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RELATIONSHIP BETWEEN ETHYLENE AND SEED DORMANCY RELEASE IN ECHINACEA SPECIES

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ABSTRACT OF THESIS

RELATIONSHIP BETWEEN ETHYLENE AND SEED DORMANCY RELEASE IN *ECHINACEA* SPECIES

Inconsistent seed germination poses a problem for efficient seedling production of *Echinacea* species. Evidence suggests that ethylene can be effective for improving germination in *Echinacea* species. The objectives of this research were: to develop an ethylene pre-germination treatment that enhances germination in *Echinacea* species that is retained following drying and storage, and to determine if the ethylene effect on enhanced germination was an important mode of action for dormancy release. Four species of *Echinacea* (*E. purpurea*, *E. tennesseensis*, *E. angustifolia* and *E. simulata*) treated with 1-aminocyclopropane-1-carboxylic acid (ACC) or ethephon resulted in faster and generally higher germination. Pre-treatment of seeds with ACC or ethephon followed by drying was as effective as chilling stratification for enhancing germination depending on the species. While ethylene pretreatments did increase germination to some extent depending on species, it was concluded that 60-day stratification alone was a more commercially-viable treatment.

Ethylene production or perception was not necessary for germination in untreated or stratified seeds as shown by aminoethoxyvinylglycine (AVG), silver thiosulfate (STS), and 1-methylcyclopropene (MCP) treatments. Both stratification and ACC treatment reduced *Echinacea* seed sensitivity to ABA and could be a common mechanism for enhanced germination. However, it does not appear that the increased germination seen after stratification was mediated through ethylene production because final germination percentages were generally unchanged following inhibition of ethylene production or action. In contrast, inhibition of ethylene production and perception reduced early 3-day germination suggesting that ethylene was more involved in seed vigor than final germination. It was determined that there is no physiological significance of ethylene for dormancy release in these *Echinacea* species.

KEYWORDS: *Echinacea* species, seed dormancy, ethylene, germination, ethylene inhibitors.

Laura Anne Wood

July 25, 2007

RELATIONSHIP BETWEEN ETHYLENE AND SEED DORMANCY RELEASE IN *ECHINACEA* SPECIES

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THESIS

Laura Anne Wood

The Graduate School

University of Kentucky

2007

RELATIONSHIP BETWEEN ETHYLENE AND SEED DORMANCY RELEASE IN *ECHINACEA* SPECIES

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

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CHAPTER 1: INTRODUCTION

Echinacea are achenes that belong to the Asteraceae and are indigenous to North America (Gleason and Cronquist, 1991). *Echinacea* species are herbaceous perennials used commonly as an ornamental, cut flower, and medicinal purposes (Foster, 1985). Inconsistent seed germination is posing a problem for efficient seedling production of *Echinacea* species (Thompson et al., 2002; Smith-Jochum and Albrecht, 1987; Smith-Jochum and Albrecht, 1988). Seed dormancy is one of the factors affecting the germination rate, but it is also a way of survival for plant seeds in general. Whenever there is a favorable environment for the seed of wild flowers (which includes *Echinacea*), biochemical mechanisms in the seed allow germination to occur (Phillips, 1985). Fast and consistent germination in species with dormant seeds can usually be achieved if moist chilling is used (Wartidingingsih et al., 1994). Even species with non-dormant seeds can have enhanced germination rate if the seeds are exposed briefly to moist chilling (Wartidingingsih et al., 1994).

Besides moist chilling, evidence suggests that ethylene can be effective in relieving dormancy in *Echinacea* species (Sari et al., 2001). During germination, ethylene appears to have two roles. Ethylene can first of all break seed dormancy in some species. It can also reduce the time to radicle emergence in non-dormant seeds. These two activities are probably related to making the seeds less sensitive to abscisic acid which keeps the seeds dormant (Ketring and Morgan, 1972; Abeles et al., 1992). The actual part ethylene plays in seed germination is obscure (Nascimento, 2003). In some cases, ethylene has been shown to be necessary for germination upon usage of ethylene inhibitors while in other instances it has not been needed.

The objectives of this study were to obtain a better understanding of the relationship between ethylene production and dormancy release in *Echinacea* and to develop a method of delivering ethylene to seeds that would be retained after seed drying. Specific objectives of this research were as follows: 1) Develop a pre-germination treatment that enhances germination in *Echinacea* species and 2) Determine if the effect of ethylene on enhanced germination in *Echinacea* seeds is an important mode of action for dormancy release.

CHAPTER 2: LITERATURE REVIEW

Echinacea belong to the Asteraceae, and they are indigenous to North America (Gleason and Cronquist, 1991). *Echinacea purpurea* (L.) Moench, known as purple coneflower, can be found growing throughout the United States and Canada (Gleason and Cronquist, 1991). Examples of cultivars are 'New Colewall Strain,' 'Bright Star,' 'Sombrero,' and 'The King' (Foster, 1985). It is an herbaceous perennial that can be used as a cut flower, and all parts of the plant are used for medicinal purposes (Foster, 1985).

The Tennessee purple coneflower (*E. tennesseensis* (Beadle) Small) is native to Tennessee and is found in cedar glades of the middle part of the state (Gleason and Cronquist, 1991). It is only found in Tennessee which causes it to be placed on the federally endangered list (Foster, 1985). Besides being a good ornamental plant in landscapes, phytotherapy (herbal medicine) may be another use for this species (Walck et al., 2002).

The narrow-leafed purple coneflower (*E. angustifolia* DC.) is also native to North America and grows in plains and rocky prairies (Foster, 1985). This species is commonly used for medicinal purposes, even though it is becoming rarer to access on the market (Foster, 1985).

Echinacea simulata McGregor, also known as wavyleaf purple coneflower and glade coneflower, grows in the region of western Kentucky, southern Illinois, northeast Arkansas, and southeast Missouri (Gleason and Cronquist, 1991). This species, like the other species, can be used as a landscape plant.

Seed Dormancy

Dormancy levels

As seeds form and mature on the mother plant, primary dormancy is initiated. This is the type of dormancy that keeps the seeds from germinating as they are being formed on the plant (Kępczyński and Kępczyńska, 1997). Primary dormancy includes exogenous, endogenous, and combination dormancy (Hartmann et al., 2002). In exogenous dormancy, germination is hindered by a structure which surrounds the

embryo, such as fruit walls, seeds coats, or endosperm (Baskin and Baskin, 1998). With endogenous dormancy, which is broken down into physiological, morphological, and morphophysiological, germination is inhibited by something associated with the embryo (Baskin and Baskin, 1998). Combinational dormancy combines exogenous and endogenous dormancy, and in order to break dormancy these different dormancy conditions must be relieved in a sequence (Hartmann et al., 2002).

After dormancy release, seeds may become dormant again (secondary dormancy) upon being exposed for a long period of time to an environment having conditions insufficient for germination. Secondary dormancy includes thermodormancy and conditional dormancy (Hartmann et al., 2002). With thermodormancy, seeds that are exposed to an ideal temperature following a high temperature will not germinate (Hartmann et al., 2002). An example of thermodormancy can be found in lettuce (*Lactuca sativa* L.) seeds which are also members of Asteraceae. Conditional dormancy is a point in which there is a small temperature span that the seed will germinate upon leaving dormancy or going into secondary dormancy (Baskin and Baskin, 1998; Vegis, 1964).

Endogenous physiological dormancy

Echinacea species have endogenous physiological dormancy. This kind of dormancy is broken down into three types, which are nondeep, intermediate, and deep dormancy (Nikolaeva, 1977). *Echinacea* seeds fall into the nondeep category.

When a seed has physiological dormancy, the embryo's growth potential needs to increase before germination can occur; therefore, the embryo must undergo physiological changes (Baskin and Baskin, 1971). The radicle is able to break through the seed coverings after this increased growth potential (Hartmann et al., 2002). If the embryo is excised, normal germination will take place in seeds of numerous species that have physiological dormancy (Hartmann et al., 2002).

Retention of Pre-sowing Treatments in Dry Seeds

Using different methods to treat seeds before sowing is one way of relieving dormancy and/or improving germination. This can involve cold stratification, priming,

and growth regulators.

Cold stratification

One method of bringing seeds out of endogenous, physiological dormancy is to submit the seeds to cold stratification, sometimes called chilling stratification or moist-chilling stratification. In cold stratification, seeds are exposed to low (chilling) temperatures for an allotted time in a moist, aerated medium. These low temperatures and moist environment allow the radicle to protrude through the seed coat following stratification and occasionally during stratification (Hartmann et al., 2002). Stratification is mainly influenced by temperature (Hartmann et al., 2002). The most effective temperature range is from 1°C to 7°C; seeds that are subjected to lower and higher temperatures need to be stratified longer (Seeley and Damavandy, 1985).

Cold stratification has been used successfully to improve the germination of *Echinacea* species. Wartidiningsih et al. (1994) showed that moist chilling *E. purpurea* seeds for 10 to 30 days increased the early and final germination of the low germinating seed lots. According to Wees (2004), germination of *E. purpurea* reached 83% following 4-week stratification in comparison to the untreated control which had a germination of 44%. Baskin et al. (1993) found that 12 weeks of cold stratification at 5^{o}C resulted in *E. tennesseensis* and *E. simulata* germination at 25/15^{o}C of about 68% and 74%, respectively.

In the *Echinacea* species commonly used for medicinal purposes, Parmenter et al. (1996) observed an increase in *E. angustifolia* germination from 10 to 48% in one experiment and from 48 to 80% in another experiment after more than 2 weeks of moist chilling. Baskin et al. (1992) reported *E. angustifolia* reaching 80-100% germination after the seeds underwent cold stratification for 12 weeks. This high germination was only achieved, though, in the 1986 lot at 15/6°C and 20/10°C, the 1987 lot at 20/10°C, and the 1988 lot at 15/6°C, 20/10°C, 25/15°C, and 30/15°C (Baskin et al., 1992). Based on these studies, high germination in this species may be achieved by cold stratifying the seeds for somewhere between 2 and 12 weeks.

Such high laboratory germination was contradicted, however, by a greenhouse study conducted by Wees (2004). Wees (2004) reported *E. angustifolia* seeds having a

germination in a greenhouse in 1997 of 22% following moist stratification for 4 weeks compared to the control of 12%. In 1999, the germination of *E. angustifolia* was 2% for untreated and 9.3% for moist stratified for 3 weeks. Different seed sources were used each of the 2 years, so the quality of the seeds might have been a factor in the decrease in germination in 1999 (Wees, 2004). This greenhouse study demonstrated that the high germination results of cold stratified seeds obtained in a laboratory environment may not necessarily be duplicated in an actual field setting, at least with *E. angustifolia*.

It has also been found that cold stratification for too many weeks may actually decrease germination in *Echinacea*. In a study by Bratcher et al. (1993), moist stratification at 5±2°C for 4 weeks provided the greatest germination percentage of *E. purpurea* seeds, while 6 to 10 weeks of stratification resulted in a decrease in germination. There may, therefore, be a limit to how long *Echinacea* seeds should be stratified, but this again could be species dependent.

Light has been claimed to be a factor in *Echinacea* germination, but results vary. Subjecting the seeds of *E. purpurea*, *E. pallida* (Nutt.) Nutt., and *E. angustifolia* during 4 weeks of cold-moist stratification at 4°C with 24 hours of light resulted in an average germination of 82% over all three species (Romero et al., 2005). However, little effect of 7- or 11-day prechilling on *E. angustifolia* germination percentage in light or dark was found by Macchia et al. (2001). There was, though, a significant improvement in germination percentage in light upon prechilling the *E. angustifolia* seeds for 15 days (Macchia et al., 2001). No matter whether the seeds were germinated in the light or dark, prechilling greatly increased the rate of germination. When prechilling was not used, *E. angustifolia* seeds that were germinated in the light reached a germination percentage of 42.7% while seeds germinated in the dark had a higher germination percentage of 53.4% (Macchia et al., 2001). This suggested that light is not needed for germination, and *E. angustifolia* seeds actually germinated better without light.

A study by Baskin et al. (1992), however, showed that light was needed. *Echinacea angustifolia* seeds that were stratified and incubated in darkness had strikingly lower germination percentages than those stratified and incubated in light (Baskin et al., 1992). Seeds that were stratified in the dark and incubated in the light also had lower germination percentages than those stratified and incubated in the light over all

thermoperiods, with the exceptions of 15/6°C and 25/15°C (Baskin et al., 1992). Germination percentages of *E. angustifolia* seeds that were stratified in light and germinated in dark and seeds that were stratified in dark and germinated in light were about the same over all thermoperiods, with the exception of 20/10°C (Baskin et al., 1992). In this particular case, the lack of light could have been a factor resulting in low germination, or stratification could have been too long thus decreasing germination.

Furthermore, it was found that chilling stratification was not always necessary. Smith-Jochum and Albrecht (1987) found that germination of *E. purpurea* seed lots were between 70-80% with or without chilling. These results show that while chilling was beneficial in improving germination it was not required.

Priming

Priming enables seeds to complete the first stages of germination without radicle emergence by taking up water from an osmotic solution (Heydecker, 1973). Following imbibition, seeds are then planted or dried to their former moisture contents and put into storage (Cantliffe, 2003). Priming is a desirable treatment as it provides fast and uniform seed emergence (Cantliffe, 2003).

Priming has been shown to increase germination in *Echinacea* species. *Echinacea angustifolia* seeds in a greenhouse had a germination of 24% following priming in 0.1 M KNO_3 for 5 days at 21°C compared to the control of 12% (Wees, 2004). Wees (2004) also used *E. purpurea* seeds in his study which obtained a germination of 69% with a priming solution of 0.1 M KNO_3 compared to the control of 44.1%. Chiu et al. (2006) found that seeds of *E. purpurea* had a faster mean germination rate and higher germination percentage upon undergoing osmopriming in a PEG (-0.5 MPa) solution at 25°C for 6 days and solid matrix priming than the un-primed control. These studies indicate that *Echinacea* seeds may respond differently to priming treatments, depending on species.

When *Echinacea* seed lots were divided into low- and high-germinating categories, priming had a different effect on each group. In comparison to untreated seeds, high-germinating *E. purpurea* seed lots that were osmotically primed in salt or PEG at 25°C saw an improvement in only the early germination percentages

(Wartidiningsih et al., 1994). Low-germinating seed lots that were primed had improved early and final germination percentages (Wartidiningsih et al., 1994). Priming may enable high-vigor *Echinacea* seed lots to have more uniform stand establishment sooner than non-primed seed lots, and priming may bring low-vigor seed lots up to a level that would be comparable in final germination to high-vigor lots.

Gibberellins

Gibberellin is a hormone that promotes seed germination (Hartmann et al., 2002). Tomato (*Lycopersicon esculentum* Mill) and mouse-ear cress (*Arabidopsis thaliana* (L.) Heynh.) mutants that lacked gibberellin did not germinate; this demonstrates the necessity of gibberellin (Bewley and Black, 1994).

Gibberellic acid has been used with *Echinacea* seeds as well but with conflicting results. Germination of *E. purpurea* was improved in a non-stressed environment when the seeds were exposed to a 20-hour soaking in GA_3 at a concentration of 60-600 $mg.1^{-1}$, while water-stressed seeds only responded to the same period of soaking in GA_3 at 60 $mg.1^{-1}$ (Kochankov et al., 1998). Gibberellic acid has also been used in combination with priming. A percentage increase in germination rate was seen in *E. purpurea* seeds following matric or osmotic priming used with 1 μM GA_3 (Pill and Haynes, 1996). With *E. angustifolia*, however, germination was not increased by constant exposure to 500 mg 1^{-1} GA_3 (Macchia et al., 2001).

Ethylene

In various species, dormancy is broken by exposing seeds to ethylene (Taiz and Zeiger, 2002). Germination rate is also accelerated by ethylene (Taiz and Zeiger, 2002). As seeds germinate, ethylene is produced (in numerous species), and auxin content in dormant seeds is supposedly controlled by ethylene (Copeland and McDonald, 2001).

Evidence suggests that ethylene can be effective in relieving dormancy in *Echinacea* species (Sari et al., 2001). ACC (1-aminocyclopropane-1-carboxylic acid) is the immediate biochemical precursor to ethylene, and it was shown to increase impatiens (*Impatiens wallerana*. L.) seeds' ability to make ethylene, which in turn reduced the time required for radicle protrusion (Dutt et al., 2004). Ethephon, an ethylene-releasing

compound, has also been used to improve seed germination.

Feghahati and Reese (1994) showed that germination of *E. angustifolia* seeds was about 80% upon being prechilled and treated with ethephon in the light for two weeks; this was in comparison to 1-week prechilling in the light and ethephon plus 1-week prechilling. Qu et al. (2004) found that it took only 4 days for seeds of *E. angustifolia* and *E. pallida* to reach about 90% of the final germination when ethephon and light were used. When ethephon was not applied, comparable germination percentages were obtained after 8 to 12 days (Qu et al., 2004).

It appears that at least for *E. angustifolia*, dormancy can be overcome and germination increased by exposing the seeds to ethylene-like compounds instead of stratifying the seeds for 3 weeks at 4°C (Korkmaz et al., 2004). Macchia et al. (2001) showed that the germination of *E. angustifolia* seeds reached about 90% when prechilling, light, and ethephon were used. When 11 days of chilling in light along with a 3 mM ethephon concentration were used, lowest mean germination time (2 days) and highest germination percentage (96%) of *E. angustifolia* was achieved (Macchia et al., 2001). Germination of *E. pallida* and *E. angustifolia* seeds was also increased when ethephon was included in the medium during chilling stratification. The average increase in germination was 49% for *E. pallida* and 17% for the *E. angustifolia* seed lots (Sari et al., 2001). Soaking *E. purpurea* seeds in 1445 $mg.1^{-1}$ ethephon with an osmotic potential of 0 MPa or -0.3 MPa for 20 hours obtained a germination percentage of about 94% and 83%, respectively (Kochankov et al., 1998). According to Kochankov et al. (1998), *E. purpurea* seeds that were treated with ethephon then subjected to normal and stressful conditions caused an increase in germination uniformity, germination rate, and final germination percentage. Ethylene must have not only improved the germination rate and final germination of the *Echinacea* seeds, but it must have also better enabled the seeds to withstand stressful conditions.

Physiological significance of ethylene during germination

In some plant species, breaking dormancy and initiating seed germination require ethylene (Kępczyński and Kępczyńska, 1997). In the process of ethylene formation, methionine is made into S-adenosylmethionine (SAM), and SAM is converted to 1-

aminocyclopropane-1-carboxylic acid (ACC) by ACC-synthase. Then, ACC is converted to ethylene by ACC-oxidase (Taiz and Zeiger, 2002). The physiological significance of ethylene has been studied in a number of species but not in *Echinacea*.

During germination, ethylene appears to have two roles. Ethylene can break seed dormancy in some species. It can also reduce the time required for radicle emergence in non-dormant seeds. These two activities are probably related to making the seeds less sensitive to abscisic acid, which keeps the seeds dormant (Ketring and Morgan, 1972; Abeles et al., 1992). For some seeds, like lettuce, the actual role of ethylene in seed germination is obscure (Nascimento, 2003). In some cases, ethylene has been shown to be necessary for germination upon usage of ethylene inhibitors, while in other instances it has not been needed.

Production of ethylene is initiated by certain factors, such as chilling, light, and imbibition that promote germination, and many species, such as wild oat (*Avena fatua* L.) (Adkins and Ross, 1981) and Virginia-type peanut (*Arachis hypogaea* L.) (Ketring and Morgan, 1971, 1972), manufacture ethylene during germination (Matilla, 2000). Thus, dormancy may be broken in some species by exposure to ethylene during early imbibition (Matilla, 2000). Fu and Yang (1983) found that when 1-100 ppm of ethylene were applied to lettuce seeds during imbibition it broke the dormancy that had been caused by heating the seeds at 97^{o}C for 30 hours before imbibition. During the time right before radicle emergence in the lettuce seeds, ethylene was required, but in the first part of imbibition ethylene was not needed (Fu and Yang, 1983). At the time of radicle protrusion in nondormant lettuce seeds, there was a rapid increase in ethylene production which peaked at 24 hours then decreased (Fu and Yang, 1983). For most seeds, ethylene production increases with radicle emergence, peaks soon after, and then declines (Matilla, 2000).

Lettuce, which is also an achene like *Echinacea*, has been extensively studied to see the effect that ethylene has on germination. Abeles (1986) found that there was a limited 5°C range that thermoinhibition in lettuce seeds can be surpressed by ethylene. Any temperature greater than 30°C did not permit ethylene to initiate germination (Abeles, 1986). Thermoinhibition in lettuce seeds occurs when the endosperm cannot be pierced by the embryo at temperatures greater than 25°C (Abeles, 1986). The same effect

was found in a study by Dunlap and Morgan (1977) in which ethylene had no effect on lettuce seed germination at 36°C, but it did at 32°C. Prevention of ethylene production by the seeds did not impact thermoinhibition (Burdett, 1972; Dunlap and Morgan, 1977; Abeles, 1986). However, in lettuce seeds the transformation of ACC to ethylene was hindered by high temperatures (Khan and Prusinski, 1989). In the temperature range of 20 to 25°C, lettuce seeds may only need to manufacture a small amount of endogenous ethylene for germination (Nascimento, 2003). On the other hand, ethylene was necessary when lettuce seeds were subjected to a stressful environment, such as 35°C, while undergoing imbibition; at this high temperature, ACC was not detected (Huang and Khan, 1992). ACC synthesis had less of an effect on hindering the formation of ethylene than warm, stressful temperatures did, and the warm, stressful temperatures prevented ethylene production altogether (Nascimento, 2003).

Furthermore, germination may require additional exogenous ethylene when lettuce seeds are held at high temperatures; this is because ethylene production is diminished by high temperatures (Nascimento, 2003). Abeles and Lonski (1969) reported that germination of dormant lettuce seeds was not influenced by ethylene; this indicates that ethylene action is restricted to the beginning stages of germination. It is believed that ethylene plays a role in the expansion of embryonic hypocotyl cells of lettuce seeds (Abeles, 1986). The structures that surround the embryo are not affected by ethylene as much as is the embryo itself; ACC, upon being converted to ethylene, enabled lettuce seeds to germinate at high temperatures (Dutta and Bradford, 1994).

According to Karssen (1976) and Schönbeck and Egley (1981), the point in which the seeds were exposed to ethylene (before germination or during imbibition) may also determine how the seeds will respond. Exposure to ethylene may promote germination of dormant seeds; such a result was seen when redroot pigweed (*Amaranthus retroflexus* L.) seeds were subjected, during imbibition for 20 hours, to exogenous ethylene (Kępczyński and Kępczyńska, 1997). Ethylene can also increase germination in seeds that are not dormant, such as pendant amaranth (*Amaranthus caudatus* L.) (Kępczyński and Karssen, 1985). Even when exposed to a less than ideal environment, non-dormant seeds germinated after being treated with ethylene (Kępczyński and Kępczyńska, 1997). Pendant amaranth (Kępczyński, 1986) and lamb's-quarters (*Chenopodium album* L.)

(Karssen, 1976) seeds germinated after the ABA effect was overcome by ethylene exposure (Kępczyński and Kępczyńska, 1997). Ethylene and ethephon promote germination of seeds of some species, thus allowing them to overcome, in part or completely, dormancy or the effects of an adverse environment (Kępczyński and Kępczyńska, 1997). Seeds from these species therefore may depend on a minimum amount of exogenous ethylene. A reduction of endogenous ethylene will not, therefore, prevent seeds from germinating (Kępczyński and Kępczyńska, 1997).

Effect of ethylene inhibitors

Applying ethylene inhibitors to seeds is one way to determine whether ethylene is needed for dormancy release and germination. If ethylene is necessary, ethylene inhibitors should decrease germination. According to Nascimento et al. (2004), 'Dark Green Boston' lettuce seeds did not germinate at 35°C in the presence of 20 mM silver thiosulphate (STS). Ethylene supplied to lettuce seeds as they imbibed at 35°C enabled germination to occur (Huang and Khan, 1992), demonstrating that lettuce seeds use ethylene to overcome thermoinhibition. Germination and ethylene production of lettuce seeds were prevented in the presence of aminoethoxyvinylglycine (AVG), indicating that ethylene was involved in the germination of lettuce seeds (Abeles, 1986). The effect of AVG was partly overridden by the application of exogenous ethylene (Abeles, 1986). In numerous species, seed germination will take place in the presence of ethylene in a concentration range of 0.1 to 200 µl/l (Kępczyński and Kępczyńska, 1997). Germination of lettuce seeds occurs ideally in the presence of 10 µl/l of ethylene (Burdett and Vidaver, 1971).

These studies indicate that ethylene is necessary for germination, but it has also been shown that ethylene is not needed for germination. In a study by Abeles (1986), germination of lettuce seeds at 25°C was not affected by STS, but it decreased germination at 30°C (Abeles, 1986). According to Nascimento et al. (2004), lettuce seed germination was not affected by 10 mM AVG. Germination could have resulted from the large production of ethylene in the light from endogenous ACC (Nascimento et al., 2004). Hoffman et al. (1983) and Kępczyński and Karssen (1985) found that germination in peanut (*Arachis hypogea* L. Yue-you 551) and pendant amaranth seeds, respectively,

was not affected by AVG, but the ethylene manufactured was still decreased by AVG.

Role of ABA

As seeds form, it appears that abscisic acid (ABA) plays an important role in dormancy initiation (Bewley and Black, 1982). While the embryo forms in the ovule, ABA keeps it from germinating too early ("precocious germination") (Hartmann et al., 2002). In order to initiate dormancy during seed formation, the presence of ABA is necessary, as was shown in mutants with and without ABA in tomato (Groot and Karssen, 1992) and *Arabidopsis* (Karssen et al., 1983). Primary dormancy is initiated by ABA as the fruit matures, and the level of ABA rises during this time. As seeds are stratified, the level of ABA decreases. The seed coats of dormant rose (*Rosa* L.), plum (*Prunus* L.), walnut (*Juglans* L.), apple (*Malus* Mill.), and peach (*Prunus persica* (L.) Batsch) seeds have also been found to contain ABA (Hartmann et al., 2002).

Abscisic acid not only keeps seeds dormant, but it also keeps seeds from producing ethylene. Gallardo et al. (1991) found that chickpea (*Cicer arietinum* L.) seeds failed to germinate and ethylene production was prevented by ABA. Dormant Virginia-type peanut seeds were able to germinate, however, when subjected to exogenous ethylene; ABA no longer had an effect in the presence of the ethylene (Ketring and Morgan, 1970). According to Ghassemian et al. (2000), ABA sensitivity in *Arabidopsis* seeds increased while sensitivity to ethylene declined; these two processes were caused by mutations. The sensitivity that *Arabidopsis* seeds had to ABA also declined in the presence of ACC (Ghassemian et al., 2000). A study by Karssen et al. (1983) showed that during seed development the embryos of wild-type *Arabidopsis* seeds are exposed to endogenous ABA. This exposure causes a dormancy that continues after ABA concentrations decline (Karssen et al., 1983). While the application of ACC can partially overcome the effects of ABA, it is not sufficient to stimulate germination in mature wild-type *Arabidopsis* seeds when ABA is not present. This suggests that ethylene acts by interfering with the ABA inhibition mechanism (Ghassemian et al., 2000).

CHAPTER 3: APPLICATION METHODS FOR ETHYLENE-ENHANCED GERMINATION IN *ECHINACEA* SPECIES

INTRODUCTION

Echinacea contains a small group of North American species in the Asteraceae family (Gleason and Cronquist, 1991). Each is a herbaceous perennial that can be used for garden plants, cut flowers, ecological restoration, and medicinal purposes (Whitten, 2004).

The principle method for propagating *Echinacea* is by seed (achene) germination. However, germination results can be inconsistent (Thompson et al., 2002). Greater germination in species with dormant seeds can usually be achieved with moist (chilling) stratification (Romero et al., 2005). Even species with non-dormant seeds can have enhanced germination speed if seeds are exposed briefly to moist chilling (Wartidingingsih et al., 1994). Cold-moist stratifying seeds of *E. purpurea*, *E. pallida*, and *E. angustifolia* at 4°C for 4 weeks in the presence of light increased germination rate and percentage (Romero et al., 2005). Wartidiningsih et al. (1994) showed that moist chilling *E. purpurea* seeds for 10 to 30 days increased the early and final germination of low germinating seed lots. Seed germination of *E. angustifolia* increased from 10 to 48% in one experiment and from 48 to 80% in another experiment, after more than 2 weeks of moist chilling (Parmenter et al., 1996). Baskin et al. (1993) found that 12 weeks of cold stratification at 5^{o}C resulted in *E. tennessensis* and *E. simulata* germination at 25/15^{o}C of about 68% and 74%, respectively. In all studies to date, germination was tested directly after stratification without an intervening drying or storage period.

Seed priming has also improved germination in *Echinacea*. In priming, germination occurs more rapidly and more consistently upon undergoing a controlled hydration treatment (Hartmann et al., 2002) than untreated seeds. Regardless of the type of priming used, *E. purpurea* seeds generally showed more consistent, rapid germination (Pill et al., 1994; Samfield et al., 1991; Wartidiningsih et al., 1994) than un-primed seeds. Germination percentage was also improved depending on the seed lot and germination conditions. The priming effect for *E. purpurea* was not always retained in storage (Samfield et al., 1990; Wartidiningsih, 1992). Primed *E. purpurea* seeds stored for 2

months at 12°C lost their priming effect compared to the control (Samfield et al., 1990), and those stored one month at 25 or 30°C showed significant deterioration compared to the controls (Wartidiningsih, 1992). However, after 3 months' storage at 5°C, germination of primed seeds was still high (Wartidiningsih, 1992).

GA_3 and ethephon have also been used to promote *Echinacea* germination. In general, germination percentage and speed can be enhanced by germinating *Echinacea* seeds in the presence of gibberellic acid (GA_3) or ethephon, but this can be species dependent (Sari, et al., 2001; Kochankov et al., 1998; Qu et al., 2004). Germination rate and final germination of water-deficient *E. purpurea* seeds increased following application of 60 mg/l GA_3 (Kochankov et al., 1998), while in *E. angustifolia* germination was not increased by 500 mg/l GA_3 (Macchia et al., 2001). In *E. angustifolia* and *E. pallida*, germination has been improved by either exposing seeds to a brief ethephon soak or providing it in the germination medium (Sari, et al., 2001; Qu et al., 2004). Qu et al. (2004) found that it took only 4 days for seeds of *E. angustifolia* and *E. pallida* to reach ~90% of final germination after a 10-minute exposure to 1 mM ethephon.

Growth regulators have also been included in stratification or priming solutions (Feghahati and Reese, 1994; Macchia et al., 2001; Korkmaz et al., 2004; Pill and Haynes, 1996). Germination levels of *E. angustifolia* seeds reached about 90% when ethephon was included during stratification (Macchia et al., 2001). Sari et al. (2001) observed increased germination of 49% for *E. pallida* and 17% for *E. angustifolia* when ethephon was combined with stratification. Using matric or osmotic priming, Pill and Haynes (1996) observed that it took fewer days to reach 50% of the final germination percentage in *E. purpurea* when used with 1 μM GA_3 or 10 mM ethephon plus 1 μM GA_3; this was in comparison to priming with only ethephon or not using growth regulators with priming.

The results of these studies demonstrate the effectiveness of GA_3 and ethylene treatments as substitutes for lengthy chilling stratification and priming treatments. However, to be a more useful pre-treatment, the effects of the growth regulator must be retained following a subsequent drying treatment to facilitate commercial sowing conditions. In previous studies, only the combination of priming with GA_3 was effective

as a growth regulator treatment that was subsequently dried (Pill and Haynes, 1996). However, *Echinacea* seed germination in the presence of ethylene (ethephon) has been found to equal or exceed the GA_3 response (Sari, et al., 2001; Kochankov et al., 1998; Qu et al., 2004). Therefore, the objective of this study was to develop an ethylene pre-germination treatment that enhances germination in *Echinacea* species and is retained following drying and storage.

MATERIALS AND METHODS

Plant material:

Four *Echinacea* species (*E. purpurea*, *E. tennesseensis*, *E. angustifolia*, and *E. simulata*) were used in this study. Seeds were grown on the South Farm of the University of Kentucky (*E. purpurea* and *E. tennesseensis*) and purchased from Native American Seed Farm, Junction, TX (*E. angustifolia*); Johnny Selected Seeds, Winslow, ME (*E. purpurea*); Jelitto, Schwarmstedt, Germany (*E. tennesseensis* 'Rocky Top Hybrids'); and Missouri Wildflowers Nursery, Jefferson City, MO (*E. simulata*). Initial seed germination within each species was similar across seed lots obtained from different sources.

Germination conditions:

Twenty-five seeds were germinated in four replicated Petri dishes (100 x 15 mm) containing 6 mL of deionized water (control), 5 mM ACC (Sigma-Aldrich, St. Louis, MO), or 1 mM ethephon (Sigma-Aldrich, St. Louis, MO), two pieces of Grade 8001 germination paper (Stults Scientific Co., Mt. Holly Springs, PA) about the size of the bottom of the Petri dishes, and sealed with parafilm (Pechiney Plastic Packaging, Chicago, IL). Seeds were germinated at a constant 25^{o}C in 8 hours light and 16 hours dark, and germination (radicle protrusion) was recorded after 3 and 12 days. Ethephon-treated seeds were germinated in a separate incubator at 25^{o}C to prevent ethylene exposure to control treatments.

Stratification:

Four replications of 25 seeds (which were transferred to new Petri dishes) or 80 seeds in one Petri dish (which were transferred to 25 mL Erlenmeyer flasks with four replications of 20 seeds in each flask) were put in Petri dishes with 6 mL of water, two pieces of germination paper, and sealed with parafilm. These seeds were placed in a refrigerator at 5°C for 60 days. Following the 60-day stratification, the seeds were either dried back to near their original water content in a laminar flow hood prior to germination, or seeds were germinated without drying.

Pre-germination treatments:

Prior to selecting a time for pre-treating seeds, water uptake in *E. purpurea* seeds was determined by measuring fresh weight gain of 5 seeds in four replications over 30 hours; the seeds underwent imbibition in a 25°C incubator. For the warm pretreatment, four replications of 25 seeds were pretreated with 6 mL of water, 5 mM ACC, or 1 mM ethephon in citrate buffer (pH 4.0) in Petri dishes for 24 hours at 25 or 35°C. In the case of the seeds that were to be transferred to flasks for the warm pretreatment, all 80 seeds per treatment were placed into one Petri dish. For the cold pretreatment, 180 seeds (which were transferred to new Petri dishes with four replications of 25 seeds or 25 mL Erlenmeyer flasks with four replications of 20 seeds) were placed into 250 mL mason jars with 6 mL of 1 mM ethephon in citrate buffer (pH 4.0) and two pieces of germination paper; the mouth of each jar was sealed with a screw-on lid. The seeds were kept at 5°C for 7 days. Following warm and cold pretreatment, seeds were briefly washed to remove the surface ACC or ethephon and dried to near their original water content in a laminar flow hood.

Storage:

The most effective pretreatment for *E. purpurea* and *E. tennesseensis* was determined to be ethephon for 7 days at 5°C, and the most effective pretreatment for *E. angustifolia* was determined to be ACC for 7 days at 5°C. Not enough *E. simulata* seeds were available to pretreat and store. Treated seeds were then dried to near their original water content and stored dry in 2 x 0.5 inch scintillation glass vials (Wheaton Glass,

Millville, NJ) in the dark at 5°C and 25°C for 1 and 3 months prior to germination.

Ethylene determination:

Twenty seeds that were untreated, stratified, or preatreated were placed in four replicated 25 mL Erlenmeyer flasks containing 1 mL of water, 5 mM ACC, or 1 mM ethephon, one piece of Whatman #1 Qualitative filter paper (Maidstone, England) about the size of the bottom of the flasks, and parafilm was placed over the tops of the flasks. Ethylene in the flasks was measured every 2 days for 6 days. On the day before each 2-day sample point, the parafilm was removed from the tops of the flasks and replaced with rubber stoppers for 24 hours to collect ethylene. Ethephon-treated seeds were kept in a constant light incubator. One mL from each flask was then injected into a Buck Scientific Model 910 flame ionization detector (FID) gas chromatograph, and upon taking each sample the flasks were vented. The gas chromatograph (GC) had a Supelco Custom Column 121799 packed with 80/100 Alumina F-1, the oven temperature was 100°C, the nitrogen flow rate was 1 mL per minute, and the detector temperature was 150°C. The standard used was 100 μl/l ethylene in helium.

Statistical analysis:

The design of each experiment was completely randomized with a one-way ANOVA arrangement. Germination percentages were converted to arcsin $\sqrt{x^2}$ before being analyzed statistically. Treatment means were compared by running Tukey's test using SAS software (SAS Institute, Cary, NC).

RESULTS

Water uptake curve of *E. purpurea* seeds

A water uptake curve for *E. purpurea* found that seeds reached full imbibition after 14 hours (Figure 3.1). Therefore, 1 day was selected for treating seeds with ethylene-releasing compounds at 25 and 35°C and 7 days at 5°C.

Germination of *Echinacea* seeds pretreated with ACC or ethephon

Chilling stratification increased germination speed (3-day count) and final germination percentage (12-day count) in *E. tennesseensis* and *E. simulata*, but only 3-day germination in *E. purpurea* (Tables 3.1, 3.2, and 3.4). There were no treatment effects for *E. angustifolia* (Table 3.3). The stratification effect relative to untreated seeds was retained following drying in *E. purpurea* and *E. tennesseensis*, but it was reduced in *E. simulata*. For all species, final germination percentage was the same for stratified and ACC-treated seeds. Ethephon increased 3-day and final germination of only *E. tennesseensis* and *E. simulata* (Tables 3.2 and 3.4).

The effect of seed pretreatments varied between species. For *E. purpurea*, pretreating seeds with water, ACC, or ethephon for 1-day at 25^{o}C had no statistical effect on final germination, but pretreatment increased 3-day germination over untreated seeds (Table 3.1). Pretreatment with ACC and ethephon at 35^{o}C increased 3-day germination but not final germination in comparison to the untreated control. Pretreatment at 5^{o}C was comparable to stratified seeds for 3-day and final germination, but there was no effect of including ACC or ethephon in the pretreatment solution.

Echinacea tennesseensis seeds pretreated at 25 or 35^{o}C only showed an increase in 3-day germination with the inclusion of ethephon in the pretreatment solution (Table 3.2). There was no effect of pretreatment at these temperatures on final germination compared to untreated seeds. Pretreatment at 5^{o}C also showed an increase in 3-day germination with the greatest impact with ethephon. There was also a trend for increased final germination at this temperature, but there was no significant increase by including ACC or ethephon in the pretreatment medium.

There was very little impact of pretreating *E. angustifolia* seeds (Table 3.3). However, there was a significant increase in 3-day germination by pretreating seeds with ACC at 5^{o}C and a 32% increase in final germination over untreated seeds.

Echinacea simulata seeds responded to pretreatment with ethephon at all temperatures for improved 3-day and final germination (Table 3.4). The increase in germination was comparable to stratification treatments. There was no effect of any ACC treatment, with the exception of increased 3-day germination following treatment for 7 days at 5^{o}C, compared to untreated seeds.

Ethylene production in ethephon (buffered) *E. angustifolia* seeds

More ethylene was produced by a combination of water and 1 mM ethephon in the presence of seeds than with any other combination (Table 3.5). Without seeds, water plus ethephon produced about 51 times less ethylene total than with seeds. Buffer plus ethephon alone produced about 1.5 times as much ethylene as water plus ethephon alone (Table 3.5).

Ethylene production after pretreatment with water, ACC, or ethephon

In all species, seeds that were constantly exposed to and pretreated with ethephon produced significantly more ethylene than other treatments. Over the species evaluated, ethephon-treated seeds produced from 399 to 980 times or 12 to 81 times more ethylene than the untreated control/water or ACC-treated seeds, respectively (Tables 3.6-3.8). Treatment with ethephon for 1 day at 25°C produced from 24 to 84 times or 3 to 23 times more ethylene than water or ACC at 25°C, depending on species (Tables 3.6-3.9). Seeds treated with ethephon for 1 day at 35°C produced from 20 to 77 times or from 5 to 18 times more ethylene than water or ACC at 35°C depending on the species (Tables 3.6-3.9). Ethephon for 7 days at 5°C produced from 4 to 92.5 times or from 1.6 to 14 times more ethylene than water or ACC at 5°C (Tables 3.6-3.9).

Germination in ACC or ethephon-treated seeds following storage

After 1 month at 5°C, the 60-day stratification effect was retained in both germination speed and final germination in *E. purpurea* and to a lesser degree in *E. tennesseensis* and *E. angustifolia* (Tables 3.10-3.12). The ethephon effect for the same time length and temperature was only retained in final germination of *E. purpurea* while the ACC effect was retained in both 3-day germination and final germination in *E. angustifolia*. Upon storage for 1 month at 25°C, the 60-day stratification effect was retained in *E. purpurea* and *E. tennesseensis* but lost to some degree in *E. angustifolia* (Tables 3.10-3.12). The ethephon effect for the same time length and temperature was retained in only final germination of *E. purpurea*, and the ACC effect in *E. angustifolia* was again retained. After 3 months at 5°C, the 60-day stratification effect remained in all three species, and the ethephon effect was only retained in *E. tennesseensis* and the ACC

effect in *E. angustifolia* (Tables 3.10-3.12). After 3 months at 25°C, the 60-day stratification effect remained in only *E. purpurea* and *E. tennesseensis*. The ethylene treatments for the same time length and temperature were not retained or retained to a lesser degree, depending on species (Tables 3.10-3.12).

DISCUSSION

The most effective seed enhancement treatments are those that are retained in dry seeds so that the commercial grower can easily sow them mechanically and obtain high germination. In this regard, priming has been shown to be an effective treatment for *Echinacea purpurea* (Pill et al., 1994; Samfield et al., 1991; Wartidininghsih et al., 1994), but it has not been extensively studied for other *Echinacea* species. Stratification generally improves seed germination in most *Echinacea* seed lots (Romero et al., 2005), but there is little information about long term retention of the stratification effect following drying. The results from the current study demonstrated that the stratification effect was generally retained in all *Echinacea* species evaluated immediately following drying, with the exception of *E. simulata*, (Tables 3.1-3.4) and can be retained up to 3 months for *E. purpurea, E. tennesseensis*, and *E. angustifolia* when stored at 5^{o}C (Tables 3.10-3.12). This was similar to a study by Wartidiningsih (1992) which found that *E. purpurea* seeds exposed to chilling stratification for 30 days prior to drying still retained the stratification effect after 3 months storage at 5^{o}C.

An alternative to lengthy chilling stratification was desired for *Echinacea* species. Since germination in the presence of ethylene (usually in the form of ethephon) has been used successfully to enhance germination in *Echinacea* species (Feghahati and Reese, 1994; Macchia et al., 2001; Sari et al., 2001; Qu et al., 2004), the current research investigated whether seeds could be pretreated with ethephon or ACC to improve germination and have this effect retained after drying. The presence of ACC with *Echinacea* seeds generally enhanced germination to a level comparable to stratified seeds, but ethephon only improved germination in *E. tennesseensis*, *E. angustifolia*, and *E. simulata* (Tables 3.1–3.4). This increase in germination may be due to the large increases in ethylene evolved in the constant ethephon treatment and the differences in sensitivity to ethylene between species (Tables 3.6-3.8). However, the results of this

study are in contrast to most other studies that showed increased germination in *E. angustifolia* seeds treated with ethephon (Feghahati and Reese, 1994; Macchia et al., 2001; Sari et al., 2001; Qu et al., 2004). Attempts to pre-treat seeds with ACC and ethephon were comparable to constant ethylene exposure (Tables 3.1-3.4). Ethylene production after pretreatment showed increased levels in ethylene-pretreated seeds compared to untreated controls especially in ethephon-pretreated seeds (Tables 3.6-3.9), indicating that the pretreatments were effective in getting the chemicals into seeds. However, comparing ACC or ethephon pretreatments with water pretreatments at each temperature, there were few significant differences in final germination; the exception was *E. simulata* seeds in which there were significant differences between ethephon-pretreated and water-pretreated seeds (Tables 3.1-3.4).

Three-day germination and final germination of *E. purpurea*, *E. tennesseensis*, and *E. simulata* seeds were both higher than untreated seeds upon being pretreated with ethephon for 7 days at 5°C (Tables 3.1, 3.2, and 3.4). Highest germination of *E. angustifolia* was obtained after the seeds were pretreated with ACC for 7 days at 5°C (Table 3.3). Improved germination which resulted from a combination of ethylene and stratification were similar to results from other studies. Feghahati and Reese (1994) found that germination of *E. angustifolia* seeds was about 80% upon being prechilled and treated with ethephon in the light for 2 weeks. Macchia et al. (2001) showed that germination levels of *E. angustifolia* seeds reached about 90% when chilling, light, and ethephon were used. Germination of *E. pallida* and *E. angustifolia* seeds was increased when ethephon was included in the medium during chilling stratification (Sari et al., 2001). The average increase in germination was 49% for *E. pallida* and 17% for the *E. angustifolia* seed lots (Sari et al., 2001).

As expected, storage studies with *E. purpurea* and *E. tennesseensis* pretreated with ethephon for 7 days and *E. angustifolia* pretreated with ACC for 7 days showed a significant effect on germination due to storage temperature with greater deterioration occurring at 25^{o}C (Tables 3.10-3.12). The results of this storage experiment were in partial agreement to previous purple coneflower and lettuce storage studies. The 60-day stratification effect was retained in *E. purpurea*, *E. tennesseensis*, and *E. angustifolia* after 1 month at both 5°C and 25°C, though to a lesser degree for *E. angustifolia* at both

temperatures and for *E. tennesseensis* at 5°C (Tables 3.10-3.12). The ethephon effect for the same time length was retained in only final germination of *E. purpurea* at both temperatures, and in *E. tennesseensis* it was retained to a lesser degree in 3-day germination at 5°C storage and in final germination at 25°C storage. The ACC effect was retained in *E. angustifolia* in 3-day germination and final germination after 1 month at both 5°C and 25°C. Germination speed in ethephon-pretreated *E. purpurea* and *E. tennesseensis* seeds dropped sharply after 1 month at 25°C. These results are similar to those found by Wartidiningsih (1992) after 1-month storage of primed *E. purpurea* seeds at 25°C or 30°C. This demonstrated that temperature may have had an effect on whether a stratification or ethylene treatment was retained. After 3 months at 5°C, the 60-day stratification effect remained in all three species, and the ethephon effect was only retained in *E. tennesseensis* and the ACC effect in *E. angustifolia* (Tables 3.10-3.12). This was similar to a study by Korkmaz (2006) in which after 2 months' storage at 4°C, germination was around 80% for lettuce seeds primed in -1.5 MPa KH_2PO_4 solution with 5 μM or 10 μM ACC. After 3 months at 25°C, the 60-day stratification effect remained in only *E. purpurea* and *E. tennesseensis* seeds. The ethylene treatments for the same time length and temperature were not retained or retained to a lesser degree, depending on species (Tables 3.10-3.12). Stratification for 60 days is a commercially-viable treatment for seeds of *E. purpurea* and *E. tennesseensis*. For *E. angustifolia*, pretreating with ACC for 7 days at 5°C as well as stratification for 60 days are possible commercially-viable treatments.

For *E. purpurea*, *E. tennesseensis*, and *E. angustifolia*, moist stratifying seeds followed by drying and putting them into storage at 5°C is a retainable treatment after 3 months (Tables 3.10-3.12). Ethylene in the form of ACC or ethephon increased germination, especially when the seeds were in constant exposure to it (Tables 3.6-3.9). Pretreating seeds with ethylene increased only germination speed in *E. purpurea* and *E. tennesseensis*, and it had no effect on final germination in *E. angustifolia* (Tables 3.1-3.3). However, in *E. angustifolia*, germination speed also increased upon pretreatment with ACC for 7 days at 5°C compared to the untreated control (Table 3.3). Pretreating with ethylene increased both germination speed and final germination in *E. simulata* (Table 3.4). It can be concluded for this part of the study that while ethylene

pretreatments did increase germination to some extent depending on species, 60-day stratification alone was a more commercially-viable treatment for *Echinacea* species.

Table 3.1: Three day and final germination percentage of *E. purpurea* seeds stratified for 60 days at 5°C (with or without drying), constantly exposed to water, 5 mM ACC, or 1 mM ethephon, and pretreated with water, ACC, or ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

Treatment	3-day	12-day
Untreated	3f[y]	62bc[y]
60-day stratification	83a	84abc
60-day stratification (dried)	65abc	73abc
Constant ACC	57b	86a
Constant ethephon	4f	37c
1 day at 25°C		
Water	27de	76abc
ACC	41bcde	84ab
Ethephon	44bcd	83ab
1 day at 35°C		
Water	13ef	53c
ACC	24de	55bc
Ethephon	34cde	64bc
7 days at 5°C		
Water	61abc	89a
ACC	69ab	93a
Ethephon	70ab	83abc

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.2: Three day and final germination percentage of *E. tennesseensis* seeds stratified for 60 days at 5°C (with or without drying), constantly exposed to water, 5 mM ACC, or 1 mM ethephon, and pretreated with water, ACC, or ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

Treatment	3-day	12-day
Untreated	2f[y]	52bcde[y]
60-day stratification	66ab	86a
60-day stratification (dried)	86a	87a
Constant ACC	59b	82a
Constant ethephon	41bc	81a
1 day at 25°C		
Water	0f	33def
ACC	3ef	19f
Ethephon	26cd	40cdef
1 day at 35°C		
Water	0f	57bcd
ACC	3ef	26ef
Ethephon	31cd	53bcde
7 days at 5°C		
Water	15cde	66abc
ACC	10def	70ab
Ethephon	41bc	73ab

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.3: Three day and final germination percentage of *E. angustifolia* seeds stratified for 60 days at 5°C (with or without drying), constantly exposed to water, 5 mM ACC, or 1 mM ethephon, and pretreated with water, ACC, or ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

Treatment	3-day	12-day
Untreated	48b[y]	56ab[y]
60-day stratification	50b	50ab
60-day stratification (dried)	46b	51ab
Constant ACC	57ab	71a
Constant ethephon	55ab	58ab
1 day at 25°C		
Water	40b	48b
ACC	61ab	61ab
Ethephon	52ab	54ab
1 day at 35°C		
Water	42b	42b
ACC	45b	45b
Ethephon	47b	47b
7 days at 5°C		
Water	50b	55ab
ACC	74a	74a
Ethephon	55ab	55ab

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.4: Three day and final germination percentage of *E. simulata* seeds stratified for 60 days at 5°C (with or without drying), constantly exposed to water, 5 mM ACC, or 1 mM ethephon, and pretreated with water, ACC, or ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

Treatment	3-day	12-day
Untreated	2ef[y]	26e[y]
60-day stratification	59a	78a
60-day stratification (dried)	20bcd	54bcd
Constant ACC	63a	82a
Constant ethephon	38abc	83a
1 day at 25°C		
Water	2ef	36de
ACC	15cde	30de
Ethephon	38abc	62abc
1 day at 35°C		
Water	1f	26e
ACC	15cde	23e
Ethephon	44ab	68ab
7 days at 5°C		
Water	4def	34de
ACC	22bc	43cde
Ethephon	60a	76ab

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.5: Ethylene production (µl/l over 48 hours per 20 seeds) from citrate phosphate buffer (pH 4.0), water, buffer plus 1.0 mM ethephon, and water plus 1 mM ethephon with and without *E. angustifolia* seeds.

Treatment	1-day	2-day	Total
Water (with seeds)	0.4b[y]	0.6b[y]	1.0b[y]
Buffer (with seeds)	1.1b	3.6b	4.7b
Water+Ethephon (with seeds)	3035.4a	6638.0a	9673.4a
Water+Ethephon (without seeds)	110.3b	80.4b	190.8b
Buffer+Ethephon (with seeds)	118.8b	304.7b	423.4b
Buffer+Ethephon (without seeds)	54.4b	231.4b	285.8b

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 3.6: Ethylene production (µl/l per 24 hours per 20 seeds) from *E. purpurea* seeds after being pretreated with water, 5 mM ACC, or 1 mM ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

	Days			
Treatment	2	4	6	Total
Untreated	0.7c[y]	4.2bc[y]	1.4b[y]	6.3b[y]
Constant ACC	9.7bc	38.0abc	28.8b	76.6b
Constant Ethephon	176.0	3496.9	2499.9	6172.7
1 day at 25°C				
Water	1.2c	3.2bc	3.5b	7.9b
ACC	15.6bc	94.5abc	34.1b	144.2b
Ethephon	8.6bc	121.4ab	266.1a	396.0a
1 day at 35°C				
Water	1.3c	1.9c	2.9b	6.1b
ACC	14.8bc	39.4abc	12.9b	67.1b
Ethephon	12.8bc	156.5a	299.3a	468.7a
7 days at 5°C				
Water	16.0bc	4.3bc	4.6b	24.9b
ACC	33.9ab	12.5bc	11.2b	57.5b
Ethephon	54.5a	28.3bc	7.3b	90.0b

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.7: Ethylene production (µl/l per 24 hours per 20 seeds) from *E. tennesseensis* seeds after being pretreated with water, 5 mM ACC, or 1 mM ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

	Days			
Treatment	2	4	6	Total
Untreated	1.3d[y]	0.4b[y]	4.1b[y]	5.8c[y]
Constant ACC	17.3d	110.7a	224.0a	352.0b
Constant Ethephon	189.4	1535.4	2523.2	4247.9
1 day at 25°C				
Water	7.7d	1.7b	2.2b	11.6c
ACC	24.9cd	9.1b	2.0b	36.0c
Ethephon	406.8a	121.6a	42.9b	571.3a
1 day at 35°C				
Water	20.9d	2.0b	1.1b	24.0c
ACC	16.4d	5.9b	4.3b	26.7c
Ethephon	348.1b	97.9a	26.1b	472.1ac
7 days at 5°C				
Water	3.5d	3.8b	3.1b	10.4c
ACC	19.7d	3.8b	4.6b	28.2a
Ethephon	70.7c	21.7b	7.8b	100.2c

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.8: Ethylene production (µl/l per 24 hours per 20 seeds) from *E. angustifolia* seeds after being pretreated with water, 5 mM ACC, or 1 mM ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

	Days			
Treatment	2	4	6	Total
Untreated	2.5e[y]	4.6d[y]	4.6d[y]	11.7d[y]
Constant ACC	15.8cde	78.8bcd	144.0a	238.6b
Constant Ethephon	54.0	1007.0	3612.7	4673.7
1 day at 25°C				
Water	14.9cde	5.6d	3.6d	24.1cd
ACC	66.2c	54.3cd	15.1cd	135.6bcd
Ethephon	288.0a	207.2ab	74.3bc	569.5a
1 day at 35°C				
Water	9.7de	4.2d	3.2d	17.0d
ACC	48.3cde	42.0cd	9.9d	100.2bcd
Ethephon	221.6b	216.6a	95.2ab	533.4a
7 days at 5°C				
Water	2.8e	3.3d	4.3d	10.5d
ACC	59.5cd	89.3abcd	37.2bcd	186.0bc
Ethephon	310.1a	148.7abc	40.7bcd	499.5a

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 3.9: Ethylene production (µl/l per 24 hours per 20 seeds) from *E. simulata* seeds after being pretreated with water, 5 mM ACC, or 1 mM ethephon for either 1 day at 25°C or 35°C or 7 days at 5°C.

	Days			
Treatment	2	4	6	Total
Untreated	3.0c[y]	3.6d[y]	4.8b[y]	11.5b[y]
Constant ACC	12.7c	85.7bcd	176.6a	275.0b
1 day at 25°C				
Water	1.8c	6.6d	2.3b	10.7b
ACC	25.5c	9.5d	4.6b	39.6b
Ethephon	552.3b	265.6a	85.4b	903.3a
1 day at 35°C				
Water	7.9c	2.2d	1.8b	11.8b
ACC	33.2c	36.9cd	5.7b	75.8b
Ethephon	625.1ab	138.3bc	51.1b	814.6a
7 days at 5°C				
Water	0.8c	8.3d	2.3b	11.4b
ACC	48.4c	16.2d	10.1b	74.7b
Ethephon	811.4a	187.2ab	55.9b	1054.5a

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

Table 3.10: Germination percentage of treated *E. purpurea* seeds after 1-month and 3-months storage at 5°C and 25°C.

Time	Treatment	Temperature	3-day	12-day
0 Months	Untreated	25°C	18bc[y]	86a[y]
	60-day stratification	25°C	83a	84a
	7-day ethephon	25°C	70a	83ab
1 Month	Untreated	5°C	15bc	77ab
		25°C	6c	58bc
	60-day stratification	5°C	82a	85a
		25°C	69a	85a
	7-day ethephon	5°C	24b	65ab
		25°C	27b	75ab
3 Months	Untreated	5°C	13bc	71ab
		25°C	5c	58bc
	60-day stratification	5°C	78a	81ab
		25°C	65a	75ab
	7-day ethephon	5°C	16bc	36c
		25°C	9bc	32c

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 3.11: Germination percentage of treated *E. tennesseensis* seeds after 1-month and 3-months storage at 5°C and 25°C.

Time	Treatment	Temperature	3-day	12-day
0 Months	Untreated	25°C	2.0g[y]	52.0def[y]
	60-day stratification	25°C	87.0ab	87.0ab
	7-day ethephon	25°C	41.0de	73.0bcd
1 Month	Untreated	5°C	2.0g	47.0ef
		25°C	1.0g	47.0ef
	60-day stratification	5°C	73.0bc	79.0bc
		25°C	94.0a	95.0a
	7-day ethephon	5°C	22.0ef	42.0f
		25°C	12.3fg	51.5def
3 Months	Untreated	5°C	0.0g	59.0cdef
		25°C	3.0g	68.0cde
	60-day stratification	5°C	81.0ab	81.0abc
		25°C	81.8ab	81.8abc
	7-day ethephon	5°C	48.0cd	70.0cde
		25°C	24.0def	54.0def

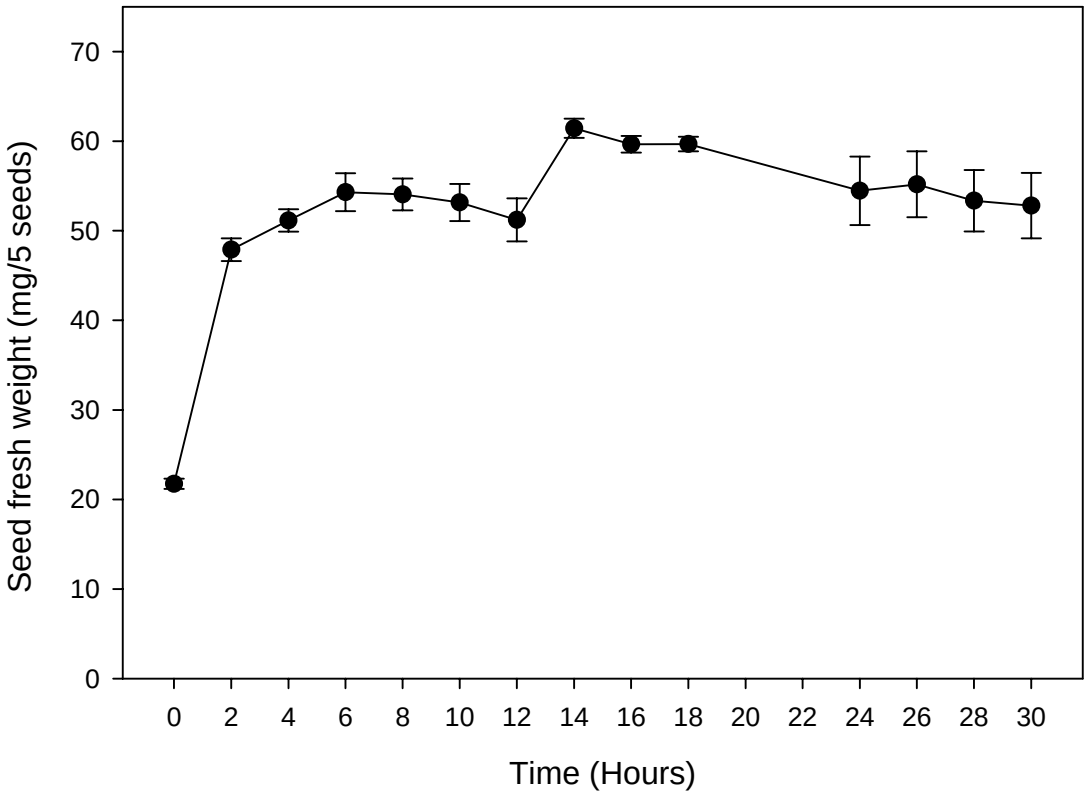
[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 3.12: Germination percentage of treated *E. angustifolia* seeds after 1-month and 3-months storage at 5°C and 25°C.

Time	Treatment	Temperature	3-day	12-day
0 Months	Untreated	25°C	48de[y]	56bc[y]
	60-day stratification	25°C	77ab	77ab
	7-day ACC	25°C	74abc	74ab
1 Month	Untreated	5°C	65abcd	65abc
		25°C	44def	44cd
	60-day stratification	5°C	53bcde	53bcd
		25°C	58bcd	58bc
	7-day ACC	5°C	68abcd	68abc
		25°C	67abcd	67abc
3 Months	Untreated	5°C	50cde	51bcd
		25°C	28ef	28d
	60-day stratification	5°C	65abcd	65abc
		25°C	22f	25d
	7-day ACC	5°C	84a	84a
		25°C	59bcd	59bc

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Figure 3.1: Water uptake curve of *E. purpurea* seeds.



CHAPTER 4: PHYSIOLOGICAL SIGNIFICANCE OF ETHYLENE PRODUCTION FOR DORMANCY RELEASE IN *ECHINACEA* SPECIES

INTRODUCTION

In some plant species, breaking dormancy and initiating seed germination require ethylene (Kępczyński and Kępczyńska, 1997). Ethylene appears to have two roles during germination. One role is that ethylene can break seed dormancy, while a second role is to reduce the time to radicle emergence in non-dormant seeds. Both of these activities are probably related to making the seeds less sensitive to abscisic acid (ABA) which keeps seeds dormant (Siriwitayawan et al., 2003). Ethylene production is initiated by certain factors, such as chilling, light, and imbibition that break dormancy and many species, such as wild oat (Adkins and Ross, 1981) and Virginia-type peanut (Ketring and Morgan, 1971, 1972), manufacture ethylene during germination (Matilla, 2000). This finding gave rise to the idea that dormancy may be broken in some species by exposure to ethylene during early imbibition (Matilla, 2000). For most seeds, ethylene production increases with radicle emergence, peaks soon after, and then declines (Matilla, 2000).

Exposure of dormant *Echinacea* seeds to ethylene can induce germination, but the physiological significance of ethylene has not been extensively studied. However, the relationship between ethylene and thermodormancy has been studied in lettuce, which is also an achene and belongs to the Asteraceae. For lettuce, the ability to produce ethylene has been associated with thermodormancy. Lettuce seeds imbibed at temperatures that induce thermodormancy (35^{o}C) show reduced ability for ACC synthesis as well as the conversion of ACC to ethylene (Huang and Khan, 1992; Khan and Prusinski, 1989). In addition, treatments that can alleviate thermodormancy (kinetin application) and those that prevent its induction (priming) result in increased ethylene production (Khan and Huang, 1988; Huang & Khan, 1992; Nascimento et al., 2004). Similarly, 'Dark Green Boston' lettuce seeds failed to germinate at 35^{o}C upon being primed with PEG + STS (STS, ethylene action inhibitor silver thiosulphate) (Nascimento et al., 2004).

One mechanism for ethylene's involvement with dormancy release is the interaction with ABA. As seeds form, it appears that ABA plays an important role in dormancy initiation (Hartmann et al., 2002). While the embryo forms in the ovule, ABA

keeps the embryo from germinating too early ("precocious germination") (Hartmann et al., 2002). To initiate dormancy during seed formation, the presence of ABA is necessary, as was shown in ABA mutants in tomato (Groot and Karssen, 1992) and *Arabidopsis* (Karssen et al., 1983). Primary dormancy is initiated by ABA as the fruit matures, and the level of ABA rises during this time. As seeds are cold-stratified the level of ABA is observed to decrease (Hartmann et al., 2002). The seed coats of dormant rose, plum, walnut, apple, and peach seeds have also been found to contain ABA (Hartmann et al., 2002).

Gallardo et al. (1991) found that chickpea seeds treated with ABA failed to germinate, and ethylene production was eliminated. Peanut seeds were released from dormancy when subjected to exogenous ethylene, and ABA had less of an effect in the presence of the ethylene (Ketring and Morgan, 1970). Ethylene and ABA share downstream elements in signal transduction, and ethylene-treated seeds show reduced sensitivity to ABA-inhibited germination (Ghassemian et al., 2000).

The objectives of this research were to study the relationship between ethylene production and dormancy release in *Echinacea* and to determine if ethylene production or perception was an important mode of action for dormancy release in *Echinacea* seeds.

MATERIALS AND METHODS

Plant material:

Four *Echinacea* species (*E. purpurea*, *E. tennesseensis*, *E. angustifolia*, and *E. simulata*) were used in this study. The seeds were grown on the South Farm of the University of Kentucky (*E. purpurea*, *E. tennesseensis*, and *E. simulata*) and purchased from Native American Seed Farm, Junction, TX (*E. angustifolia*), Johnny Selected Seeds, Winslow, ME (*E. purpurea* and *E. tennesseensis*), Everwilde Farms, Bloomer, WI (*E. tennesseensis*), and Easyliving Wildflowers, Willow Springs, MO (*E. simulata*). Initial seed germination within each species was similar across seed lots obtained from different sources.

Germination conditions:

Twenty-five seeds were germinated in four replicate Petri dishes (100 x 15 mm) containing 6 mL of deionized water (control), two pieces of Grade 8001 germination paper (Stults Scientific Co., Mt. Holly Springs, PA) about the size of the bottom of the Petri dishes, and sealed with parafilm (Pechiney Plastic Packaging, Chicago, IL). Germination occurred at a constant 25^{o}C in 8 hours light and 16 hours dark, and germination (radicle protrusion) was recorded daily for 12 days or after 3 (germination speed) and 12 days (final germination).

Ethylene inhibitors and cold stratification:

Four replications of 25 seeds were placed into Petri dishes with 6 mL of deionized water, 2 mM silver thiosulfate (STS) (Sigma-Aldrich, St. Louis), or 1 mM AVG (ReTain, Valent Biosciences, Libertyville, IL), two pieces of germination paper (Stults Scientific Co., Mt. Holly Springs, PA), and sealed with parafilm. The seeds were then placed in a refrigerator at 5^{o}C for 0, 30, or 60 days, depending on the experiment.

Following stratification, seeds from all species were transferred to new Petri dishes with 6 mL of water, 2 mM STS, or a 2-day treatment with 1 µl/l MCP (EthylBloc powder, Biotechnologies for Horticulture, Burr Ridge, IL) mixed in a pH 5 buffer prior to being moved to Petri dishes with water. For seeds treated with MCP following stratification, 25 seeds were placed into four replicate Petri dishes (60 x 15 mm) with two pieces of germination paper about the size of the bottom of the Petri dishes and 2 mL of deionized water. Open dishes were placed on top of a black plastic plug tray (to keep them level) inside a 3.9 L glass jar along with a 1.5 mL microcentrifuge tube containing the MCP powder and buffer solution. The tube containing the MCP was capped, shaken, and inserted inside the jar; the tube was then quickly uncapped, and the jar lid screwed on. The dishes were kept in the jar for 2 days, and upon removal the seeds were transferred to 100 x 15 mm Petri dishes with 6 mL of deionized water. Germination was recorded after 3 and 12 days.

In a second experiment using only *E. purpurea* and *E. tennesseensis*, seeds were treated with 2 mM STS before, during, and following 30-day stratification. Seeds of *E. tennesseensis* were treated with 1 mM AVG with and without stratification and ethylene

production and germination recorded each day for 6 days and final germination (12-day) being recorded as well. Seeds of *E. purpurea* were also treated with 1 mM AVG with and without stratification, and germination was recorded after 3 and 12 days.

ABA sensitivity:

For the experiment investigating the effect of ACC on ABA sensitivity for germination, seeds were placed in four replicate Petri dishes with 6 mL of water, 5 mM ACC, 10 μM ABA (Sigma-Aldrich, St. Louis, MO), 50 μM ABA, 5 mM ACC + 10 μM ABA, or 5 mM ACC + 50 μM ABA, and germination was recorded daily. In a second ABA experiment evaluating the effect of stratification on ABA sensitivity for germination, seeds were stratified for 0 or 60 days in the manner mentioned previously then transferred to new Petri dishes with 0, 10, or 50 μM ABA. Germination was recorded daily for 12 days.

Ethylene determination:

Twenty seeds were placed in four replicated 25 mL Erlenmeyer flasks containing 1 mL of 0 or 5 mM ACC, one piece of Whatman #1 Qualitative filter paper (Maidstone, England) about the size of the bottom of the flasks, and parafilm was placed over the tops of the flasks. Ethylene in the flasks was measured every day for 6 days. On the day before each 2-day sample point, the parafilm was removed from the tops of the flasks and replaced with rubber stoppers for 24 hours to collect ethylene. One mL from each flask was then injected into a Buck Scientific Model 910 flame ionization detector (FID) gas chromatograph, and upon taking each sample the flasks were vented. The gas chromatograph (GC) had a Supelco Custom Column 121799 packed with 80/100 Alumina F-1, the oven temperature was 100^{o}C, the nitrogen flow rate was 1 mL per minute, and the detector temperature was 150^{o}C. The standard used was 100 μl/l ethylene in helium.

Ethylene was measured in seeds receiving 0 and 60 days stratification for all species and for *E. purpurea* and *E. tennesseensis* seeds stratified for 0 and 30 days treated with or without 1 mM AVG.

Statistical analysis:

The design of each experiment was completely randomized with a one-way ANOVA arrangement, but a two-way layout for Tables 4.1-4.4. Germination percentages were converted to arcsin $\sqrt{x^2}$ before being analyzed statistically. Treatment means were compared by Tukey's test using SAS software (SAS Institute, Cary, NC).

RESULTS

Ethylene production and germination in stratified and ACC-treated seeds

For *E. purpurea* and *E. angustifolia*, stratification did not increase the capacity for seeds to make ethylene (Tables 4.1 and 4.3). There was only an effect for increased ethylene production with ACC treatment. For *E. tennesseensis* and *E. simulata*, there was very little impact of stratification on the ability to make ethylene, but there was a significant increase in the ability to convert exogenous ACC to ethylene (Tables 4.2 and 4.4).

Germination in STS- and MCP-treated seeds

STS treatment had no effect on germination in all four species regardless of stratification treatment, although for *E. angustifolia* germination of the stratified STS seeds was decreased to some degree compared to the stratified control (Tables 4.5-4.8). MCP treatment did not effect final 12-day germination in any species except *E. angustifolia* which showed a reduction in stratified seeds compared to the stratified control (Tables 4.5-4.8). This decrease in *E. angustifolia* germination could have been due to poor seed quality or fungal contamination during stratification. There was a consistent reduction in 3-day germination in MCP-treated seeds of *E. tennesseensis* and *E. simulata* following stratification compared to the stratified control (Tables 4.6 and 4.8). A decrease in germination speed was also seen in non-stratified MCP-treated seeds of *E. angustifolia* compared to the untreated control, and 3-day germination was decreased to some degree in stratified MCP-treated seeds compared to the stratified control (Table 4.7).

Germination with or without stratification in water or STS then transferred to water or STS

STS applied during or after stratification generally did not effect 3-day or final germination in *E. purpurea* and *E. tennesseensis*; it actually increased final germination of *E. tennesseensis* over the water control (Figures 4.1-4.2). With 30-day moist stratification, STS only decreased germination speed (3-day germination) of *E. tennesseensis* when the seeds were first stratified in water then germinated in STS (Figure 4.2).

Impact of AVG on germination and ethylene production

In *E. tennesseensis*, more ethylene was produced by seeds moist chilled for 30 days in water then germinated in water compared to non-stratified seeds or seeds treated with AVG (Figure 4.3). AVG-treated seeds showed a reduced amount of ethylene produced no matter whether the seeds were moist stratified for 30 days or not (Figure 4.3).

There were no significant differences in germination over the first 6 days or after 12 days between water and AVG-treated seeds without stratification (Figure 4.4). There was an increase in germination speed due to stratification, but there was no impact of including AVG in the stratification medium (Figure 4.4). However, there was slower germination when seeds were stratified in AVG and moved to AVG for germination than when seeds were stratified in water then moved to water or AVG (Figure 4.4). There was also no effect of AVG on germination in stratified or non-stratified seeds of *E. purpurea* (Figure 4.5).

Sensitivity of stratified seeds to exogenous ABA

In non-stratified seeds, ABA alone decreased 3-day germination of only *E. angustifolia*; 3-day germination decreased as the ABA concentration increased from 10 to 50 μM (Figure 4.8). Final germination of the 10 μM ABA-treated seeds of *E. purpurea*, *E. tennesseensis*, and *E. angustifolia* was comparable to that of the untreated controls, but 50 μM ABA resulted in a decrease (Figures 4.6-4.8). In *E. simulata*, 10 μM ABA gave final germination that was comparable to the untreated control (Figure 4.9). Overall,

ABA alone decreased germination in all four species. ABA also decreased germination speed following stratification in all species as the ABA concentration increased from 10 to 50 μM ABA; this was in comparison to 60-day stratification in water (Figures 4.6-4.9). Following stratification in all four species, final germination of 10 μM ABA-treated seeds was comparable to the 60-day stratified control, but there was a reduction in final germination of the 50 μM ABA-treated seeds with the exception of *E. tennesseensis* (Figures 4.6-4.9). Overall, when seeds were stratified, they were no longer inhibited to the same extent as non-stratified seeds treated with the same amount of ABA; the exception was *E. angustifolia*. This difference in germination with *E. angustifolia* could have been due to poor seed quality or fungal contamination during stratification.

Sensitivity of ACC-treated seeds to ABA

ABA at 10 and 50 μM decreased germination percentages over 12 days compared to the untreated controls for *E. tennesseensis*, *E. angustifolia*, and *E. simulata* (Figures 4.10-4.12). Including ACC in the medium reduced the sensitivity of seeds to ABA, thus reducing the overall negative impact of ABA on germination. This reduction in ABA sensitivity resulted in an increase in 3-day germination and final germination (Figures 4.10-4.12).

DISCUSSION

Echinacea species display endogenous physiological dormancy, and cold stratification can generally be used to break it (Tables 3.1-3.4). Ethylene in the form of ACC or ethephon can also substitute for stratification (Tables 3.1-3.4). However, it is not clear if there is a physiological relationship between stratification and ethylene on dormancy release.

Like many species, *Echinacea* seeds make ethylene following imbibition. It is commonly observed that ethylene production increases with radicle emergence, peaks soon after, and then declines (Matilla, 2000). However, in this study cold stratification did not dramatically increase ethylene production (Tables 4.1-4.4). In contrast, Esashi et al. (1978) found that imbibing cocklebur (*Xanthium pennsylvanicum* Wallr.) seeds in water for up to 11 days at 13°C resulted in a high rate of ethylene production. In the case

of the cocklebur study, the temperature may not have been low enough for cold stratification, which could have accounted for the increase in ethylene. Following stratification, *E. purpurea* and *E. angustifolia* showed no change in ability of seeds to convert ACC to ethylene, while *E. tennesseensis* and *E. simulata* showed dramatic increases in conversion rate (Tables 4.1-4.4). *Echinacea tennesseensis* and *E. simulata* showed the greatest response to stratification. It is possible that the sensitivity to ethylene also increased during stratification rather than ethylene production. Studies with lettuce seeds have looked at the effect of ethylene on germination under stressful temperatures. In the temperature range of 20 to 25°C, lettuce seeds may only need to manufacture a small amount of endogenous ethylene for germination (Nascimento, 2003). On the other hand, ethylene was necessary when lettuce seeds were subjected to a stressful environment, such as 35°C, while undergoing imbibition; at this high temperature, detection of ACC synthesis could not be made (Huang and Khan, 1992).

Blocking ethylene production using AVG and ethylene perception using silver and MCP suggest that ethylene action was not required for germination or dormancy release via stratification. STS treatment had no impact on germination in any *Echinacea* species regardless of stratification treatment (Tables 4.5-4.8). This is in contrast to a study by Nascimento et al. (2004) in which PEG-primed 'Dark Green Boston' lettuce seeds at 35°C did not germinate in the presence of 20 mM STS. However, in a study by Abeles (1986), germination of lettuce seeds at 25°C was not hindered by STS, but a decrease in germination was seen at 30°C. MCP treatment did not effect final 12-day germination in all species except *E. angustifolia* which showed a reduction in stratified seeds compared to the stratified control (Tables 4.5-4.8). There was no significant difference in *E. tennesseensis* germination over the first 6 days or after 12 days between water and AVG-treated seeds without stratification (Figure 4.4). There was also no effect of AVG on germination in stratified or non-stratified seeds of *E. purpurea* (Figure 4.5). These results are supported by findings in which germination in peanut (Hoffman et al., 1983) and pendant amaranth (Kępczyński and Karssen, 1985) seeds were not affected by AVG, but ethylene manufactured was still decreased by AVG. The results of these previous studies helped confirm those of this study as AVG-treated *E. tennesseensis* seeds showed a reduced amount of ethylene produced no matter whether the seeds were

moist stratified for 30 days or not (Figure 4.3). In contradiction to this, Abeles (1986) found that germination and ethylene production of lettuce seeds were prevented in the presence of AVG, indicating that ethylene is involved in germination of lettuce seeds. The effect of AVG was partly overridden by application of exogenous ethylene (Abeles, 1986).

The impact of reduced ethylene action in *Echinacea* seeds was only seen in a decrease in germination speed, but the exception was *E. angustifolia* which also had a decrease in final germination (Tables 4.5-4.8). There was a consistent reduction in 3-day germination in MCP-treated seeds of *E. tennesseensis* and *E. simulata* following stratification compared to the stratified control (Tables 4.6 and 4.8). STS only decreased 3-day germination of *E. tennesseensis* when seeds were first stratified for 30 days in water then germinated in STS (Figure 4.2). *Echinacea tennesseensis* seeds also germinated slower when seeds were stratified in AVG then moved to AVG for germination (Figure 4.4). According to a study on lettuce seeds by Sharples (1973), the 24 hours following ethephon imbibition resulted in the highest peak in germination rate overall. Age of seeds also plays a role in the effect of ethylene on seed vigor. Lettuce seeds had higher ethylene production at 35°C prior to aging (Nascimento et al., 1999). Ethylene production appears to decrease as seeds age (Nascimento, 2003).

Based on these two pieces of evidence, one conclusion of this study is that ethylene is not involved in germination and dormancy release. Alternatively, the membrane around the *Echinacea* seeds could have reduced uptake of the inhibitors. The embryo of Asteraceae species is enclosed in an endosperm that is made up of thick-walled cells found in sheets (Schulthess et al., 1991). This layer may be semipermeable as has been found in muskmelon and cucumber seeds (Singh, 1953; Sreenivasulu and Amritphale, 1999). In cucurbits, this envelope is made of three layers which are as follows: a thin layer consisting of lipids, a thick, non-cellular layer consisting of callose substances, and a layer of endosperm cells is below these two layers (Yim and Bradford, 1998). It is common that seed coverings (fruit, testa, or endosperm) act as semipermeable barriers that allow water uptake but restrict solute penetration (Taylor et al., 1997). Therefore, it is possible that significant amounts of ethylene-action inhibitors (STS and MCP) could have been prevented from entering the seed and affecting the embryo.

However, endogenous ethylene production in ACC- (Table 4.2) and AVG- (Figure 4.3) treated seeds demonstrated that these chemicals are being delivered to the embryo to some degree. Still, ethylene production in AVG-treated *E. tennesseensis* seeds showed that ethylene production was reduced but not completely eliminated (Figure 4.3). Therefore, we must consider that *Echinacea* seeds could only require a small amount of ethylene to stimulate dormancy release and germination, and the degree of ethylene response has greater impact on speed (vigor) rather than total germination percentage.

There is a change in *Echinacea* seeds sensitivity to ABA following stratification (Figures 4.6-4.9). Based on the role of ABA on maintaining seed dormancy, this could be the major physiological change that occurs during stratification to improve germination speed and total germination percentage. Likewise, non-stratified ACC-treated seeds also showed reduced sensitivity to ABA (Figures 4.10-4.12). Gallardo et al. (1991) found that chickpea seeds failed to germinate, and ethylene production was prevented by ABA. According to Ghassemian et al. (2000), as seeds germinate the effects of ABA are reduced by ethylene. This was seen in this study as including ACC in the medium reduced sensitivity of the seeds to ABA, reducing the overall negative impact of ABA on germination (Figures 4.10-4.12). Other examples of this effect were found in pendant amaranth (Kepczynski, 1986) and lamb's-quarters (Karssen, 1976) seeds that germinated after the ABA effect was overcome by ethylene exposure (Kępczyński and Kępczyńska, 1997).

The conclusion from this study is that it appears that both stratification and ethylene treatments share the same mode of action related to dormancy release. There could be a physiological relationship between the two, and data from the current study suggests that these are not strongly related, but additional evidence would be required to definitively determine that relationship.

Table 4.1: Ethylene production (µl/l per 24 hours per 20 seeds) of *E. purpurea* seeds after being stratified or not stratified for 60 days at 5°C then germinated on water or 5 mM ACC.

Treatment					
ACC	Stratification	2-day	4-day	6-day	Total
0	0	1.3b[y]	1.4b[y]	2.3a[y]	4.9b[y]
5	0	8.6b	44.3a	44.8a	97.7a
0	60	7.0b	5.9b	8.6a	21.5b
5	60	42.2a	38.6a	20.0a	100.9a

ANOVA	F values			
Main effects:				
ACC	24.02**	7.38*	2.47ns	7.19*
Stratification	20.57**	0.00ns	0.29ns	0.10ns
Interaction:				
ACC x Stratification	10.31**	0.13ns	0.82ns	0.04ns

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

*, **, ns indicate significant at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively.

Table 4.2: Ethylene production (µl/l per 24 hours per 20 seeds) of *E. tennesseensis* seeds after being stratified or not stratified for 60 days at 5°C then germinated on water or 5 mM ACC.

Treatment					
ACC	Stratification	2-day	4-day	6-day	Total
0	0	3.4c[y]	5.6c[y]	1.0c[y]	10.0c[y]
5	0	13.2b	20.5b	18.1b	51.7b
0	60	1.2c	8.6c	2.7c	12.5c
5	60	116.8a	287.1a	307.2a	711.2a

ANOVA	F values			
Main effects:				
ACC	140.83**	40.76**	39.39**	84.92**
Stratification	92.10**	34.44**	32.23**	67.89**
Interaction:				
ACC x Stratification	100.42**	32.91**	31.49**	66.88**

[y]Means followed by the same letter in a column were not different at $\alpha = 5\%$ by Tukey's HSD test.

*, **, ns indicate significant at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively.

Table 4.3: Ethylene production (µl/l per 24 hours per 20 seeds) of *E. angustifolia* seeds after being stratified or not stratified for 60 days at 5°C then germinated on water or 5 mM ACC.

Treatment					
ACC	Stratification	2-day	4-day	6-day	Total
0	0	1.1b[y]	2.6b[y]	2.1b[y]	5.9c[y]
5	0	13.3b	89.3a	215.5a	318.1a
0	60	5.9b	7.2b	28.8b	41.9bc
5	60	68.5a	89.0a	92.9ab	250.3ab

ANOVA	F values			
Main effects:				
ACC	65.09**	23.88**	17.65**	24.32**
Stratification	41.75**	0.02ns	2.11ns	0.09ns
Interaction:				
ACC x Stratification	29.49**	0.02ns	5.11*	0.97ns

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

*, **, ns indicate significant at P ≤ 0.05, P ≤ 0.01, or non-significant, respectively.

Table 4.4: Ethylene production (µl/l per 24 hours per 20 seeds) of *E. simulata* seeds after being stratified or not stratified for 60 days at 5°C then germinated on water or 5 mM ACC.

Treatment					
ACC	Stratification	2-day	4-day	6-day	Total
0	0	1.7b[y]	2.0b[y]	2.7c[y]	6.4c[y]
5	0	12.7b	85.7b	176.6b	275.0b
0	60	5.7b	10.7b	7.2c	23.5c
5	60	124.7a	347.1a	761.1a	1233.0a

ANOVA	F values			
Main effects:				
ACC	95.54**	62.42**	105.31**	100.13**
Stratification	75.86**	25.81**	42.43**	43.58**
Interaction:				
ACC x Stratification	65.89**	22.59**	41.15**	40.57**

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

*, **, ns indicate significant at $P \leq 0.05$, $P \leq 0.01$, or non-significant, respectively.

Table 4.5: Germination percentage of *E. purpurea* seeds either stratified or not stratified for 60 days at 5°C then germinated in the presence of water or 2 mM STS or treated with 1 µl/l MCP for 2 days.

Treatment			
Ethylene inhibitors	Stratification	3-day	12-day
Untreated	0	5b[y]	57a[y]
	60	45a	58a
STS	0	3b	50a
	60	57a	72a
MCP	0	1b	60a
	60	32a	57a

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 4.6: Germination of *E. tennesseensis* seeds either stratified or not stratified for 60 days at 5°C then germinated in the presence of water or 2 mM STS or treated with 1 µl/l MCP for 2 days.

Treatment			
Ethylene inhibitors	Stratification	3-day	12-day
Untreated	0	2c[y]	47c[y]
	60	66a	86a
STS	0	0c	57bc
	60	49a	77ab
MCP	0	0c	58bc
	60	23b	76ab

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 4.7: Germination of *E. angustifolia* seeds either stratified or not stratified for 60 days at 5°C then germinated in the presence of water or 2 mM STS or treated with 1 µl/l MCP for 2 days.

Treatment			
Ethylene inhibitors	Stratification	3-day	12-day
Untreated	0	57a[y]	71a[y]
	60	50ab	50ab
STS	0	36ab	64a
	60	33ab	33bc
MCP	0	7c	71a
	60	24bc	24c

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Table 4.8: Germination of *E. simulata* seeds either stratified or not stratified for 60 days at 5°C then germinated in the presence of water or 2 mM STS or treated with 1 µl/l MCP for 2 days.

Treatment			
Ethylene inhibitors	Stratification	3-day	12-day
Untreated	0	2c[y]	26b[y]
	60	59a	78a
STS	0	1c	40b
	60	58a	87a
MCP	0	0c	21b
	60	23b	76a

[y]Means followed by the same letter in a column were not different at α = 5% by Tukey's HSD test.

Figure 4.1: Germination percentage of *E. purpurea* seeds after being stratified or not stratified for 30 days at 5°C in either water or 2 mM STS. Means (±S.E.) followed by the same letter for 3-day and 12-day bars, respectively, were not different at α = 5% by Tukey's HSD test.

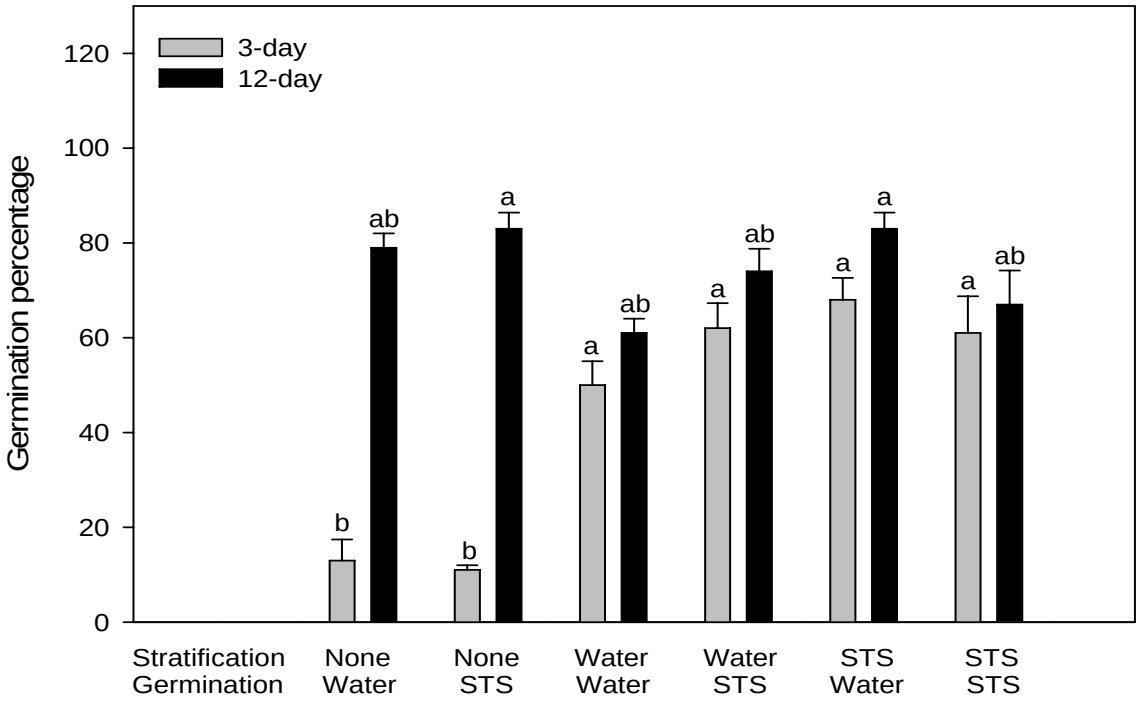


Figure 4.2: Germination percentage of *E. tennesseensis* seeds after being stratified or not stratified for 30 days at 5°C in either water or 2 mM STS. Means (±S.E.) followed by the same letter for 3-day and 12-day bars, respectively, were not different at α = 5% by Tukey's HSD test.

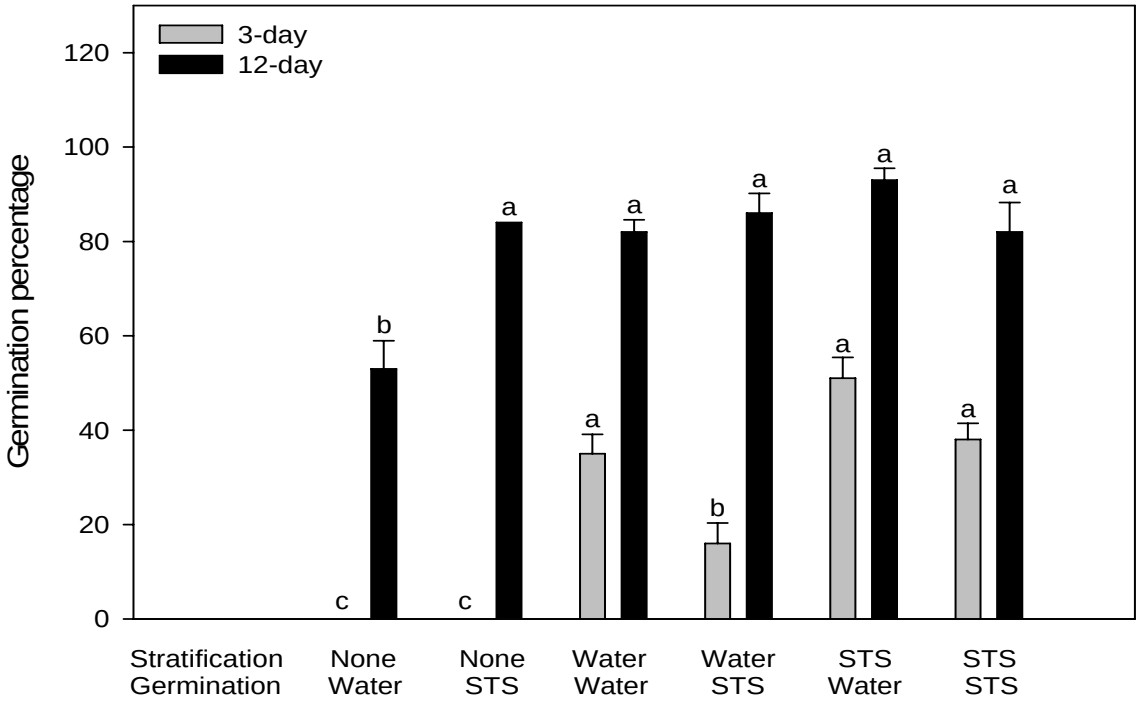


Figure 4.3: Ethylene production (µl/l per 24 hours per 20 seeds) of *E. tennessseensis* seeds stratified or not stratified for 30 days at 5°C in either water or 1 mM AVG then transferred to water or 1 mM AVG. Means (±S.E.) for each day were analyzed by Tukey's HSD test at $\alpha = 5\%$.

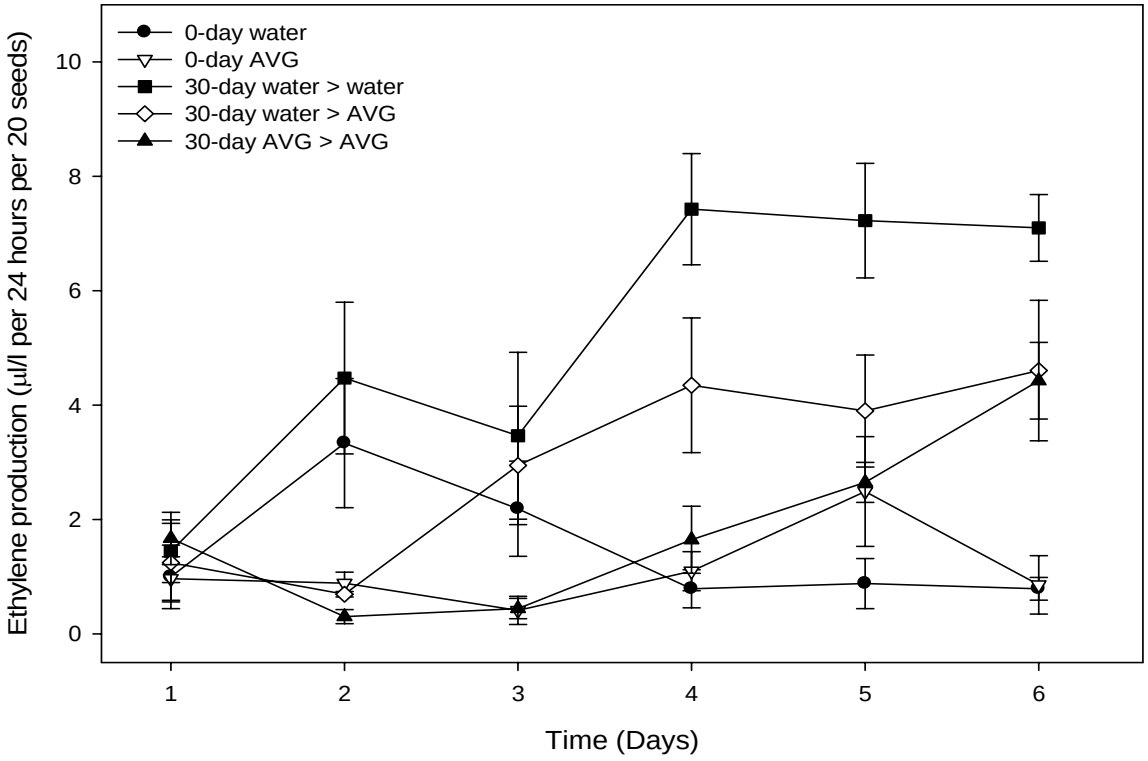


Figure 4.4: Germination percentage of *E. tennesseensis* seeds stratified or not stratified for 30 days at 5°C in water or 1 mM AVG then transferred to water or 1 mM AVG. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

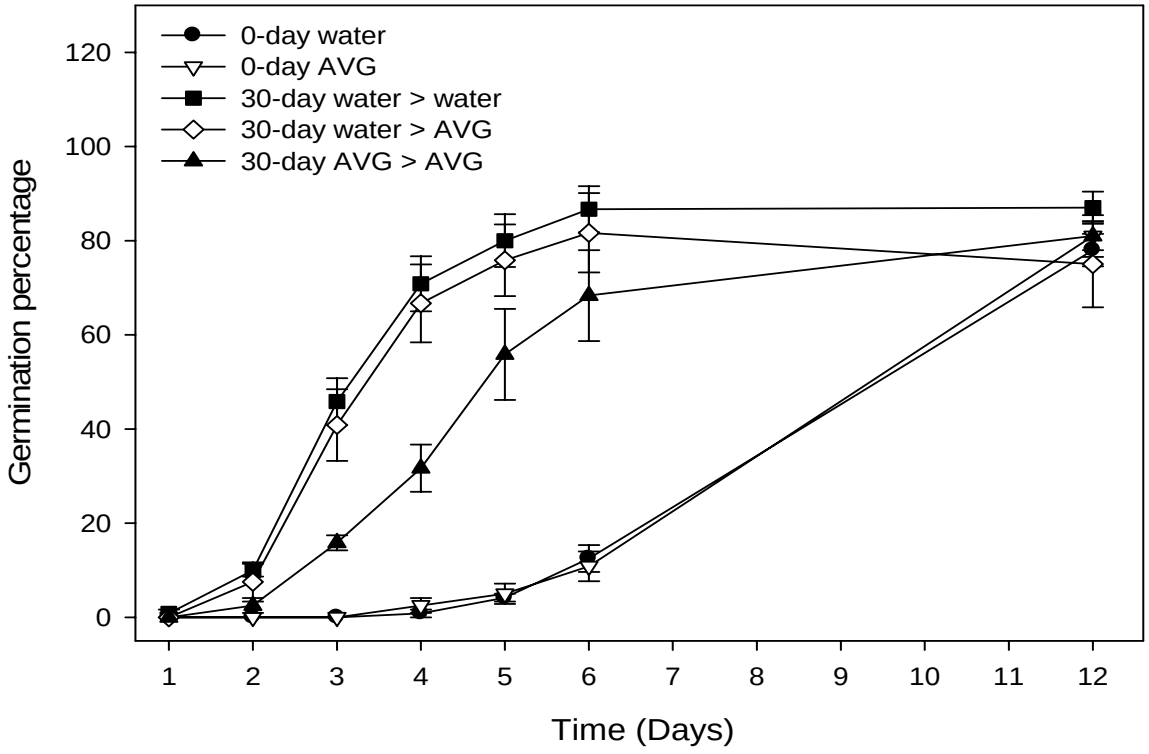


Figure 4.5: Germination percentage of *E. purpurea* seeds after being stratified or not stratified for 30 days at 5°C in either water or 1 mM AVG. Means (±S.E.) followed by the same letter for 3-day and 12-day bars, respectively, were not different at α = 5% by Tukey's HSD test.

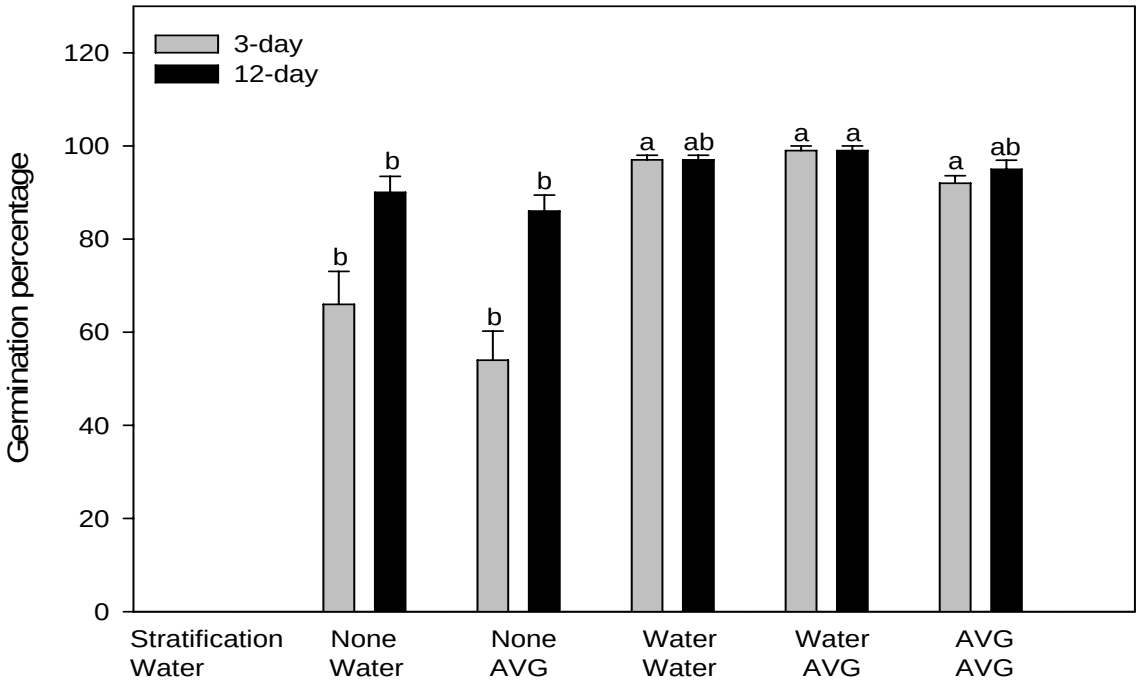


Figure 4.6: Germination percentage of *E. purpurea* seeds stratified or not stratified for 60 days at 5°C then treated with water, 10 μM ABA, or 50 μM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

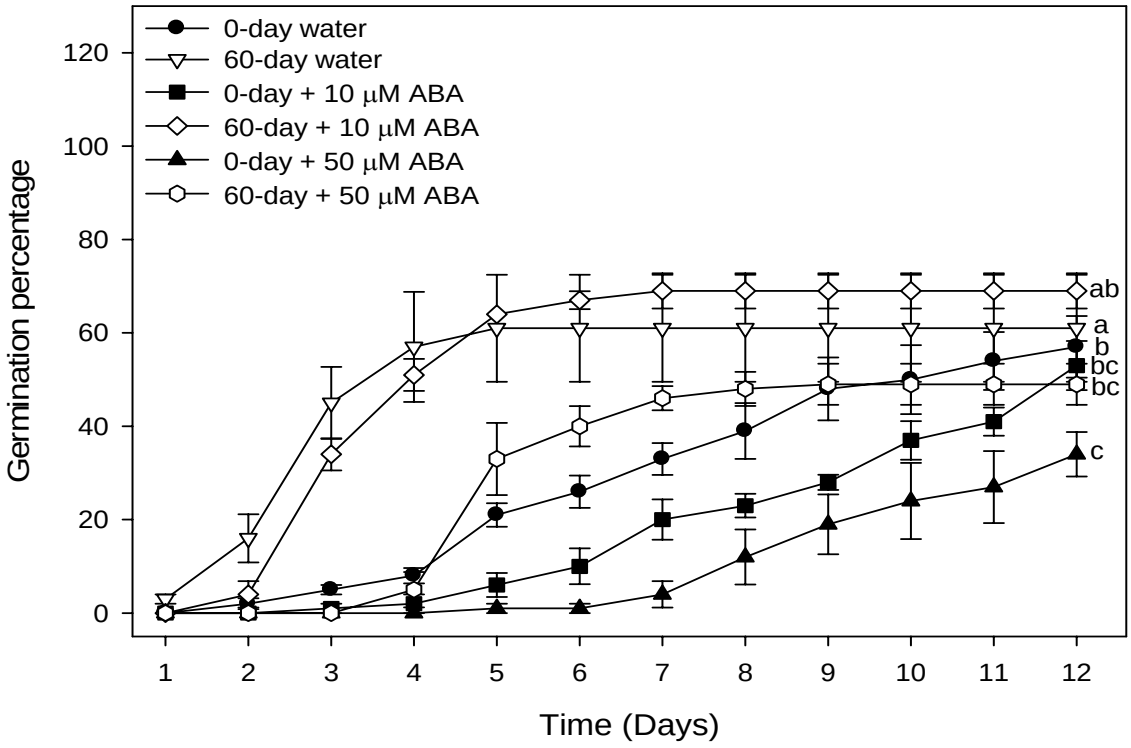


Figure 4.7: Germination percentage of *E. tennesseensis* seeds stratified or not stratified for 60 days at 5°C then treated with water, 10 µM ABA, or 50 µM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

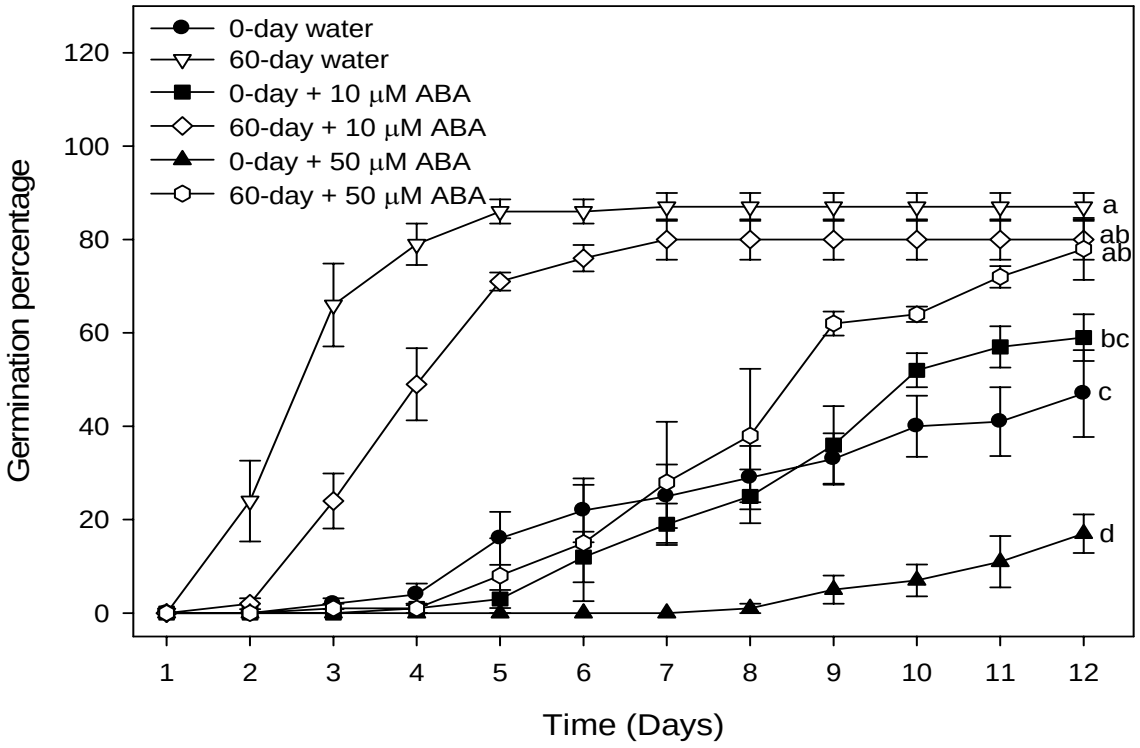


Figure 4.8: Germination percentage of *E. angustifolia* seeds stratified or not stratified for 60 days at 5°C then treated with water, 10 μM ABA, or 50 μM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

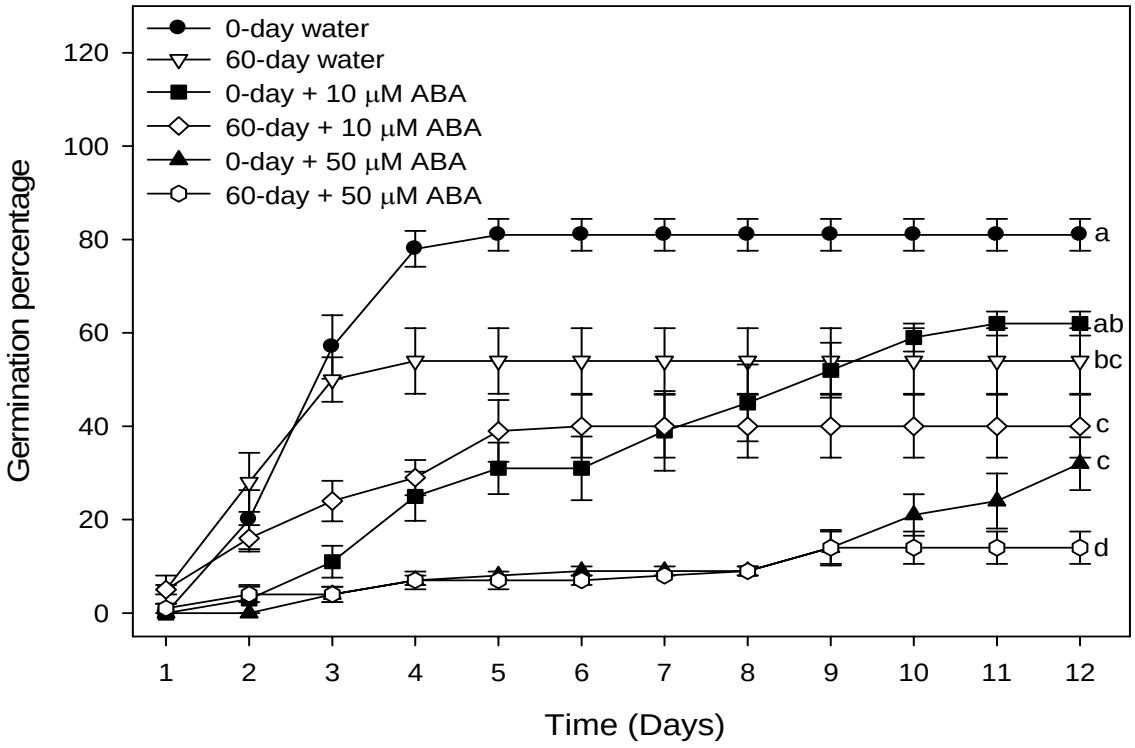


Figure 4.9: Germination percentage of *E. simulata* seeds stratified or not stratified for 60 days at 5°C then treated with water, 10 μM ABA, or 50 μM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

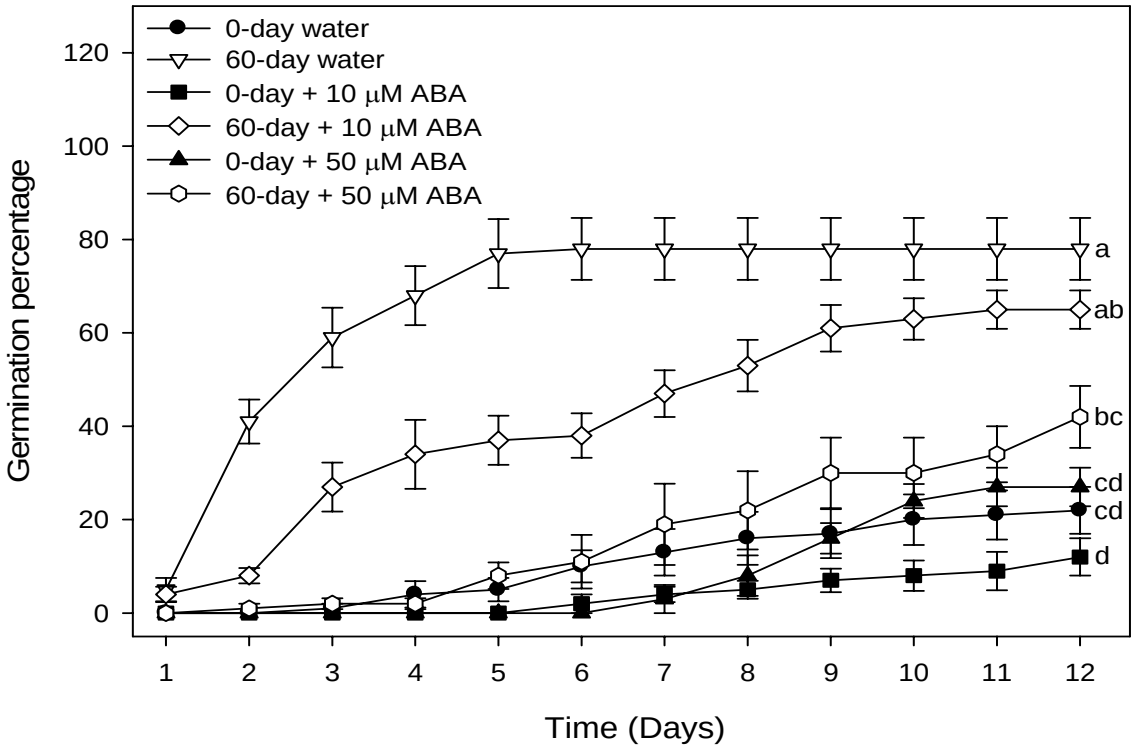


Figure 4.10: Germination percentage of *E. tennesseensis* seeds treated with water, 5 mM ACC, 10 μM ABA, 50 μM ABA, 5 mM ACC + 10 μM ABA, and 5 mM ACC + 50 μM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

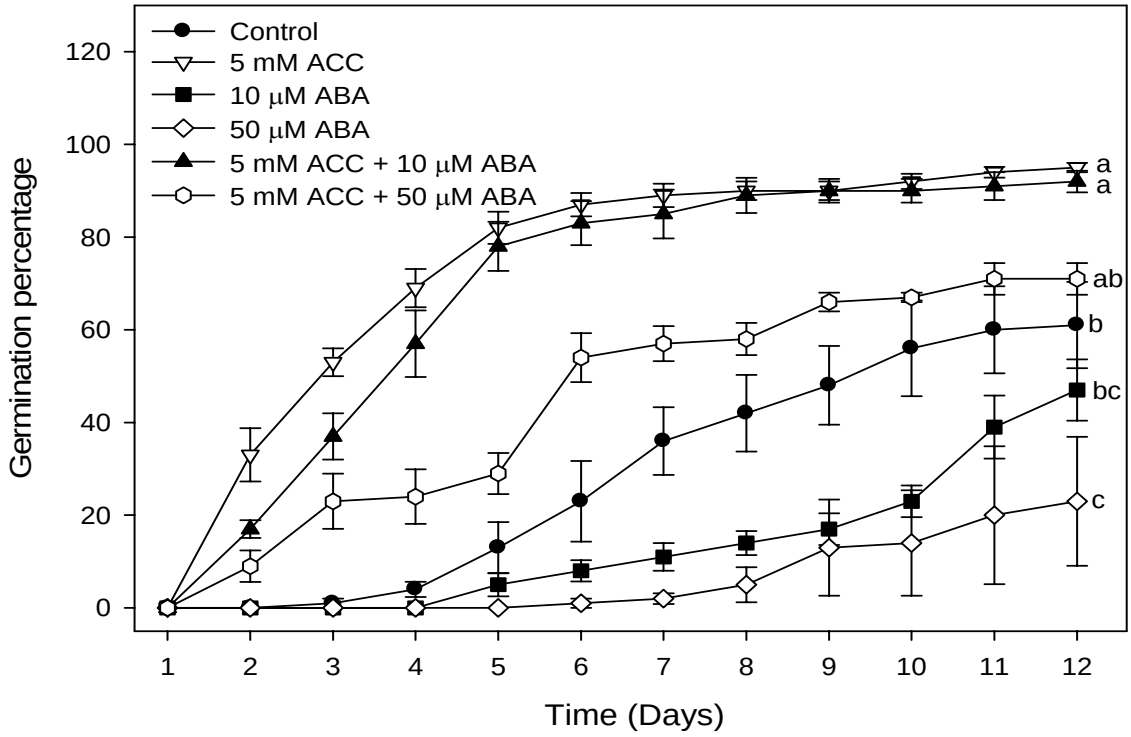


Figure 4.11: Germination percentage of *E. angustifolia* seeds treated with water, 5 mM ACC, 10 μM ABA, 50 μM ABA, 5 mM ACC + 10 μM ABA, and 5 mM ACC + 50 μM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.

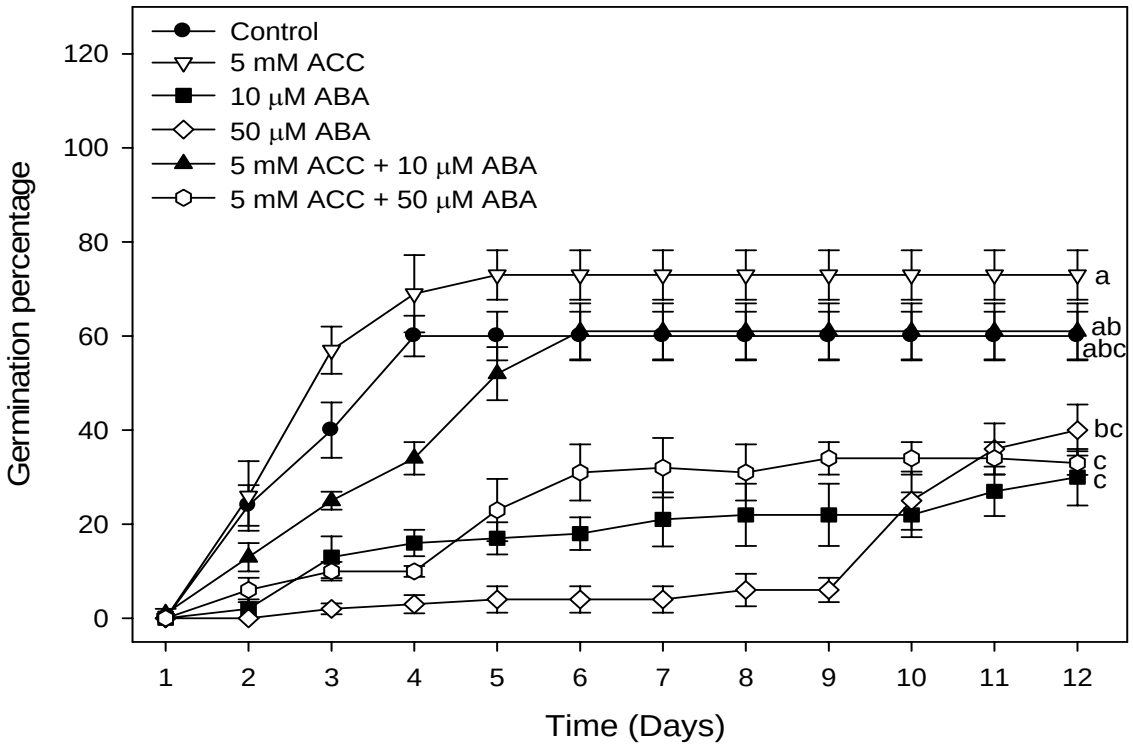
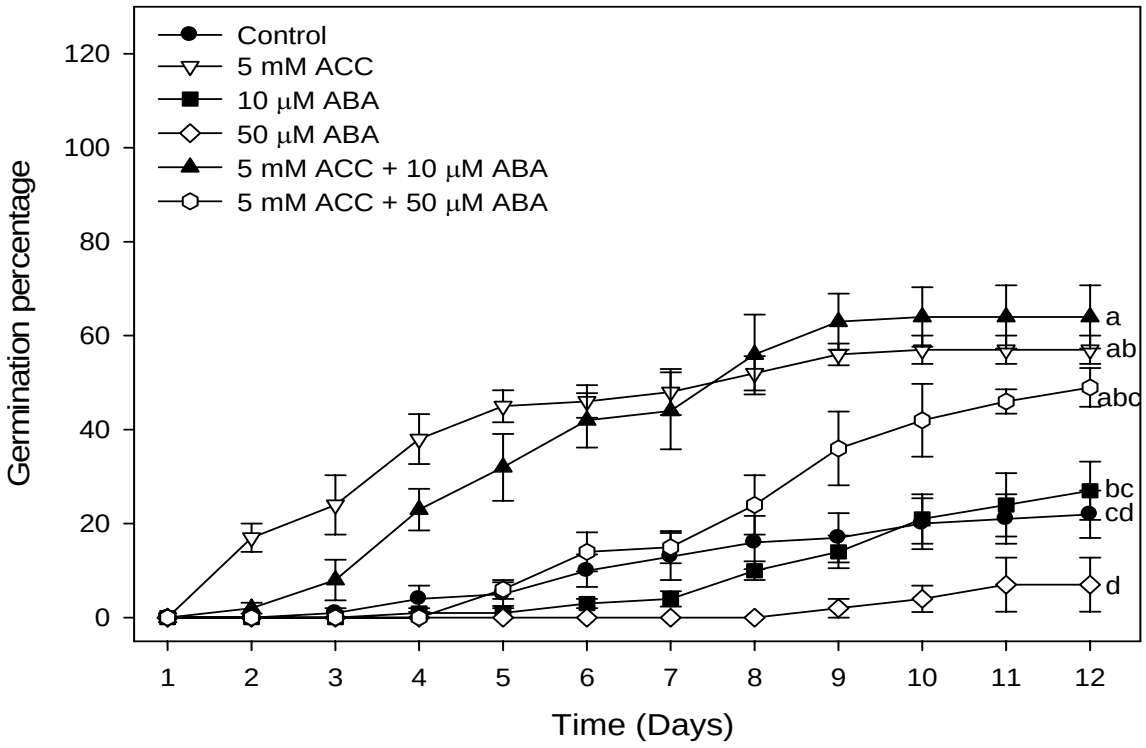


Figure 4.12: Germination percentage of *E. simulata* seeds treated with water, 5 mM ACC, 10 μM ABA, 50 μM ABA, 5 mM ACC + 10 μM ABA, and 5 mM ACC + 50 μM ABA. Means (±S.E.) for each day were analyzed by Tukey's HSD test at α = 5%.



CHAPTER 5: SUMMARY AND CONCLUSIONS

Two studies were conducted looking at the relationship between ethylene, germination, and dormancy release in *Echinacea* species.

In the first study, *E. purpurea*, *E. tennesseensis*, *E. angustifolia*, and *E. simulata* were stratified for 0 or 60 days and either dried or not dried back to near their original water content. Seeds were then treated with water, 5 mM ACC (1-aminocyclopropane-1-carboxylic acid), or 1 mM ethephon (2-chloroethylphosphoric acid). Ethylene production was recorded after 2, 4, and 6 days in non-stratified seeds exposed to water, ACC, or ethephon. Seeds were also pretreated with water, ACC, or ethephon for 24 hours at 25^{o}C or 35^{o}C and 7 days at 5^{o}C then dried back to near their original water content; ethylene production was again recorded after 2, 4, and 6 days. Untreated, 60-day stratified then dried, and ACC- or ethephon-pretreated seeds of *E. purpurea*, *E. tennesseensis*, and *E. angustifolia* were stored for 0, 1, and 3 months as well.

The results showed that 60-day stratification and constant ethephon and ACC were effective in increasing germination speed and final germination of all four species to be greater than or comparable to the untreated control. Stratification and ethylene were therefore effective treatments in relieving dormancy and increasing germination. The effect of seed pretreatments varied between species. For *E. purpurea*, including ACC or ethephon in the pretreatment solution did not increase 3-day germination, and it increased final germination to some degree although the increase was not statistically different. *Echinacea tennesseensis* seeds pretreated at 25 or 35^{o}C only showed an increase in 3-day germination with the inclusion of ethephon in the pretreatment solution. Pretreatment at 5^{o}C also showed an increase in 3-day germination with the greatest effect with ethephon. There was very little effect of pretreating *E. angustifolia* seeds. However, there was a significant increase in 3-day germination by pretreating seeds with ACC at 5^{o}C and a 32% increase in final germination compared to untreated seeds. *Echinacea simulata* seeds responded to pretreatment with ethephon at all temperatures for improved 3-day and final germination. The increase in germination was comparable to stratification treatments. In all four species, ethylene production after pretreatment with ethylene showed increased levels in pretreated seeds compared to untreated controls. This was

seen especially in ethephon-pretreated seeds indicating that they were effective in getting the chemicals into seeds.

The 7-day ethephon effect in *E. purpurea* seeds was lost upon 3 months of storage, but the 60-day stratification effect was retained after 3 months thus demonstrating that it can potentially be used in commercial production. In *E. tennesseensis* seeds the 7-day ethephon effect was still present after storage for 3 months at 5°C and to a lesser degree at 25°C, and the 60-day stratification effect was also retained at both 5°C and 25°C. Pretreating with ethephon for 7 days at 5°C as well as stratification for 60 days are possible commercially-viable treatments for this species. Both the 7-day ACC effect and the 60-day stratification effect were retained in *E. angustifolia* seeds after 3 months at 5°C; the ACC effect was also retained to a lesser degree after 3 months at 25°C. Pretreating with ACC for 7 days at 5°C as well as stratification for 60 days are possible commercially-viable treatments for this species. While ethylene pretreatments did increase germination to some extent depending on species, it was concluded that 60-day stratification alone was a more commercially-viable treatment.

In the second study, *E. purpurea*, *E. tennesseensis*, *E. angustifolia*, and *E. simulata* were stratified for 0 or 60 days then treated with 0 or 5 mM ACC. Ethylene production was recorded after 2, 4, and 6 days. In another set of experiments, seeds were stratified for 0, 30, or 60 days in the presence of water, 2 mM STS, or 1 mM AVG then germinated in water, STS, or AVG. Seeds, either stratified for 60 days or not stratified, were treated with 1 µl/l MCP mixed in a pH 5 buffer for 2 days. Ethylene was also measured as well as germination every day for 6 days in *E. tennesseensis* seeds following combinations of 0- or 30-day stratification and water or 1 mM AVG. In addition, seeds were stratified for 0 or 60 days then germinated in water, 10 µM ABA, or 50 µM ABA, and in another experiment non-stratified seeds were germinated in water, 5 mM ACC, 10 µM ABA, 50 µM ABA, 5 mM ACC + 10 µM ABA, or 5 mM ACC + 50 µM ABA.

For *E. purpurea* and *E. angustifolia*, stratification did not increase the capacity for seeds to produce ethylene. There was only an effect for increased ethylene production with ACC treatment. For *E. tennesseensis* and *E. simulata*, there was very little impact of stratification on the ability to make ethylene, but there was a significant increase in the

ability to convert exogenous ACC to ethylene. Stratification was, therefore, not necessary to increase ethylene production, but ACC did increase production of ethylene.

STS treatment had no effect on germination regardless of stratification treatment, although for *E. angustifolia* germination of the stratified STS seeds was decreased to some degree compared to the stratified control. MCP treatment did not effect final 12-day germination in all species except *E. angustifolia*, which showed a reduction in stratified seeds compared to the stratified control. This difference in germination with *E. angustifolia* could have been due to poor seed quality or fungal contamination during stratification. There was a consistent reduction in 3-day germination in MCP-treated seeds of *E. tennesseensis* and *E. simulata* following stratification compared to the stratified control. A decrease in germination speed was also seen in non-stratified MCP-treated seeds of *E. angustifolia* compared to the untreated control, and 3-day germination was decreased to some degree in stratified MCP-treated seeds compared to the stratified control. Blocking ethylene with these two inhibitors showed that ethylene action was not required for germination or dormancy release via stratification.

AVG-treated *E. tennesseensis* seeds showed a reduced amount of ethylene whether the seeds were stratified or not stratified. An increase in germination speed of *E. tennesseensis* seeds was due to stratification, but there was no impact of including AVG in the stratification medium. However, seeds that were stratified in AVG and moved to AVG germinated slower than when seeds were stratified in water then moved to water or AVG for germination. There was also no effect of AVG on germination in stratified or non-stratified seeds of *E. purpurea*. This again suggested that ethylene was not required for germination or dormancy release.

Both stratification and ACC treatment reduced *Echinacea* seed sensitivity to ABA and could be a common mechanism for enhanced germination. However, it does not appear that the increased germination seen after stratification was mediated through ethylene production because final germination percentages were generally unchanged following inhibition of ethylene production or action. In contrast, inhibition of ethylene production and perception reduced early 3-day germination suggesting that ethylene was more involved in seed vigor than final germination. It was determined that there is no physiological significance of ethylene for dormancy release in these *Echinacea* species.

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