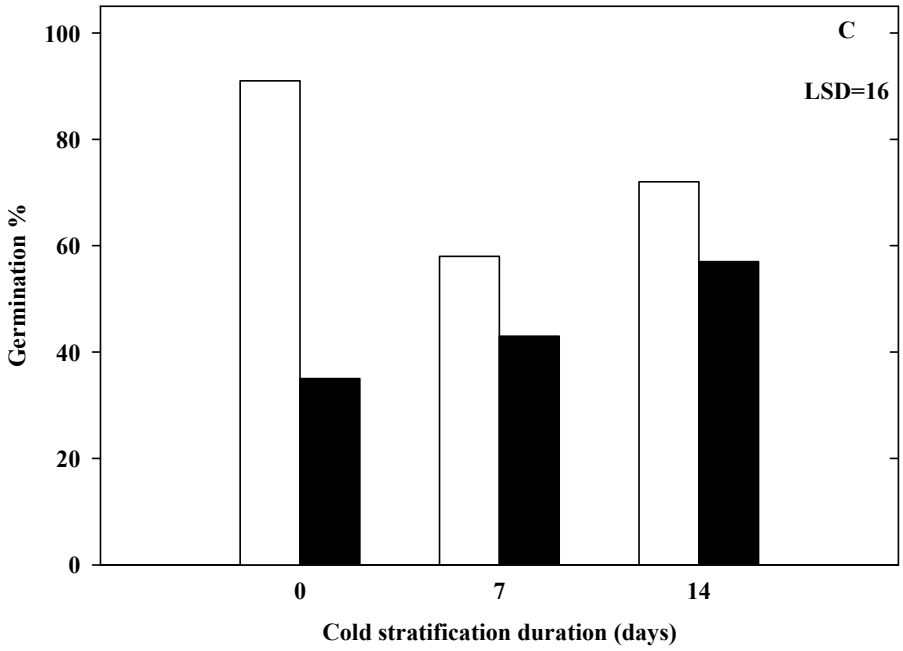
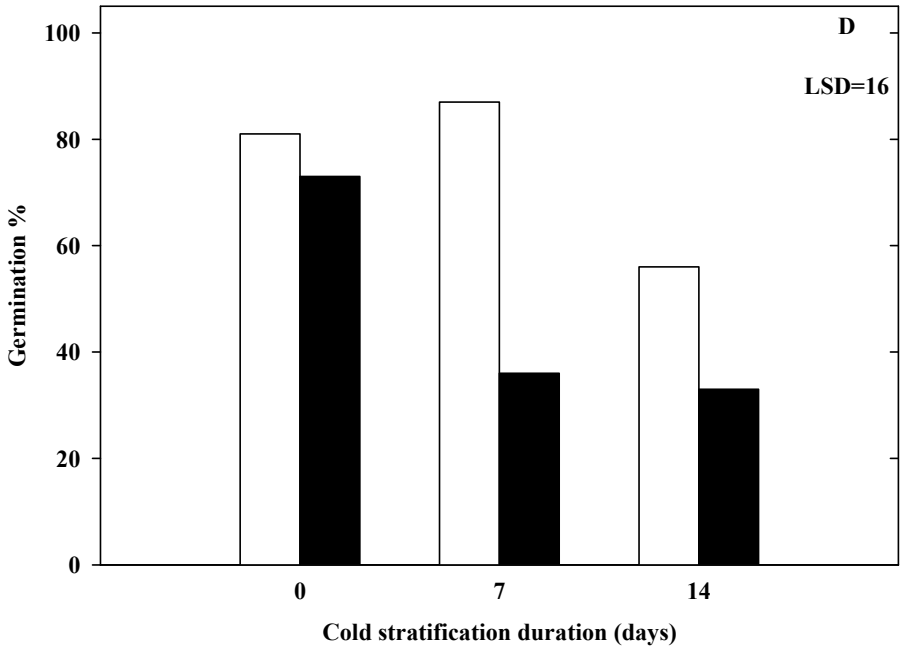


Figure 4-3. Germination of various *Coreopsis* species at constant 20°C in light (white bars) and dark (black bars) after dark, cold (5°C) stratification. A) C. *basalis.* B) *C. floridana*. C) *C. leavenworthii*. D) *C. pubescens*. E) *C. lanceolata*, North Florida ecotype. F) *C. lanceolata*, North Carolina ecotype. G) *C. lanceolata*, dwarf type. H) *C. lanceolata*, lanceleaf type.





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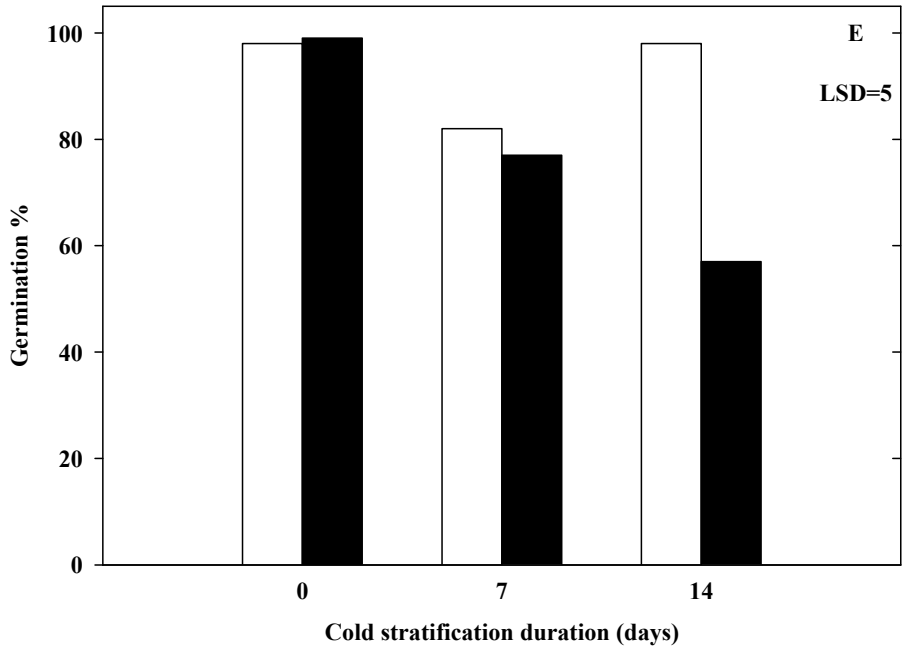

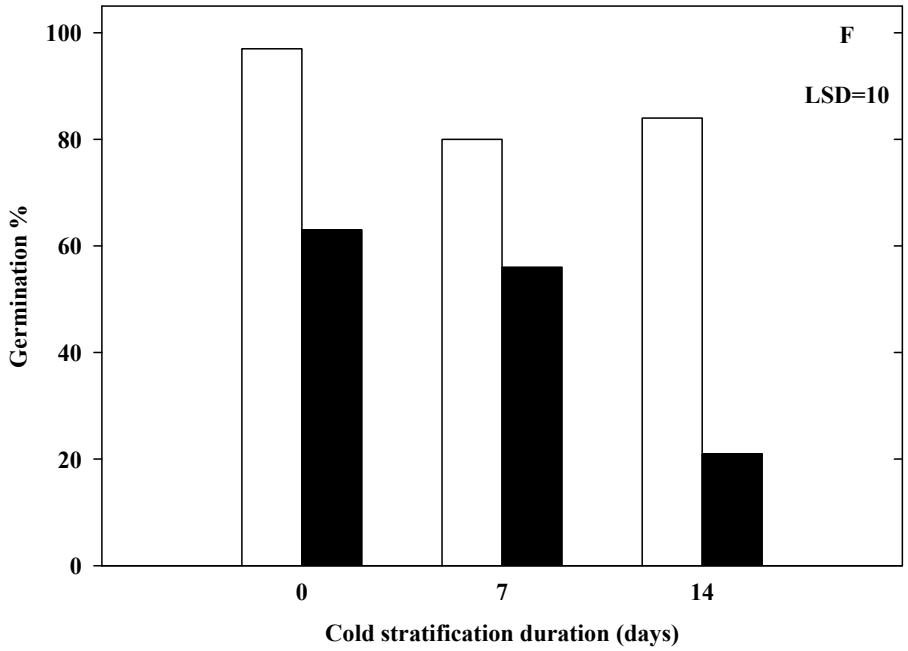

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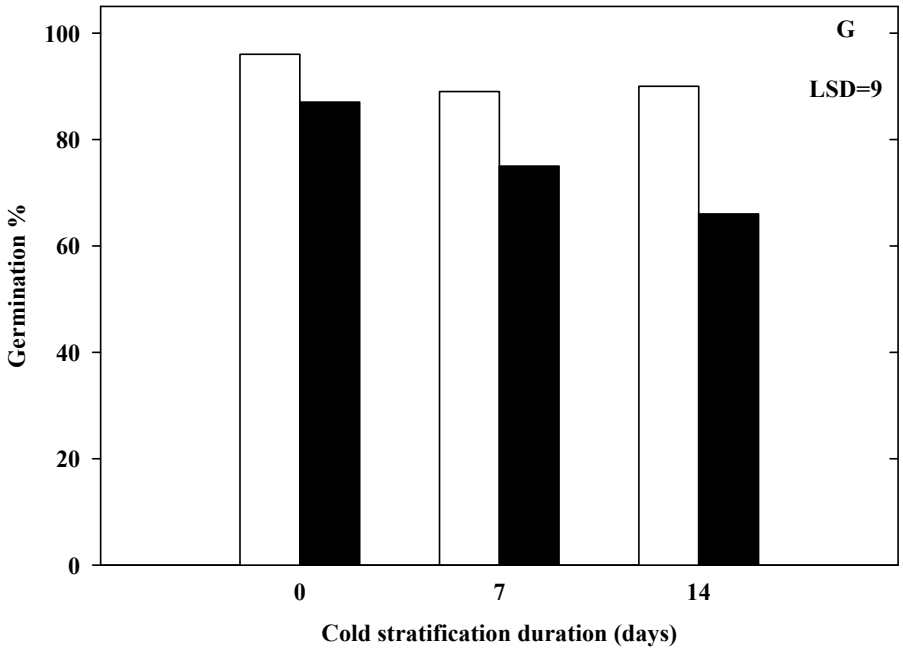

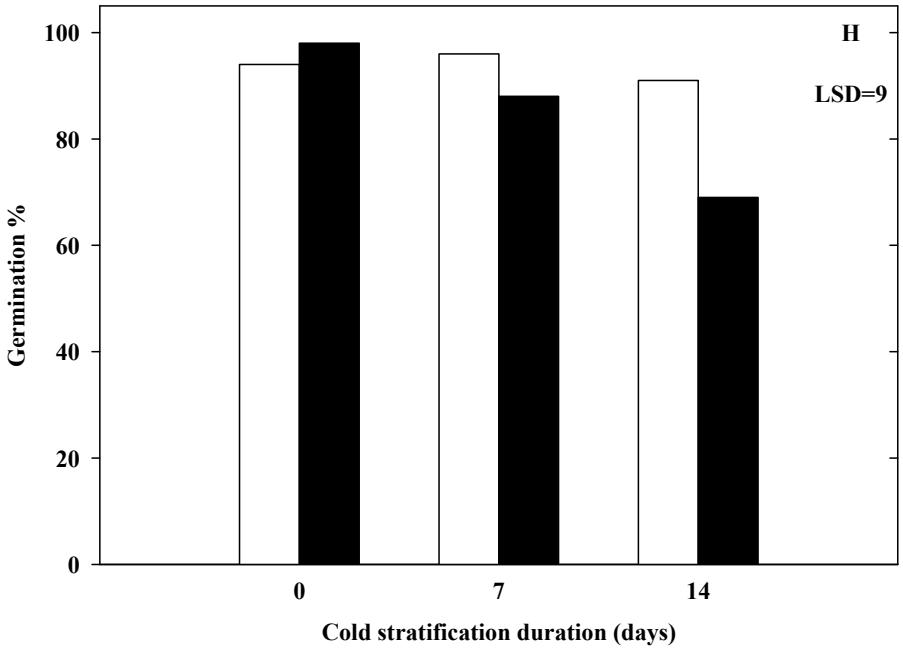

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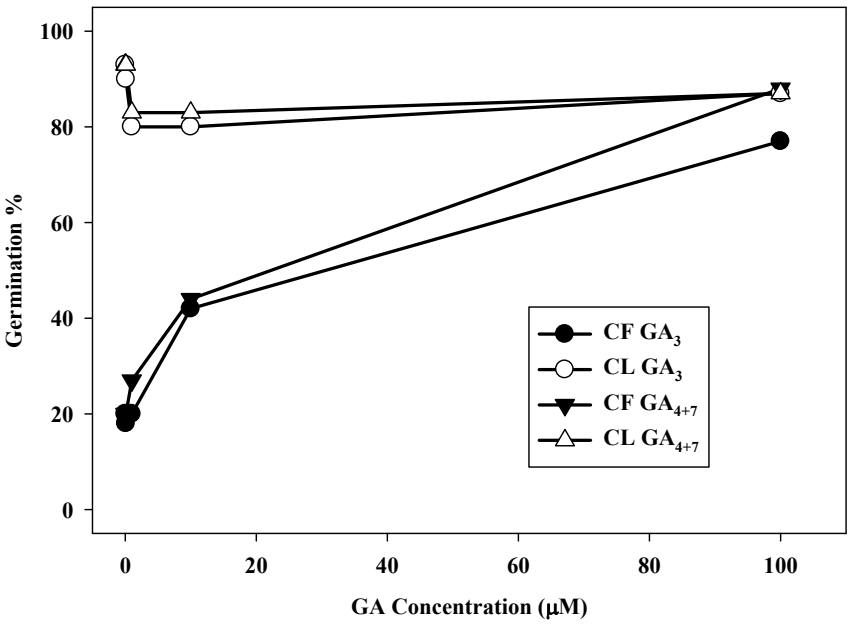


Figure 4-4. Response of *Coreopsis* species seeds to GA_3 or GA_{4+7}. Germination tests were conducted in dark at 20°C. CF, *C. floridana* (LSD=14); CL, *C. lanceolata* (LSD=11). Means comparison was within species.

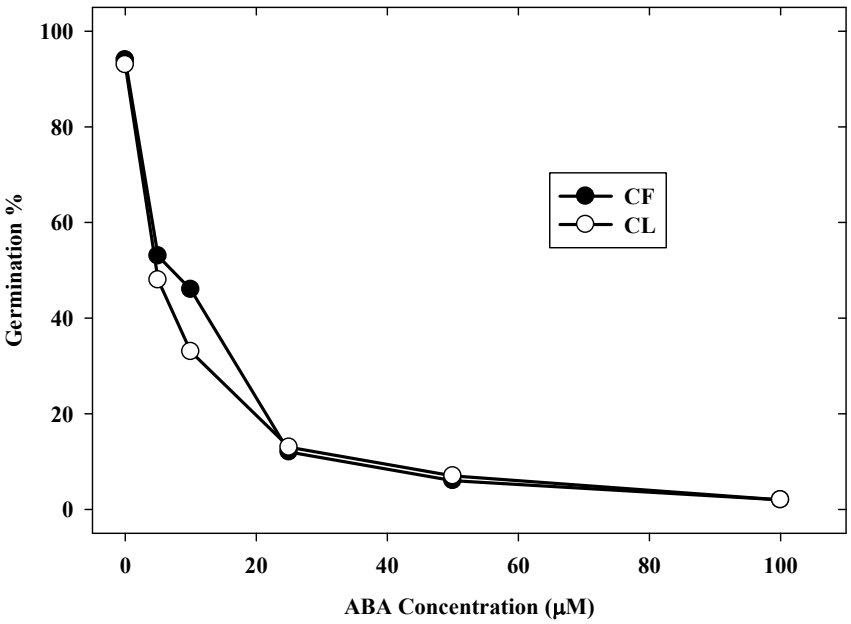


Figure 4-5. Response of *Coreopsis* seeds to ABA. Germination tests were conducted under white fluorescent light at 20°C. CF, *C. floridana* (LSD=13); CL, *C. lanceolata* (LSD=6). Means were compared within each species.

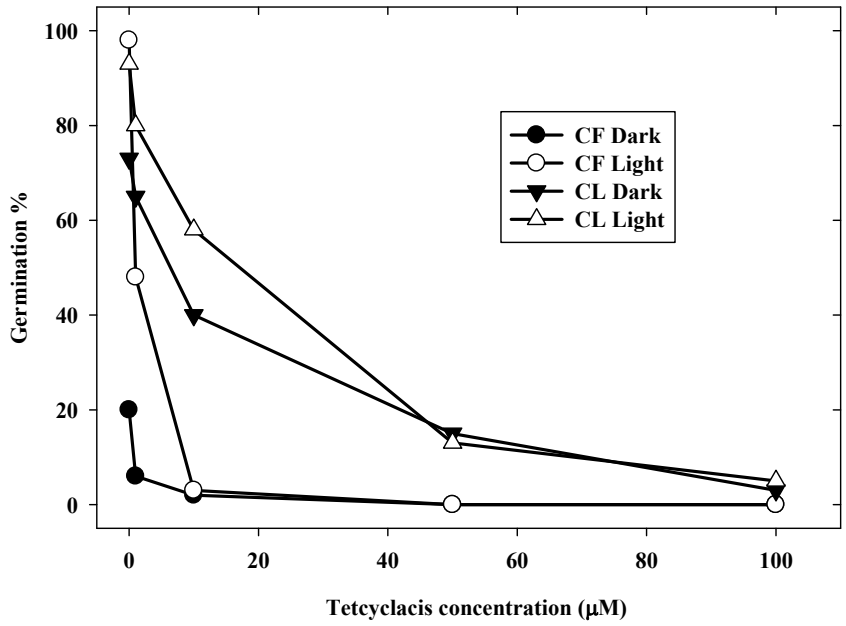


Figure 4-6. Response of *Coreopsis* seeds to tetcyclacis, a GA biosynthesis inhibitor, (inhibits the oxidation of *ent*-kaurene to *ent*-kaurenol in the GA biosynthesis pathway). Germination tests were conducted under white light or in dark at 20°C. CF, *C. floridana* (LSD=8); CL, *C. lanceolata* (LSD=9). Means comparison was within species.

CHAPTER 5
AFTERRIPENING ALLEVIATES DORMANCY IN FRESH *COREOPSIS* SEEDS

5.1 Introduction

Indigenous *Coreopsis* species are important in Florida for ecological restoration and aesthetic reasons. However, large sowing activities can be hampered by poor germination because of dormant seeds. Past research identified afterripening as one of the factors influencing dormancy (Kabat, 2004; Norcini *et al.,* 2006; Kabat *et al.*, 2007; Norcini and Aldrich, 2007a), but other factors such as embryo envelopes were not examined. Afterripening (dry) is a natural process that occurs in dry seeds, which allows seeds to germinate after a certain period from physiological maturity (Widrlechner, 2006). The process, which can take a few weeks to years depending on species, occurs below a certain seed moisture content and is delayed when seeds are too dry; it is accelerated by high temperatures and elevated oxygen levels (Baskin and Baskin, 1976, 1998; Bewley and Black, 1994; Steadman *et al.*, 2003). Dry afterripening is associated with non-enzymatic biochemical reactions among carbohydrates (sugars) and proteins, called Maillard and Amadori reactions (Wettlaufer and Leopold, 1991; Berma-Lugo and Leopold 1992; Murthy *et al.* 2000, 2003).

Afterripening in *Asteraceae* occurs at seed moisture contents of 5 to 12% (Schutz *et al.*, 2002). Prediction of afterripening duration is confounded by initial dormancy level since seed lots of one species stored under the same conditions lose dormancy at different rates, and require distinct dormancy breaking treatments (Steadman *et al.,* 2003; Benech-Arnold *et al.,* 1999; Taylor *et al.*, 2000; Ramagosa *et al.*, 2001; Chono *et al.*, 2006).

Temperature controls germination through its inherent regulation of metabolic rates. The degree of afterripening determines the temperature range at which germination occurs (Leon and Knapp, 2004). Non-dormant seeds generally germinate in a wide temperature range (Bewley

and Black, 1994; Baskin and Baskin, 1998; Pritchard *et al.*, 1999), but germination is inhibited above or below certain limits. Germination failure at supraoptimal temperatures (thermoinhibition) is associated with *de novo* abscisic acid (ABA) biosynthesis in lettuce (Gonai *et al*, 2004) and *Arabidopsis* (Toh *et al*, 2008) seeds. Abscisic acid is known to impose and maintain dormancy (Finch-Savage and Leubner-Metzger, 2006). Fluridone, an ABA biosynthesis inhibitor, generally increases the high limit temperature for germination (Yoshioka *et al.,* 1998).

In many seeds, light regulates germination (Loercher, 1974; Shinomura *et al.,* 1994; Poppe and Schafer, 1997; Milberg *et al.,* 2000; Yamaguchi *et al.*, 2004; Leon and Owen, 2003). Lettuce seeds respond to light at seed moisture content from 4-32% (Vertucci *et al.,* 1987). Gibberellins have been used to substitute for light in dark-imbibed, light-requiring seeds (Braun and Khan, 1975; Hilhorst and Karsen, 1988; Yoshioka *et al.,* 1998; Yamaguchi and Kamiya, 2002; Yamaguchi *et al*, 2004; Mollard *et al.*, 2007). Cold stratification is associated with enhanced *de novo* GA biosynthesis and sensitivity, and substitutes for light requirement in positively photoblastic lettuce genotypes (Lewak and Khan, 1977; van der Woude and Toole, 1980). In many species nitrate breaks dormancy (ISTA, 1985; Hilhorst and Karsen, 1988; AOSA, 1998; Alboresi *et al.,* 2005); Giba *et al.* (2003) reported that nitrate action is enhanced by light. Nitrates break down and donate nitric oxide (NO), a signaling molecule that plays an important role in seed dormancy loss (Bethke *et al.,* 2006a; 2006b).

Dormancy alleviation through afterripening could be due to an acquired capacity to degrade ABA during imbibition (Romagosa *et al.*, 2001; Debeaujon and Koornneef, 2000; Olszewski *et al.,* 2002; Yamaguchi and Kamiya, 2002; Yamaguchi *et al* 2004; Kucera *et al.,* 2005). ABA biosynthesis in imbibed seeds is associated with non-afterripened seeds (Schopfer

and Plachy, 1984, 1985; Leon-Kloosterziel *et al.,* 1996; Leung and Giraudat, 1998; Toorop *et al.*, 2000; Schmitz *et al.*, 2002; Leubner-Metzger 2003; Benech-Arnold *et al.,* 1999, 2006; Kucera *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006). Abscisic acid specifically inhibits endosperm rupture in most seeds as observed in lettuce (Argyris *et al.*, 2008), *Lepidium sativum* and *Arabidopsis thaliana* (Muller *et al.,* 2006). Embryo envelopes are effective in imposing dormancy (Jones, 1974; Watkins and Cantliffe, 1983; Groot and Karsen, 1992; Welbaum *et al.*, 1998; Sung *et al.,* 1998; Finch-Savage and Leubner-Metzger, 2006).

Although dormancy in native *Coreopsis* seeds can be partly modulated by dry afterripening (Norcini and Aldrich, 2007a), other abiotic factors are involved as well (Kabat *et al.*, 2007). To elucidate the roles of these factors, effects of temperature, light regimen, GA, nitrate, cold stratification and embryo envelopes on dormancy were examined during afterripening under dry conditions.

5.2 Materials and Methods

5.2.1 Plant Material

Previously described seed lots of *C. floridana* and *C. lanceolata* (Chapter 3, section 3.2.1.2) were used. When seeds were stored at 10^{o}C and 50% relative humidity seed moisture content ranged from 7 to 9% in both species. Experiments with *C. lanceolata* seeds began on 20 June 2007, and with *C. floridana,* on 1 December 2007. Germination tests were carried out bi-weekly in each species for 8 weeks in *C. floridana* and 12 weeks in *C. lanceolata*. Cold stratification and potassium-nitrate dormancy-breaking experiments were conducted after 60 days of dry storage of each species.

5.2.2 Germination Tests

A thermo-gradient table, Type db 5000 (Van dok and de Boer Machinefabriek BV, Enkhuizen, The Netherlands) provided temperatures of 10, 15, 20, 25 and 30^{o}C in continuous

light or dark. An Isotemp incubator model 304R (Fisher Scientific, Fair Lawn, NJ) set at 20°C in continuous light or dark was used for other assays.

Four replications of 25 seeds each were placed on two blue blotter papers (Anchor Paper Company, St. Paul, MN) moistened with de-ionized water in 5 cm glass Petri dishes. Dark conditions were achieved by wrapping Petri dishes with aluminum foil. Number of germinated seeds (radicle protrusion of 2 mm or greater) were recorded at 7 and 14 days. Intermediate dark-germination counts were made under a dim-green light (25W, A19, Specialty 90912; General Electric Company, Cleveland, OH).

5.2.3 Gibberellic Acid and Potassium Nitrate

Except for moistening blotter papers with 100 μM GA_{4+7} (Plant Protection Limited, Yalding, United Kingdom) or KNO_3 (Fisher Scientific Company, Fair Lawn, NJ) at 1, 10, 50 and 100 mM, germination tests were conducted at 20°C as described previously. At intermediate counts, the blotter papers were re-moistened with GA or the appropriate nitrate solution.

5.2.4 Cold Stratification

Seeds were sown in Petri dishes as in dark germination tests described previously, and placed in dark at 5°C in a Precision incubator (model 816; Precision Scientific Group, Chicago, IL). Four replicates of 25 seeds each were then transferred to 20°C in light or dark for germination tests. Germination tests were conducted every 7 days up to 42 days for *C. floridana* and up to 28 days for *C. lanceolata*. Germination evaluations were performed as described previously.

5.2.5 Removal of Seed Coverings

Pericarps, testa and endosperms of *C. floridana* and *C. lanceolata* seeds imbibed for 24 hours were excised from embryos using a dissecting microscope (Model, ASZ37L3, Bausch and

Lomb, Rochester, NY) and tweezers. Fifteen embryos per replicate and three replicates per treatment were used in germination tests. Intact seeds were used as controls.

5.2.6 Data Analysis

Data from each species was analyzed separately. Germination percentages were adjusted for viability based on pre-germination TZ tests. This correction was done for *C. lanceolata* only because *C. floridana* had 100% viable seeds. If necessary, an arcsine-square root transformation was done. Corrected data were analyzed using PROC GLM (SAS Version 9.1; SAS Institute, Cary, NC). Treatment means were separated using the least significant difference (LSD) test at $\alpha = 0.05$.

5.3 Results

5.3.1 Temperature and Light

At harvest, *C. floridana* seed germination in light was 100% at 10 to 25°C and declined to 83% at 30°C. In dark it was consistently about 20% at 10 to 20°C, dropped to about 10% at 25°C before it further declined to about 5% at 30°C (Figure 5-1).

In *C. lanceolata* seeds incubated at 10 to 25°C, germination progressively increased with increasing dry storage duration at each sampling time (Figure 5-2). Until seeds were totally non-dormant, that is fully afterripened, germination in light was greater than in dark. Germination was greatest at 15 and 20°C and peak germination (99%) in intact seeds was attained after 150 days of afterripening (Figures 5-3 and 5-4). At 30°C, germination was low in light and dark at all sampling times regardless of the degree of afterripening (Figures 5-3 and 5-4).

5.3.2 Gibberellic Acid, Potassium Nitrate and Cold Stratification

In intact dark-imbibed *C. floridana* seeds, GA totally overcame dormancy. In *C. lanceolata,* GA only marginally alleviated dormancy in light or dark (Figure 5-2). Potassium nitrate did not alleviate dormancy in dark-imbibed, 60-day afterripened *C. floridana* seeds, but

10 mM potassium nitrate promoted germination from 43 to 61% in light-imbibed *C. lanceolata* seeds (Figure 5-5); but 50 and 100 mM KNO_3 inhibited germination. Forty-two days of cold stratification in 60-day afterripened *C. floridana* seeds promoted germination from 20 to 60% in dark (Figure 5-6), but in *C. lanceolata*, cold stratification reduced germination in dark-imbibed, 60-day afterripened seeds (Figure 5-7).

5.3.3 Germination After Removal of Embryo Coverings

In *C. floridana* seeds imbibed and incubated in the dark, intact seeds had 20% germination, seeds without pericarp 50% germination, and naked embryos 100% germination (Figure 5-8); however in light, all three seed treatments had 100% germination. Germination in dark-imbibed *C. floridana* seeds did not improve as the afterripening period increased. After 30 days afterripening, *C. lanceolata* intact seeds had 26% germination in dark and 65% in light, seeds without pericarp germinated 37% in dark and 67% in light, and excised embryos had 90% germination in dark and 97% in light. Seeds of *C. lanceolata* imbibed intact lost dormancy after 150 days of afterripening, when pericarp plus testa were removed seeds lost dormancy in 90 days, whereas naked embryos were virtually non-dormant at harvest (Figure 5-9).

5.4 Discussion

When imbibed in light or dark, *C. lanceolata* seeds required 150 days dry afterripening for complete dormancy loss. *Coreopsis lanceolata* seeds afterripened in 180 days (Norcini and Aldrich, 2007a), and a similar period was reported for *C. basalis* (Carpenter and Ostmark, 1992). Differences in afterripening duration within a species could be caused by environmental conditions during seed maturation, storage conditions and genetics (El-Keblawy and Al-Ansari, 2000; Leubner-Metzger, 2002; Steadman *et al.,* 2003; Cristaudo *et al.*, 2007; Tarasoff *et al.*, 2007). Termination of dormancy was coincident with a loss of response to GA and light (Figure 5-2). Light effects on dormancy relief were dependent on afterripening period in *Polygonum*

aviculare seeds (Batlla and Benech-Arnold, 2005). Afterripening conditions used in this study were similar to those used by Shutz *et al.* (2002) in four *Asteraceae* species (*Millotia myosotidifolia, Podotheca gnaphalioides, P. chrysantha* and *Ursinia anthemoides*), and by Norcini and Aldrich (2007a) in *C. basalis, C. floridana, C. lanceolata* and *C. leavenworthii* seeds. Afterripening alleviates dormancy in other *Asteraceae* such as *Artermisia tridentate* (Meyer *et al.*, 1990) and *Tagetes minuta* (Karlson *et al.*, 2008).

Potassium nitrate (10 mM) diminished dormancy in partially afterripened (60 days afterripening) *C. lanceolata* seeds imbibed in light (Figure 5-5). Similarly, nitrates alleviated dormancy in *Arabidopsis* but the response to nitrate and light was dependent on afterripening duration (Giba *et al.*, 2003; Alboresi *et al.*, 2005; Ali-Rachedi *et al.*, 2004; Finch-Savage *et al.*, 2007). Although nitrate often alleviates dormancy (ISTA, 1985; AOSA, 1998), it only partially did so in non-afterripened *C. lanceolata* seeds. In the natural environment, nitrate sensing by seeds could be an adaptation for detecting water availability and/or soil fertility (Giba *et al.*, 2003). Since *C. lanceolata* is a colonizer, soil water and nutrient availability may indicate gaps in vegetation to insure seedling survival and rapid plant establishment in a competition-free microsite.

Cold stratification reduced germination in partially afterripened (60 days afterripening) *C. lanceolata* seeds (Figure 5-7). In previous experiments (Chapter 4), similar behavior was observed in seeds of four *C. lanceolata* accessions, *C. basalis* and *C. pubescens*. Cold stratification at 5^{o}C induced secondary dormancy in northern USA accessions of *C. lanceolata* seeds (Banovetz and Scheiner, 1994). Inhibition of germination under cold conditions is a probable mechanism to avoid germination during cold winters which are likely not favourable to plant establishment.

Removal of the pericarp, testa and endosperm in *C. floridana* seeds alleviated dormancy in dark. Intact seeds exhibited no dormancy in light (100% germination). In light-requiring lettuce genotypes, endosperm imposed dormancy in dark (Jones, 1974; Psaras *et al.,* 1981; Nijsse *et al.*, 1998), and endosperm enhanced dormancy in many *Asteraceae* (Leubner-Metzger, 2003; Finch-Savage and Leubner-Metzger, 2006). The most frequent ways by which primary dormancy is imposed are by the seed coat (physical) and embryo (physiological) dormancy (Baskin *et al.*, 2003; Leubner-Metzger, 2003; 2005; Ali-Rachedi *et al.*, 2004; Muller *et al.,* 2006; Donohue *et al.*, 2008). In *C. floridana* and *C. lanceolata* seeds imbibed at supraoptimal temperatures the endosperm imposed dormancy by acting as a physical barrier to radicle protrusion (Chapter 3). In many *Asteraceae* species, dormancy is alleviated by light (Karlson *et al.*, 2008). Although initial germination in dark-imbibed excised embryos was high, slight afterripening effects were observed (Figure 5-8), and this was consistent with reported seed behavior in *C. floridana* (Norcini and Aldrich, 2007a).

Exogenous GA relieved dormancy of dark-imbibed *C. floridana* seeds. Likewise GA relieved dormancy in light-requiring lettuce seeds (Braun and Khan, 1975; Psaras *et al.*, 1981; Vertucci *et al.,* 1987; Nijsse *et al.*, 1998; Yoshioka *et al.,* 1998). In light-requiring species, light is postulated to overcome dormancy by stimulating *de novo* GA biosynthesis (Shinomura *et al.*, 1994; Poppe and Schafer, 1997; Milberg *et al.,* 2000; Yamaguchi *et al.*, 2004; Leon and Owen, 2003) and enhancing embryo sensitivity to GA (Hilhorst and Karsen, 1988; Yamaguchi and Kamiya, 2002; Roth-Bejerano *et al.*, 1999; Leubner-Metzger, 2003).

Cold stratification of *C. floridana* seeds partially relieved dormancy when germinated in dark (Figure 5-6). Cold stratification also alleviated dormancy in other *Asteraceae* species, *Guizotia scabra*, *Parthenium hysterophorus* and *Verbesina encelioides* (Karlson *et al.,* 2008), in

Arabidopsis thaliana (Ali-Rachedi *et al.*, 2004; Finch-Savage *et al.*, 2007), as well as other genera (Noronha *et al.,* 1997; Leon and Owen, 2003; Leon *et al.,* 2006; 2007). Light-requirement for germination in some lettuce genotypes could be substituted for by cold stratification (Lewak and Khan, 1977; van der Woude and Toole, 1980). *Coreopsis floridana* flowers and produces seeds in fall, and ecologically, cold stratification is likely to promote germination when soil temperatures rise in spring, as occurs with temperate species (Bewley and Black, 1994). In *C. floridana* seeds, the extent of dormancy alleviation is dependent on cold stratification duration.

Potassium nitrate did not relieve dormancy of dark-imbibed *C. floridana* seeds. Nitrate is used to break dormancy in seeds of several species (ISTA, 1985; Hilhorst and Karsen, 1988; AOSA, 1998; Giba *et al.,* 2003; Alboresi *et al.*, 2005). Nitric oxide alleviated dormancy in light-requiring dark-imbibed lettuce seeds (Beligni and Lamattina, 2000). Nitrates break dormancy in *Arabidopsis thaliana* (Alboresi *et al.*, 2005; Bethke *et al*., 2006a, 2007) seeds probably by donating the NO molecule to the nitric oxide pathway (Javanovic *et al*., 2005; Bethke *et al*., 2006a, 2006b, 2007). The disparity in potassium nitrate effects between *C. floridana* and *C. lanceolata* may be attributed to natural habit differences. *Coreopsis floridana* seeds lack responsiveness to nitrate probably because water and nutrients are not likely to be limiting in their wetland habitat. Being an upland colonizer species, *C. lanceolata* seeds might be sensitive to nitrate as a means to detect gaps in vegetation where water and nutrients would not be limiting for seedling establishment.

Optimal germination temperatures were 15 and 20^{o}C in fresh and afterripened *C. floridana* and *C. lanceolata* seeds (ISTA, 1985; Carpenter and Ostmark, 1992; Banovetz and Scheiner, 1994; AOSA 1998). Germination of *C. floridana* seeds at elevated temperatures

(30°C) was promoted by light (Figure 5-1), but in *C. lanceolata* (at 25°C), afterripening duration dictated germination response (Figures 5-2, 5-3 and 5-4). Light-requiring seeds tolerate higher imbibition temperatures (Taylorson and Hendricks 1972; Shinomura *et al.,* 1994; Poppe and Schafer, 1997; Steadman, 2004). In earlier studies with *Coreopsis* seeds (Chapter 3), results suggested that pericarp, testa and endosperm inhibited germination at supraoptimal temperatures. Reduced germination at supraoptimal temperatures was associated with endosperm resistance in seeds of lettuce (Cantliffe *et al.*, 1984; Abeles, 1986; Sung *et al*, 1998; Nascimento *et al*, 2001; Leubner-Metzger, 2003; Gonai *et al.*, 2004) and *Arabidopsis thaliana* (Yoshioka *et al.,* 1998; Ramagosa *et al.*, 2001; Tamura *et al.*, 2006; Toh *et al.*, 2008). Although the two species had a common optima, germination at elevated temperatures seems to be species dependent and could be an ecological adaptation because *C. floridana* is a wetland and *C. lanceolata* an upland species.

Disparities in seed dormancy behavior between *C. floridana* and *C. lanceolata* might be related to differences in natural ecological habitats. In *C. floridana,* like in most wetland species, soil disturbance and exposure of seeds to adequate light is necessary for germination (Kettenring *et al*., 2006). *Coreopsis lanceolata* is an upland species that flowers in spring but seeds germinate in late summer and fall when soil temperatures are low, thus seeds may afterripen in the warm summer soil temperatures. Germination at optimal and elevated temperatures in *C. floridana* seeds is regulated by light, but in *C. lanceolata,* this is modulated by afterripening duration. Afterripening requirement of *C. lanceolata* seeds has been reported under different storage conditions (Norcini and Aldrich, 2007a), but this study demonstrated that these seeds can also be afterripened under low temperatures (10°C) at a seed moisture content of 8.1%. Removal of pericarp, testa and endosperm relieves dormancy in *C. floridana* and *C. lanceolata* seeds,

indicating that there is potential to manipulate embryo covers to overcome dormancy. Seed producers should have prior knowledge of afterripening requirements for *Coreopsis* seeds to enable them to devise marketing plans and deliver non-dormant seeds to end users.

5.5 Summary

Effects of temperature and light, embryo envelopes, potassium nitrate (KNO_3), gibberellic acid (GA_{4+7}) and cold stratification were examined to determine influence on dormancy alleviation during afterripening (dry storage). Seeds were produced in a greenhouse, harvested and bulked over 4 weeks, dried to 8.7% (*C. floridana*) and 8.1% (*C. lanceolata*) moisture content and stored in moisture-proof plastic bags in dark at 10°C. Freshly harvested *C. floridana* seeds germinated at 100% in light but only 20% germinated in dark. Gibberellic acid overcame dormancy in dark-imbibed seeds. Cold stratification for 42 days at 5°C partially overcame (60% germination) dormancy in dark-incubated seeds. Potassium nitrate did not affect germination in *C. floridana* seeds. Removal of pericarp in *C. floridana* led to 50% germination while naked embryos germinated 100% in the dark. In intact seeds of *C. lanceolata*, afterripening (dry storage) for 150 days relieved dormancy. Removal of endosperm overcame dormancy in fresh *C. lanceolata* seeds. Potassium nitrate (10 mM) partially overcame dormancy in 60-day afterripened *C. lanceolata* seeds, germination increasing to 61% from 43%. In *C. lanceolata*, afterripened seeds germinated normally and light or GA did not substitute for afterripening, whereas cold stratification enhanced dormancy. Light is required to break dormancy in *C. floridana* while in *C. lanceolata*, dry afterripening is paramount before germination can be optimized.

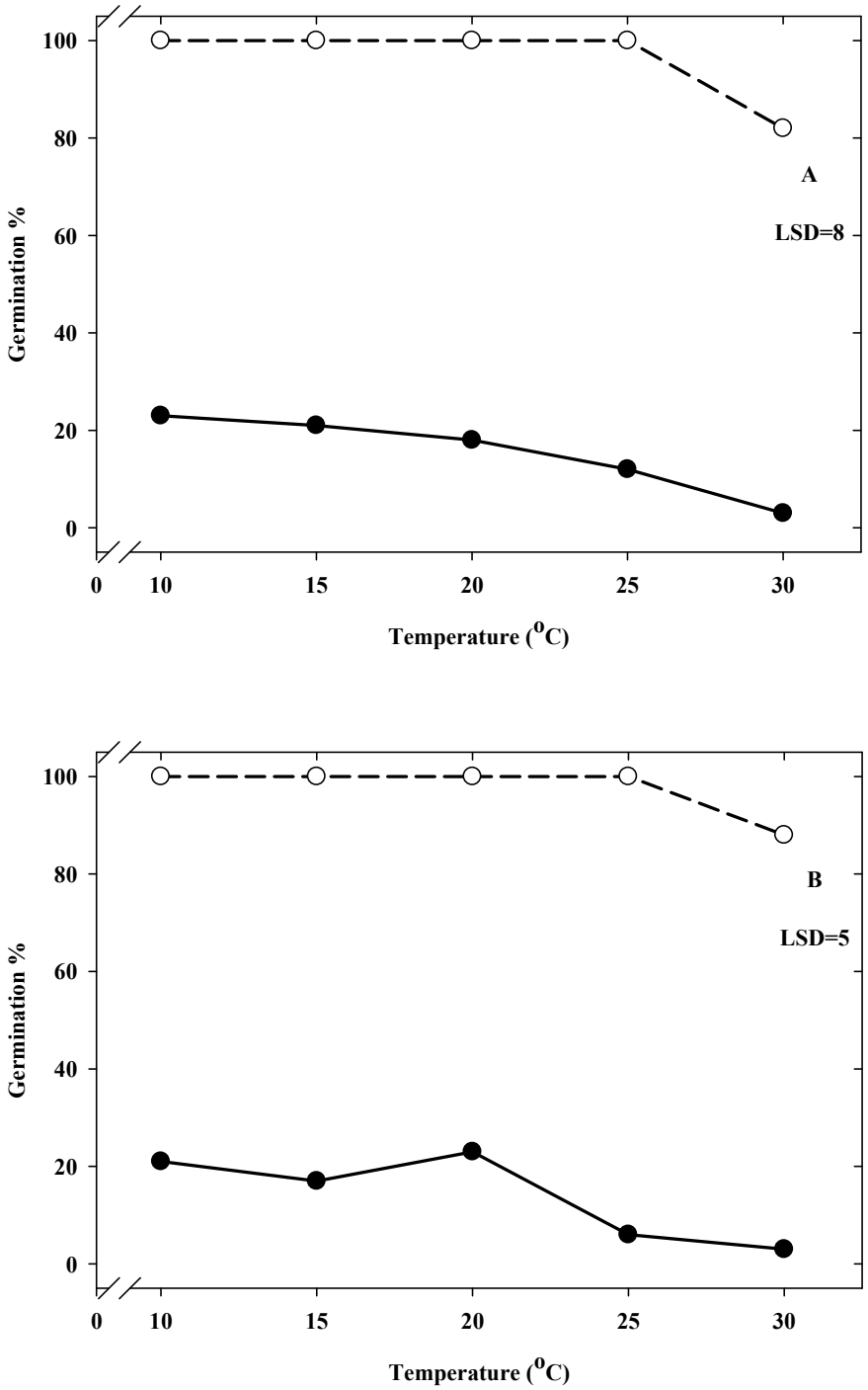


Figure 5-1. Effects of five incubation temperatures at two dry afterripening periods on germination in *C. floridana* seeds in light (broken lines) and dark (solid lines). A) At zero days afterripening. B) At 75 days afterripening. LSDs are as displayed in each graph.

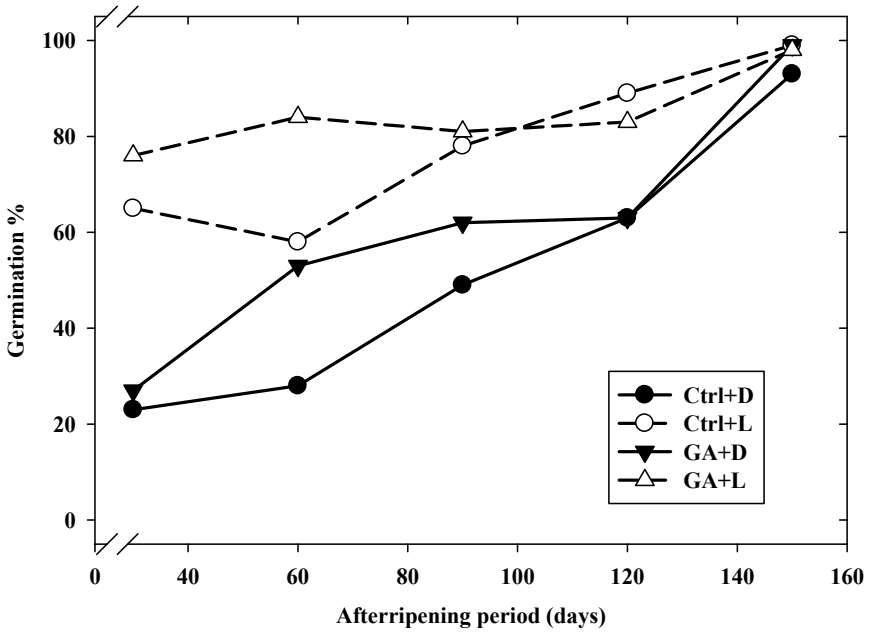


Figure 5-2. Progressive dormancy loss with increasing dry afterripening duration in *C. lanceolata* seeds. Seeds were imbibed in light or dark in water (control) or on GA substrate in light or dark. Germination tests were conducted at 20°C. L, light; D, dark; Ctrl, control; GA, gibberellic acid. LSD=12.

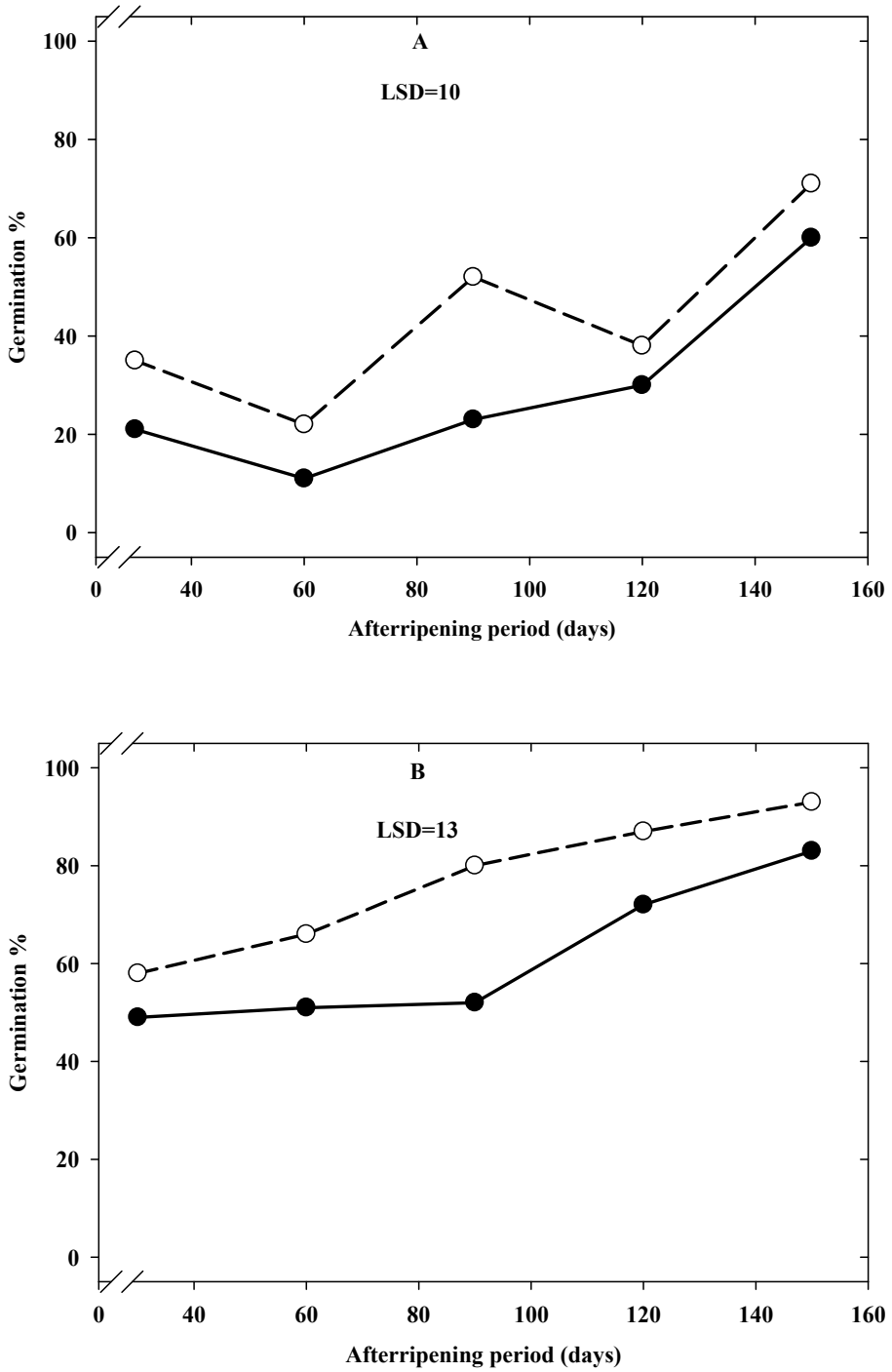
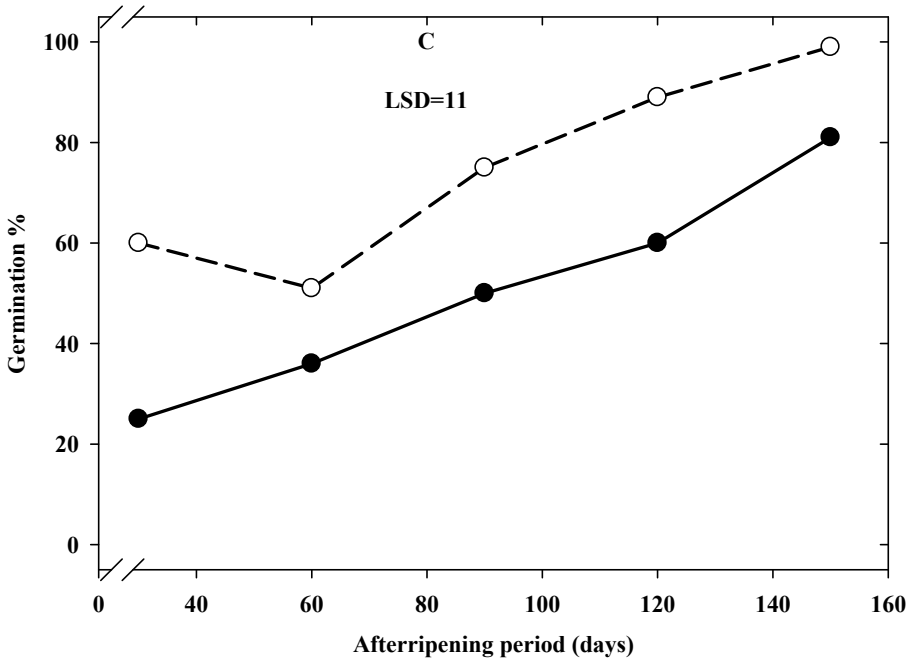


Figure 5-3. Effect of dry afterripening duration and imbibition temperature on germination in *C. lanceolata* seeds in light (broken lines) or dark (solid lines). A) 10°C. B) 15°C. C) 20°C. D) 25°C. E) 30°C. LSDs are displayed in each graph.



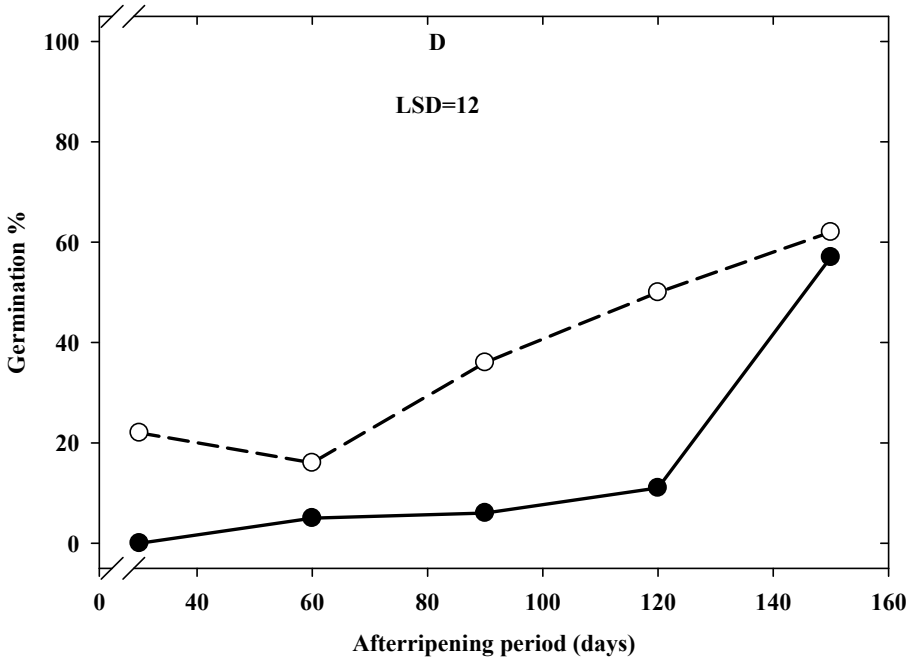


Figure 5-3. Continued

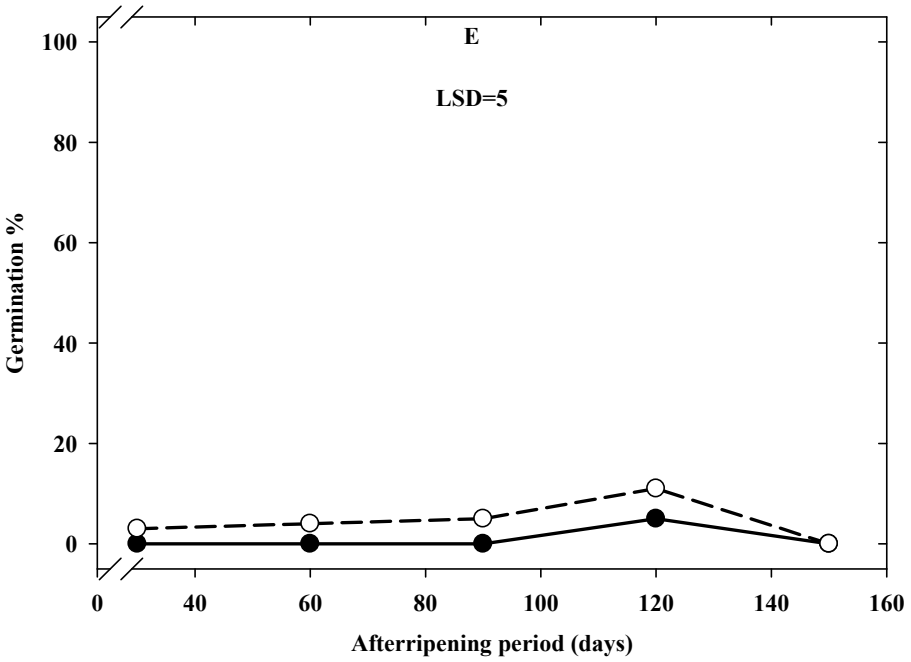


Figure 5-3. Continued

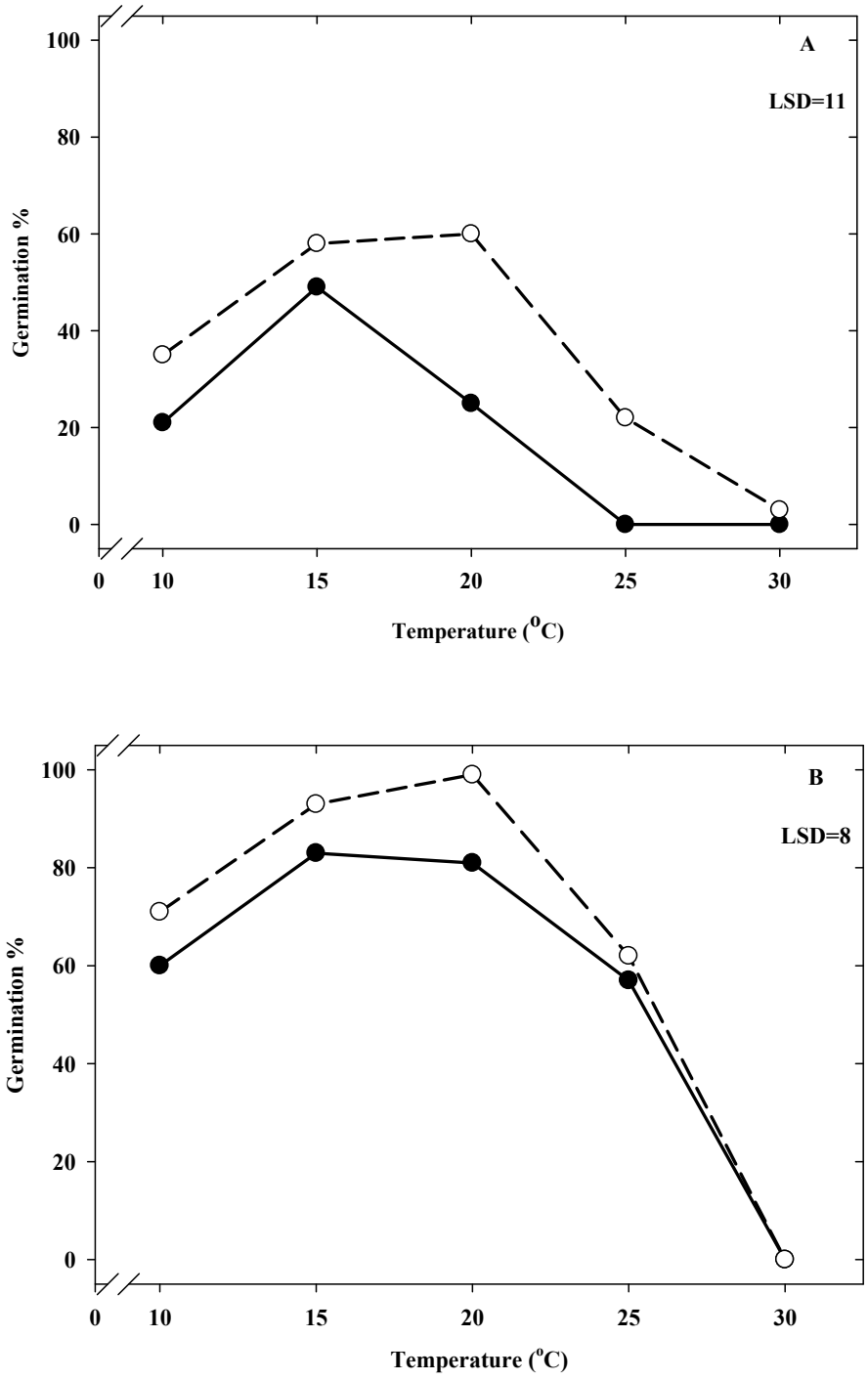


Figure 5-4. Influence of various incubation temperatures on dormancy at two dry afterripening periods in *C. lanceolata* seeds imbibed in light (broken lines) or dark (solid lines). A) After 30 days, and B) 150 days afterripening. LSDs are shown in each graph.

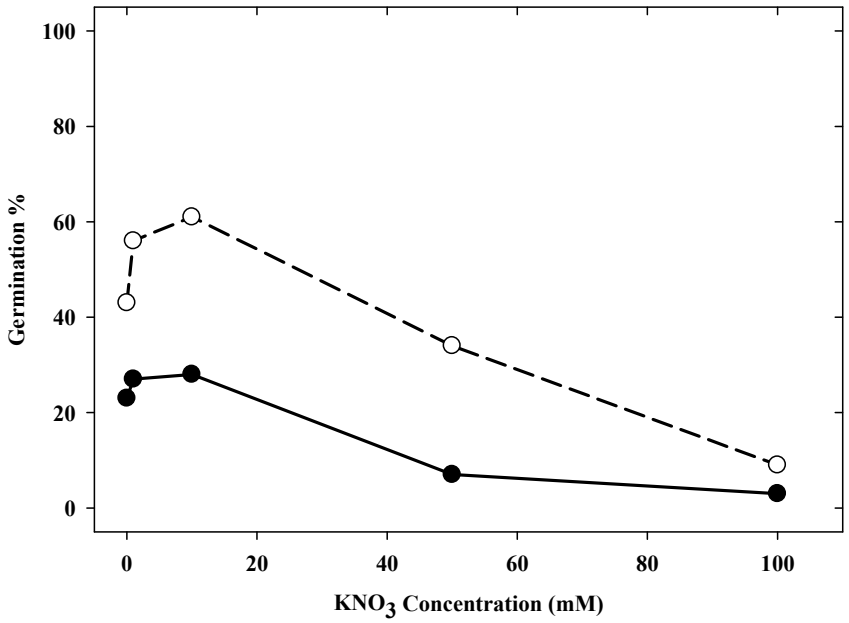


Figure 5-5. The effect of increasing concentrations of KNO_3 on dormancy relief in 60-day dry afterripened *C. lanceolata* seeds in light (broken line) and dark (solid line). Germination tests were conducted at 20°C. Non-germinated seeds were dormant. LSD = 10.

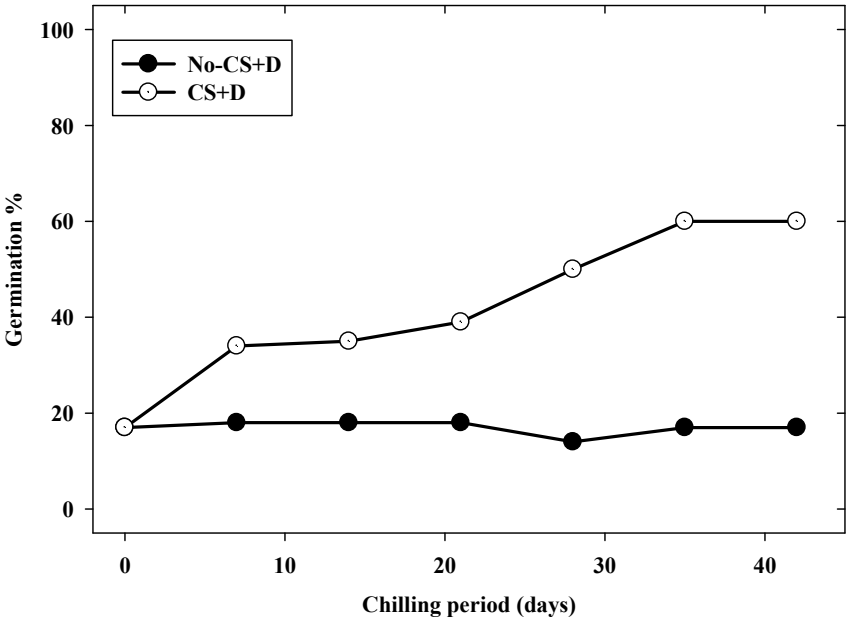


Figure 5-6. Germination response of 60-day dry afterripened *C. floridana* seeds after various cold stratification (5°C) durations in dark. Cold stratified and non-cold stratified seeds had 100% germination in light. CS, cold stratification; No-CS, no cold stratification; D, dark. LSD = 13.

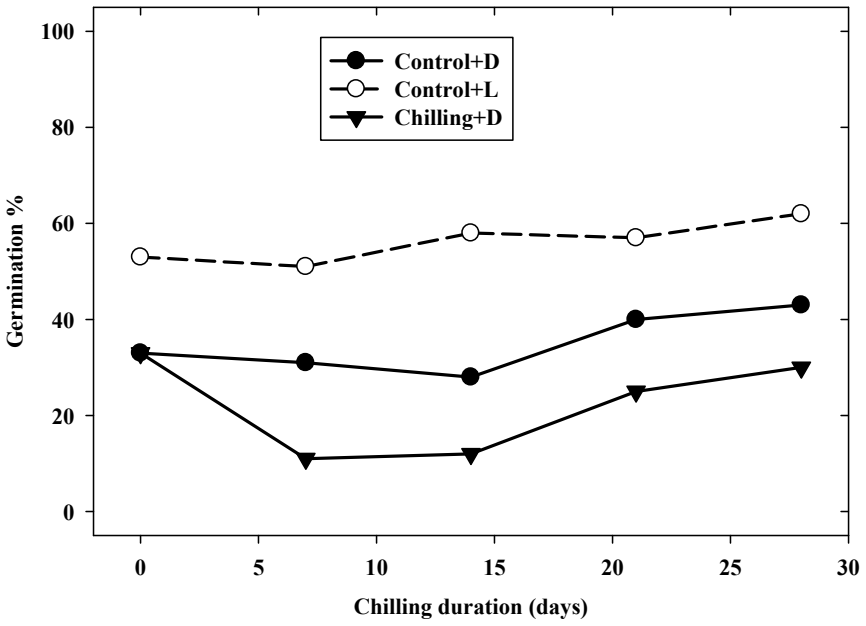


Figure 5-7. Cold stratification (5°C) effects on 60-day dry afterripened *C. lanceolata* seeds. Germination of non-chilled seeds in light (Control+L) or dark (Control+D); or chilled seeds in dark (Chilling+D). In chilled seeds incubated in light, total germination after 14 days was similar to light control but germination was slower. LSD = 11.

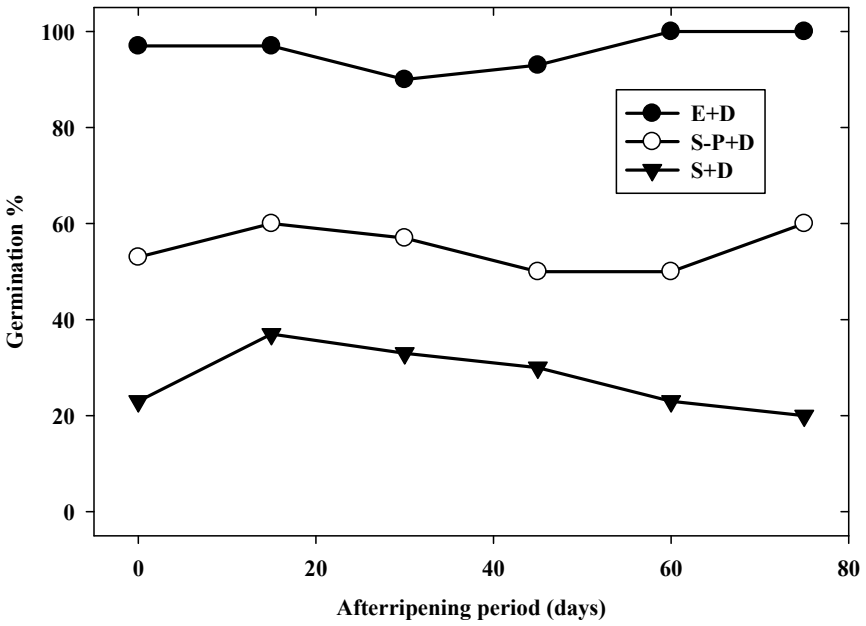


Figure 5-8. Influence of increasing dry afterripening duration on germination (at 20°C) of intact *C. floridana* seeds in dark; or when pericarp was removed in dark; or when naked embryos were imbibed in dark. In light, all treatments germinated to 100%. D, dark; S, intact seeds; P, pericarp, E, naked embryo. LSD = 14.

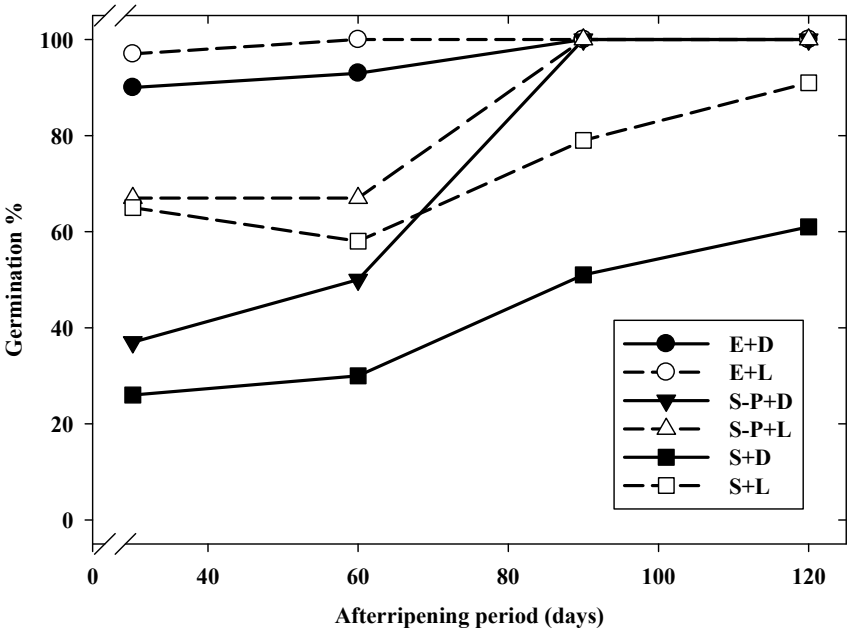


Figure 5-9. Influence of embryo envelopes on *C. lanceolata* seed germination at increasing dry afterripening periods of intact seeds imbibed in light or dark; or when pericarp was removed in light or dark; and in naked embryos in light or dark. Germination tests were conducted at 20°C. L, light; D, dark; S, intact seeds; P, pericarp, E, naked embryo. LSD = 15.

CHAPTER 6
SEED PRIMING ALLEVIATES DORMANCY IN *Coreopsis floridana* AND PERMITS GERMINATION AT HIGH TEMPERATURES IN *C. floridana* AND *C. lanceolata*

6.1 Introduction

Coreopsis floridana and *C. lanceolata* are important native wildflowers used in Florida for ecological restoration, reclamation, and beautification of roadsides and public parks. Some seeds are expensive if used in large quantities for roadside restoration ($110 and $220 per kilogram; Norcini and Aldrich, 2008), increasing seeding rates to achieve acceptable field establishment will increase costs for public use areas. Seeds of these native species remain dormant under many environmental conditions (Norcini and Aldrich, 2007; 2008) and germinate poorly at supraoptimal temperatures (thermoinhibition) found in the southeastern USA resulting in reduced stand establishment. In *C. floridana*, light overcomes dormancy and in *C. lanceolata* dry afterripening for 5 to 6 months is often required (Norcini and Aldrich, 2007a; Chapter 5). Optimal germination temperatures are 15 to 20^{o}C for both species (ISTA, 1985; Banovetz and Scheiner, 1994; AOSA, 1998). Daily soil temperatures at 10 cm depth exceed 30^{o}C during the summer in Florida (FAWN, 2008), which means that soil temperatures in the seed zone are likely much higher.

Seed priming is a technology that improves germination uniformity, seedling vigor and stand establishment under a variety of field environments (Cantliffe, 1981). Priming regulates seed hydration to permit pre-germinative biological activity, but stops prior to radicle emergence. Seeds are primed in various liquids (osmopriming or osmoconditioning) or in hydrated inert solid-matrix materials (solid matrix priming [SMP] or matriconditioning) (Cantliffe, 2003). Use of priming technology to mitigate dormancy and/or promote germination at elevated temperatures facilitates efficient sowing programs in restoration projects (Walmsley and Davy, 1997). While priming could increase costs per seed, stand establishment under diverse

environments is maximized and thus total seed costs many times are reduced (Cantliffe, 1981; Cantliffe *et al*., 1984; Cantliffe and Abebe, 1993; Cantliffe, 2003).

From anatomical studies, *C. floridana* and *C. lanceolata* seeds consist of an outer dry pericarp, testa and a single cell-layer of endosperm surrounding an embryo (Chapter 3). Lettuce seeds have similar anatomy (Borthwick and Robbins, 1928; Jones, 1974; Psaras *et al*. 1981; Nijsse *et al*., 1998). In previous studies (Chapter 3), thermoinhibition in *C. floridana* and *C. lanceolata* seeds was alleviated by removal of endosperm. Additionally, removal of these same tissues relieved dormancy in dark-imbibed *C. floridana* seeds. Lettuce (same family as *Coreopsis*) seeds exhibited thermoinhibition (Cantliffe *et al*., 1984; Abeles, 1986; Sung *et al*, 1998; Nascimento *et al*, 2000; 2001, 2005; Leubner-Metzger, 2003; Gonai *et al*., 2004), and endosperm removal relieved this problem (Guedes *et al*., 1981; Psaras *et al.,* 1981; Dutta *et al.,* 1997; Sung *et al*., 1998; Nonogaki and Morohashi, 1999; Leubner-Metzger, 2003; Nascimento *et al*, 2005). Endosperm removal alleviates dormancy in dark-imbibed light-requiring lettuce seeds (Jones, 1974; Psaras *et al.,* 1981; Nijsse *et al*., 1998). Priming overcame thermoinhibition in seeds of lettuce (Guedes and Cantliffe, 1980; Cantliffe *et al*., 1984; Cantliffe, 1991; Sung *et al*., 1998; Nascimento and Cantliffe, 1998; Nascimento *et al*, 2000, 2001; Nascimento, 2003), and *Tagetes minuta* (Taylor, 2007). Priming induces endosperm weakening in lettuce seeds (Sung *et al*, 1998; Nascimento *et al*, 2000; 2001, 2005), before irreversible cell elongation and division occur (Cantliffe *et al*., 1984).

Since removal of tissues enveloping the embryo is not economically practical, seed priming techniques that weaken embryo envelopes and/or promote embryo growth might be utilized to overcome dormancy and hasten germination of *Coreopsis* seeds. This study explored

the possible beneficial effects of osmopriming and SMP techniques in alleviating dormancy of *C. floridana* and for circumventing thermoinhibition of both *C. floridana* and *C. lanceolata* seeds.

6.2 Materials and Methods

6.2.1 Plant Material

The same seed lots of *C. floridana* and *C. lanceolata* as described in Chapter 5, section 5.2.1 were used. Seed priming experiments for both species were conducted in May and June 2008.

6.2.2 Osmotic Priming Protocol

Seed samples (0.5 g) were primed in 30 ml of priming solution in 50 ml test tubes in a 30% (wt:vol) aerated solution of polyethylene glycol 8000 (PEG) (Fisher Scientific, Fair Lawn, NJ) (-1.2 Mpa) for 3, 4, 5, 6 or 7 days at 15°C with constant cool white fluorescent light (10 $\mu mol \bullet m^{-2} \bullet s^{-1}$). Seeds were primed in PEG only, PEG plus gibberellic acid (GA_{4+7}) (Plant Protection Limited, Yalding, United Kingdom), or PEG plus 6-benzyladenine (BA) (Sigma Chemical Company, St. Louis, MO). Gibberellic acid and BA were added at 100 mg/liter PEG priming solution. A Tetratec aquarium pump (Tetra Sales, Blacksburg, VA) provided aeration. Air from the aquarium pump was hydrated by bubbling through de-ionized water to minimize evaporation from the priming solution. Primed seeds were washed thoroughly in de-ionized water, damp dried on paper towel, and placed on raised wire-gauze in a container for further drying at 15°C and 50% RH for 3 days.

6.2.3 Solid Matrix Priming (SMP) Protocol

Emathlite clay (0.5 g, Cat Litter, Publix Supermarkets Inc. Lakeland, FL) was mixed with 0.25 g seeds and various volumes (0.2, 0.5, 1.5 or 2.5 ml) of distilled water, 100 mg BA/liter water, or 100 mg GA/liter water in clear plastic bottles. Seeds were primed at 15°C for 4 days under white light (10 $\mu mol \bullet m^{-2} \bullet s^{-1}$) on a vertically rotating (1 cycle/5 minutes) wheel (Lab-line

Instruments, Inc., Melrose Park, IL). Bottles were held in horizontal slots on the rotating wheel to ensure adequate light exposure. Primed seeds were washed and dried as described previously.

6.2.4 Germination Tests

Constant temperatures of 20 or 30°C were provided in an Isotemp incubator (model 304R, Fisher Scientific, Fair Lawn, NJ) in continuous dark. Darkness was achieved by wrapping Petri dishes with aluminum foil and maintaining darkness in the incubator. Four replications of 25 seeds were placed on two blue blotter papers (Anchor Paper Company, St. Paul, MN) moistened with de-ionized water in 5 cm glass Petri dishes. Germination was recorded daily for 14 days. Intermediate dark-germination counts were made under a green light (25W, A19, Specialty 90912, General Electric Company, Cleveland, OH) in a dark room. Germination was defined as 2 mm protrusion of the radicle.

6.2.5 Statistical Analysis

Germination data were analyzed separately for each species. Data were adjusted for viability as determined by a TZ test (ISTA 2003). If necessary data were arcsine-square root transformed and analyzed by general linear model methods (SAS Version 9.1; SAS Institute, Cary, NC). Treatment means were separated by the least significant difference (LSD; α=0.05) test. Mean germination time (MGT, average time to total germination) was computed using Seed Calculator Version 3.0 (Plant Research International B.V., Wageningen, The Netherlands).

6.3 Results and Discussion

6.3.1 *Coreopsis floridana*

Seed priming for 6 to 7 days in PEG improved germination (23 to 63% after 7 days) of *C. floridana* in dark at 20°C (Figure 6-1). However, germination at 30°C in dark was not improved by PEG priming (Figure 6-1). Non-primed seeds when imbibed in light at 20°C had 100% germination, and at 30°C germination was 67%. In dark, non-primed seeds had 23%

germination at 20°C and 12% at 30°C (Figure 6-1). In celery (*Apium graveolens*), the cultivar 'Earlybelle' failed to germinate at supraoptimal temperatures after priming in PEG alone (Tanne and Cantliffe, 1989).

Adding GA to the PEG (100 mg GA/L PEG) priming solution promoted germination at 20°C from 23 to 67% after 3 days of priming, and from 12 to 31% at 30°C after 4 days priming (Figure 6-2). Increasing priming duration past 3 days did not improve germination at 20°C, but at 30°C germination was promoted. Priming celery seeds in PEG plus GA_{4+7} improved germination in dark and at supraoptimal temperatures (Tanne and Cantliffe, 1989), and in dark-imbibed lettuce seeds, GA stimulated hypocotyl elongation and cotyledon expansion (Ikuma and Thimann, 1963), thereby overcoming the restricting endosperm.

When *C. floridana* seeds were primed in PEG plus BA (100 mg BA/L PEG), germination was 100% after 3 days priming at both 20 and 30°C in dark (Figure 6-3). Priming duration past 3 days was not necessary. Priming seeds in various concentrations of BA at 25, 50, 75 and 100 mg BA/L PEG for 4 days led to 100% germination when seeds were imbibed and germinated at 20 or 30°C in dark. Mean germination time (MGT) was reduced from 4.6 days at 20°C to 3.7 days at 30°C for non primed seeds, to less than 2 days at both temperatures when seeds were primed in PEG+BA (Figure 6-4). Increasing BA concentration from 25 to 100 mg/liter PEG had no influence on further reduction of MGT. Less than 5% of *C. floridana* seeds germinated with cotyledons protruding first (abnormal). This has been common in other species. Benzyladenine promoted dark-germination of other *Asteraceae* seeds such as *Bidens pilosa* (Valio *et al*., 1972). When BA was added to PEG priming solution, celery seeds germinated in dark and at supraoptimal temperatures (Tanne and Cantliffe, 1989), while in lettuce seeds, a similar priming treatment promoted germination at supraoptimal temperatures (Cantliffe, 1991). In the case of

lettuce, at high BA concentrations cotyledons expanded and ruptured endosperm first followed by radicle protrusion. Due to its chemical composition and structure (N^6- substituted aminopurine), synthetic BA used in these experiments mimics cytokinins (aminopurine derivatives); cytokinins regulate cell cycle (Taiz and Zeiger, 2002), and promote cell division and expansion (Pozsar and Matolcsy, 1968; Sussex *et al.,* 1975; Khan, 1971; Taiz and Zeiger, 2002). It is noteworthy that BA has been reported to occur naturally in some plant species as an aromatic cytokinin (Sakakibara, 2006; Strnad, 1997). Kinetin stimulates cotyledon expansion in imbibed lettuce seeds (Ikuma and Thimann, 1963).

Solid matrix priming with BA and osmopriming in PEG+BA both improved germination of *C. floridana* seeds in dark at both 20 and 30°C (Figures 6-3 and 6-5). When using GA, however, *C. floridana* seeds had 85% germination at 20°C in dark when solid matrix primed, and 65% germination when osmoprimed (Figure 6-2 and 6-5). Germination was 71% at 30°C after SMP plus GA and 31% after osmopriming plus GA. Increasing the volume of BA or GA priming solution in SMP reduced MGT when seeds were primed for 4 days (Figure 6-6). At both 20 and 30°C, MGT was similar for 0.5 to 2.5 ml BA priming solution, but when using GA, 0.5 ml had the shortest MGT at 20°C while at 30°C it was 1.5 ml. Priming celery seeds using SMP overcame thermoinhibition (Parera *et al*., 1993).

6.3.2 *Coreopsis lanceolata*

In *C. lanceolata* seeds, priming in 100 mg BA/L PEG for 5, 6 or 7 days, produced the same final germination (~86%) at 30°C, but the MGT was shortest at the longest priming duration (Figure 6-7). When imbibed at 20°C, non-primed *C. lanceolata* seeds germinated 98% but at 30°C only 2% germinated. Optimizing seed priming factors leads to rapid uniform germination and seedling emergence in *C. lanceolata*. When other seed priming conditions were

held constant, priming effect was determined by priming duration in different lettuce cultivars (Cantliffe, 1981).

Seed priming technologies have potential to enhance native *Coreopsis* seed germination under diverse environmental conditions, and this study is the first to report success in priming *C. floridana* and *C. lanceolata* seeds. To overcome dormancy, and promote maximum germination and seedling uniformity in *C. floridana*, it is recommended that seed producers and sellers adopt seed priming technology for the benefit of end users. Primed seeds of *C. floridana* and *C. lanceolata* will also produce maximal germination at high soil temperatures. Using SMP with emathlite clay and BA at a ratio of 1:1:0.5 (clay [weight]:BA [volume]:seed [weight]) produced better results, and is recommended. This SMP method will be sustainable because the clay is easy to separate from seeds and is locally available, and disposal may not be an environmental hazard. Use of primed *Coreopsis* seeds maximizes genetic diversity by permitting most seeds to germinate, and would facilitate successful sowing programs even during the hot Florida summers. The goal of this research is to identify priming methods that result in acceptable field establishment, thus promoting widespread use of these native wildflowers in revegetation of roadsides and ecological restoration sites.

6.4 Summary

Germination of the wildflowers *C. floridana* and *C. lanceolata* is erratic due to seed dormancy. *Coreopsis floridana* seeds do not germinate in dark, and both species have reduced germination above 25^{o}C. Osmopriming and SMP techniques were used to circumvent these problems. Seeds of both species were produced in a passively ventilated greenhouse, harvested and cleaned before storage in moisture-proof bags in darkness at 10^{o}C for 6 months (*C. floridana*) or 12 months (*C. lanceolata*). Osmopriming was accomplished using PEG-8000 at a water potential -1.2 Mpa. Emathlite clay was used for SMP. Gibberellic acid (GA_{4+7}) or BA

were added at 100 mg/liter PEG to some osmopriming treatments, while 100 mg GA or BA per liter water were added to emathlite in SMP to evaluate possible enhancement of the priming effect. *Coreopsis floridana* seeds primed in PEG improved germination from 23 to 63% at 20°C but at 30°C germination only increased from 12 to 33%. When BA was added to PEG, 100% germination was promoted at both 20 and 30°C, but addition of GA to PEG priming solution promoted germination from 23 to 67% at 20°C and from 12 to 31% at 30°C in *C. floridana* seeds imbibed in dark. SMP of *C. floridana* seeds with BA led to similar positive effects on germination as osmopriming, while SMP seeds with GA had 85% germination, compared to osmoprimed plus GA which led to 63% germination. Using SMP and BA at a ratio of 1:1:0.5 (clay [weight]:BA [volume]:seed [weight]) produced the best results. In *C. lanceolata*, osmopriming with BA increased germination percentage from 2 to 86% at 30°C. Priming reduced the MGT in both species. In *C. floridana* and *C. lanceolata,* seed priming can maximize germination and thus improve stand establishment under diverse environments.

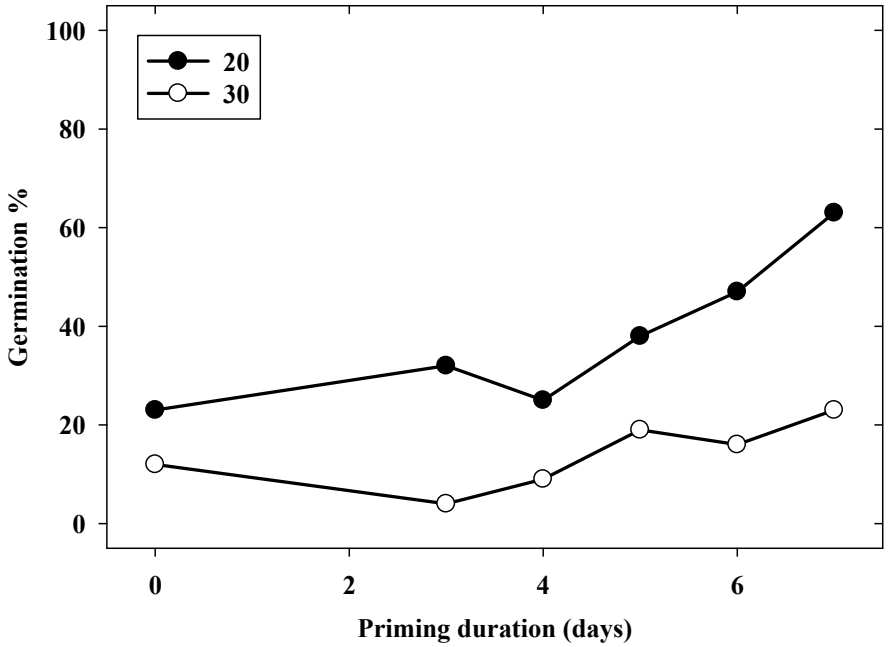


Figure 6-1. Germination of *C. floridana* seeds in dark after priming in PEG for 3, 4, 5, 6 or 7 days in light. Germination tests were conducted at 20°C (LSD = 20) or 30°C (LSD = 9) for 14 days.

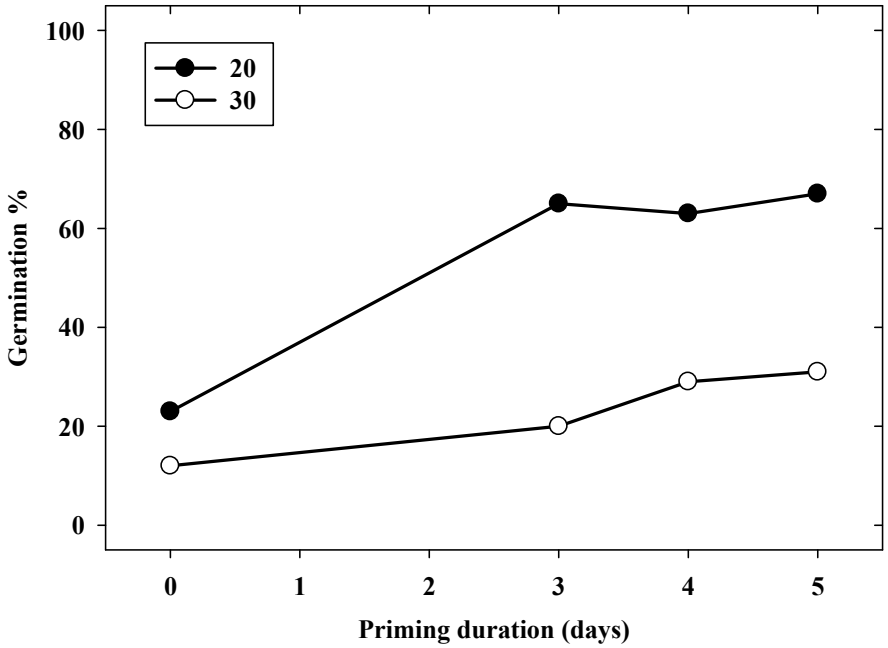


Figure 6-2. Germination of *C. floridana* seeds in dark at 20°C (LSD = 20) or 30°C (LSD = 9) after priming in 100 mg GA_{4+7}/L PEG for 3, 4 or 5 days in light. Non-primed seeds are denoted by zero days priming duration. Non-primed seeds imbibed on 100 mg GA_{4+7}/L water substrate led to 97% germination at 20°C and 68% at 30°C. Germination test duration was 14 days.

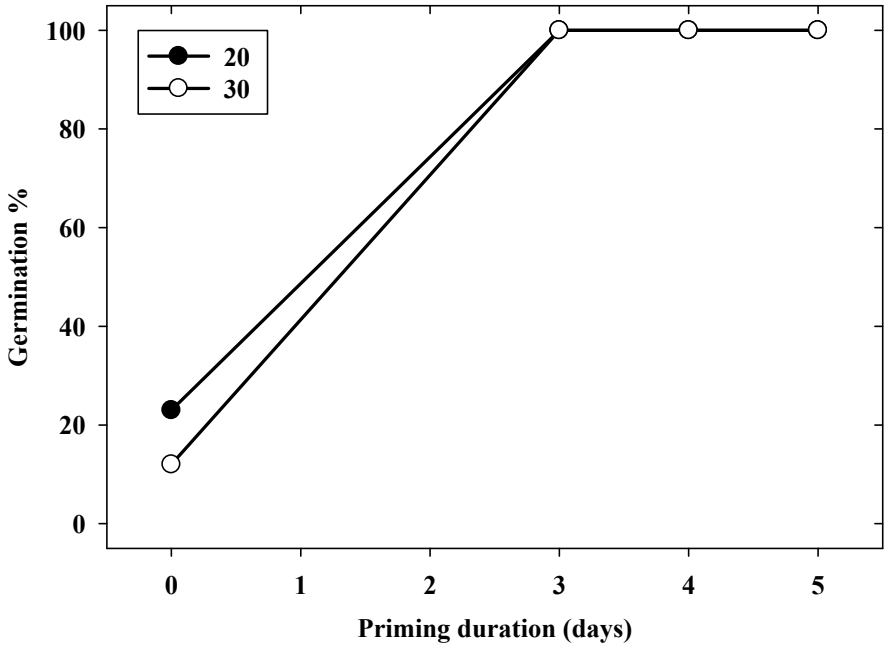


Figure 6-3. Germination of *C. floridana* seeds in dark at 20 or 30°C after priming in 100 mg BA/L PEG for 3, 4 or 5 days in light. Non-primed seeds are denoted by zero days priming duration. LSD = 9. Non-primed seeds imbibed on 100 mg BA/L water substrate had 54% germination at 20°C and 34% at 30°C. Test duration was 14 days.

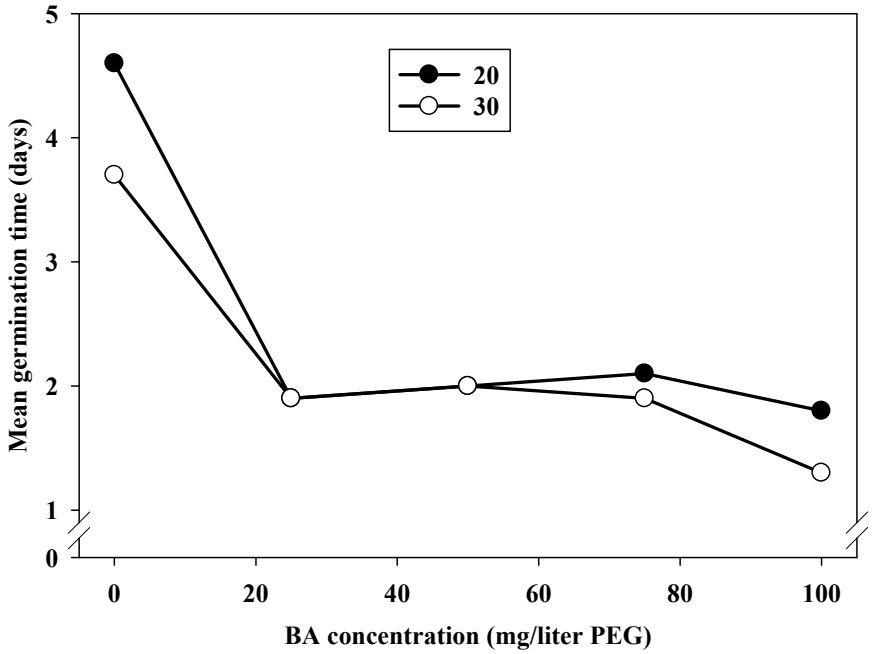


Figure 6-4. Mean germination time (MGT) in *C. floridana* seeds in dark after osmopriming in PEG plus various BA concentrations for 4 days in light. LSD = 1.0. BA treatments germinated 100%, and non-primed seeds 23% at 20°C and 12% at 30°C. Germination tests were conducted for 14 days.

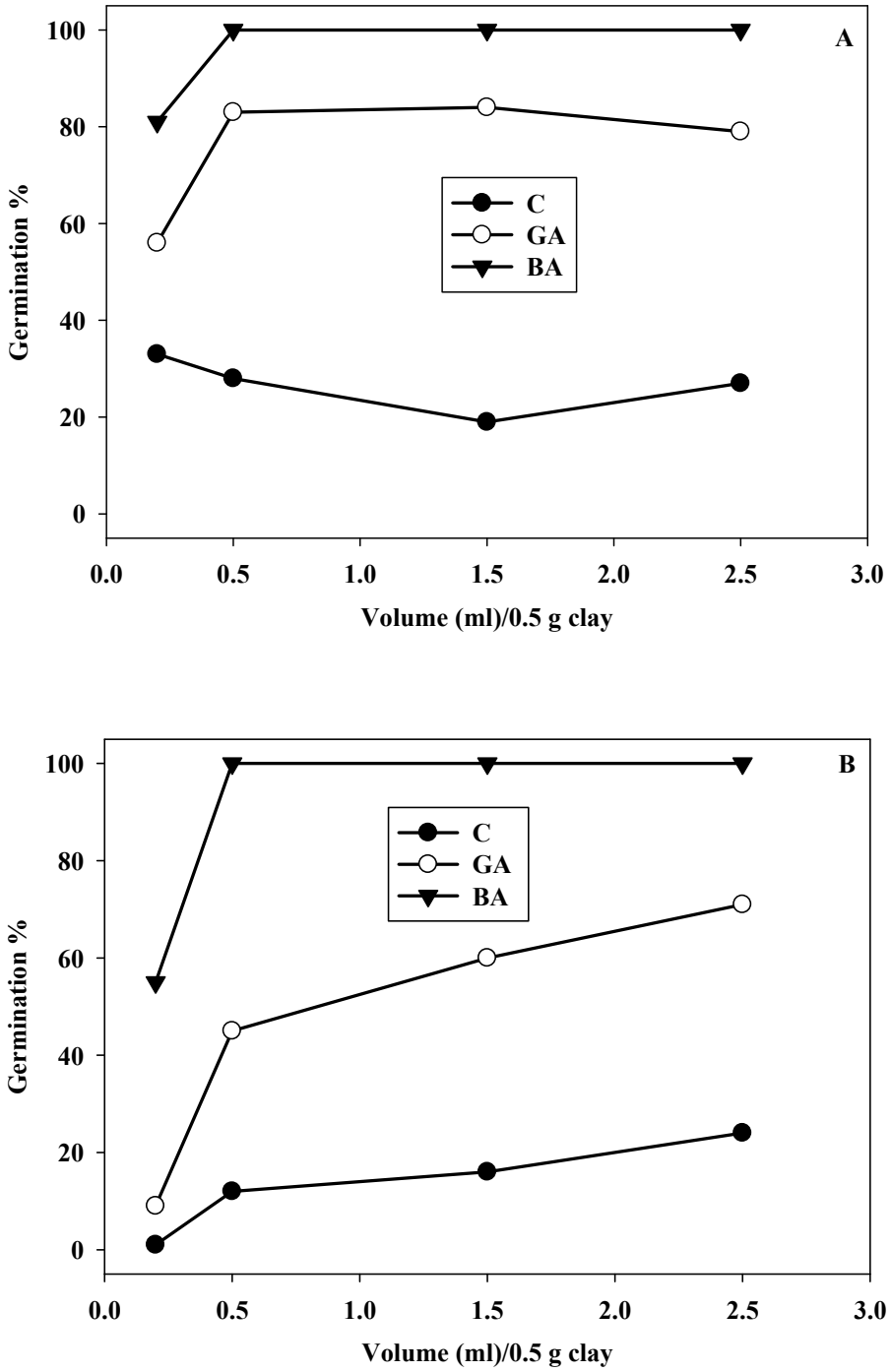


Figure 6-5. Germination of *C. floridana* seeds in dark after 4 days of SMP in various volumes of solutions in light. A) Germination at 20°C, LSD = 19. Non-primed seeds had 23% germination at 20°C. B) Germination at 30°C, LSD = 22. Non-primed seeds had 12% germination at 30°C. SMP was conducted with emathlite clay plus 100 mg BA/L water (BA) or 100 mg GA/L water (GA), or water control (C). Regardless of volume, BA or GA concentrations were maintained the same. Germination test duration was 14 days.

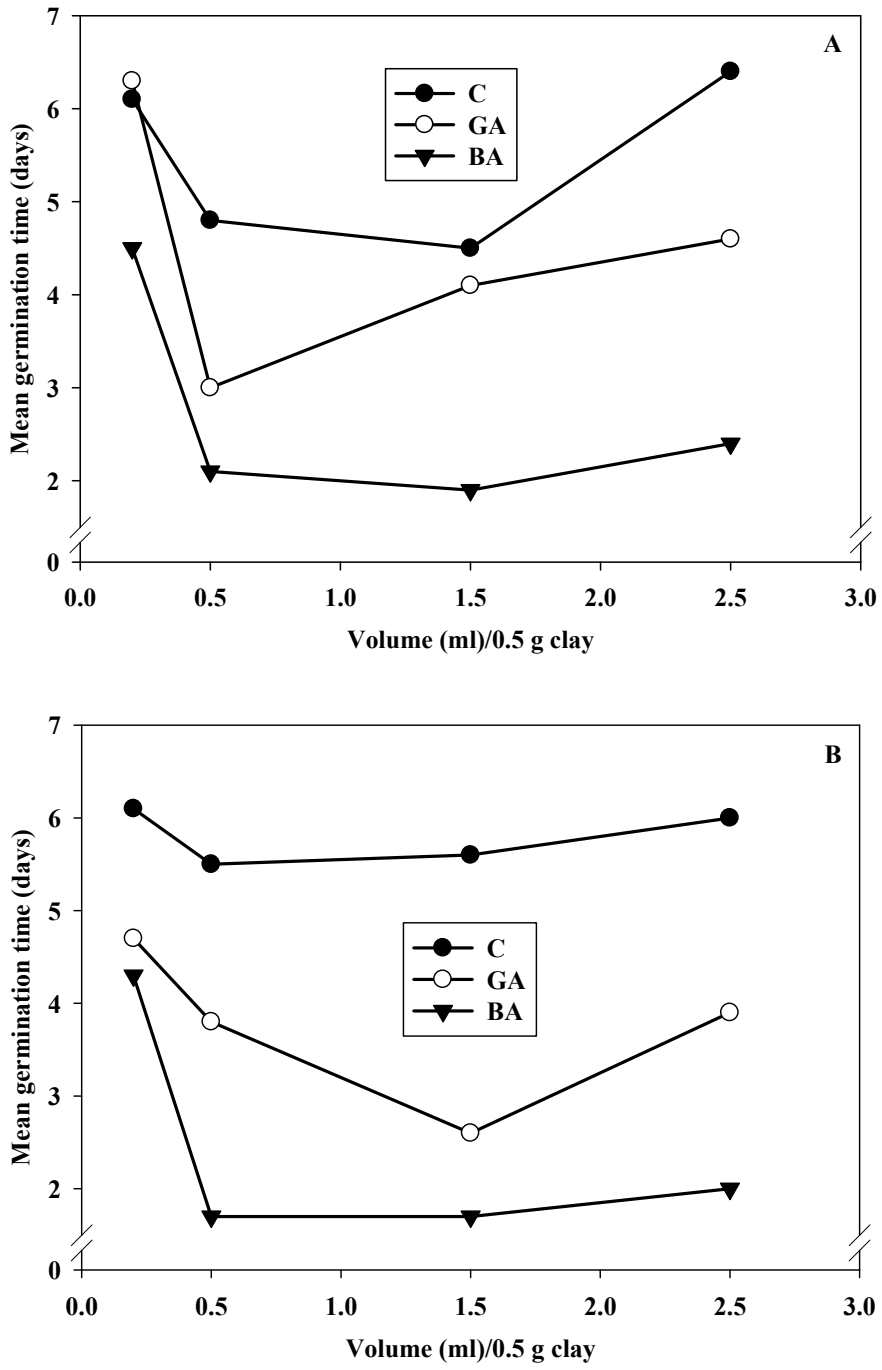


Figure 6-6. Mean germination time (MGT) of *C. floridana* seeds in dark after SMP in clay (0.5 g) with various volumes of benzyladenine (BA, 100 mg/L water) or gibberellic acid (GA, 100 mg/L water) or water control (C) in light. Regardless of volume, BA or GA concentrations were maintained the same. A) Germination at 20°C, LSD = 1.1. Non-primed seeds had a MGT of 7.2 days at 20°C. B) Germination at 30°C, LSD = 2.5. Non-primed seeds had a MGT of 6.3 days at 30°C. Germination test duration was 14 days.

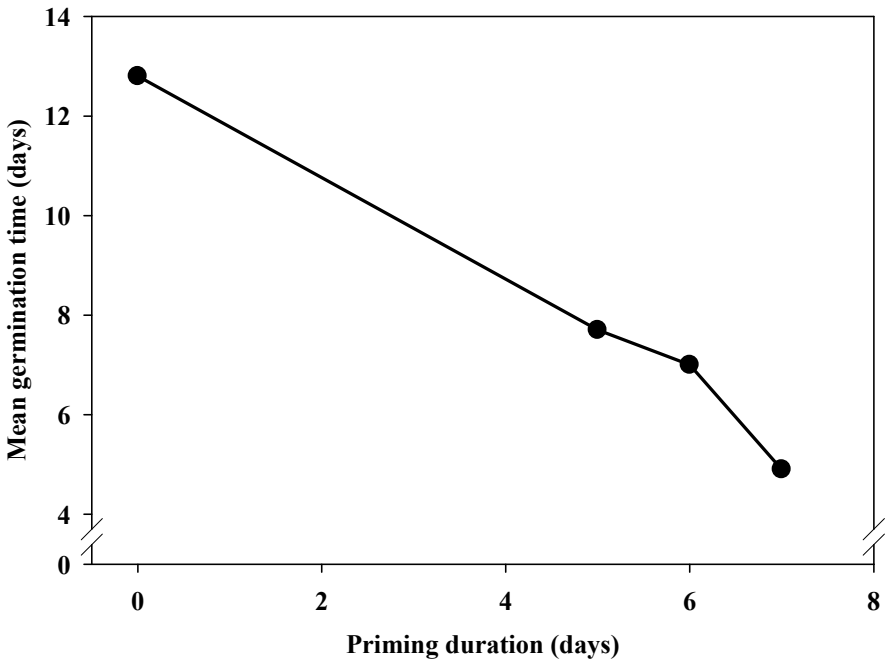


Figure 6-7. Mean germination time (MGT) of *C. lanceolata* seeds in dark at 30°C after osmopriming in PEG plus BA (100 mg/L PEG) for various durations in light. LSD = 1.1. All BA priming treatments had 86% final germination and non-primed seeds had 2%. Germination tests were conducted for 14 days.

CHAPTER 7
CONCLUSION

In Florida, there is growing demand for premium value, pre-variety germplasm of locally or regionally specific ecotype seeds of native *Coreopsis* (*Asteraceae*) for use in ecological restoration, reclamation and along roadsides. Demand of this seed has led to a concomitant rise in seed production; however, unlike seeds of domesticated crops, moderate to substantial dormancy is common among seeds of pre-variety germplasm of native flowers. Seed dormancy continues to present a major challenge to seed producers, testing agencies and end users, because it affects seed quality assessment and therefore value to producers and the amount purchased by end users (Norcini and Aldrich, 2008).

Coreopsis species, commonly known as tickseed, have a wide distribution range from North to South America (Smith, 1975; Tadesse *et al.*, 1995). There are 28 *Coreopsis* species native to the U.S. (USDA-NRCS, 2007), and ecotype seeds of at least eight species are being commercially produced (Norcini and Aldrich, 2007a). Dormancy in cultivated populations is variable within and among, freshly harvested or stored pre-variety seed lots of native *Coreopsis* (Kabat *et al*., 2007; Norcini and Aldrich, 2007a; Norcini *et al*., 2004, 2006). To gain insight about seed dormancy in pre-variety germplasm of native *Coreopsis* species, anatomical and physiological studies were conducted.

Coreopsis seeds (achenes) were comprised of a dicotyledonous embryo enclosed by an outer pericarp, testa and endosperm (Chapter 3). The ploidy ratio of endosperm to embryo was 3:2 as determined by DNA fluorescence techniques. While this is consistent with other angiosperms, this has not been reported elsewhere for *Coreopsis*. In *C. floridana* and *C. lanceolata* the lateral endosperm was a single cell-layer, but micropylar endosperm was 2-3 cell-layers thick in *C. lanceolata*. This is the first such report detailing the anatomy of *Coreopsis*

seeds, and paves way for future research in seed physiology of this species. Germination results showed that pericarp, testa and endosperm modulated dormancy of *C. floridana* and *C. lanceolata*, and especially so at elevated temperatures. In dark-imbibed *C. floridana* seeds, the same tissues imposed dormancy (Chapters 3 and 5).

The different germination behavior between the two species might be linked to their natural ecological habitat. *Coreopsis floridana* and *C. lanceolata* germination requirements were consistent with those of wetland and upland species, respectively. Dormancy is important for the survival of some plant species because it enables them to evade harsh and unsuitable environmental conditions which do not support seedling growth and establishment. Intact seeds of *C. floridana* tolerated germination at higher temperatures than *C. lanceolata* seeds, a possible adaptation for wetland species. These results on anatomy and germination are important for devising pre-germination seed treatments that weaken embryo envelopes for overcoming dormancy.

In *C. lanceolata* seeds, EBM activity was associated with germination, and activity was detected at 90 hours imbibition while germination occurred at 96 hours (Chapter 3). Conditions not favorable to germination such as abscisic acid (ABA), tetcyclacis (gibberellic acid [GA] biosynthesis inhibitor) and high imbibition temperature (30^{o}C) inhibited this enzyme's activity. *Coreopsis floridana* seeds did not exhibit EBM activity at any time throughout germination, implying that another system might be involved in endosperm digestion during germination in this species, however, this was not explored further. This is the first report of EBM activity in *C. lanceolata* seeds, and this mechanism may be important to circumventing dormancy in non-afterripened *C. lanceolata* seeds.

Optimum germination temperatures for *Coreopsis* seeds were 15 and 20°C in light and germination was low above 25°C (Chapters 3, 4 and 5). In roadside sowings, germination will be reduced and erratic when soil temperatures exceed 25°C and sowing should be confined to periods when soil temperatures are below this threshold. Gibberellic acid and cold stratification (5°C) promoted germination in dark-imbibed *C. floridana* and *C. leavenworthii* seeds but maximum germination was not achieved. However, in *C. basalis, C. lanceolata* and *C. pubescens* GA had no effect while cold stratification reduced germination (Chapters 4 and 5), indicating that seeds of these species should not be sown into cold soils during Florida winters to avoid inducing dormancy, and precautions should be taken since *C. lanceolata* is normally sown in fall. *Coreopsis floridana* flowers and produces seed in fall and seeds might be adapted to germinate in spring after cold stratification during the winter. Potassium nitrate (10 mM) promoted germination of non-afterripened *C. lanceolata* seeds in light, but did not totally overcome dormancy. All this information is important to nursery managers and other end users, because it enables them to know what germination conditions are required to optimize germination.

Coreopsis lanceolata seeds required 150 days of dry afterripening at 10°C to overcome dormancy, but fresh (non-afterripened), naked *C. lanceolata* embryos germinated 100% (Chapter 5). Seed producers desiring seed lots with minimal dormancy should allow *C. lanceolata* seeds to dry afterripen for at least this period before they sell the seeds. They should also rely on viability tests such as the tetrazolium (TZ) or excised embryo test to ascertain the suitability of a seed lot for afterripening, otherwise non-viable seeds may be mistakenly left to afterripen with no consequent improvement in germination. Afterripened seeds should be stored under low humidity (<50%) and low temperatures (~10°C) to promote longevity. Seed producers should

therefore have prior knowledge of afterripening requirements so that they can devise marketing plans. While *C. floridana* seeds had 100% germination in light when freshly harvested, these seeds had 23% germination in dark; however, naked embryos germinated 100% in dark. For species like *C. floridana* which do not require afterripening (Chapter 5), low temperature storage soon after conditioning is advisable to maintain viability. Pre-germination seed treatments that help weaken endosperm have potential to alleviate dormancy in dark-imbibed *C. floridana* and non-afterripened *C. lanceolata* seeds. In lettuce, another *Asteraceae*, endosperm removal alleviated dormancy (Leon-Kloosterziel *et al.,* 1996; Debeaujon and Koorneef, 2000; Leubner-Mezger, 2003). Afterripening requirement has been reported before in *C. lanceolata* although under different storage conditions (Norcini and Aldrich, 2007a), and this study demonstrated that seeds of this species can dry afterripen under low temperatures (~10^{o}C) at a seed moisture content of 8.1%. Norcini and Aldrich (2007a) reported an afterripening period of 6 months whereas in this study *C. lanceolata* seeds afterripened in 5 months, a minor discrepancy which could be due to the influence of different storage conditions and initial dormancy level.

Naked embryos of *C. floridana* seeds germinated 100% in dark, and germination of naked embryos was also 100% at 30^{o}C in both *C. floridana* and C. *lanceolata*. Whole non-primed *C. floridana* seeds had 23% germination at 20^{o}C and 12% at 30^{o}C when imbibed in dark, while whole non-primed *C. lanceolata* seeds had 2% germination at 30^{o}C. However, removal of these tissues in large sowing programs is not practical. This study suggested that technology such as seed priming might be used to maximize and promote germination of *Coreopsis* seeds under diverse environments (Chapter 6). *Coreopsis floridana* seeds germinated 100% in dark after priming, and in both *C. floridana* (100%) and *C. lanceolata* (86%), germination was maximized at high temperature (30^{o}C). While priming would increase costs slightly, the benefits

far outweigh any extra costs, because germination and plant establishment is maximized (Cantliffe, 2003). Results here suggest that using solid matrix priming (SMP) with emathlite clay and 100 mg benzyladenine (BA) per liter water solution gives the best results at a ratio of 1:1:0.5 (clay [weight]:BA [volume]:seed [weight]). This study is the first to report success in priming *C. floridana* and *C. lanceolata* seeds, a technology that could be adopted by seed producers or sellers to benefit end users.

Based on this study, recommendations to promote germination and establishment of native *Coreopsis* species include: 1) sowing *Coreopsis* seeds when soil temperatures are below 25^{o}C in order to avoid thermoinhibition, 2) *C. floridana* seeds require light for germination, and should be sown on the soil surface to maximize light perception, 3) testing for viability and dormancy at harvest in *C. lanceolata* seeds and allowing for afterripening under appropriate conditions such as 10^{o}C at a seed moisture content of 8.1%, if necessary, and 4) use of seed priming techniques to overcome dark-dormancy in *C. floridana* and maximize germination in high soil temperatures for both *C. floridana* and *C. lanceolata*. Additionally, future research is recommended on: 1) investigating seed maturation conditions that favor production of high quality *Coreopsis* seeds with low dormancy, 2) studying pre-sowing seed treatment methods that weaken the endosperm to overcome dormancy in freshly harvested *C. lanceolata* seeds, and 3) further exploration of seed priming technology to promote germination under diverse environments.

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BIOGRAPHICAL SKETCH

Dzingai Rukuni was born in Bulawayo, Zimbabwe. He graduated with a Bachelor of Science honors degree, in agriculture, from the University of Zimbabwe in 1989. He worked for the Forestry Commission of Zimbabwe's Research and Development Division as a seed physiologist from January 1991 to July 1995. In July 1995 he left for New Zealand and started a Master of Applied Science honors degree program in seed and crop science at Massey University in Palmerston North. He specialized in Seed Technology and completed in July 1997, and left for Zimbabwe to rejoin the Forestry Commission in the same capacity until September 2001. In October 2001 he joined the Tobacco Research Board as a senior seed physiologist until January 2006, when he left to begin his doctoral studies in seed physiology at the University of Florida in Gainesville. His research focused on seed dormancy in Florida native wildflower *Coreopsis* species.