

Environment and hormone effects on seed biology of kochia (*Kochia scoparia*)

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## Abstract

Understanding the impact of early spring environmental conditions on kochia seed biology as well as time from flowering to seed maturation could suggest effective control practices. Kochia seed accessions were exposed to moisture stress or different phytohormone combinations across a range of temperature conditions to evaluate cumulative seed germination. Base temperature ( $T_b$ ) and base water potential ( $\psi_b$ ) were determined and used to calculate thermal (TT), hydro (HT), and hydrothermal (HTT) time to describe cumulative percent kochia germination across the environmental conditions. Base temperature was 2.7 C and  $\psi_b$  was -1.70, -1.62, and -0.92 MPa at 11, 6, and 4 C, respectively. To reach 50% cumulative seed germination, more days (2 and 11 d) and more TT (16.7 and 74.8 Cd) were needed as temperature decreased from 11 to 4 C and as water potential decreased from 0.0 to -1.2 MPa. Using the HT and HTT scale helped to account for effect of moisture stress. A small fraction of seeds under low temperature and high moisture stress germinated faster (10% at 6 C and -1.2 MPa) compared to more seed germinated at higher temperature and less moisture stress (91% at 11 C and -0.0 MPa). Addition of abscisic acid (10  $\mu$ M ABA) decreased germination by 16% at both 23 and 6 C, while addition of gibberellic acid (10  $\mu$ M GA) increased germination by 14% at both 23 and 6 C. Addition of ABA delayed time to 50% germination at 6 C compared to water, with 3 days or 10 Cd. Similarly, addition of GA shortened time to 50% germination at 6 C as it took 8.9 d and 29.3 Cd as compared to 10 d and 33 Cd in water. Kochia seed development was observed visually in greenhouse conditions after controlled and natural pollination. Kochia flower buds changed color from light green to dark brown by 36 days after pollination (DAP). At 36 DAP, buds had seeds enclosed in them but a germination study showed they were not yet viable. Hence, seed germination decreased and seeds needed more time to accumulate enough thermal

and hydro unit before germinating as temperature and moisture stresses increased from 11 to 4 C and -0.0 to -1.2 MPa. We conclude that kochia seeds can germinate under very low temperature and moisture potential. Hence, should be controlled very early in the growing season.

Phytohormones ABA and GA decrease and increase the germination of seeds, respectively.

Kochia takes more than 36 DAP to produce viable seeds. Information on flower bud changes after pollination have implications for both kochia management and research.

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# Chapter 1 - Review of Literature

## Kochia: A troublesome weed

Kochia (*Kochia scoparia* (L.) Schrad) is a summer annual broadleaf weed introduced in North America from Europe belonging to the goosefoot (*Chenopodiaceae*) family. It escaped cultivation and started naturalizing (Georgia 1914). It was grown as an ornamental hedge around gardens because of its attractive red color. Now, it is the most troublesome weed growing everywhere in North America but is most adapted to arid and semi-arid areas of the Canadian Prairies and the Great Plains of the United States (Friesen et al. 2009; Leeson et al. 2005; Nussbaum et al. 1985; Wiese 1970). Kochia is a C<sub>4</sub> plant. It utilizes water more efficiently and fixes CO<sub>2</sub> via more effective C<sub>4</sub> pathway (Friesen et al. 2009). Because of this pathway, it effectively tolerates low soil temperature, grows rapidly and tolerates heat, drought, saline soil and other form of stresses (Coxworth et al. 1969; Evetts and Burnside 1972; Pafford and Wiese 1964; Schwingamer and Van Acker 2008; Weatherspoon and Schweizer 1970). Kochia is an aggressive competitor with crops because of its early and continual emergence, extreme environment tolerance, growth habit and high seed production. Studies have shown that kochia densities of 0.5 and 0.2 plants/m reduced sugar beet yields up to 32 and 18%, respectively (Mesbah et al. 1994; Schweizer 1973;). Season long competition of kochia in soybean has been shown to reduce yield up to 30% (Forcella 1985a). Wicks et al. (1994) documented that for every kg/ha of kochia dry weight, corn grain yields decreased 0.33 kg/ha.

Kochia is known to be a very prolific seed producer (Nussbaum et al. 1985) and has been documented to produce up to 30,000 seeds per plant (Stallings et al. 1995). In a summary of small-plot studies, Watson et al. (2001) listed kochia maximum seed production at 15,000 to 25,000 seeds per plant. Kochia seeds are nearly oval and measure 1.5 to 2.0 mm long, and are

often enclosed by a fragile star-shaped hull (Friesen et al. 2009). Kochia 1000-seed weights vary widely and have been documented to range from 0.47 g (Nussbaum et al. 1985) to 0.85 g (Stevens 1932) to 1.2 g (Liebman and Sundberg 2006). The dispersal of kochia seed is aided by its tumbleweed-like structure, such that as the plant matures, seed rain can occur around the mother plant followed by stem breakage after senescence that allows the whole aboveground plant to tumble freely spreading seed for long distances (Becker 1978). Kochia is known to have the highest rate of spread among alien weeds in the western United States from 1880 to 1980 due to its tumbleweed mode of seed dispersal (Forcella 1985a).

### **Seed germination in kochia**

Kochia seed germination is highly tolerant of moisture stress, salinity, and extremes in pH. In a Petri-dish experiment, germination was reduced by simulated moisture stress (polyethylene glycol solutions) at -0.8 MPa however, germination was still approximately 50% at -0.13 MPa (Evetts and Burnside 1972) to -1.6 MPa (Everitt et al. 1983). Germination was not greatly affected by NaCl concentrations up to 10,000 ppm (170 mM) nor by the presence of six different salts in aqueous solution (Evetts and Burnside 1972). Few kochia seeds germinated at 1000 mM NaCl but germination was not permanently inhibited as seeds germinated when transferred to a distilled-water treatment after 20-d exposure to salt solutions (Khan et al. 2001). In a Saskatchewan study, germination in soil declined with increasing salinity, averaging 3.3% per deci Siemens/m between 12 and 30 deci Siemens/m (Steppuhn and Wall 1993). Like the results of Petri-dish germination experiments, exposure to high soil NaCl concentrations did not inhibit germination permanently, i.e., germination occurred after leaching of salts by precipitation and subsequent infiltration. Over a pH range of 2 to 8, kochia seed germination was 60 to 70% but declined to 25% at pH 10 (Evetts and Burnside 1972) and was only slightly

reduced (10%) at the extremes in pH of 2 and 12 (Everitt et al. 1983). These results suggest that seed germination was not severely affected by extremes of pH or saline conditions.

In Petri-dish experiments, kochia seed was observed to germinate at temperatures ranging from 3.5 to 40 C (Eberlein and Fore 1984), with an optimum temperature of 25 C (50% germination within 24 h) (Al-Ahmadi and Kafi 2007). For 10-d, 90% or greater germination occurred over a relatively wide temperature range (i.e., constant 5, 10, 15, 20, and 25 C treatments); however, germination was markedly reduced above 30 C, declining to 10% at 40 C (Everitt et al. 1983). Khan et al. (2001) reported maximum germination of a Utah population at 25/35 C night/day temperatures, whereas cooler temperatures of 5 to 15 C significantly inhibited germination. Everitt et al. (1983) found that percentage germination was not affected by alternating versus constant temperatures. Light was not required for germination as the percentage of kochia seeds that germinated in constant darkness did not differ from those exposed to a daily 8-h photoperiod (Maguire and Overland 1959). Alternatively, Zorner et al. (1984) recommended incubation at 16 C with a 16-h photoperiod to maximize germination.

Kochia seed can overwinter on the plant or below the soil surface (Frankton and Mulligan 1987; Georgia 1914). Kochia seed generally does not exhibit a high degree of innate dormancy (Dyer et al. 1993; Everitt et al. 1983; Thompson et al. 1994). Any innate or induced dormancy in kochia seed can be effectively released if seed is stored at 15 C for 3 month, followed by treatment of seed on filter paper moistened with 0.1 mM potassium nitrate (KNO<sub>3</sub>) and 500 ppm gibberellic acid, and incubated in the dark for 2 wk at 5C (Zorner et al. 1984). Kochia seed does not have physical dormancy but has physiologically-based dormancy which is the most abundant type of dormancy (Finch-Savage and Leubner-Metzger 2006) and prevents germination until a chemical change takes place in the seed (Fenner and Thompson 2005). Schwingamer and Van

Acker (2008) confirmed that even shallow burial (10 mm) of kochia seeds in pots under controlled environment conditions significantly reduced emergence. No seedlings emerged from depths greater than 40 mm, although radical emergence was evident indicating failed germination. The authors suggested that using inversion tillage to deeply bury seed might be an effective method to manage dense patches of kochia (Schwingamer and Van Acker 2008). Generally, kochia seed is relatively short lived in the soil with seed bank longevity lasting between one to two years. In the absence of seed return, the germinable end-of-season kochia seed bank is typically less than 10% of total kochia seedlings that emerged throughout the growing season (Schwingamer and Van Acker 2008). This means kochia populations are seed limited and if seed return is prevented populations will not be persistent (Schwingamer and Van Acker 2008). Previous studies suggest that kochia seeds can germinate when the upper 5 to 10 cm of soil becomes frost-free (Becker 1978).

In a field study of the emergence periodicity of kochia at 12 sites in southern Manitoba, Schwingamer and Van Acker (2008) reported that seedlings began emerging very early in the spring at about 50 cumulative growing degree-days (GDD; base temperature 0 C. More than 1000 kochia seedlings/m<sup>2</sup> emerged throughout the growing season at most sites, with an observed maximum density of 12,700 seedlings/m<sup>2</sup>. Most of these seedlings emerged early in the season prior to crop seeding. In Colorado, the minimum temperature for kochia emergence was 2.2 C (Anderson and Nielsen 1996). The predicted cumulative GDD needed for 10, 50, and 90% cumulative kochia emergence in Kansas were 168, 308, and 692 GDD, respectively (Dille et al. 2017). Studies carried out by Werle et al. (2014) in Iowa found that kochia was the first summer annual that emerged. Kochia germinated very early in spring as soon as there was enough moisture (Werle et al. 2014). Kochia emergence appears to require less moisture than what was



required for soil-applied herbicides to get activated for kochia control (Sebastian et al. 2017).

Early emergence offers kochia distinct survival and competitive advantages in cropping systems (Evetts and Burnside 1972) by exploiting the spring soil moisture in arid and semiarid regions (Eberlein and Fore 1984). Although kochia germinates early, additional flushes can occur sporadically throughout the growing season, inevitably following post-emergence herbicide applications, resulting in substantial uncontrolled populations (Mickelson et al. 2004).

### **Temperature and moisture effects on weed seed germination**

Weed seed germination and seedling establishment is a very complex physiological phenomenon that is responsive to many signals like temperature, moisture condition, light, nutrients, and phytohormones. Out of many such factors temperature and water potential are the most important environmental signals that directly guide germination and emergence, and regulate dormancy in weed seeds (Baskin and Baskin 1988).

Temperature has a primary influence on seed germination and dormancy (Baskin and Baskin 1988). There are three cardinal temperatures for seed germination: the minimum or base temperature below which no germination occurs ( $T_b$ ), optimum temperature ( $T_o$ ) where most of the germination occurs, and maximum or ceiling temperature ( $T_c$ ) above which no seeds germinate (Bewley and Black 1984). In a study carried out in Iran,  $T_b$ ,  $T_o$  and  $T_c$  for kochia were determined to be 3.5, 24, and 50 C, respectively (Al-Ahmadi and Kafi, 2007). The range between base and ceiling temperatures are the most important for either maintaining dormancy status or breaking it. Summer annual and winter annual species experience a cyclic seasonal change based on temperature. For summer annual species seed dispersal is at the end of the summer and seedling emergence is at the beginning of following spring. Germination is alleviated by the low temperatures experienced by seeds during winter, while high temperatures during summer induce

a decrease in germination levels. Thus the emergence period is restricted to the time window when the population has reached its minimum dormancy and maximum germination. Reverse is experienced by a winter annual species (Batlla and Benech-Arnold 2010).

This degree of germination establishes the width of the range of environmental conditions that allow germination. A high germination level is characterized by a wide range of environmental conditions permissive for seed germination, while a narrow range of environmental conditions result in low germination levels (Batlla and Benech-Arnold 2010). This relationship between seed germination level and the range of environmental conditions permissive for seed germination was first proposed by Vegis (1964).

More recent findings showed that not only the range of temperatures under which germination is possible changes in relation to seed germination level, but also the range of water potentials within which seed germination can proceed (Batlla et al. 2004). Seeds germinate as the range of water potential and temperature widen and a narrow range is observed for dormant seeds (Batlla and Benech-Arnold 2007; Vegis 1964). For example, during dormancy loss for the summer annual prostrate knotweed (*Polygonum aviculare*) the range of temperatures and water potentials permissive for seed germination widened because of a decrease in the lower limit temperature for seed germination ( $T_b$ ) and the base water potential ( $\psi_b$ ). On the other hand, dormancy induction occurred through a narrowing of these ranges due to an increase in  $T_b$  and  $\psi_b$  (Batlla and Benech-Arnold 2003, 2004). Field germination takes place when the range of temperatures and water potentials permissive for seed germination overlaps with the variation in soil temperature and soil water potential. This interaction between temperature and water potential thresholds values is very crucial in predicting extent and timing of weed emergence.

## **Models used to study weed seed germination and emergence**

Within the range of available weed control practices, mechanical and chemical control are most commonly used methods under field conditions (Altieri and Liebman 1988). The success of these control methods depends on reaching the highest number of individuals in the seedling stage (Fenner 1987; Kruk and Benech-Arnold 1998). Therefore, to increase their effectiveness we must be able to predict not only the date time at which certain weed species emerge, but also what proportion of the seed bank would emerge at that moment. Attempts have been made to model dormancy changes of weed seeds and predict emergence patterns in the field. Different models were developed to simulate changes in dormancy of prostrate knotweed seeds in relation to soil temperature and moisture (Batlla and Benech-Arnold 2003; 2004; 2005). The first-generation models for predicting weed emergence were based on the thermal time (growing degree days) concept (Bewick et al. 1988). In these models, weed emergence dynamics were described by considering temperature as the only factor influencing germination phase. Gummerson (1986) introduced the concept of hydrothermal time (HTT), which integrated thermal time (TT) above a base temperature ( $T_b$ ) and hydro time (HT) above a base water potential ( $\psi_b$ ) in the same algorithm. Similarly, Werle et al. (2014) made use of HT and TT models to predict emergence of 23 summer annual weed species where they found  $T_b$  and  $\psi_b$  for kochia emergence to be 12 C and -0.75 MPa respectively. Guillemain et al. (2013) determined  $T_b$  and  $\psi_b$  for 14 different weed species common to north-western European cropping system but did not have any kochia seed for their study. Parameters such as  $T_b$  and  $\psi_b$  for kochia germination have not been documented for KS. Such information would help directly in predicting kochia emergence and in designing better management plans in future

## Hormonal effects on seed germination

Phytohormones are chemical messengers that coordinate cellular activities in plants (Koornneef and Karsen 1994). The five classic hormones discovered until the 20<sup>th</sup> century were abscisic acid (ABA), gibberellic acid (GA), auxin, cytokinins and ethylene. Recently, other classes of plant hormones such as jasmonates and salicylates have been characterized (Finkelstein 2004). Hormones play an important role in each stage of vegetative and reproductive development, cellular activities and stress responses. Plant hormones are extremely important for the regulation of seed dormancy and germination; ABA and GA are directly involved in seed maturation, germination, and induction of dormancy (Finkelstein 2002; Koornneef et al. 2002).

Abscisic acid is a positive regulator of dormancy induction and most likely dormancy maintenance, while it is a negative regulator of germination. Abscisic acid accumulates during seed maturation, leading to the production of proteins and compounds that protect the embryo during seed desiccation. Gibberellic acid releases dormancy, promotes germination, and counteracts ABA effects. Apart from GA, ethylene and brassinosteroids promote seed germination and counteract ABA effects (Kucera et al. 2005).

Deficiency of ABA during seed development was associated with absence of primary dormancy in the mature seed, whereas over-expression of ABA biosynthesis genes increased seed ABA content and enhanced seed dormancy or delayed germination (Finkelstein et al. 2002; Nambara and Marion-Poll 2003). High ABA contents were present in imbibed seeds of the strongly dormant *Arabidopsis thaliana* ecotype Cape Verde Island (Cvi) and decreased as dormancy was lost (Ali-Rachedi et al. 2004). Strongly dormant *A. thaliana* ecotype Cvi showed that dormancy depended on an intrinsic balance of GA and ABA biosynthesis and catabolism,

which determined the dominance of either of the hormones (Ali-Rachedi et al. 2004; Cadman et al. 2006).

The hormone-balance theory explained seed dormancy by the simultaneous operation of hormones promoting (GA) and inhibiting (ABA) germination (Nambara and Marion-Poll 2003). The study of numerous mutant plants impaired in GA and/or ABA synthesis or sensitivity (Hilhorst and Karssen 1988; Hilhorst 1995) demonstrated definitively the essential roles of ABA and GA in seed dormancy and germination. According to Karssen and Laçka (1986), ABA and GA act at different times and sites during 'seed life'. ABA induced dormancy during seed maturation and GA played a key role in dormancy release and in the promotion of germination. Newer evidence suggests that it is likely that this revised theory went too far. Experiments with sorghum (*Sorghum bicolor*) (Steinbach et al. 1997) and with ABA-deficient and -insensitive mutants of maize (*Zea mays*) (White and Rivin 2000; White et al. 2000) demonstrated that GA and ABA acted at the same time on dormancy and germination. It appeared to be the ratio of ABA:GA, not the absolute hormone content, that controlled germination. Thus, it seemed that GA directly antagonized ABA signaling during dormancy induction of sorghum and maize seed. Experiments with other species are needed to determine whether this is a general phenomenon. While dormancy maintenance also depends on high ABA:GA ratios, dormancy release involves a net shift to increased GA biosynthesis and ABA degradation resulting in low ABA:GA ratios (Ali-Rachedi et al. 2004; Cadman et al. 2006). This supports the proposal of Le Page-Degivry and Garello (1996) that ABA is the primary hormone involved in any step during dormancy maintenance and release, and that GA are present at sufficient concentrations to promote germination as soon as ABA biosynthesis is inhibited. There is further support from genetic work with wild oat (*Avena fatua*) (Fennimore and Foley 1998) showing that GA itself, although

its addition to the medium can cause germination of dormant seeds, was not involved in (embryo) dormancy loss but in stimulating seed germination. Thus, dormancy release was characterized by the capacity for enhanced ABA degradation and increased GA biosynthesis, which was followed by GA promotion of seed germination.

### **Pollination in kochia and seed maturation biology**

Kochia typically flowers about 8 to 10 weeks after early spring emergence (Thill and Mallory-Smith 1996). It is a short-day plant that initiates flowering when the light period is less than its critical period of 13 to 15 hours (Eberlein and Fore 1984). Flowers on the plant are found in clusters in the axils of upper leaves and in terminal spikes (Eberlein and Fore 1984). Kochia produces a protogynous flower where the stigma emerges and can accept foreign pollen, and then deteriorates before pollen is shed and anther dehiscence which prevents self-pollination within the same flower (Stallings et al. 1995). Pollen grains are spheroidal, 20 to 40  $\mu\text{m}$  in diameter, and granular with 100 to 130 pores uniformly distributed over the grain surface (Stallings et al. 1995). Mulugeta et al. (1994) collected pollen from greenhouse and field-grown kochia plants and measured viability during storage at three temperatures and five relative humidity levels. Pollen stored at low temperature and high relative humidity retained at least 10% viability after 9 d. Pollen stored under high temperature and low humidity conditions retained 10% viability after about 2 d. In another study of field-collected kochia pollen, viability was 68, 27, and 9% at 0, 24, and 48 h after dehiscence, respectively, when stored at 23 C and 18% relative humidity (Stallings et al. 1995). Pollen counts from traps at 0.5 to 1.0 m above the soil surface height were highly correlated with prevailing wind direction. Up to 23 pollen grains/cm<sup>2</sup> were recovered 50 m from the pollen source (Mulugeta 1991). Mulugeta et al. 1994 estimated that 0.1% of shed pollen could be deposited more than 150 m from the source. This

type of flowering system and the copious amount of pollen produced for extended periods is an indication that the plant species is highly outcrossing but also allows for self-pollination within the same plant (Friesen et al. 2009; Mulugeta 1991).

Many weed species are short-day plants with flowering beginning after the summer solstice as daylength decreases (Deen et al. 1998; Dekker et al. 1996; Huang et al. 2001; Warwick and Black 1988). In greenhouse research, Benvenuti et al. (1994) reported growing degree day (GDD) requirements for flowering and seed formation (defined as fruits beginning to mature) for johnsongrass (*Sorghum halepense*), jimsonweed (*Datura stramonium*) and velvetleaf (*Abutilon theophrasti*) and reported shading lengthened GDDs by 10%. For the weed species studied in above mentioned literatures, the 2- to 3 wk period after anthesis is the critical time for management interventions to stop weeds from producing viable seed (Bell and Tranel 2010). Experiments showed that waterhemp (*Amaranthus tuberculatus*) seeds become mature less than 2 weeks after pollination occurred. Seed color darkens and seed weight increases linearly until approximately 12 days after pollination. Waterhemp seeds exhibited high levels of dormancy at an early age, and these dormancy levels began to drop between 15 and 30 days after pollination (Bell and Tranel 2010). Similarly, seed maturation has been studied in some other weedy species, including prickly sida (*Sida spinosa*), pitted morningglory (*Ipomoea lacunosa*), and purple moonflower (*Ipomoea turbinata*). Prickly sida had viable seed 12 days after anthesis (Egley 1976) and morningglory had viable seed 20 days after anthesis (Chandler et al. 1977; Jayasuriya et al. 2007). Winter (1960) reported mature velvetleaf seeds within 20 days after pollination. However, there is no information on time after pollination for seed maturation in kochia.

An important focus of any weed management strategy should be the prevention of seed production, which entails having some knowledge of the seed maturation biology of that species (Bell and Tranel 2010) which is lacking for kochia. Impacts of lower temperature regimes and drier moisture condition in kochia seed germination is very limited. Additional impact of hormones has never been studied. Hence, the research objectives were to: 1) determine seed germination of kochia under low temperature and high moistures stress situations under controlled conditions in growth chambers, 2) determine whether exogenously-applied hormones increase or decrease seed germination of kochia, and 3) document the seed maturation process in kochia after pollination based on visual analysis of flowering buds at different times after pollination in greenhouse conditions.



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## **Chapter 2 - Effect of temperature, moisture stress, and hormones on kochia seed germination**

### **ABSTRACT**

Kochia seed readily germinates under cool temperature and low moisture conditions. This study was done to determine base temperature ( $T_b$ ) and base water potential ( $\psi_b$ ) for kochia seed germination, rate of seed germination under varying temperature and water potentials, and influence of phytohormones on total seed germination. Seeds were collected from a common garden field experiment near Manhattan, KS in the fall of 2015. Seeds from two accessions were incubated in Petri dishes in growth chambers at one of three temperature levels (4, 6, and 11 C) and included five moisture stress levels (-0, -0.4, -0.8, -1.2 and -1.6 MPa) with 50 seed per Petri dish. Seven combinations of either water, abscisic acid (ABA) + water, ABA + fluridone + water at four concentrations and ABA + GA + water were studied on low dormant seeds and seven combinations of either water, gibberellic acid (GA) + water, GA + paclobutrazol + water at four concentrations and ABA + GA + water were studied on moderately dormant seeds in growth chambers at either 23 or 6 C. Germination counts were taken daily. Base temperature was determined to be 2.7 C and  $\psi_b$  were determined to be -1.70 MPa at 11 C, -1.62 MPa at 6 C, and -0.92 MPa at 4 C. These base values were used to calculate thermal time (TT), hydro time (HT) and hydro thermal time (HTT) and used to describe cumulative percent kochia germination across temperature and moisture stress levels. Days to 50% germination was delayed as moisture stress increased at all temperatures. Cumulative TT required for 50% seed germination in water at 11 C was 17 Cd which increased to 31 Cd at -1.2 MPa. Cumulative HT for 50% seed germination showed that seeds under greater moisture stress required less cumulative HT, that is

3.35 MPa\*d at -1.2 MPa and 4.63 MPa\*d in water. At low temperature and high moisture stress, only 10% of seed germinated quickly while at high temperature, 90% of seed germinated as they accumulated enough HT and HTT. The addition of 10  $\mu$ M of ABA decreased germination from 98 to 76% of low dormant seeds at both 23 and 6 C, seeds required 141.8 Cd cumulative TT compared to 109.1 Cd in water at 23 C. The addition of 10  $\mu$ M GA increased germination of moderately dormant seed from 74 to 88% and the addition of paclobutrazol restored the effects of GA at both 23 and 6 C. We conclude that kochia seeds can germinate under very low temperature and moisture potential. Hence, should be controlled very early in the growing season. Phytohormones ABA and GA decrease and increase the germination of seeds respectively.

## INTRODUCTION

Kochia is an increasingly problematic weed in most parts of United States and Canada. It is an early spring-emerging summer annual species that can germinate at cold temperatures. It makes use of cool spring temperatures and low moisture conditions to germinate and establish before most other summer annual weed species (Werle et al. 2014). Research carried out by Al-Ahmadi and Kafi (2007) looked at the impact of temperature on kochia seed germination and determined the base temperature or  $T_b$  as 3.5 C. Recently, Werle et al. (2014) predicted kochia emergence pattern using concepts such as growing degree days (GDD) and hydrothermal time (HTT) analysis and also quantified the additional impact of moisture on cumulative percent emergence of kochia among 23 other weed species where they determined base temperature ( $T_b$ ) and base water potential ( $\psi_b$ ) for kochia emergence to be 12 C and -0.75 MPa respectively. Kochia has been documented to germinate at soil water potentials between -1.3 and -1.6 MPa, which are considered to be dry soil moisture conditions (Everitt et al. 1983; Weise 1970).

Typically, pre-emergence soil-applied herbicides can be used to control kochia in early spring. Efficacy of pre-emergence herbicides are affected by soil physicochemical properties and available soil moisture. In a recent study, Sebastian et al. 2017 showed reduced phytotoxicity of indaziflam and flumioxazin, two broad spectra pre-emergence herbicides labeled for kochia control, under low water potential levels of -0.2 and -0.4 MPa. Previous research showed that kochia can germinate at water potentials of -0.75 MPa (Werle et al. 2014) and at -1.6 MPa (Everitt et al. 1983), which are below the moisture required for herbicide activation. Information on water potential requirement for weed seed emergence is very important in locations where water is limiting during the emergence period (Leguizamon et al. 2005). In typical field conditions of western KS, there might not be enough soil moisture available to activate these pre-

emergence herbicides, but enough soil moisture for kochia to germinate and escape herbicidal control. This may contribute to the difficulty of controlling kochia with pre-emergence herbicides. Hence, determination of  $T_b$  and  $\psi_b$  for kochia seed germination in western KS is very important, as this can suggest to farmers how to plan effective cultural practices.

Furthermore, hormones have widely been used in seed germination studies of other species where GA promoted and ABA reduced seed germination (Koorneef et al. 2002). Abscisic acid accumulates during seed maturation, leading to the production of proteins and compounds that protect the embryo during seed desiccation, and repress germination. Gibberellins release dormancy, promotes germination, and counteracts ABA effects (Kucera et al. 2005). Research on using this option to regulate germination patterns in weed seeds is a relatively new concept. No data have been reported on how kochia responds to these phytohormones. Applying hormones to kochia plants or seeds could provide new control options.

The overall goal of this research was to determine the role of low temperature, high moisture stress, and phytohormones on kochia seed germination. The specific objectives were to 1) determine base temperature and base water potential for kochia seed germination, 2) determine the rate of seed germination under range of temperature and water potentials, and 3) determine the influence of phytohormones on total kochia seed germination.

## **MATERIALS AND METHODS**

### **Effect of temperature and moisture stress**

Seeds were collected from a common garden field experiment near Manhattan, KS in fall of 2015. Two kochia accessions, identified as KS1 and KS2, were used to study the effect of low temperature and high moisture stress on seed germination. Maternal parent for KS1 accession

was from Colby, Thomas County, KS and maternal parent for KS2 accession was from Garden City, Finney County, KS while pollen was from diverse sources surrounding each maternal parent. All the experiments were carried out in growth chambers located in Department of Agronomy, Kansas State University, Manhattan, KS. Each germination test was conducted using 100 mm by 15 mm Petri dishes with 24 hour dark period. Three growth chambers were set at either 4, 6, or 11 C. Five moisture stress levels of 0.0, -0.4, -0.8, -1.2, and -1.6 MPa were created using polyethylene glycol-8000 (PEG, Sigma Aldrich) by solving quadratic equation for each temperature of 4, 6, and 11 C according to Michel (1983). These conditions were created in Petri dishes to depict a field situation where 0.0 MPa represented pure water. The moisture environment becomes drier, that is increasing moisture stress levels, as water potential decreases from -0.4 to -1.6 MPa. Each moisture level within a temperature treatment had five replications with 50 seeds per Petri dish for each accession and repeated in time. The Petri dishes were covered with parafilm throughout the experiment to avoid moisture loss. First run of the experiment was set up in December 16, 2015 and the second run was set up in January 18, 2016. Daily counts of germinated seeds were taken by opening each Petri dish to count, removed germinated seeds, re-sealed. A seed was regarded as germinated as soon as radicle was visible. This procedure was continued until no more germination was observed or 21 days. All seeds that did not germinate at the end of the experiment had presence of mold and were considered dead. Cumulative percent seed germination was determined based on total seed germination at the end of the experiment. Analysis of variance was carried out using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC) to determine if total seed germinated was influenced by main effects and interactions among two runs, three temperature levels, five moisture stress levels within each

temperature level, and two accessions. Significant effects were compared based on least significant difference (LSD) value among the means at  $\alpha = 0.05$ .

To describe the germination response over time, a sigmoidal function was fit to the data using SigmaPlot v.12 (Systat Software Inc, San Jose, California):

$$G = a/(1+\exp(-(X-X_0)/b)) \quad \text{Eq. 2.1}$$

where  $G$  is total germination (#/dish),  $X$  is days or thermal time (TT), hydro time (HT), hydro thermal time (HTT),  $a$  is maximum germination,  $b$  is slope at the inflection point, and  $X_0$  is time required for 50% germination.

Initial F-test conducted using sum of squares of residuals and degrees of freedom for combined model (both accession combined) and individual model (each accession) showed that accession was not a significant factor ( $P > 0.05$ ) influencing germination of kochia, hence, data were pooled across accessions.

In order to estimate the  $T_b$  and  $\psi_b$  for kochia seed germination, the reciprocal time to 50% germination ( $1/X_0$ ) is believed to be the most statistically robust and biologically relevant (Steinmaus et al. 2000). A linear regression was performed with the reciprocal of number of days required for 50% germination ( $T_{50}$ ) against incubation temperature. The minimum temperature threshold ( $T_b$ ) was estimated as the intercept of the specific regression line with the temperature (x-axis) axis. Similarly, reciprocal of  $T_{50}$  when regressed against moisture stress levels gave the base water potential value ( $\psi_b$ ). The estimated base values were used to convert days of observation for each temperature and moisture stress level treatment to thermal time (TT), hydro time (HT), and hydrothermal time (HTT) as follows:

$$TT = (T-T_b) \quad \text{Eq 2.2}$$

$$HT = (\psi-\psi_b) \quad \text{Eq 2.3}$$

$$\text{HTT} = (T - T_b) * (\psi - \psi_b) \quad \text{Eq 2.4}$$

where T is daily air temperature within growth chamber (C),  $\Psi$  is daily water potential value within Petri dish (-MPa),  $T_b$  is estimated base temperature for kochia germination, and  $\psi_b$  is estimated base water potential for kochia germination. Cumulative percent germination was regressed on cumulative TT (Cd), HT (MPa\*d), or HTT (MPa\*Cd) using the sigmoidal three-parameter model (equation 2.1) using SigmaPlot v.12 (Systat Software Inc, San Jose, CA).

### **Effect of phytohormones**

Several kochia accessions were collected from above mentioned field experiment and were subjected to germination tests to identify an accession with greatest germination rate (for ABA portion of study) and one with least germination rate (for GA portion of study). A total of five accessions were screened. Five replications of 50 seeds per Petri dish per accession were placed in a dark growth chamber set at 25 C on January 20, 2016. Daily germination counts were taken for three weeks. Total percent germination was determined at the end of the experiment (Appendix B.3), with KS3 accession having 98% total germination and KS4 accession having least germination rate of 75%. These two accessions were used in following experiments.

Seeds from KS3 were exposed to seven different phytohormone combinations including tap water, 10  $\mu\text{M}$  abscisic acid (ABA) alone in water, 10  $\mu\text{M}$  ABA + 2.5  $\mu\text{M}$  fluridone (FL) in water, 10  $\mu\text{M}$  ABA + 5  $\mu\text{M}$  FL in water, 10  $\mu\text{M}$  ABA + 15  $\mu\text{M}$  FL in water, 10  $\mu\text{M}$  ABA + 20  $\mu\text{M}$  FL in water, and 10  $\mu\text{M}$  ABA + 10  $\mu\text{M}$  gibberellic acid (GA) in water. Seeds from KS4 accessions were exposed to seven different combinations of water, 10  $\mu\text{M}$  GA alone in tap water, 10  $\mu\text{M}$  GA + 2.5  $\mu\text{M}$  paclobutrazol (PL) in water, 10  $\mu\text{M}$  GA + 5  $\mu\text{M}$  PL in water, 10  $\mu\text{M}$  GA + 15  $\mu\text{M}$  PL in water, 10  $\mu\text{M}$  GA + 20  $\mu\text{M}$  PL in water, and 10  $\mu\text{M}$  GA + 10  $\mu\text{M}$  ABA in water.



Each dish had 20 mL tap water + other chemicals. All the above listed chemicals were purchased from Sigma Aldrich (St Louis, MO). Four replications of 50 seed per Petri dish were evaluated for each hormone treatment in growth chambers set at one of two temperature levels (6 and 23 C) and repeated. Petri dishes were covered with parafilm throughout the experiment to avoid moisture loss. Daily counts of germinated seeds were taken by opening each Petri dish to count and remove germinated seeds and re-sealed. A seed was regarded as germinated as soon as radicle was 2 mm long. This procedure was continued until no more germination was observed or 21 days. All seeds that did not germinate at the end of the experiment had presence of mold and were considered dead.

Total number of seeds germinated for each accession was used in an ANOVA to determine if main effects and interactions of runs, temperature levels, and respective phytohormone treatments were significant using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC). Differences were determined based on an LSD of 0.05. Germination rate over time was modeled using a sigmoidal function (equation 2.1) to describe the germination response of each accession using SigmaPlot v.12 (Systat Software, San Jose, CA).

## **RESULTS AND DISCUSSION**

A significant three-way interaction among temperature, moisture stress, and kochia accessions on total seed germination was determined (Table 2.1). Number of seeds that germinated across temperature and moisture stress treatments ranged from 3 to 44 out of 50 seed. Fewer seeds germinated in the low temperature and high moisture stress treatment, while almost all seeds germinated in the high temperature and low moisture stress treatment across both accessions.

## **Base temperature and base water potential**

Base temperature ( $T_b$ ) for kochia seed germination under present experimental conditions was 2.7 C and base water potentials ( $\psi_b$ ) were -1.70 MPa at 11 C, -1.62 MPa at 6 C, and -0.92 MPa at 4 C (Figures 2.1 and 2.2). Al-Ahmadi and Kafi (2007) observed that kochia from fields in Iran germinated under a wide range of temperature conditions (10 to 40 C) with  $T_b$  of 3.5 C. Our results suggest that kochia can germinate at cooler temperatures and we tested temperature response at much lower values of 4 and 6 C. Nussbaum et al. (1985) observed in Texas that kochia seedlings could emerge when daily minimum and maximum soil temperatures were only 3 and 8 C, respectively, and that kochia seedlings commonly emerged immediately following the last night of frosts in the spring. Schwingamer and Van Acker (2008) have used 0 C as an even lower soil  $T_b$  in Manitoba, Canada, where they used cumulative emergence data of kochia seedlings in field situations.

Everitt et al. (1983) reported that cold to moderate temperatures improved kochia germination levels. The mechanism behind cold temperature germination was reported by Hóla et al. (2004) who suggested greater photosystem I activity in kochia biotypes grown under low-temperature conditions. Also, some kochia biotypes resistant to sulfonylurea herbicides had rapid cell division and growth during early germination at low temperatures (Dyer et al. 1993; Subramanian et al. 1991). Also, greater production of isoleucine, valine and oxalate in kochia seeds may play a role both in early germination ability and salt tolerance (Curtin and Wen 2004; Dyer et al. 1993). Werle et al. (2014) modeled the  $T_b$  for kochia and estimated to be 12 C, which was high compared to what others observed, but was the best fit model that they were using to predict emergence patterns of kochia in Iowa. A study carried out in Italy by Masin et al. (2010)

determined  $T_b$  values for nine common weed species, such that velvetleaf (*Abutilon theophrasti* Medik.) had the lowest  $T_b$  value of 3.9 C

Masin et al. (2010) determined  $\psi_b$  values for nine common weed species, such that all species had  $\psi_b$  value of -1.0 MPa except barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) whose value was -0.97 MPa. Werle et al. (2014) found  $\psi_b$  for kochia to be -0.75 MPa. Our results showed that kochia germinated at a much lower water potential based on growth chamber situations whereas results from Werle et al. (2014) were modeled and based on field situations.

### **Effect of temperature and water potential on kochia germination**

No germination was observed at lowest water potential level of -1.6 MPa at any temperature studied. At 4 C, none of the seeds germinated at -1.2 MPa. Greatest germination was in water ( $\psi = -0.0$  MPa) across temperatures in both accessions (Table 2.1). The number of seeds that germinated at 11, 6, and 4 C decreased as the water potential decreased from -0.0 to -0.4 MPa in both accessions. At -1.2 MPa, 24 and 4 out of 50 seeds germinated at temperatures 11 and 6 C, respectively

Overall number of kochia seed that germinated was reduced as temperature decreased from 11 to 4 C. Greatest germination was observed across the two accessions when temperature was high and moisture stress was low and, if germination occurred, it was least when temperature decreased to 6 C and when water potential was -1.2 MPa (Table 2.1). At 11 C, total seed germination at each water potential level was significantly different from each other where more seeds germinated at -0.0 than -1.2 MPa across both accessions. No differences in total seed germination were observed between the two water potential levels of -0.0 and -0.4 MPa as

temperature decreased from 6 to 4 C. Number of seed germinated at 11 C and -1.2 MPa and 4 C and -0.8 MPa were not different from each other.

A sigmoidal model described the germination response over actual days very well (Table 2.2). As the moisture stress level increased, more days were required to reach 50% kochia seed germination across all temperature levels (Table 2.2). For example, when temperature decreased from 11 to 6 to 4 C at a water potential of 0 MPa, number of days to reach 50% germination increased from 2 to 6 to 11 days, respectively, while at -0.4 MPa, it took 3 to 7 to 16 days to reach 50% germination, respectively (Table 2.2). The response to temperature of time taken to 50% germination followed a typical path of the temperature reaction of many developmental processes where a lag is observed as temperature decreases (Oliver and Annandale 1998). Flores and Briones (2001) studied the effect of three temperatures and five water potentials (0, -0.12, -0.2, -0.41 and -0.66 MPa) in six desert species. As temperature increased from 12 to 20 and 26 C, the onset of germination was earlier and the time required for 50% germination decreased across all six desert species. Germinability decreased and time to 50% germination decreased as moisture stress increased from -0.0 to -0.66 MPa.

### **Thermal time (TT), hydro time (HT), and hydrothermal time (HTT) for kochia germination**

To further determine the influence of temperature and water potential on rates of kochia seed germination,  $T_b$  and  $\psi_b$  were used to calculate cumulative TT, HT, and HTT to place germination rates on the same time scale. The response of cumulative percent germination was described using equation 2.1 using these different values of 'X' for time. As temperature decreased and water potential decreased, seeds required more time to accumulate enough TT and moisture to germinate. As temperatures decreased from 11 to 6 to 4 C, more cumulative TT was

needed for 50% seed germination in water, that is, from 17 to 22 to 14 Cd, respectively (Table 2.3). As water potential decreased, cumulative TT needed for 50% cumulative percent germination increased from 16.7 Cd at 0 MPa to 74.8 Cd at -1.2 MPa (Table 2.3). Seeds needed more heat units before germinating as moisture stress increased at all temperature levels studied. There was no difference in rate of seed germination at 11 C for water potentials of -0.4 and -0.8 MPa (Figure 2.3). Moisture had less effect on germination as temperature at 11 C but at lower temperatures of 4 C (Figure 2.2) and 6 C (Figure 2.3), moisture played a more important role than temperature on rate of seed germination.

Cumulative HT required for 50% of seeds to germinate at 11 C in water was 4.6 MPa\*d which was reduced to 3.3 MPa\*d as water potential decreased to -1.2 MPa (Table 2.3). Similarly seeds at temperatures 6 and 4 C accumulated more HT for 50% germination in water than at -1.2 MPa. Within the range of experimental treatments, decreasing the water potential (increasing moisture stress) in all constant temperatures caused a delay in germination and a decrease in the final germination percentages (Table 2.2). For example, at a water potential of -1.2 MPa at 11 C, 24 out of a total 50 seeds germinated in 10 days, whereas 23 seeds germinated in only 2 days in water. It is hypothesized that in a typical field situation, a very small fraction of seeds accumulate enough HT and germinate early whereas a very large fraction of seeds in soil still need more HT to germinate. Therefore, the curves for high moisture stress were located above the curves of low moisture stress (Figures 2.3, 2.4 and 2.5).

Cumulative HTT required for 50% germination of kochia seeds decreased as moisture stress increased at each temperature situation (Table 2.3). Germination data across all moisture stress treatments were pooled together at each temperature (Figure 2.5) and was seen that seeds

germinated much faster at 4 C than at 11 C. But of course, greater fraction of seed germinated at 11 C than 4 C.

### **Effect of abscisic acid on kochia germination**

Kochia seed germination was investigated in Petri dishes containing 10 mL of either water, or combinations of hormones ABA, GA, or the inhibitor FL, added to the water and incubated at either 23 or 6 C. Seed germination was expected to decrease when ABA alone was exogenously applied to kochia. Fluridone or GA, when combined with ABA, were expected to reverse the effect of ABA and restore original germination levels (i.e. germination in water).

Analysis of variance on total number of germinated seed showed a significant two-way interaction between ABA hormone levels and temperature levels, and between ABA hormone levels and two runs (Table 2.4). Total seed germination for each phytohormone treatment was greater at high vs. low temperature. At 23 C, greatest germination was 46 seed/dish in water and least germination was 33 seeds/dish in 15  $\mu$ M of FL added to 10  $\mu$ M of ABA (Table 2.5). Addition of only 10  $\mu$ M ABA to water significantly reduced the germination from 46 to 38 seeds/dish. None of the FL inhibitor treatments could restore original seed germination level to that of water, because germination was still reduced and not different than that of ABA alone. The combination of ABA + GA increased germination from 38 to 43 seeds/dish and was similar to that of water. Hence, GA played some role in reversing the effect of ABA on seed germination levels. At 6 C, ABA reduced the germination from 39 to 33 seeds/dish and none of the inhibitors could reverse the effect of ABA. At 6 C, ABA + GA treatment was not able to restore the germination level, but remained lowest compared to all other treatments at 6 C. When temperature decreased from 23 to 6 C, GA could not reverse the effect of ABA.

Similar response of hormone levels was observed in run 1 and run 2 where germination was highest in water with 44 and 41 seeds/dish, respectively. Germination was reduced with ABA but the inhibitor did not restore the germination level to that of water. ABA+GA treatment was not different than water, since germination increased from 36 to 43 seeds/dish in run 1 and from 34 to 41 seeds/dish in run 2 (Table 2.4). Hence, ABA alone decreased the germination but was not able to increase when its inhibitor was added at various concentrations. In a study carried out by Yoshioka et al. (1998), FL restored the germination of *Arabidopsis* seeds at higher temperatures and FL caused a decrease in ABA content in seed endosperm at 33 C.

The model describing seed germination explained the data very well (Table 2.6). At 23 C, seeds required less TT (109 Cd) and germinated fastest when in water. When ABA alone or in combination with FL were added to water, rates of germination decreased as seeds required more TT to achieve final germination. None of the concentrations of inhibitors used were able to reverse the effect of ABA. Germination rate in 10  $\mu$ M ABA + 10  $\mu$ M GA was slowest (Figure 2.6). Addition of GA restored seed germination to that of water but required more TT (194.2 Cd) to achieve it. At 6 C, similar trend was seen where ABA alone or in combination with FL reduced the seed germination. However, since smaller fraction of seeds germinated when temperature was reduced from 23 to 6 C, seeds required 22 Cd to germinate in water at 6 C which was very less than what was required for seeds to germinate in water at 23 C.

### **Effect of gibberellic acid on kochia germination**

Kochia seed germination was investigated in Petri dishes containing 10 mL of either water, or combinations of hormones of GA, ABA, or the inhibitor PL when added to the water and incubated at either 23 or 6 C. Seed germination was expected to increase when GA alone

was added to water. Paclobutrazol and ABA were expected to reverse the effect of GA and restore original germination (i.e. germination in water).

Analysis of variance showed three-way interaction among temperature, runs, and GA hormone levels. The addition of GA increased the total germination of seeds in both runs at 23 and 6 C. At 23 C, germination in water was lowest with 37 seeds/dish in run 1 and 33 seeds/dish in run 2 (Table 2.5). When GA was added germination increased to 44 and 41 seeds/dish in runs 1 and 2, respectively. Studies carried out by Khan and Rizvi (1994) on seed germination of the desert species saltbush (*Atriplex griffithii*), also a member of Chenopodiodeae, found that combination of GA (28.9  $\mu\text{M}$ ) and kinetin (0.46  $\mu\text{M}$ ) alleviated salinity-induced germination inhibition. A much higher concentration of GA was required to increase germination of these seeds compared to kochia because their seeds were germinating in a salt stress situation. Addition of increasing amounts of PL to 10  $\mu\text{M}$  GA reduced kochia germination. Hence, PL could reverse the germination of seeds to that of water, and 20  $\mu\text{M}$  of PL was most effective in reducing germination to 35 and 30 seeds/dish in run 1 and run 2, respectively. Germination was still higher when 10  $\mu\text{M}$  of ABA was combined with 10  $\mu\text{M}$  of GA compared to pure water + 10  $\mu\text{M}$  GA in both runs indicating ABA could not reverse the effect of GA. At 6 C, highest germination was 34 seeds/dish in run 1 and 40 seeds/dish in run 2 in GA only treatment. Germination was still higher when in combination with 10  $\mu\text{M}$  ABA. Lowest germination was seen in run 1 when 15  $\mu\text{M}$  of PL was added to 10  $\mu\text{M}$  of GA. At 6 C, every combination of GA + PL reduced the germination significantly.

Parameters for the relationship between TT and cumulative germination response to GA are in Table 2.7 and regression fits are shown in Figure 2.8. At 23 C, TT requirement to germinate that fraction of seed in pure water (123.5 Cd) and 10  $\mu\text{M}$  GA alone was similar (122.3



Cd). Thus, both curves were located close to each other in figure 2.8. At 23 C, all concentration of PL counteracted with GA to reduce total germination and rate by increasing the TT requirement to 50 % germination. Similar was the trend at 6 C, although very less heat unit was acquired by seeds across all treatments before germination compared to seeds at 23 C.

Hence, kochia seed germination was highly affected by temperature, moisture and hormones. Temperature and moisture played a very important role in seed germination. Percent seed germination was reduced when temperature decreased from 11 to 4 C and moisture stress increased from -0.0 to -1.2 MPa, evident from the increase in number of days or thermal time (TT) required to reach 50% germination. In TT scale, seeds under high temperature and low moisture stress germinated faster than seeds under low temperature and high moisture stress. In HT scale, smaller fraction of seeds germinated quickly under low temperature and high moisture stress compared to seeds under high temperature and low moisture stress. When both temperature and moisture were combined in HTT scale, a small fraction of seeds germinated faster at 4 C than 11 C. In this study base temperature ( $T_b$ ) was 2.7 C and base water potential ( $\psi_b$ ) values -1.70 MPa at 11 C, -1.62 MPa at 6 C, and -0.92 MPa at 4 C. Thus, kochia can germinate at very low temperature and high moisture stress. Most studies carried out on kochia seed germination have documented the impact of temperature but results from this study has investigated additional impact of water potential. Results from this study have great importance in developing models to predict kochia emergence. The hormone ABA decreased the germination of highly germinable seeds while GA increased the germination of low germinable kochia seeds. Further research on combining ABA and GA with other chemicals to impact weed seed germination is required.

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## TABLES AND FIGURES

**Table 2.1 Observed total number of germinated seed (n=50 seeds / dish) as influenced by the three-way interaction among temperature, water potential and kochia accession. Means followed by same letter are not significantly different at LSD of 1.2**

Temperature	Water potential	KS1	KS2
C	-MPa	seeds/dish	
11	0.0	44 a	41 b
11	0.4	39 cb	39 c
11	0.8	35 ed	30 f
11	1.2	24 kj	20 l
11	1.6	0 n	0 n
6	0.0	34 ed	32 d
6	0.4	33 e	33 e
6	0.8	28 gh	25 ij
6	1.2	4 m	3 m
6	1.6	0 n	0 n
4	0.0	29 f	23 kj
4	0.4	27 fih	23 kj
4	0.8	18 fl	23 k
4	1.2	0 n	0 n
4	1.6	0 n	0 n

**Table 2.2 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination using sigmoidal function where a is maximum cumulative germination, b is slope of the inflection, and  $X_0$  is days required for 50% germination.**

Temperature	Water potential	Parameter estimates			$R^2$
		a (se)	b (se)	$X_0$ (se)	
C	-MPa	%		days	
11	0.0	98 (2.1)	2 (0.2)	2 (0.2)	0.87
11	0.4	96 (1.5)	1 (0.1)	3 (0.1)	0.92
11	0.8	93 (1.1)	1 (0.1)	4 (0.1)	0.92
11	1.2	99 (1.7)	2 (0.1)	10 (0.2)	0.94
6	0.0	96 (0.8)	1 (0.1)	7 (0.1)	0.97
6	0.4	95 (1.7)	2 (0.1)	8 (0.2)	0.95
6	0.8	97 (3.6)	2 (0.2)	11 (0.3)	0.91
6	1.2	92 (10.4)	3 (0.7)	16 (1.1)	0.98
4	0.0	97 (1.3)	1 (0.1)	12 (0.1)	0.97
4	0.4	109 (2.6)	2 (0.1)	17 (0.2)	0.98
4	0.8	101 (1.7)	2 (0.1)	16 (0.1)	0.98

**Table 2.3 Time to 50% germination of kochia seeds when cumulative percent germination of each combination of temperature and moisture were plotted against actual days of observation, cumulative thermal time (TT), hydro time (HT), and hydrothermal time (HTT)**

Temperature	Water potential	Actual days	TT	HT	HTT
C	-MPa	d	Cd	MPa*d	MPa*Cd
11	0	2	17	5	39
11	0.4	3	30	5	38
11	0.8	4	31	3	29
11	1.2	10	75	3	28
6	0	6	22	10	35
6	0.4	7	25	9	31
6	0.8	11	36	9	30
6	1.2	16	52	7	22
4	0	11	15	10	14
4	0.4	16	21	9	12
4	0.8	17	23	2	3

**Table 2.4 Observed total germination of kochia seeds (n=50 seeds/dish) in response to water, abscisic acid (ABA), fluridone (FL), and gibberellic acid (GA) at two temperature conditions and two runs. Means in a column followed by same lowercase letters for temperature or uppercase letters for runs were not significantly different at LSD of 2.2**

Hormone	Inhibitor	Other	Total germinated seed			
( $\mu$ M)	( $\mu$ M)	( $\mu$ M)	seeds/dish			
ABA	FL		23 C	6 C	Run 1	Run 2
0	0	Water	46 a	39 b-d	44 A	41 AB
10	0	0	38 c-e	33 e-g	36 BC	34 CD
10	2.5	0	36 c-f	29 gh	33 CD	33 CD
10	5	0	34 d-g	30 f-h	32 CD	32 CD
10	15	0	33 d-g	27 h	29 D	31 CD
10	20	0	34 d-g	26 h	28 D	33 CD
10	0	10 GA	43 ab	26 h	43 A	41 AB



**Table 2.5 Observed total germination of kochia seeds (n=50 seeds/dish) in response to water, gibberellic acid (GA), paclobutrazol (PL), abscisic acid (ABA) at two temperatures and two runs. Means in a column followed by same lowercase letters for 23 C or uppercase letters for 6 C were not significantly different at LSD 2.3**

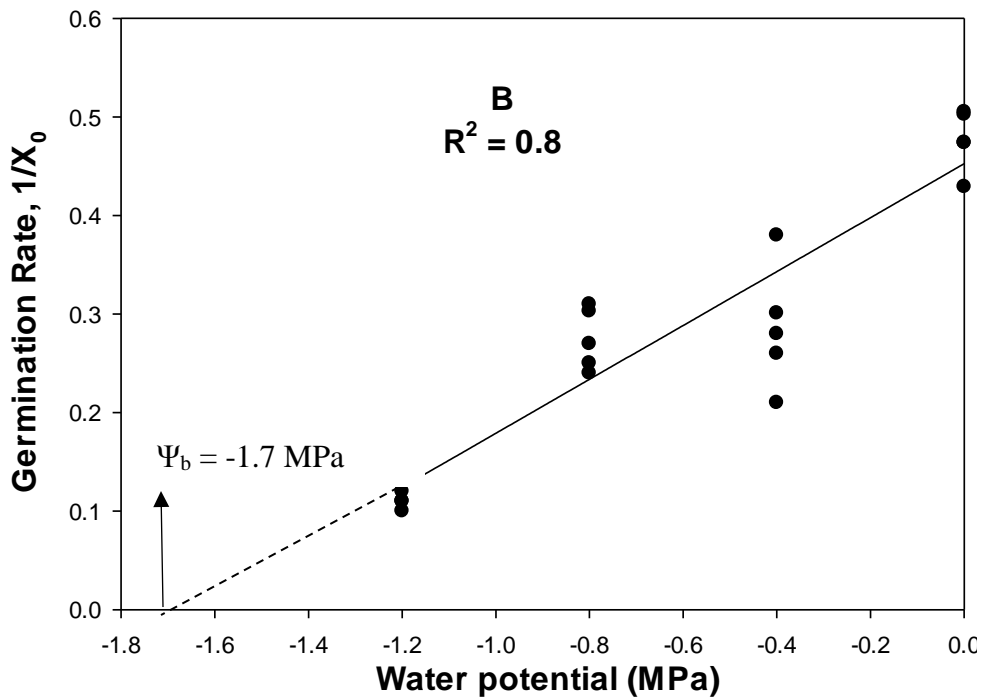
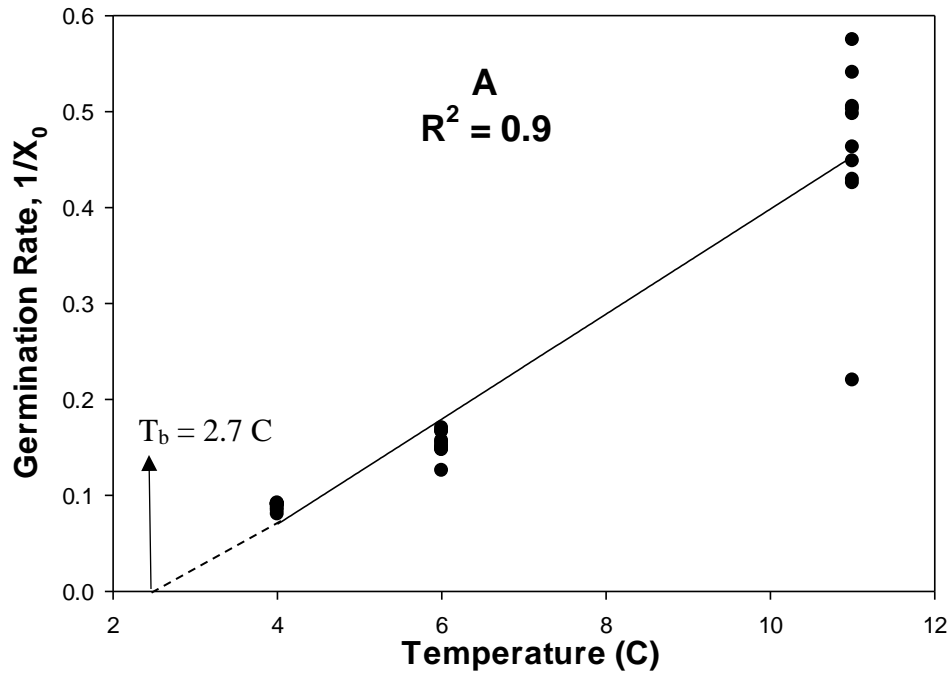
Hormone	Inhibitor	Other	Total germinated seed				
GA ( $\mu$ M)	PL ( $\mu$ M)	( $\mu$ M)	Run	23 C		6 C	
				seeds/dish			
0	0	Water	1	37	a-h	29	H-I
0	0	Water	2	33	d-i	29	H-I
10	0	0	1	44	a	34	B-I
10	0	0	2	41	a-c	40	A-E
10	2.5	0	1	38	a-g	32	E-I
10	2.5	0	2	40	a-d	33	C-I
10	5	0	1	38	a-f	34	B-I
10	5	0	2	39	a-e	30	F-I
10	15	0	1	36	a-h	28	I
10	15	0	2	33	d-i	30	G-I
10	20	0	1	35	b-i	30	F-I
10	20	0	2	30	f-i	30	G-I
10	0	10 ABA	1	41	a-d	40	A-D
10	0	10 ABA	2	41	ab	33	C-I

**Table 2.6 Observed number of days and thermal time (TT) required for 50% germination of kochia seeds in response to water, abscisic acid (ABA), fluridone (FL), gibberellic acid (GA) at two temperatures pooled across two runs**

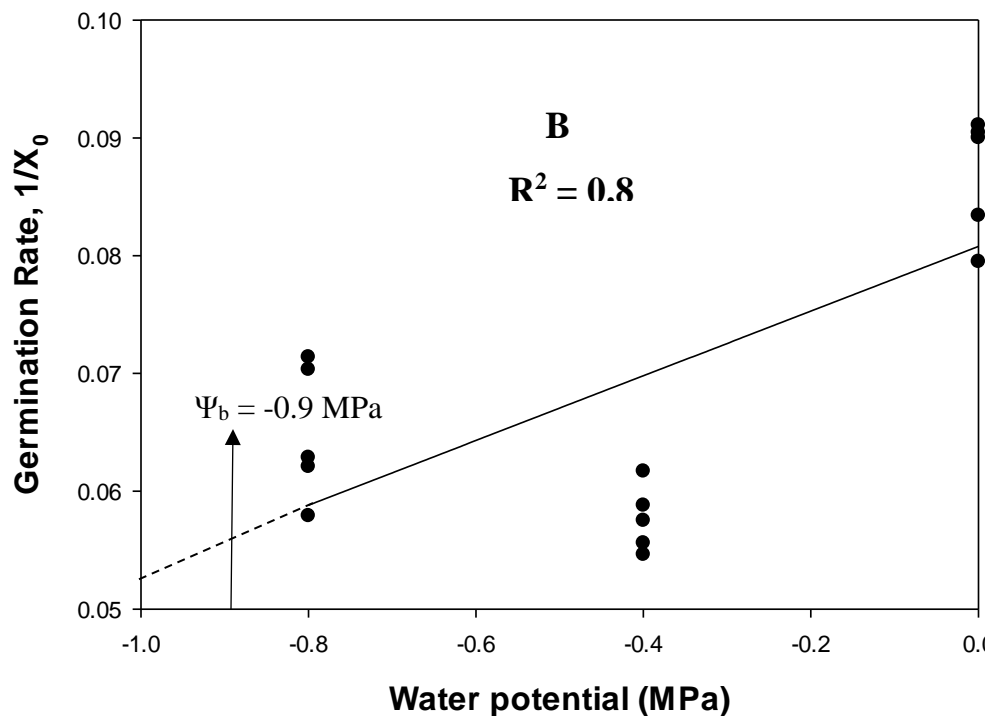
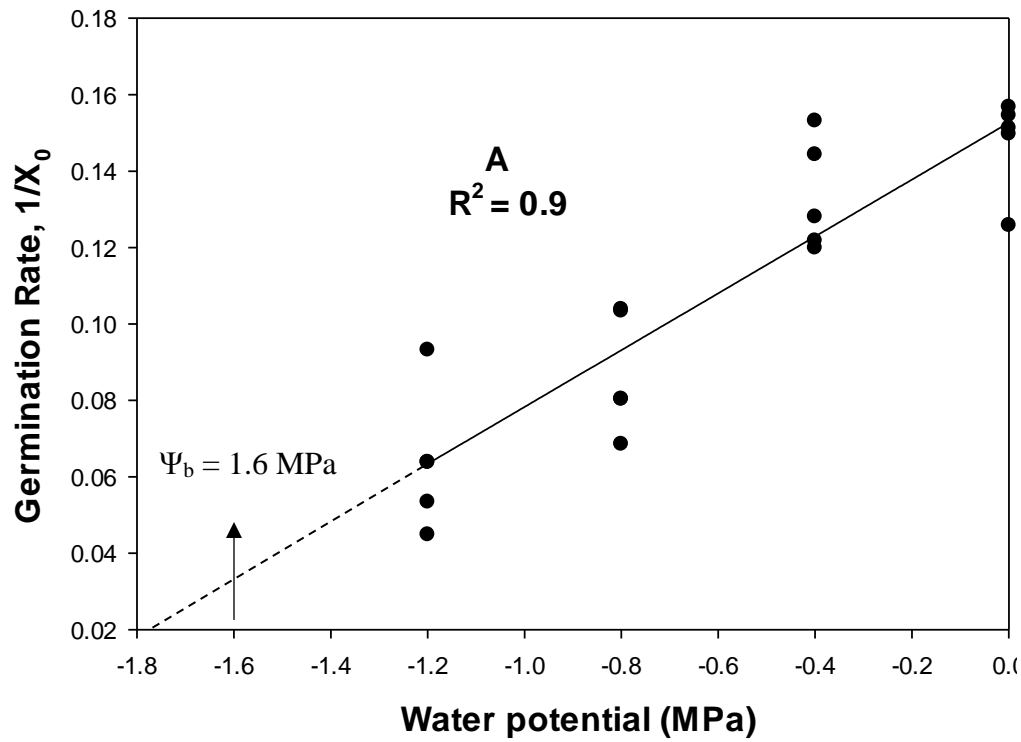
Hormone	Inhibitor	Other	Days to 50% germ		TT to 50% germ	
( $\mu\text{M}$ )	( $\mu\text{M}$ )	( $\mu\text{M}$ )	d		Cd	
ABA	FL		23 C	6 C	23 C	6 C
0	0	Water	5	7	109	22
10	0	0	7	10	142	32
10	2.5	0	7	7	148	21
10	5	0	9	7	176	24
10	15	0	8	10	160	34
10	20	0	6	12	128	41
10	0	10 GA	10	9	194	30

**Table 2.7 Observed number of days and thermal time (TT) required for 50% germination of kochia seeds in response to water, gibberellic acid (GA), paclobutrazol (PL) and abscisic acid (ABA) at two temperatures and two runs**

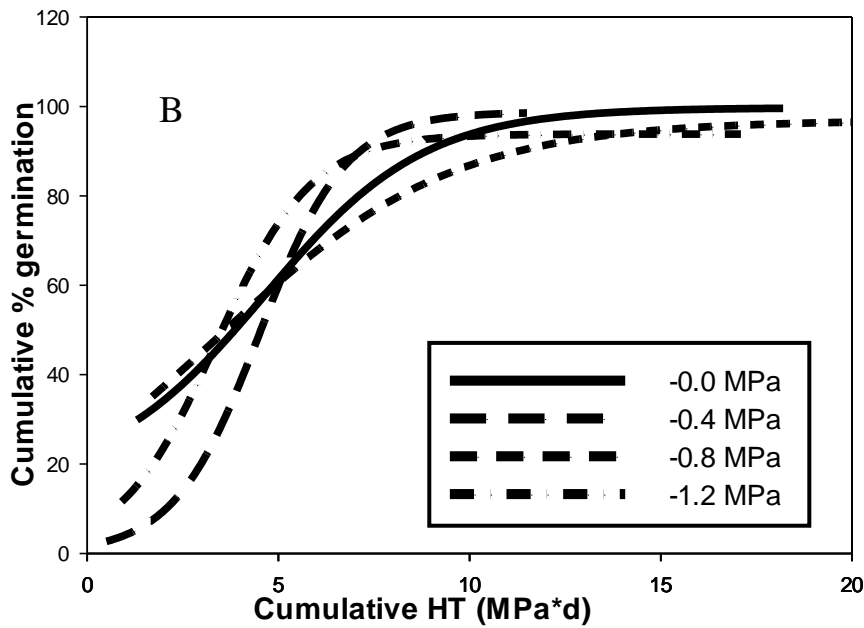
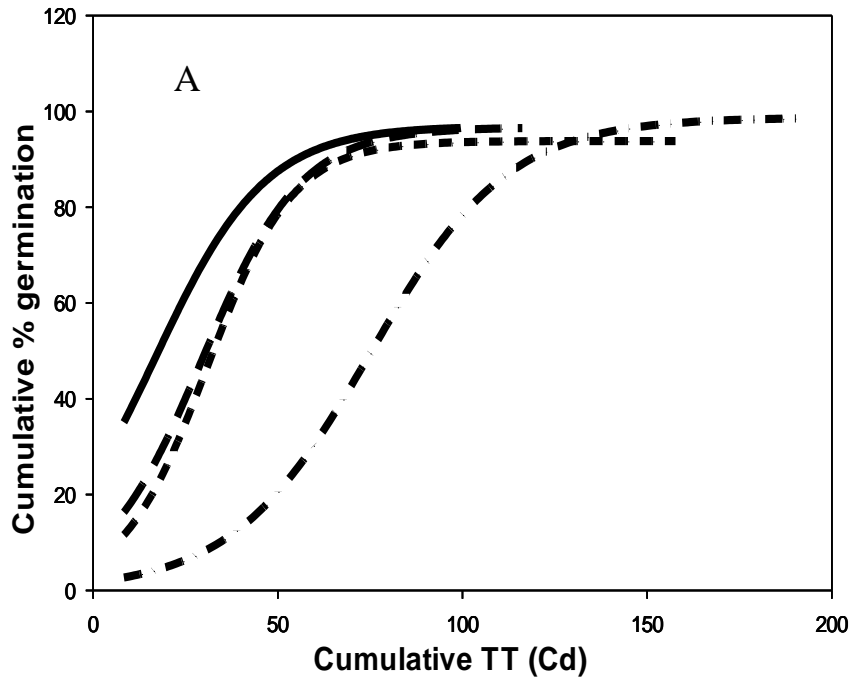
Hormone	Inhibitor	Other	Days to 50% germ		TT to 50% germ	
			23 C	6 C	23 C	6 C
( $\mu\text{M}$ )	( $\mu\text{M}$ )	( $\mu\text{M}$ )	d		Cd	
0	0	Water	6	9	123	30
10	0	0	6	10	122	33
10	2.5	0	7	9	152	27
10	5	0	5	13	101	43
10	15	0	7	10	141	32
10	20	0	10	10	193	32
10	0	10 ABA	13	13	274	32



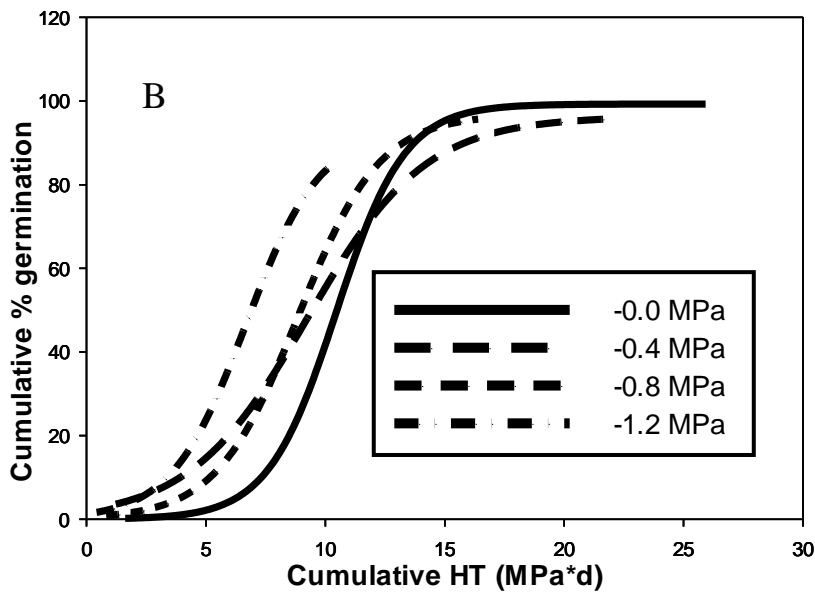
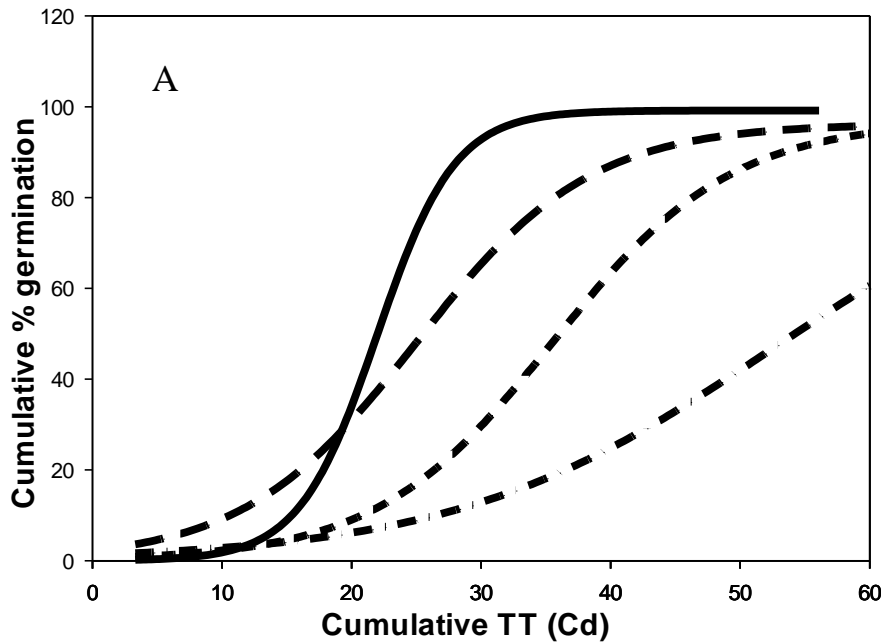
**Figure 2.1 Estimation of base temperature ( $T_b$ ) (A) and base water potential ( $\psi_b$ ) (B) at 11 C of kochia seed germination using the method of reciprocal time to 50% germination. Solid line represents the linear regression and the points are the calculated germination rate ( $1/X_0$ ).**



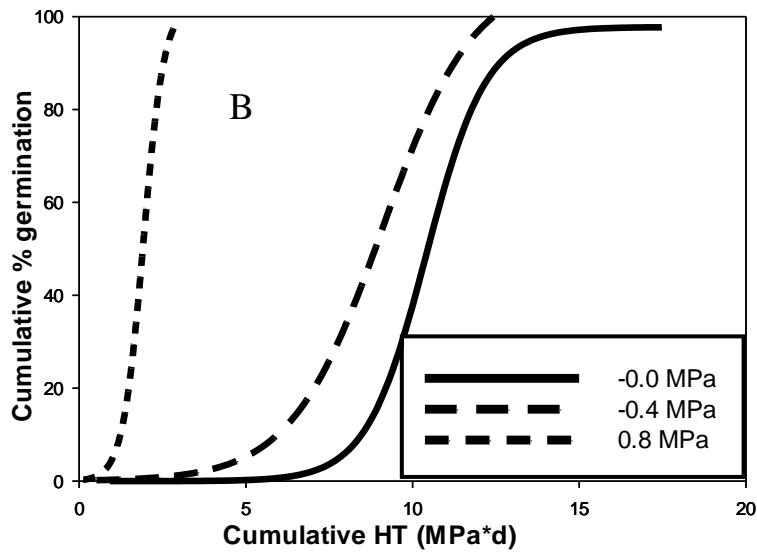
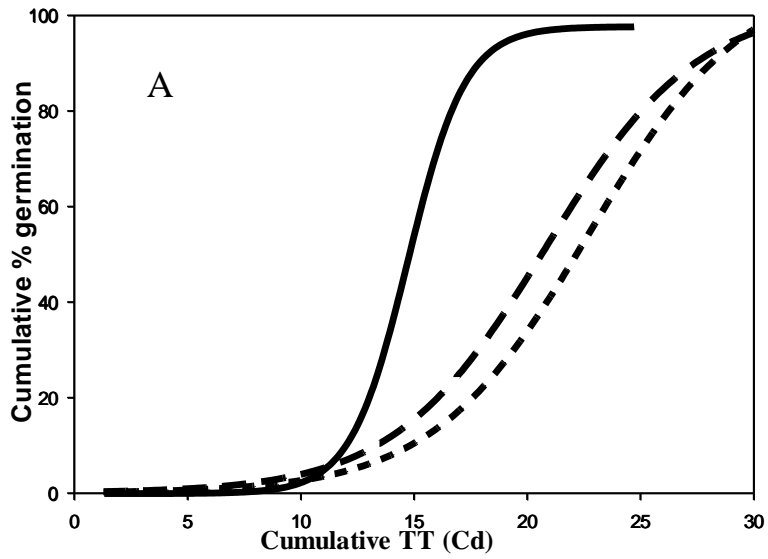
**Figure 2.2** Estimation of base water potential ( $\psi_b$ ) (A) at 6 C and  $\psi_b$  (B) at 4 C of kochia seed germination using the method of reciprocal time to 50% germination. Solid line represents the linear regression and the points are the calculated germination rate ( $1/X_0$ )



**Figure 2.3** Final cumulative percentage germination of kochia seeds vs cumulative thermal time (TT) (A) and hydro time (HT) (B) under various water potential levels at 11 C. Parameter estimates for graphs A and B are shown in Appendix B.4 and B.5 respectively

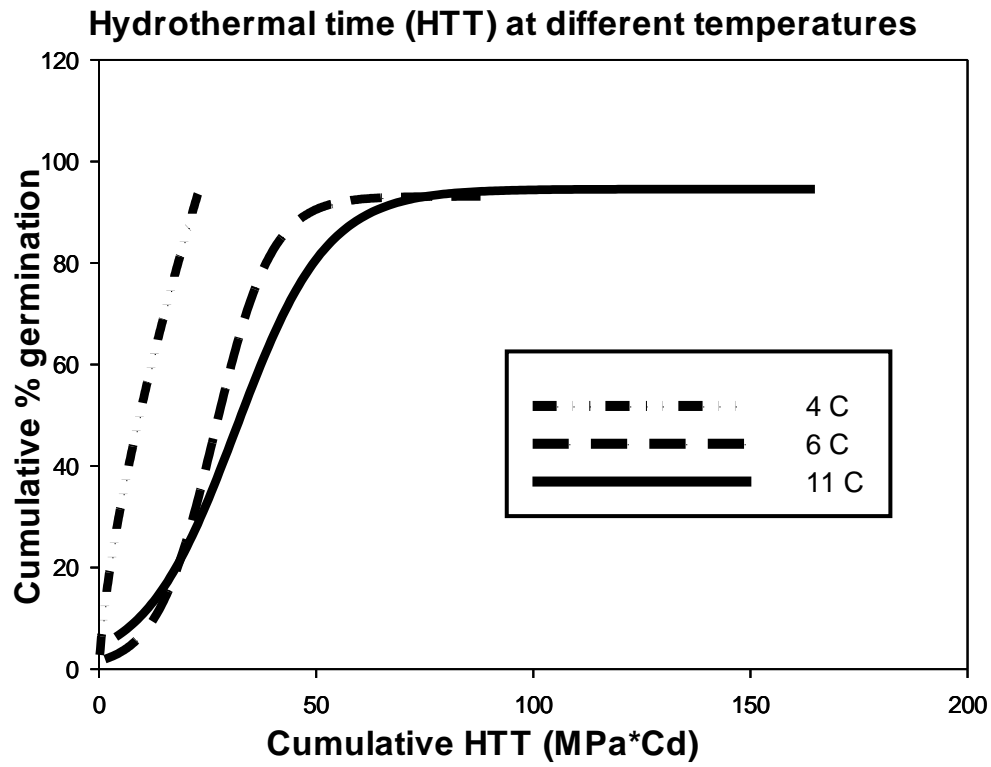


**Figure 2.4 Final cumulative percentage germination of kochia seeds vs cumulative thermal time (TT) (A) and hydro time (HT) (B) under various water potential levels at 6 C. Parameter estimates for graphs A and B are shown in Appendix B.4 and B.5 respectively**



**Figure 2.5** Final cumulative percentage germination of kochia seeds vs cumulative thermal time (TT) (A) and hydro time (HT) (B) to germination under various moisture stress levels at 4 C. Parameter estimates for graphs A and B are shown in Appendix B.4 and B.5 respectively





**Figure 2.6 Hydrothermal time (HTT) accumulated by kochia seeds at all moisture treatments in each growth chamber set at one of the temperature treatments. Parameter estimates for graph are shown in Appendix B.6**

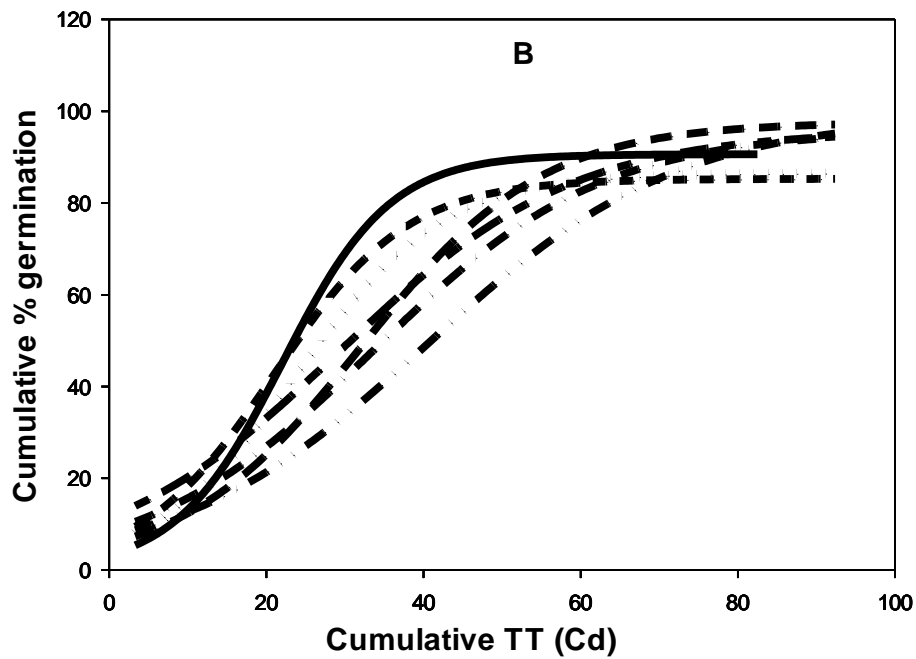
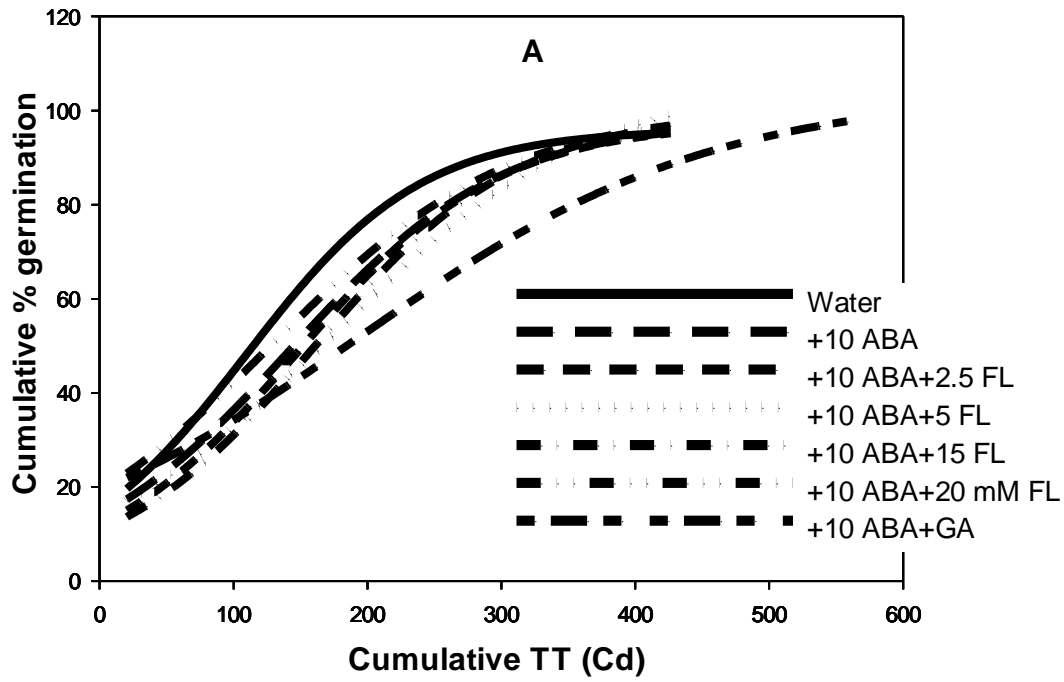
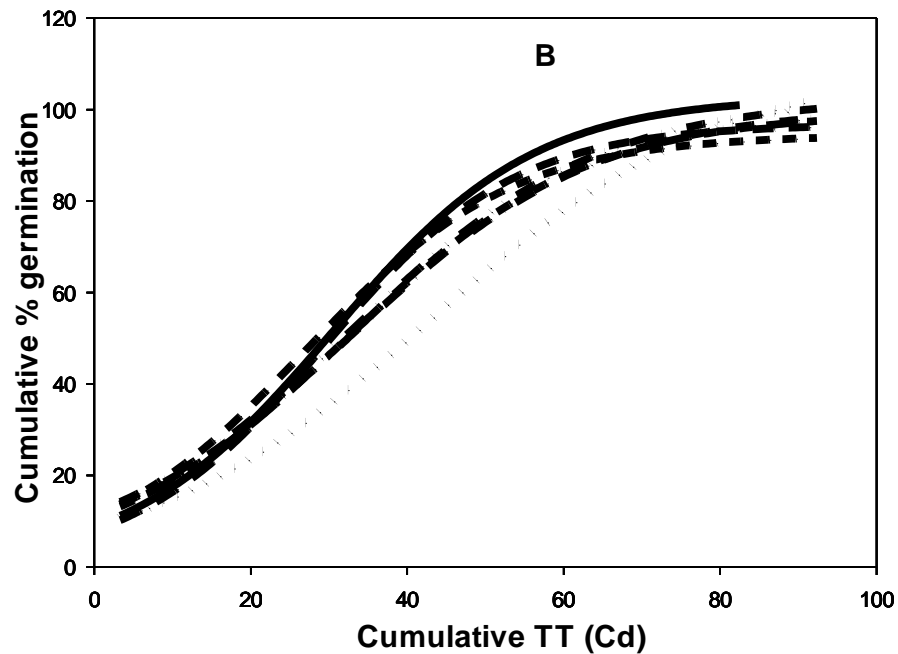
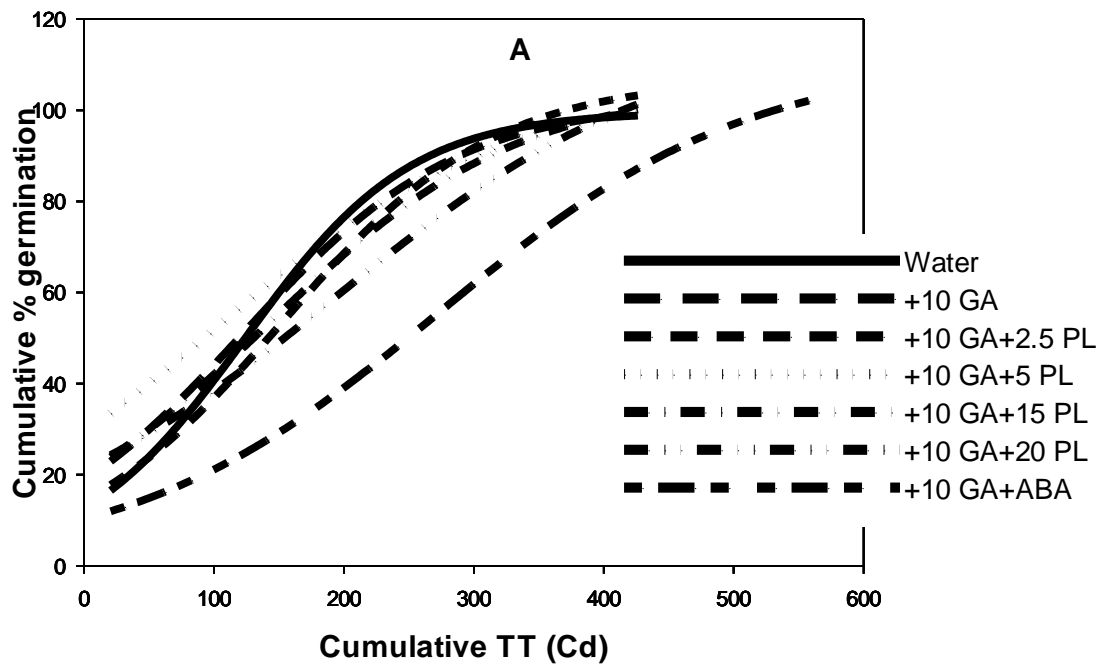


Figure 2.7 Thermal time (TT) accumulated by kochia seeds in response to water, abscisic acid (ABA), fluridone (FL), ABA + gibberellic acid (GA) at two temperatures 23 (A) and 6 (B) C. Parameter estimates for graphs A and B are shown in Appendix B.7



**Figure 2.8 Thermal time (TT) accumulated by kochia seeds in response to water, gibberellic acid (GA), paclobutrazol (PL), ABA + gibberellic acid (GA) at two temperatures 23 (A) and 6 C (B). Parameter estimates for graphs A and B are shown in Appendix B.7**

## **Chapter 3 - Seed development in kochia**

### **ABSTRACT**

Experiments were conducted to determine the length of time required for kochia to produce mature seeds after pollination and the length of seed-filling period based on seed weight under greenhouse conditions. In 2015, a total of 14 branches from one plant were selected for controlled pollination by removing all the leaves and exposing the flower buds when stigmas became receptive. The branches were pollinated for three consecutive days. Time after pollination on fourth day was designated as 0 days after pollination (DAP) and harvests began up to 36 DAP. At each harvest time, 15 to 20 seeds were collected, fresh weight of 10 seeds was measured, photograph of a single seed was taken, and all seeds stored in a tube for 48 hours in a freezer at -4 C. In a separate experiment in 2016, flower buds were photographed on the plant itself to observe different flowering stages. In 2015, kochia buds turned completely black or dark brown in color by 36 DAP. Buds had a black-colored oval shaped seed enclosed at 36 DAP but did not germinate in Petri dishes. Some buds did not produce pollen while in some buds, anthesis occurred. In 2016 on kochia plants, similar bud color was observed but only at 40 to 45 DAP when directly observed under natural pollination. In 2016, anthers were visible 8 to 9 days after stigma emergence, and these anthers shed pollen for 8 to 9 days and then turned brown. More information on whether viable seeds capable of germination under favorable environmental conditions are present inside those buds at different stages of flower development is required to determine the actual length of time to produce mature seeds.

## INTRODUCTION

*Kochia* (*Kochia scoparia*) can be found in crop fields, pastures, rangeland, roadsides, ditch banks, and wastelands. It can be a major weed problem in chemical fallow systems and causes severe yield losses when occurring with crops. *Kochia* produces a protogynous flower where the stigma emerges and can accept foreign pollen, and then deteriorates before pollen is shed and anther dehiscence which prevents self-pollination within the same flower (Stallings et al. 1995). It initiates flowers about 8 to 10 weeks after early spring emergence (Thill and Mallory-Smith 1996). Flower buds consist of two receptive hairy stigmas that receive pollen from other flowers of the same plant or a different plant. Stigma soon deteriorates and bright yellow or orange colored anthers are visible after a week (Stallings et al. 1995). This type of flowering mechanism increases the likelihood of genetic exchange among the flowers and prevents self-pollination within flower (Stallings et al. 1995). Stallings et al. (1995) found that sulfonylurea herbicide-resistant *kochia* pollen can spread the sulfonylurea-resistant trait at least 30 meter with each growing season. Pollen grains are spheroidal, 20 to 40  $\mu\text{m}$  in diameter, and granular with 100 to 130 pores uniformly distributed over the grain surface (Stallings et al. 1995). *Kochia* biotypes or ecotypes can exhibit large variability in days to flowering, which, in turn, may affect synchrony of pollination (Beckie et al. 2013). In a field study in North Dakota, Bell et al. (1972) observed that it took 57 to 100 d from emergence to flowering among 13 *kochia* accessions collected from across the western United States. Two *kochia* biotypes from North Dakota, which differed markedly in plant structure, also differed in time to flower initiation by as much as 24 d (Stallings et al. 1995). Understanding the flowering biology of *kochia* helps in developing management strategies for the weed.

Kochia is prone to evolving resistance to herbicides with different modes of action. Photosystem II-inhibitor (e.g. atrazine) resistance in kochia was first reported in 1976 in Kansas. Subsequently, ALS-inhibitor and auxinic herbicide resistances were reported in other US Great Plains states in 1987 and 1995, respectively (Cranston et al. 2001; Morrison and Devine, 1994). Kochia was first confirmed resistant to glyphosate in Kansas in 2007; since then GR kochia has been reported and confirmed in six additional US states and three Canadian provinces (Beckie et al 2013; Godar 2014; Heap 2017; Waite et al. 2013). If herbicide susceptible ovules are fertilized by pollen containing herbicide-resistance alleles and those ovules develop into mature seeds, management of herbicide-resistant kochia plants from a field will become very difficult. But from what we know is that about 80% of kochia seed that is shed in any one year germinates the next year, and the following year only 3% germinate and the year after that only 1% (Schwingamer and Van Acker 2008). Hence, if seed production is controlled in the first year itself chances of it being a problem the following year is very low (Schwingamer and Van Acker 2008).

In order to reduce seed production, it is important to schedule chemical control tactics prior to flowering when the plant is not producing viable seeds. Seed biology, in particular, time to flowering, time seeds take to mature after first being pollinated, and pollen viability can be useful for both management and research purposes. Hence, the main objective of this study was to document the seed maturation process of kochia based on visual analysis of seeds and flowers taken at different times after pollination in greenhouse conditions.

## MATERIALS AND METHODS

### Controlled pollination study

One experiment was conducted in Department of Agronomy Weed Science greenhouse with a 12 hr day/night cycle at  $27/22 \pm 2$  C day/night to determine the time required from pollination to produce mature seeds in kochia. In May 2015, about 100 kochia seeds from Wichita County, KS (collected in 2006) were sown in 28 by 19.5 by 6 cm flats with 480 g of commercial potting mix (Metro-mix 360, Sun-Gro Horticulture Canada Ltd, Agawam, MA) with a hope that at least ten seeds would germinate and emerge and at least two of them would flower at the same time so that enough pollen would be present at the time of controlled pollination. After two weeks, ten seedlings that were 5 to 10 cm tall were selected and transplanted to individual pots of 1.5 L with 430 g of commercial potting mix. The plants were watered as needed and fertilized once a week with water soluble all-purpose plant food at 4.0 g/L to allow the plants to be robust and produce as many branches as possible.

Fourteen branches from one plant that had initiated flowering were selected in mid-July 2015. Prior to stigma emergence, leaves and apical meristems that would produce secondary branches were removed from main branches with the help of sterile forceps and each branch was covered with a pollination bag (Lawson '217' bags, Lawson Bags, Northfield, IL). The branches were covered for 6 to 7 days and 10 to 12 flower buds from each branch were targeted. When receptive stigmas were visible on those branches, they were pollinated with pollen from other plant of same population. Collection of pollen was done in the morning between 8 and 10 am because kochia produces maximum pollen at that time (Shamsutdinov and Khamidov 1984). Tiny yellow-colored pollen grains were collected in wax paper with the help of small make-up

brush and dusted on receptive stigmas for three consecutive days. Those buds were immediately covered with same pollination bags.

Evaluation of seed development began immediately after pollination on fourth day. First harvest was done on fourth day from first dusting and designated as 0 days after pollination (0 DAP). The ones harvested after three days of first harvest designated as 3 DAP pollination and at other different times designated as 6, 9, 12, 15, 18, 21, 25, 32, and 36 DAP. Harvesting was done between the months of July and September 2015. At each harvest time, 15 to 20 seeds were collected in a agar plate to avoid moisture loss during weighing, fresh weight of 10 seeds was taken, a photograph of a single seed was taken, all seeds stored in 5 ml micro centrifuge plastic tubes in a freezer at -4 C. Seeds harvested at 9, 15, 18, 25, and 36 DAP were placed in 100 by 15 mm Petri dishes with 10 mL of water in growth chambers in April 2017 to assess germination.

### **Photographs of Flower Development**

For 2016 study, kochia seeds were collected from a common garden field experiment near Manhattan, KS in fall of 2015 where the pollen was from mixed sources. Seeds were sown in mid-April in the greenhouse with similar growing conditions as above. About 20 seeds were planted in 28 by 19.5 by 6 cm flats with 480 g of commercial potting mix (Metro-mix 360). Six seedlings that were 5 to 10 cm tall were chosen and transplanted into individual pots of 1.5 L with 430 g of potting mix. They were watered and fertilized regularly and allowed to grow into a robust plant.

When the plants initiated flowering in July 2016, they were left to self-pollinate as they would do under natural conditions. The stages from stigma emergence to seed formation were photographed on the plant itself. Three branches were photographed from three different plants



daily in the morning to document the changes that take place in a specific bud from stigma emergence to pollen shedding and finally seed formation. To get clear pictures of different phases of seed development, leaves were removed from some of the branches.

## **RESULTS AND DISCUSSION**

### **Seed Appearance**

Photographs of individual flower buds are shown in Figure 3.1. All the flower buds harvested on a given day appeared similar under a microscope, hence, a representative photograph was chosen. At 0 DAP flower buds appeared yellow in color with two hairy stigmas protruding out (Figure 3.1). The buds on 0 DAP looked green and juicy with slightly brown-colored stigmas, an indication of a successful pollination since these female flowers were pollinated for 3 consecutive days and kochia pollen remains viable for 12 days after shedding (Mulugeta 1991). The buds also appeared empty and hollow. At 3 DAP the buds still appeared green and juicy. Slight change in kochia flower bud color began at 6 DAP, when the buds turned slightly brown at the base (Figure 3.1). At 9 DAP, flower buds appeared swollen and fleshy. By 12 DAP, the base of the kochia bud turned brown. After this point of time, the buds changed its appearance from green to brown and by 15 DAP, the color changed to light brown with stigma still intact. Six out of ten buds at 18 DAP had only female parts while rest had both male and female parts. At 36 DAP, all flower buds appeared completely brown in color. Four out of ten buds at this stage had black-colored seeds inside them when placed in Petri dish for germination (Figure 3.1). However, none of them germinated.

The seed weight was similar from 0-6 DAP which was 0.0065 g (Table 3.1). At 9 DAP, seed weight decreased slightly to 0.0064 g. After 18 DAP, weight increased with final weight of 0.0070 g at 36 DAP.

In contrast to kochia that had not changed color by 3 DAP, waterhemp ovules were a light tan color (Bell and Tranel 2010). Waterhemp seeds at 7 DAP showed increase in weight and change in color to reddish brown and by 12 DAP appeared to finish darkening, reaching a terminal color of black (Bell and Tranel 2010). Tests done on waterhemp seeds showed that germination occurred as soon as 7 DAP (Bell and Tranel 2010). Waterhemp developed seed faster than kochia with progression of color from light yellow to dark brown or black and seed weight from 0.002 to 0.008 grams/10 seeds.

Time required for seed maturation after pollination has been studied in many important weeds in agronomic cropping systems and varies widely. For instance, Egley (1976) reported that prickly sida (*Sida spinosa* L.) seeds were incapable of germination until 12 DAP and that the seeds reached their maximum dry weight by 14 DAP. Prickly sida seeds were capable of 80% germination at 12 to 16 DAP, after which time to germination dropped to nearly 0%, presumably due to the onset of dormancy (Egley 1976). Chandler et al. (1977) reported that in purple moonflower (*Ipomoea turbinata* Lag.), seeds were able to germinate at 20 DAP, reaching maximum germination at 26 DAP, and maximum dry weight at 34 DAP. Similarly Jayasuriya et al. (2007) reported that pitted morningglory (*Ipomoea lacunosa* L.) germination does not occur until at least 20 DAP. These seeds reached their maximum dry mass by 22 DAP and their maximum germination percentage at 24 DAP, but by 30 DAP the seeds no longer germinated unless manually scarified, indicating that seed were dormant due to impermeable seed coats (Jayasuriya et al. 2007). Even in mouse-ear cress (*Arabidopsis thaliana* (L.) Heynh.), a species

known for its short life cycle, Koorneef et al. (1989) found that seeds do not become viable until at least 13 DAP when dried immediately after harvest.

Given that kochia seed do not possess any type of dormancy just after being shed from the plant (Zorner et al. 1984) it is suspected that they are viable very early after pollination. Based on above discussion on seed maturation process of other plants, seeds become viable 2 to 3 weeks after pollination. This period after pollination is the critical time for management interventions to stop weeds from producing viable seed. Hill et al. (2016) found that viable seed production was reduced by 64 to 100% when weeds were terminated when immature seeds were present compared to weeds terminated when mature seeds were present. Kochia seeds did not appear to be completely black or dark brown in color until 36 DAP and they were not yet viable. Hence, a next step in this research would be to produce more replicates to determine when kochia seeds become viable after pollination.

### **Photographs of Flower Development**

To document changes occurring with kochia flowering after stigma emergence, photographs of buds from three branches from three different plants were taken daily in greenhouse. A representative set of pictures of flower buds from branch-1 were taken over a series of 45 days (Figure 3.2). Stigmas were protruding out of the buds which indicated that this flower was receptive and soon would get fertilized upon receiving foreign pollen (Figure 3.2 A). Stigmas were visible 8 to 9 days before anther and filament appeared in all buds observed (Figure 3.2 B). Color of the anther varied from yellow to orange. Mature anthers dehisced pollen only during morning hours from 8 to 11 am. Flower buds shed pollen for 8 to 9 days after anther and filaments were visible and turned brown after that (Figure 3.2D). In the present study, kochia flowered indeterminately for an extended period (around 2 months). This is because axils

produced new flower buds as it grew vertically and continuously shed pollen from new flowers. Flower buds that were under observation had flower longevity, a concept introduced by Primack (1985) indicating the length of time an individual flower remains open with fresh appearing stigma and stamens which in our case is the length of time between stigma emergence and browning of pollen was 19 to 25 days.

Male parts from old flower buds from branch-1 appeared light brown after 10 to 12 days of anther browning (Figure 3.2E). The same bud turned dark brown 7 to 9 days after anther browning (Figure 3.2F). Further, investigation is required to determine if complete browning is an indication of seed formation and if they are capable of germinating under favorable environmental conditions.

In kochia, others have observed stigmas to emerge 7 days before pollen is shed and are receptive to foreign pollen at that time. The stigma deteriorated before pollen was shed from the same flower which prevented self-pollination within the same flower (Stallings et al. 1995). But in our study, it took 8 to 9 days for anthers and filaments to appear after stigma emergence. Color variation in kochia anthers observed by Stallings et al. (1995) were associated with environmental conditions where high soil moisture resulted in cream to light pink anthers whereas low soil moisture resulted in bright orange colored anthers. In our study, most of the anthers were bright yellow colored. Kochia plants in this study were greenhouse grown whereas Stallings et al. (1995) grew plants in the field. This difference might have affected the timing of stigma emergence and color of anthers. Anther dehiscence prefers temperature above 21 C and the phenomenon ceases as temperature goes above 30 C (Shamsutdinov and Khamidov 1984). In our study kochia plants shed pollen only in morning hours when temperature was relatively low compared to higher daytime temperature. The phenomenon of browning of pollen after shedding

is commonly observed in Chenopodiaceae family (Blackwell and Powell 1981). Following pollination anthers senesced rapidly and turned brown in Russian thistle (*Salsola kali*) (Blackwell and Powell 1981). Kochia flowered for a long period of time in our study (19 to 25 days). Longer flower longevity increases the probability of it being visited by foreign pollen for a longer period of time. Longer flower longevity quality when combined with ability of kochia pollen to spread sulfonylurea-resistant trait over a far distance (Stallings et al. 1995) increase the chances of susceptible ovule to result in seeds that facilitate the further spread of herbicide-resistance. However, further scientific evidence on flower longevity causing further spread of herbicide-mechanisms is required to fully support this hypothesis.

Based on visual observations using photographs of kochia flower buds, it took 36 DAP, from the first sight of a receptive stigma in flower buds for buds to turn black or dark-brown under controlled pollination. Seeds enclosed in buds at 36 DAP appeared brown in color but did not germinate in Petri dish. Similarly, under natural greenhouse conditions, it took 40 to 45 days for buds to appear dark brown, but do not know if these buds have viable seeds enclosed. Results from this study can be used to further investigate time to mature and viable seed formation in kochia after flowering to effectively schedule cultural, chemical, and other management tactics for its control.

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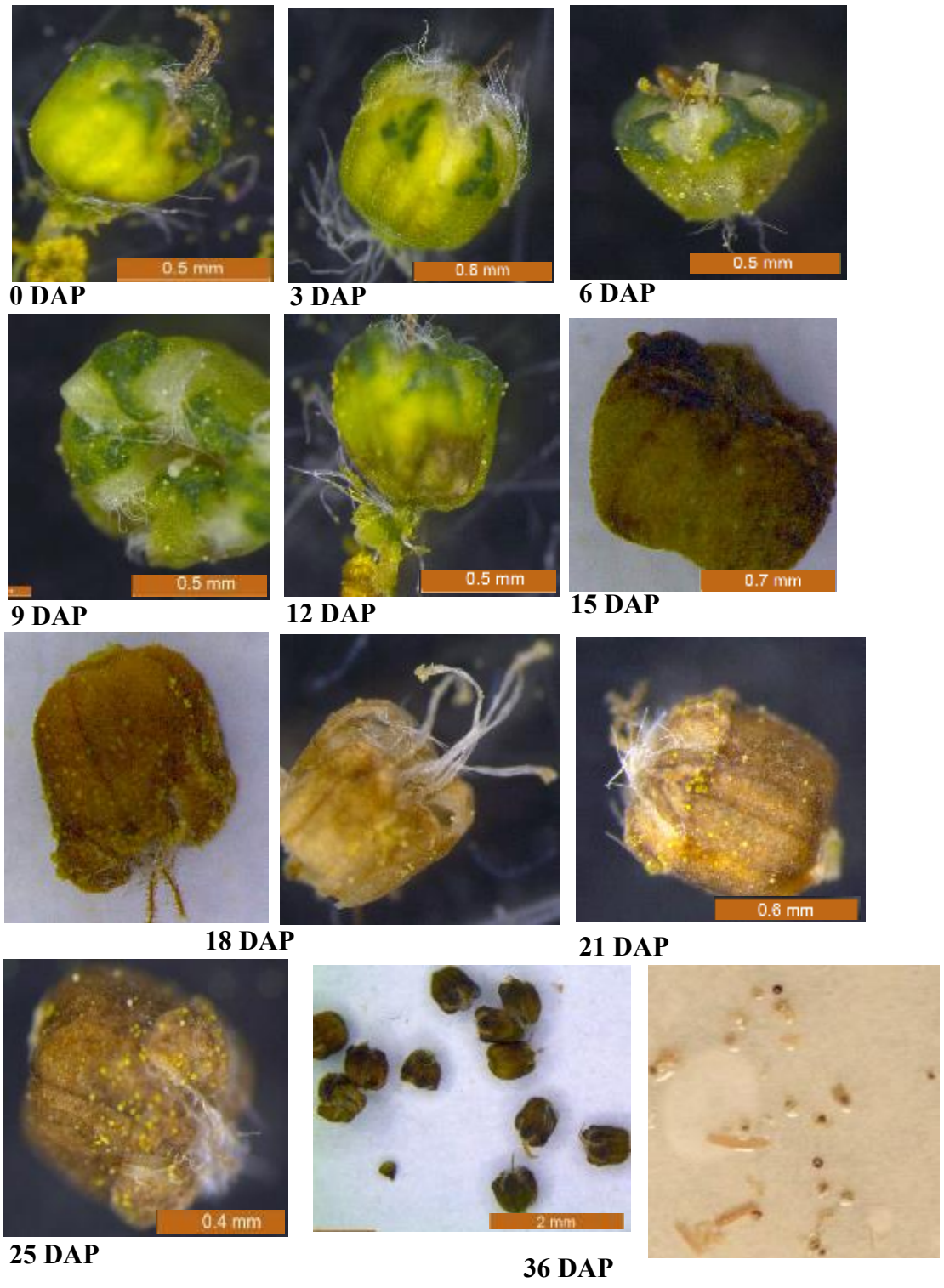
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## TABLES AND FIGURES

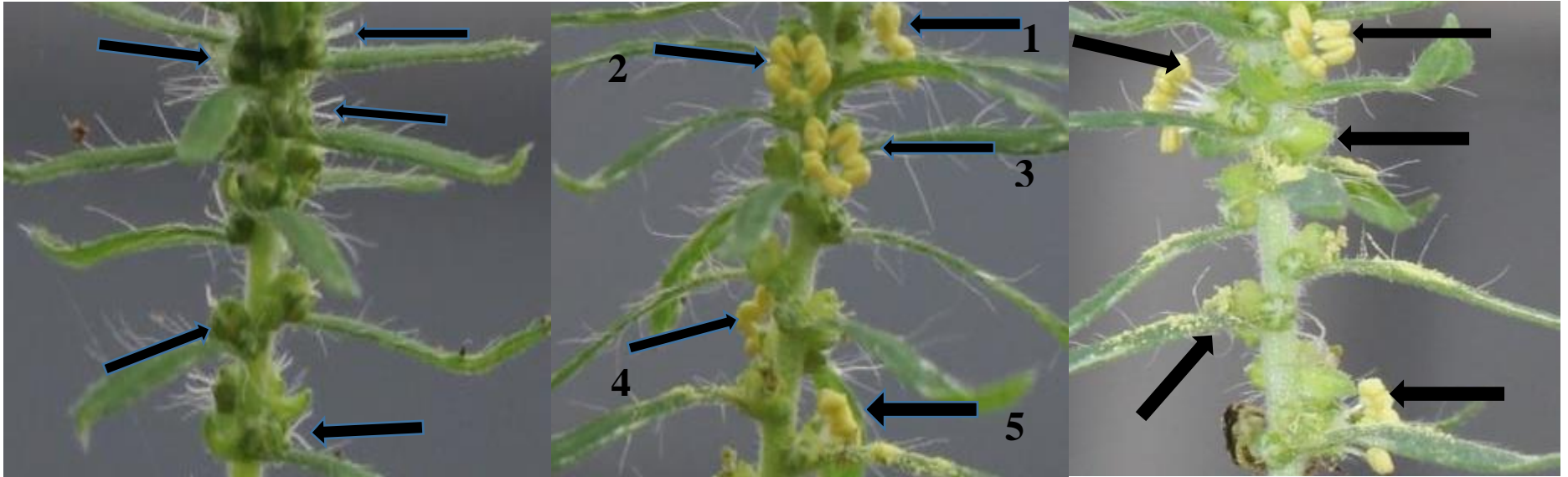
**Table 3.1 Seed weight of flower buds from controlled pollination taken at different times after pollination**

Days after pollination	Seed weight (g/10seeds)
0	0.0065
3	0.0065
6	0.0065
9	0.0064
12	0.0064
15	0.0065
18	0.0066
21	0.0068
36	0.0070





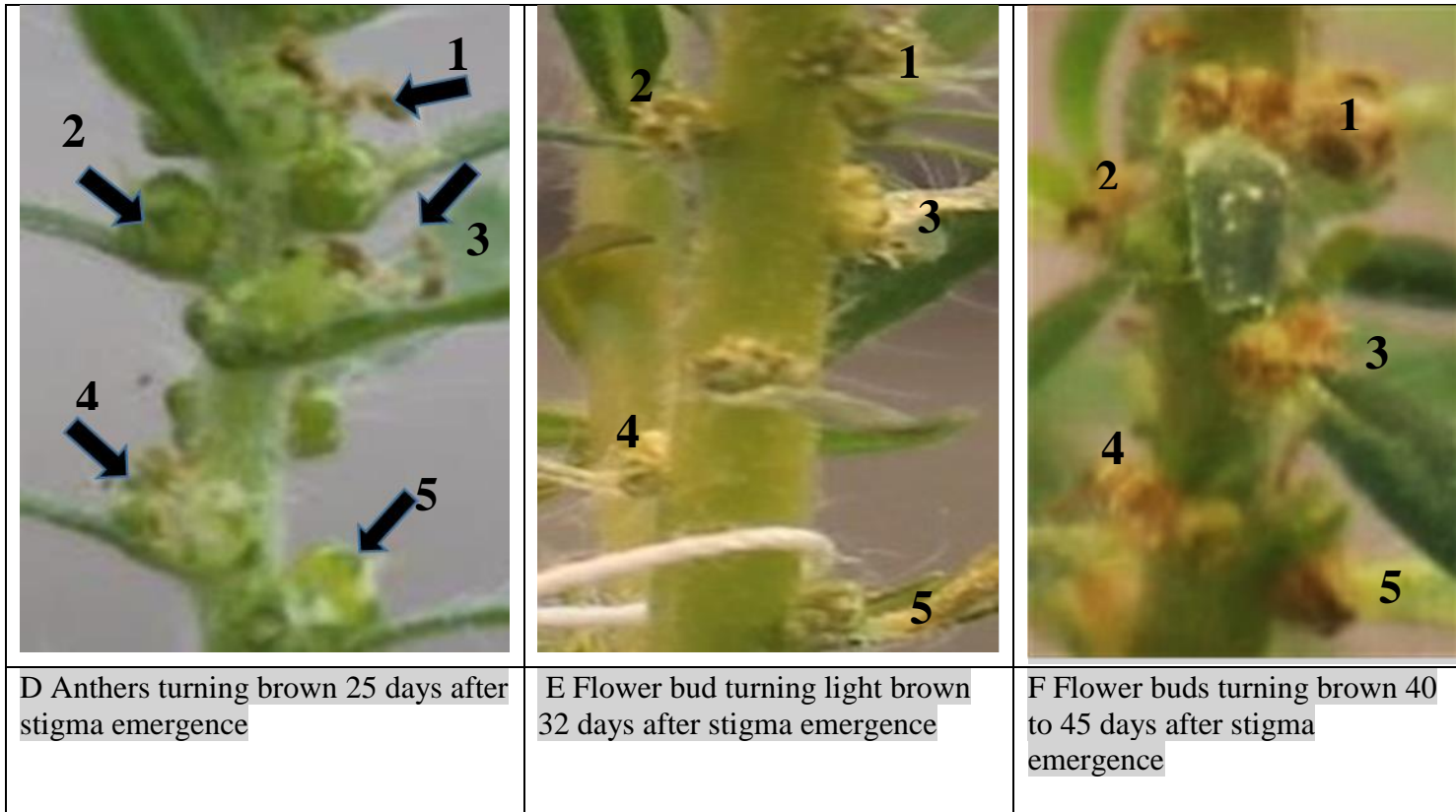
**Figure 3.1** Photographs of individual kochia flower bud development from cross pollination study in greenhouse at different times after pollination.



**A** Stigma emerging and receptive at each bud

**B** Male parts visible 6 to 7 days after stigma emergence, only anther visible

**C** Male parts with both anther and filament shedding dust like, yellow colored pollen 8 to 9 days after stigma emergence



**Figure 3.2 Photographs of individual kochia flower bud development from natural pollination study in greenhouse at different times after pollination. Stages of flower bud development from stigma emergence to browning of flower bud has been represented by letters A-F. Numbers 1-5 in the photographs represent the flower buds tracked throughout the experiment duration**

## Appendix A - ANOVA results

**Table A.1 Analysis of variance (ANOVA) table showing three-way significant interaction between temperature levels, moisture levels and accessions on total number of seed germination**

**Significant three-way interaction between temp, moist and accession**

<b>Effect</b>	<b>Num DF</b>	<b>Den DF</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>Run</b>	1	16.1	3.9	0.1
<b>Rep(Run)</b>	8	16.1	0.7	0.7
<b>Temp</b>	2	16	780.2	<.0001
<b>Moist</b>	4	32.1	2000.4	<.0001
<b>Temp*Moist</b>	8	206	85.0	<.0001
<b>Run*Temp</b>	2	16	1.7	0.2
<b>Run*Moist</b>	4	32.1	1.0	0.4
<b>Accessions</b>	1	206	24.8	<.0001
<b>Temp*Accessions</b>	2	206	3.5	0.0
<b>Moist*Accessions</b>	4	206	2.1	0.1
<b>Temp*Moist*Accession</b>	8	206	10.3	<.0001

**Table A.2 Analysis of variance (ANOVA) table showing NS interactions (1) and significant interactions (2) between ABA treatments, temperature levels and two runs on total number of seed germination**

**Table A.2 (1): Three-way interaction between run, temp and ABA**

<b>Effect</b>	<b>Num DF</b>	<b>Den DF</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>Run</b>	1	8.3	0.0	1.0
<b>Rep(Run)</b>	6	8.3	0.3	0.9
<b>Temp</b>	1	6	34.8	0.0
<b>Run*Temp</b>	1	6	1.4	0.3
<b>ABA</b>	6	36	36.6	<.0001
<b>Run*ABA</b>	6	36	2.9	0.0
<b>Temp*ABA</b>	6	36	2.8	0.0
<b>Run*Temp*ABA</b>	6	36	0.9	0.5

**Table A.2 (2): Two-way interaction between temp and ABA**

<b>Effect</b>	<b>Num DF</b>	<b>Den DF</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>Run</b>	1	9.5	0.0	1.0
<b>Rep(Run)</b>	6	9.5	0.3	0.9
<b>Temp</b>	1	7	33.1	0.0
<b>ABA</b>	6	36	36.6	<.0001
<b>Run*ABA</b>	6	36	2.9	0.0
<b>Temp*ABA</b>	6	42	2.8	0.0

**Table A.3 Analysis of variance (ANOVA) table showing significant three-way significant interaction between GA treatments, temperature levels and two runs on total number of seed germination**

**Significant three-way interaction between run, temp and GA**

<b>Effect</b>	<b>Num DF</b>	<b>Den DF</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>Run</b>	1	6.72	1.01	0.3499
<b>Rep(Run)</b>	3	6.72	0.47	0.7106
<b>Temp</b>	1	6	54.25	0.0003
<b>Run*Temp</b>	1	6	1.01	0.3540
<b>GA</b>	6	36	23.66	<.0001
<b>Run*GA</b>	6	36	1.75	0.1378
<b>Temp*GA</b>	6	36	1.23	0.3135
<b>Run*Temp*GA</b>	6	36	5.13	0.0007

## Appendix B - Calculations for PEG and hormones

Polyethylene glycol- 8000 concentration required for particular temperature (T) and water potential ( $\psi$ ) was obtained by using following equation given Michel (1983):

$$[\text{PEG}] = (4 - (5.16 \psi T - 560 \psi + 16^{0.5}) / (2.58T - 280))$$

where, [PEG] = concentration of polyethylene glycol in grams, T = temperature (either 4, or 11 C) and  $\psi$  = water potential (either 0.0, -0.4, -0.8, -1.2 or -1.6).

Based on this equation following grams of PEG was used given by Table B.1 below:

**Table B.1 Table showing grams of polyethylene glycol added in 1 L of water at each temperature and water potential**

	PEG concentration in g/1 L of water				
	0.0 MPa	-0.4 MPa	-0.8 MPa	-1.2 MPa	-1.6 MPa
	----- g -----				
11 C	0	155	235	296	348
6 C	0	148	228	289	341
4 C	0	67	71	111	171

Following mass molarity calculator from <http://www.sigmaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/mass-molarity-calculator.html> was used to calculate the mass of abscisic acid (ABA), ABA inhibitor fluridone (FL), gibberellic acid (GA) and GA inhibitor paclobutrazol (PL) required to achieve a specific molar concentration. Table B.2 shows grams of hormones added in each Petri dish to 20 mL of water.

**Table B.2 Table showing grams of hormones and inhibitors added to 20 mL of water in each Petri dish**

..... $\mu$ M.....		.....grams.....	
Hormone	Inhibitor	Hormone	Inhibitor
10 ABA	None	0.008	None
10 ABA	2.5 FL	0.008	0.03
10 ABA	5 FL	0.008	0.08
10 ABA	15 FL	0.008	0.10
10 ABA	20 FL	0.008	0.20
10 GA	None	0.01	None
10 GA	2.5 PL	0.01	0.002
10 GA	5 PL	0.01	0.004
10 GA	15 PL	0.01	0.017
10 GA	20 PL	0.01	0.023



**Table B.3 Total number (SE or SD?) and average percentage of seed germination out of 50 seeds in Petri dish for five accessions (5 replications) used for initial germination screening to determine accessions with highest and lowest germination rates to be used in hormone study.**

Accessions	Total # germinated	Total % germination
1	44 (1.2)	88
KS3	49 (0.9)	98
3	44 (1.1)	89
4	40 (0.9)	80
KS4	38 (1.0)	75

**Table B.4 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection, and  $X_0$  is thermal time (TT) required for 50% germination**

Temperature	Water potential	a (se)	b (se)	$X_0$ (se)	$R^2$
C	-MPa	%		Cd	
11	0.0	92 (2.1)	2 (0.2)	17 (0.1)	0.87
11	0.4	98 (1.5)	1 (0.1)	30 (0.2)	0.92
11	0.8	92 (1.1)	1 (0.1)	31 (0.3)	0.92
11	1.2	98 (1.7)	2 (0.1)	75 (0.4)	0.94
6	0.0	94 (0.8)	1 (0.1)	22 (0.3)	0.97
6	0.4	93 (1.7)	2 (0.1)	25 (0.2)	0.95
6	0.8	97 (3.6)	2 (0.2)	36 (0.1)	0.91
6	1.2	98 (10.4)	3 (0.7)	52 (0.2)	0.98
4	0.0	99 (1.3)	1 (0.1)	15 (0.1)	0.97
4	0.4	99 (2.6)	2 (0.1)	21 (0.1)	0.98
4	0.8	92 (1.7)	2 (0.1)	23 (0.2)	0.98

**Table B.5 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection, and  $X_0$  is hydro time (HT) required for 50% germination**

Temperature	Water potential	a (se)	b (se)	$X_0$ (se)	$R^2$
C	-MPa	%		MPa*d	
11	0.0	93 (1.1)	2 (0.2)	5 (0.1)	0.98
11	0.4	97 (1.3)	2 (0.1)	5 (0.2)	0.92
11	0.8	93 (2.1)	3 (0.1)	3 (0.2)	0.92
11	1.2	99 (1.8)	1 (0.1)	3(0.1)	0.89
6	0.0	98 (1.8)	1 (0.1)	10 (1.1)	0.87
6	0.4	95 (1.6)	1 (0.1)	9 (1.2)	0.95
6	0.8	98 (2.6)	2 (0.2)	9 (0.1)	0.81
6	1.2	92 (1.4)	2 (0.7)	7 (0.2)	0.98
4	0.0	99 (1.3)	3 (0.1)	10 (0.1)	0.87
4	0.4	115 (2.2)	1 (0.1)	9 (0.1)	0.92
4	0.8	92 (1.8)	2 (0.1)	2 (0.2)	0.99

**Table B.6 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection, and X<sub>0</sub> is hydro thermal time (HTT) required for 50% germination**

Temperature	Water potential	a (se)	b (se)	X <sub>0</sub> (se)	R <sup>2</sup>
C	-MPa	%		MPa*Cd	
11	0.0	95 (1.3)	2 (0.2)	39 (0.2)	0.88
11	0.4	96 (1.3)	1 (0.1)	38 (0.1)	0.92
11	0.8	94 (1.1)	2 (0.1)	29 (0.2)	0.92
11	1.2	98 (2.8)	1 (0.1)	28 (1.1)	0.89
6	0.0	98 (1.8)	1 (0.1)	35 (1)	0.87
6	0.4	99 (1.6)	1 (0.1)	31(0.8)	0.95
6	0.8	98 (2.6)	3 (0.2)	30 (0.9)	0.92
6	1.2	93 (2.4)	2 (0.7)	22 (0.8)	0.98
4	0.0	99 (1.3)	1 (0.1)	14 (0.9)	0.97
4	0.4	99 (1.2)	1 (0.1)	12 (0.7)	0.82
4	0.8	98 (1.8)	1 (0.1)	3 (0.8)	0.99

**Table B.7 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination in response to water, abscisic acid (ABA), fluridone (FL) and gibberellic acid (GA) at 23 C using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection, and  $X_0$  is thermal time (TT) required for 50% germination**

Hormone	Inhibitor	Other	a (se)	b (se)	$X_0$ (se)	$R^2$
ABA	FL		%		Cd	
( $\mu$ M)	( $\mu$ M)	( $\mu$ M)				
0	0	Water	96 (1.2)	2 (0.2)	109 (0.1)	0.98
10	0	0	96 (1.1)	3 (0.1)	142 (0.2)	0.92
10	2.5	0	94 (1.1)	2 (0.1)	148 (0.1)	0.99
10	5	0	98 (1.8)	2 (0.1)	176 (0.2)	0.89
10	15	0	98 (1.7)	1 (0.1)	160 (1.1)	0.97
10	20	0	98 (1.6)	2 (0.1)	128 (0.9)	0.95
10	0	10 GA	98 (1.6)	1 (0.2)	194 (0.8)	0.92

**Table B.8 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination in response to water, abscisic acid (ABA), fluridone (FL) and gibberellic acid (GA) at 6 C using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection, and  $X_0$  is thermal time (TT) required for 50% germination**

Hormone	Inhibitor	Other	a (se)	b (se)	$X_0$ (se)	$R^2$
ABA	FL		%		Cd	
( $\mu$ M)	( $\mu$ M)	( $\mu$ M)				
0	0	Water	96 (1.2)	2 (0.2)	22 (0.1)	0.98
10	0	0	96 (1.1)	3 (0.1)	32 (0.2)	0.92
10	2.5	0	94 (1.1)	2 (0.1)	21 (0.1)	0.99
10	5	0	98 (1.8)	2 (0.1)	24 (0.2)	0.89
10	15	0	98 (1.7)	1 (0.1)	34 (1.1)	0.97
10	20	0	98 (1.6)	2 (0.1)	41 (0.9)	0.95
10	0	10 GA	98 (1.6)	1 (0.2)	30 (0.8)	0.92

**Table B.9 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination in response to water, gibberellic acid (GA) abscisic acid (ABA), paclobutrazol (PL), abscisic acid (ABA) at 23 C using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection,  $X_0$  is thermal time (TT) required for 50% germination**

Hormone	Inhibitor	Other	a (se)	b (se)	$X_0$ (se)	$R^2$
GA	PL		%		Cd	
( $\mu$ M)	( $\mu$ M)	( $\mu$ M)				
0	0	Water	98 (1.1)	1 (0.1)	123 (0.1)	0.99
10	0	0	97 (1.1)	2 (0.1)	122 (0.3)	0.97
10	2.5	0	98 (1.1)	1 (0.3)	152 (0.1)	0.98
10	5	0	99 (1.6)	2 (0.2)	101 (0.1)	0.98
10	15	0	97 (1.7)	3 (0.1)	141 (1.1)	0.98
10	20	0	99 (1.7)	2 (0.3)	193 (0.7)	0.97
10	0	10 ABA	97 (1.6)	3 (0.1)	274 (0.6)	0.99

**Table B.10 Parameter estimates ( $\pm$ SE) of total percent kochia seed germination in response to water, gibberellic acid (GA) abscisic acid (ABA), paclobutrazol (PL), abscisic acid (ABA) at 6 C using sigmoidal function (Equation 2.1) where a is maximum cumulative germination, b is slope at the inflection,  $X_0$  is thermal time (TT) required for 50% germination**

Hormone	Inhibitor	Other	a (se)	b (se)	$X_0$ (se)	$R^2$
GA	PL		%		Cd	
( $\mu$ M)	( $\mu$ M)	( $\mu$ M)				
0	0	Water	98 (1.1)	1 (0.1)	30 (0.1)	0.99
10	0	0	97 (1.1)	2 (0.1)	33 (0.3)	0.97
10	2.5	0	98 (1.1)	1 (0.3)	27 (0.1)	0.98
10	5	0	99 (1.6)	2 (0.2)	43 (0.1)	0.98
10	15	0	97 (1.7)	3 (0.1)	32 (1.1)	0.98
10	20	0	99 (1.7)	2 (0.3)	32 (0.7)	0.97
10	0	10 ABA	97 (1.6)	3 (0.1)	32 (0.6)	0.99