

**Multi-tactic ecological strategies to manage *Bassia scoparia* and *Amaranthus tuberculatus* in the cropping systems of Great Plains and Midwestern U.S.**

by

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A dissertation submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of  
DOCTOR OF PHILOSOPHY

Major: Crop Production and Physiology (Weed Science)

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Iowa State University

Ames, Iowa

2021

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**DEDICATION**

I dedicate my dissertation work to my loving parents and in memory of my late brother.

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## ACKNOWLEDGMENTS

A very special thanks to my committee chair, Prashant Jha for his guidance, support and patience throughout the course of this research. I would like to thank my committee members, Matt Liebman, Bob Hartzler, Sotirios Archontoulis, and Ajay Nair for their patience and constructive feedbacks.

I wish to thank my extended family members who have been a constant source of perspectives, distractions, and levity. I would also like to thank my friends and teachers who have helped me to move through this academic career. Finally, a big thanks to all the farmers around the world for their dedication to the agriculture.



**ABSTRACT**

Kochia (*Bassia scoparia* [L.] A.J. Scott) and waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) are one of the most troublesome weeds in the cropping systems of Great Plains and Midwestern United States, respectively. A widespread occurrence of multiple herbicide-resistant (MHR) *B. scoparia* and *A. tuberculatus* populations across the region has seriously limited herbicide options to control these weed species. Therefore, there is an immediate need for ecologically based, multi-tactic strategies to manage these weeds. Laboratory and field experiments were conducted during 2017 to 2019 at the Montana State University Southern Agricultural Research Center, Huntley, MT: 1) to quantify moisture and thermal requirements for germination of *B. scoparia* and 2) to quantify the effect of winter wheat cover crop and irrigation on *B. scoparia* emergence patterns across the U.S. Great Plains. Forty-four *B. scoparia* accessions were collected from northern (Huntley, MT; Powell, WY) and southern (Lingle, WY; Scottsbluff, NE) regions of the Great Plains. Moisture requirements for germination of *B. scoparia* accessions did not differ between northern and southern regions. It required a water potential of -0.85 MPa and -1.9 MPa to reduce *B. scoparia* germination by 10 and 90%, respectively. The germination rate was highest at temperatures between 14 and 26 C for all accessions and did not differ among locations. Winter wheat cover crop and irrigation treatments did not influence *B. scoparia* emergence in the northern region. In the southern region, the winter wheat cover crop did not reduce cumulative emergence of *B. scoparia*. However, irrigation prompted earlier and faster emergence of *B. scoparia*. This indicates that in the southern region of the Great Plains, *B. scoparia* emergence can effectively be stimulated by one to two irrigations during early spring and then controlled with either tillage or non-selective herbicides before planting late-season crops. Field experiments were conducted during 2019 and

2020 at two sites (Iowa State University Curtiss Farm in Ames, IA and Bruner Farm in Boone, IA) to design multi-tactic strategies to manage *A. tuberculatus* in a corn-soybean rotation. Effect of three herbicide programs (HP) on *A. tuberculatus* seed inputs was tested in the 2019 corn. The effects of previous year's seed inputs, cereal rye cover crop, and narrow-row soybean on *A. tuberculatus* density, biomass, and seed production were tested in the 2020 soybean. A weed seed destructor was used to prevent seed inputs by *A. tuberculatus* escapes at soybean harvest. In corn, an HP with two sites of action provided only 35% control of *A. tuberculatus* compared with  $\geq 97\%$  control by an HP with three sites of action. In soybean, no new seed inputs from the previous year's corn crop reduced *A. tuberculatus* density and biomass by  $>30\%$  compared with seed inputs from the previous year's corn crop. Including a cereal rye cover crop or using a narrow-row soybean reduced *A. tuberculatus* density by at least 13% and biomass by 50%, compared with a no cover crop or a wide-row soybean. The weed seed destructor physically destroyed 90% of *A. tuberculatus* seeds at soybean harvest. A combination of all four-control tactics (spanned over two years) reduced *A. tuberculatus* seed inputs by 95% at the time of soybean harvest. These results indicate that diverse control tactics targeting *A. tuberculatus* at different life-cycle stages can successfully manage MHR populations.

## CHAPTER 1. GENERAL INTRODUCTION

### *Amaranthus tuberculatus* biology, ecology, and herbicide resistance

Waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) is a dioecious, summer annual weed species native to the Midwestern United States (U.S.) where it is known to occur naturally along riverbanks and in floodplains (Pammel 1913). Despite first report of the presence of this weed species in 1910s (Pammel 1913) and then history of invasions in croplands during 1950s (Sauer 1957), *A. tuberculatus* was not a significant weed in the Midwestern U.S. until 1990s (Hager et al. 1997). Several factors such as reductions in tillage, increased use of herbicides, differential susceptibility to herbicides, and rapid evolution of herbicide-resistant (HR) populations (Hager et al. 1997; Jasieniuk et al. 1996; Patzoldt et al. 2002; Sprague et al. 1997) contributed to the rise of *A. tuberculatus* as a driver weed in corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) rotations of the Midwest. Currently, *A. tuberculatus* is the most dominant and troublesome weed of soybean in the region (Van Wychen 2019). In 2017, *A. tuberculatus* was ranked third most common and second most troublesome weed of corn in the U.S. (Van Wychen 2017).

Waterhemp (*A. tuberculatus*) in the Midwestern U.S. was traditionally considered as two closely related dioecious species comprising tall waterhemp (*A. tuberculatus*) and common waterhemp (*A. rudis*) (Sauer 1955). This classification was primarily based on fruit dehiscence and presence/absence of a sepal. However, due to a lack of clear distinction between morphological and molecular characteristics of these two species, a single polymorphic species with the name *A. tuberculatus* was proposed (Costea and Tardif 2003; Pratt and Clark 2001). Currently, *A. tuberculatus* has been reported in 42 states of the U.S., five Canadian provinces, 15 European countries, and two Western Asian countries (Centre for Agriculture and Bioscience

International 2021). Contaminated soybean shipment from the U.S. has been a major cause for the emigration of this species outside the U.S.

*Amaranthus tuberculatus* thrives in a wide range of soil type and textures and grows in non-disturbed and disturbed habitats (Costea et al. 2005). It is very well adapted to no-till cropping systems and can have a serious negative impact on crops, if left uncontrolled.

*Amaranthus tuberculatus* can grow 2 to 3 m tall with a height-increase rate of 0.11 to 0.16 cm per growing degree-day under favorable conditions (Horak and Loughin 2000). A single female *A. tuberculatus* plant can produce >1 million seeds in the absence of crop competition (Hartzler et al. 2004; Sellers et al. 2003; Steckel et al. 2003). A season-long infestation of *A. tuberculatus* can reduce corn and soybean grain yields by >40% (Hager et al. 2002; Steckel and Sprague 2004a, 2004b). Similarly, *A. tuberculatus* density as low as 8 plants m<sup>-1</sup> of row can reduce soybean grain yield by >55% (Bensch et al. 2003).

One of the most important factors which influence the efficacy of weed management practices is the emergence periodicity of a weed species (Buhler et al. 1997; Forcella et al. 1992). Knowledge of the timing of weed seedling emergence enables a control tactic to be utilized effectively. Factors such as crop residue on the soil surface, soil moisture and temperature, and interactions between these factors can influence emergence periodicity of a weed species. For instance, presence of corn residues on the soil surface reduced both the total emergence and the emergence rate of *Amaranthus* spp. (Buhler et al. 1996). A crop residue can influence weed seed emergence through physical impedance and allelopathy, and changes in soil moisture, temperature, light, microbial decomposition, and predation. Stoller and Wax (1973) reported that while soil warming was the primary factor for the initiation of emergence in early-emerging weed species, availability of soil moisture was the primary factor for late-emerging weed species

(after May 1) like *A. tuberculatus*. Weise and Vandiver (1970) also reported that *Amaranthus* spp. had a high soil moisture requirement. Rainfall events >1.5 cm are more likely to initiate the emergence of *A. tuberculatus* later in the growing season (Cordes et al. 2004).

*Amaranthus tuberculatus* is one of the last weed species to emerge among the problematic weeds of corn and soybean in the Midwestern U.S. (Hartzler et al. 1999; Werle et al. 2014). Furthermore, *A. tuberculatus* has an extended emergence period, with several cohorts emerging during a growing season (Hartzler et al. 1999). Typically, in Iowa, *A. tuberculatus* starts emerging in the month of May and continue to emerge until late July or early August, with a peak emergence period during the month of June (Hartzler et al. 1999; Leon and Owen 2006; Refsell and Hartzler 2009).

Adoption of no-till systems has favored small-seeded species such as *A. tuberculatus* by keeping the weed seed near the soil surface; hence, providing optimum conditions for germination and establishment (Buhler 1992; Buhler and Oplinger 1990; Felix and Owen 1999). Germination of small-seeded weed species is higher on the soil surface or within 1 to 2 cm of the surface where maximum diurnal temperature fluctuations, availability of oxygen, and exposure to light occurs (Alm et al. 1993; Sauer and Struik 1964; Taylorson and Hendricks 1977). Emergence of *A. tuberculatus* was 3 to 4 times higher in no-till than in chisel or moldboard plow conditions (Leon and Owen 2006; Refsell and Hartzler 2009). Similar to the presence of crop residues on soil surface, no-till conditions prolonged the emergence of *A. tuberculatus* compared to chisel or moldboard plow conditions (Leon and Owen 2006). Moreover, under no-till conditions, the emergence peak occurred at the end of June compared to end of May or the first week of June under tilled conditions.

Seeds of *A. tuberculatus* can persist in soil for a significant amount of time (>4 yr) since only a small portion of the original seedbank emerges each year. Weed seed persistence is a complex phenomenon and is influenced by many factors such as environmental conditions, burial depth in soil, and intrinsic characteristics of the seed (Baskin and Baskin 1989). Less than 22% of *A. tuberculatus* seeds buried to a depth of 5 cm emerged annually over a period of three to four years (Buhler and Hartzler 2001; Hartzler et al. 1999). Steckel et al. (2007) reported that only 0.004% of a naturally established *A. tuberculatus* seedbank remained in the soil after four years of no additional seed input. However, at least 40 plants m<sup>-2</sup> emerged in the fourth year. This implies that integrated control tactics targeting seed prevention can deplete *A. tuberculatus* soil seedbank in a relatively short period (low seed persistence), but a few escapes can replenish the soil seedbank because of the ability of this weed species to produce copious amount of seeds (Hartzler et al. 2004).

Temperature plays an important role in weed seed germination by regulating both seed dormancy and the initiation/rate of germination (Forcella 1998; Roberts 1988). A minimum mean temperature of 10 C is required for germination of *A. tuberculatus* seeds (Guo and Al-Khatib 2003; Leon et al. 2004; Steckel et al. 2004). Alternating temperatures increase total germination of *A. tuberculatus* (Leon et al. 2004; Steckel et al. 2004). Additionally, increase in thermal amplitudes can reduce the temperature requirements for *A. tuberculatus* germination (Leon et al. 2004). Nonetheless, no germination occurred at alternating temperatures of 14/8 C (Leon et al. 2004) and 15/10 C (Guo and Al-Khatib 2003).

Timing of weed seedling emergence relative to the crop is important for crop-weed competition, and their response to environmental stresses and control tactics. Generally, weed cohorts emerging earlier in the growing season produce more aboveground biomass and seeds,

and cause greater crop yield losses than late-emerging cohorts (Bensch et al. 2003; Dieleman et al. 1996; Knezevic et al. 1997). For instance, *A. tuberculatus* seedlings that emerged after the V5 growth stage of soybean and corn did not reduce grain yields of these crops significantly (Bensch et al. 2003; Murphy et al. 1996). In a research conducted by Hartzler et al. 2004, survival of seedlings that emerged with soybean was 90% compared with only 13% survival when emerged 50 days after soybean planting. Furthermore, biomass accumulation in late-emerging plants was reduced by  $\geq 90\%$  compared with early-emerging plants. However, those late emerging plants were still able to produce about 3,000 seeds plant<sup>-1</sup>. Similarly, Steckel et al. (2003) reported that even though *A. tuberculatus* biomass was reduced by 52% and seed production by 87% under reduced light conditions (68% shading), shaded plants were still able to produce about 90,400 seeds plant<sup>-1</sup>. Therefore, late-emerging *A. tuberculatus* plants may not affect crop yields, but can contribute significant amount of seeds to the soil seedbanks, implying the need to manage the seedbank.

Weeds may grow more successfully than crops under resource limiting conditions such as shading because of their greater genetic variation and phenotypic plasticity (Baker 1965). For example, redroot pigweed (*Amaranthus retroflexus* L.) plants allocated more dry matter to their upper portion of the canopy in response to low-light conditions created by corn crop (McLachlan et al. 1993). *Amaranthus retroflexus* plants attained this by reducing allocations to branch components and increasing allocations to main-stem leaves, main-stem inflorescence, and main-stem itself. Similarly, the time required for flowering or maturity in *A. retroflexus* was delayed with a delay in emergence relative to the corn planting date (Vengris 1963). This plasticity in flowering phenology has also been observed in *A. tuberculatus*. For instance, in a field study, Wu and Owen (2014) reported that late-emerging cohorts of *A. tuberculatus* took less time to

initiate flowering and had a smaller flowering duration compared with early-emerging cohorts. In addition, late-emerging cohorts showed a higher resource-allocation efficiency, indicating high reproductive fitness. *Amaranthus tuberculatus* had an extended flowering period ranging from 40 to 60 days, with 7 to 8 distinct flowering pulses (Wu and Owen 2014). In another study, differences in maternal environment experienced by *A. tuberculatus* plants (that emerged over an extended period) did not affect maturation time and mass of seed produced by those plants (Wu and Owen 2015). These results indicate that *A. tuberculatus* possesses an extended flowering time and can produce viable seeds under varying environmental conditions. This warrants the implementation of season-long control tactics for effective management of this weed species.

Because of an extended emergence period and the ability to produce viable seeds from late-emerging cohorts, *A. tuberculatus* is difficult to control with a single control tactic. Therefore, multiple applications of herbicides within a growing season were needed for the chemical control of *A. tuberculatus* in no-till systems (Hager et al. 1997; Steckel et al. 2002; Wax 1995). However, high herbicide use within a season has resulted in widespread evolution of HR *A. tuberculatus* populations, which has made the control of this species even more difficult.

Currently, *A. tuberculatus* has developed resistance to seven different herbicide groups including glyphosate, synthetic auxins, and inhibitors of acetolactate synthase (ALS), photosystem II (PSII), protoporphyrinogen oxidase (PPO), 4-hydroxyphenylpyruvate dioxygenase (HPPD), and very long chain fatty acid synthesis (Tranel 2020). The first occurrence of HR *A. tuberculatus* was reported from Nebraska in 1990 (Anderson et al. 1996). The population was resistant to PSII-inhibitor herbicide, namely atrazine. However, in subsequent years, more populations resistant to PSII-inhibitors or/and ALS-inhibitors were reported in other Midwestern states (Tranel et al. 2011). The first ALS-inhibitor resistant *A.*



*tuberculatus* population was identified from Kansas in 1992 (Horak and Peterson 1995).

*Amaranthus tuberculatus* became the first weed species in the world to develop resistance to PPO-inhibitors in 2000 (Shoup et al. 2003) and HPPD-inhibitors in 2009 (Hausman et al. 2011). Populations resistant to glyphosate were first reported in 2004 in Missouri from a soybean field which had been exposed to glyphosate for at least six consecutive years (Legleiter and Bradley 2008). Most importantly, *A. tuberculatus* populations resistant to multiple herbicides from five to six different sites of action have been evolved and are becoming widespread (Heap 2021; Schultz et al. 2015; Shergill et al. 2018). Currently, HR *A. tuberculatus* has been reported in 20 U.S. states (Heap 2021), and in Canada (Heap 2021), Italy (Milani et al. 2020), and Israel (Yadid et al. 2021). Characteristics of *A. tuberculatus* such as flowering plasticity, long-distance gene flow, multiple modes of convergent evolution and limited fitness penalty in HR individuals have fueled the rapid evolution and spread of resistant biotypes (Duff et al. 2009; Kreiner et al. 2019; Liu et al. 2012; Wu et al. 2018; Wu and Owen 2014; Yadid et al. 2021). Therefore, integrated weed management strategies targeting *A. tuberculatus* at different life-cycle stages are needed to successfully manage the resistant biotypes.

### ***Bassia scoparia* biology, ecology and herbicide resistance**

Kochia (*Bassia scoparia* [L.] A.J. Scott) is one of the most troublesome weed across the northern Great Plains of U.S. and Canadian Prairies (Kumar et al. 2019). *Bassia scoparia* is a summer annual broadleaf weed in Amaranthaceae family (formerly Chenopodiaceae), and native to central-eastern Europe and western Asia (Georgia 1914; Whitson et al. 1991). European immigrants first introduced *B. scoparia* to North America in the mid- to late-1800s as an ornamental plant (Friesen et al. 2009). It was grown in gardens because of its colorful appearance in autumn, but later escaped to prairies possibly by the tumbling mechanism of seed

dispersal (Georgia 1914). In late fall, a senesced *B. scoparia* plant breaks off at the base of the stem (near the soil surface) and tumbles (rolls) with the prevailing wind across landscapes. This aided *B. scoparia* in quickly invading and establishing in new land areas (Forcella 1985). During the period from 880 to 1980, *B. scoparia* was the fastest spreading alien weed species in the U.S. (Forcella 1985). Although *B. scoparia* is more problematic in the western region of the U.S. (Kumar et al. 2019), it has been reported throughout the U.S. except five states, namely Arkansas, Alabama, Georgia, Florida, and Hawaii (EDDMapS 2021). *Bassia scoparia* can be found in agricultural lands, rangelands, pastures, ruderal sites, gardens, roadsides, railroads, and ditchbanks (Frankton and Mulligan 1987; Whitson et al. 1991).

Genera *Kochia* and *Bassia* have been differentiated based on the morphology of the perianth; taxa with wings were named *Kochia*, whereas taxa with spines were named *Bassia* (Friesen et al. 2009). First suggested by Scott (1978) based on the existence of taxa with intermediate perianth morphology and later confirmed by Kadereit and Freitag (2011) using molecular phylogenies, *Kochia* was included into the genus *Bassia*. Aggregation of genera *Kochia* and *Bassia* removed most  $C_3$  species from the combined group, leaving primarily  $C_4$  species in the genus *Bassia* (Scott 1978; Wilson 1975). Currently, there are about 20 species in the genus *Bassia* (Kadereit and Freitag 2011). *Bassia scoparia* is a diploid species with chromosome number of  $2n = 18$ , and is self-compatible and primarily self-pollinated (Beckie et al. 2016; Lee et al. 2005). However, occurrence of protogynous flowers and some level of self-incompatibility allows *B. scoparia* to outcross, thereby ensuring a high level of genetic variability within and among populations (Mengistu and Messersmith 2002; Stallings et al. 1995). This genetic variation allows a population to sustain and evolve under changing environmental conditions and cropping practices (Mengistu and Messersmith 2002).

*B. scoparia* is one of the first species to emerge in cropping systems of the Northern Great Plains, and starts emerging in fields as soon as winter snow melts in the spring, typically in March and continues until early-summer, June (Dille et al. 2017; Eberlein and Fore 1984; Kumar et al. 2018). *Bassia scoparia* can germinate at soil moisture levels lower than that required for activation of some soil residual herbicides (Sebastian et al. 2017). Early emergence in the spring allows *B. scoparia* to utilize a limited soil moisture in arid regions of the Great Plains and provides a competitive advantage against crops (Dyer et al. 1993). Since *B. scoparia* has a  $C_4$  photosynthetic pathway (Chu and Sanderson 2008), it has a high water use efficiency. The plant has a taproot system, which can go deep as 4 m, and the foliage can grow up to 2 m in height and form a woody stem (Phillips and Launchbaugh 1958).

Sugar beet (*Beta vulgaris* L.) is the most susceptible crop to *B. scoparia* infestation in North America (Kumar et al. 2019; Mesbah et al. 1994; Mosqueda et al. 2020). This is partly because of concurrency in emergence timing of *B. scoparia* and planting of sugar beet, and initial slow growth and short-stature of sugar beet canopy. Mesbah et al. (1994) found that minimum *B. scoparia* density to reduce sugar beet root yield was 0.3 plants  $m^{-1}$  row of the crop, with a minimum duration of 3.5 weeks after sugar beet emergence. In that research, a season-long infestation of *B. scoparia* at 1.5 plants  $m^{-1}$  of crop-row intercepted 76% of the sunlight reaching the sugar beet canopy and reduced sugar beet root yield by 67%. *B. scoparia* reduced sunflower (*Helianthus annuus* L.) grain yield by 76% when both species emerged at the same time (Lewis and Gulden 2014). It also reduced soybean, sorghum, and maize (*Zea mays* L.) grain yields by 30%, 38%, and 40%, respectively (Waite et al. 2013; Wicks et al. 1993, 1994). Small-grain cereal crops (drill-seeded) such as oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) are good competitors with *B. scoparia*. A high density of *B.*

*scoparia* at 30 plants m<sup>-2</sup> reduced oat grain yield by only 31% (Manthey et al. 1996). Similarly, winter wheat grain yield was reduced by only 15% at a density of 4 plants m<sup>-2</sup> (Dahl et al. 1982), which would otherwise cause >67% yield reductions in sugar beet (Meshbah et al. 1994).

*Bassia scoparia* plant produces copious amount of viable seeds. Seed weight and number of seeds produced per plant depends on the interspecific and intraspecific competition (Becker 1978; Stallings et al. 1995). In a multi-location study in the north-western U.S., *B. scoparia* plants produced 24,000 seed plant<sup>-1</sup> when competing with sugar beet compared to only 1,500 or 1,000 seeds plant<sup>-1</sup> when competing with maize or spring wheat, respectively (Mosqueda et al. 2020). However, these numbers still pose a significant threat to the subsequent crops grown in a rotation; therefore, diversified management practices are required to prevent further spread of this most troublesome weed species of the U.S. Great Plains.

*Bassia scoparia* starts emerging much earlier than most other weed species in the U.S. Great Plains region (Schwingamer and Van Acker 2008). *Bassia scoparia* seeds are either non-dormant or exhibit very little dormancy (Dyer et al. 1993); hence, fully-matured seeds can potentially germinate as soon as the germination requirements are met. Because of these characteristics, seeds do not persist in soil for a longer period. More than 95% of seeds germinate in less than two years (Dille et al. 2017; Schwingamer and Van Acker 2008; Zorner et al. 1984). Viability of *B. scoparia* seeds declined by 20% within 6 months of burial in the soil and by >95% following 12 months of burial (Dille et al. 2017). *Bassia scoparia* emergence is higher at or near the soil surface and starts to decline as burial depth increases. Although seeds germinated at burial depths below 8 cm, seedlings did not reach to the soil surface, indicating a fatal germination (Schwingamer and Van Acker 2008). Anderson and Nielsen (1996) also found that *B. scoparia* emergence was four times greater in no-till compared to tilled systems.

Lack of seed dormancy and low temperature requirements for germination enable *B. scoparia* to emerge early in the spring season. For example, in a field observation at Manitoba province of Canada, Schwinghamer and Van Acker (2008) noted that *B. scoparia* accessions at 12 different sites emerged at 50 cumulative growing-degree days (GDD starting January 1, base temperature 0 C). Similarly, in a field observation in Colorado, *B. scoparia* initiated emergence when the daily minimum and maximum air-temperature was 2.2 and 17.2 C, respectively (Anderson and Nielsen 1996). Unlike many weed species, *B. scoparia* germination was not found to be affected by alternating vs. constant temperatures (Everitt et al. 1983). Similarly, light is not required for germination (Maguire and Overland 1959).

Although *B. scoparia* starts emerging primarily in early spring (March), it can continue to emerge until June in two to three major flushes (Dille et al. 2017; Kumar et al. 2018). It can still emerge, though in small proportions, as late as mid-August (Anderson and Nielsen 1996). In field observations by Schwinghamer and Van Acker (2008) in southern Canada, majority of *B. scoparia* seeds emerged by the end of May, a small percentage of seeds continued to emerge throughout the growing season. In a more recent multi-location study including 17 site-years across the Great Plains of the U.S., majority of *B. scoparia* emerged before May and little emerged after June (Dille et al. 2017). Furthermore, *B. scoparia* accessions from Kansas required 690 GDD to achieve 90% emergence, whereas Nebraska and Wyoming accessions required only 230 GDD. These differences in emergence patterns might be attributed to a substantial amount of genetic/phenotypic variation between the populations/ecotypes (Bell et al. 1972). Differential emergence patterns might also be attributed to differences in agronomic practices and herbicide use patterns across the region (Mengistu and Messersmith 2002).

Herbicides used to control *B. scoparia* influence not only the selection of HR individuals but also the adaptive traits, which benefits this species within a given environmental condition (Sbatella and Wilson 2010). For example, *B. scoparia* seeds collected from a field where isoxaflutole herbicide was used for eight years had a greater seed dormancy and delayed germination than seeds collected from adjacent fields, which never received isoxaflutole. In addition, those dormant seeds also required fluctuating 35/25 C day/night temperatures to break seed dormancy. These phenomena were not due to differences in sensitivity to isoxaflutole, instead due to the selection of cohorts that emerged later in the growing season when isoxaflutole had degraded, implying an ecological adaptation to selection pressure exerted by the herbicide (Sbatella and Wilson 2010).

Evolution of HR can also alter germination characteristics in resistant compared to susceptible populations. This may be due to physiological changes associated with the mechanism of resistance in the weed species. For instance, acetolactate synthase (ALS) inhibitor-resistant *B. scoparia* accessions from Montana germinated rapidly and at a higher percentage than the susceptible accessions at 4.6 C (Dyer et al. 1993). This early, rapid germination of the ALS-resistant accessions was attributed to a two-fold increase in the levels of branched chain amino acids in seeds from resistant compared to susceptible accessions. Similarly, ALS inhibitor-resistant *B. scoparia* accessions from Kansas and North Dakota had higher germination rate and total germination than susceptible accessions at 8 C (Thompson et al. 1994). These results indicate that ALS inhibitor-resistant populations will have competitive advantage over susceptible populations by emerging early in the spring. However, this trait can be exploited to reduce the R:S ratio of the seedbank by using early-season weed control practices.

Unlike *B. scoparia* populations resistant to ALS inhibitors, populations resistant to glyphosate or dicamba had lower germination rates and total germination than the susceptible populations (Beckie et al. 2018; Kumar and Jha 2016; Kumar and Jha 2017; Osipitan and Dille 2017). Kumar and Jha (2017) reported that four out of seven glyphosate-resistant populations from Montana had lower germination rate, delayed germination, and total germination compared with glyphosate-susceptible populations at 5 or 10 C. Osipitan and Dille (2017) reported similar results at 15 C. Beckie et al. (2018) observed similar patterns in two out of three glyphosate-resistant populations. Seeds of *B. scoparia* inbred lines resistant to dicamba and fluroxypyr had delayed onset of germination and lower total germination compared to susceptible populations at low temperatures (Kumar and Jha 2016). In addition, seeds of resistant lines were more dormant; hence likely to persist in soil for a longer period of time compared with those of susceptible lines (Kumar and Jha 2016). It is not clear whether these differential germination characteristics are due to physiological changes associated with the resistance mechanism or due to selection by long-term intensive cropping practices. However, Beckie et al. (2018), and Kumar and Jha (2017) suggested selection of resistance and avoidance (management practices) mechanisms might have contributed to these differences. Nonetheless, diversified multi-tactic practices are needed to control both early-emerging susceptible and late-emerging resistant *B. scoparia* populations.

Herbicides have been used as a primary tool to control *B. scoparia* the past several decades. There has been an increase in herbicide selection pressure especially with an increased adoption of no-till systems to control this species (Kumar et al. 2019). Heavy reliance on herbicides and unique characteristics of this species have resulted in widespread evolution of HR *B. scoparia* biotypes across the Great Plains region. Significant outcrossing, high genetic

variation, high seed production, tumble mechanism of dispersal, and short seed longevity are the key biological characteristics that have contributed to this rapid evolution of HR *B. scoparia* biotypes (Gressel and Segel 1978; Kumar et al. 2019). The first case of HR in *B. scoparia* was reported to atrazine in 1976 in Kansas (Bandeem et al. 1982). Currently, HR *B. scoparia* biotypes have been reported in 20 states of the U.S. (Heap 2021). *Bassia scoparia* populations resistant to four herbicide sites of action, including inhibitors of ALS, PS II, and EPSPS, and synthetic auxins was first identified from a chemical fallow field in 2012 in Kansas. Since then multiple-HR *B. scoparia* populations resistant to ALS and PS II inhibitors, ALS inhibitors and glyphosate, or glyphosate and synthetic auxins have been reported in several other states in the U.S. Great Plains (Kumar et al. 2019). Most importantly, the presence of *B. scoparia* populations resistant to ALS-inhibitors and glyphosate (two most widely used herbicides in sugar beet) has become a norm in production fields of the northern Great Plains (Heap 2021). Therefore, ecologically based diverse strategies should be used for the management of *B. scoparia*, specifically in crops grown in rotation with sugar beet since viable herbicide options are critically limited in sugar beet.

Knowledge of weed biology and ecology is a prerequisite for the development of sound integrated weed management (IWM) strategies. Differences in growth and development of *B. scoparia* and crops provide opportunities to incorporate nonchemical weed management strategies. Therefore, tactics such as tillage, crop rotation, competitive crops, cover crops, and harvest weed seed control, which can manipulate life history traits of *B. scoparia* should be utilized in favor of the crop. When combined together, these tactics can suppress *B. scoparia* to a greater extent, compared to when used alone (Anderson 2003).



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## CHAPTER 2. MULTI-TACTIC STRATEGIES TO MANAGE GLYPHOSATE-RESISTANT WATERHEMP (*AMARANTHUS TUBERCULATUS*) IN CORN-SOYBEAN ROTATIONS OF THE MIDWEST

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Modified from a manuscript to be submitted to *Weed Science*

### Abstract

In response to the mounting cases of multiple herbicide-resistant (MHR) waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) populations in the Midwestern United States, field experiments were conducted to design multi-tactic strategies to manage this weed. The study spanned over two years in a corn-soybean rotation during 2019 to 2020. Effect of three herbicide programs (HP) on *A. tuberculatus* seed inputs was tested in the 2019 corn. Whereas, the effect of previous year's percent control/ seed inputs, cereal rye cover crop, and narrow-row soybean on *A. tuberculatus* density, biomass, and seed production was tested in the 2020 soybean. A weed seed destructor was tested to prevent seed inputs by *A. tuberculatus* survivors/escapes in 2020 soybean. In corn, an HP consisting herbicides from two sites of action provided only 35% control of glyphosate-resistant (GR) *A. tuberculatus* compared with  $\geq 97\%$  control by an HP which included herbicides from three sites of action. In soybean, no new seed inputs (HP consisting three sites of action plus weed seed removal) from the previous year's corn crop reduced *A. tuberculatus* density and biomass by  $>30\%$  compared with seed inputs from the HP consisting two sites of action. Including a cereal rye cover crop or using narrow-row soybean reduced *A. tuberculatus* density by at least 13%, and biomass by 50% compared with no cover crop or wide-row soybean at 9 week after planting. Combination of either of these two non-

chemical control tactics reduced *A. tuberculatus* density and biomass by at least 40%. Combination of all three tactics (HP consisting three sites of action, cover crop, and narrow-row soybean) reduced *A. tuberculatus* density and biomass by at least 77% and seed production by 85%. The weed seed destructor physically destroyed 90% of *A. tuberculatus* seeds at soybean harvest. Finally, combination of all the four control tactics (spanned over two years) reduced *A. tuberculatus* seed inputs by 95% at the time of soybean harvest. These results indicate that diverse control tactics targeting *A. tuberculatus* at different life-cycle stages can successfully manage the MHR populations.

### Introduction

Waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) is a summer annual broadleaf weed species native to the Midwestern United States (U.S.) (Pammel 1913; Waselkov and Olsen 2014). It is one of the most common and troublesome weeds in corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) (Van Wychen 2017; Van Wychen 2019). The key characteristics which make this species troublesome in crops include an extended emergence period (Hartzler et al. 1999), high growth rate (Horak and Loughin 2000), phenotypic plasticity (Wu and Owen 2014), dioecy (Costea et al. 2005), and copious amount of seed production (Hartzler et al. 2004). It is very well adapted to no-till cropping systems, and can reduce corn and soybean grain yields by >40% (Hager et al. 2002; Steckel and Sprague 2004).

*Amaranthus tuberculatus* has developed resistance to seven different herbicide groups including synthetic auxins, and inhibitors of enolpyruval shikimate phosphate synthase (glyphosate), acetolactate synthase, photosystem II, protoporphyrinogen oxidase, 4-hydroxyphenylpyruvate dioxygenase, and very long chain fatty acid synthesis (Tranel 2020). Additionally, widespread occurrence of multiple herbicide-resistant (MHR) *A. tuberculatus* populations across the midwestern US states (Heap 2021) has seriously limited soybean growers'

ability to use postemergent (POST) herbicides to control this weed (Sarangi et al. 2019). Since *A. tuberculatus* has high propensity to evolve resistance against any single control tactic (Tranel 2020), herbicide rotation — commonly recommended strategy to counter herbicide resistance — will not work for this species (Wu et al. 2018). Therefore, ecologically based multi-tactic strategies are needed for sustainable management of this weed species.

Cover crops have been beneficial in preventing soil erosion by providing surface residue (Mohler and Teasdale 1993), trapping residual nitrate which otherwise would leach out in drainage (Kaspar et al. 2012), improving soil organic matter and physical properties (Moore et al. 2014), and suppressing weeds (Teasdale et al. 2007). Cereal rye (*Secale cereale* L.) is the most widely used cover crop in the Midwest US due to its winter hardiness, ease of establishment, and high biomass accumulation (Snapp and Surapur 2018; Teasdale 1996). Cereal rye suppresses weeds primarily through physically impeding emergence and growth (Teasdale and Mohler 1993) and also by inhibiting seed germination by release of allelochemicals (Teasdale et al. 2012). Cornelius and Bradley (2017) reported that a cereal rye cover crop reduced *A. tuberculatus* emergence by >35% when terminated two wk before soybean planting. However, the level of weed suppression by a cereal rye cover crop is directly correlated with cover crop biomass at the time of termination and weed emergence patterns (Mirsky et al. 2011; Mirsky et al. 2013).

Crop competitiveness is an important factor in determining the outcomes of crop-weed interference and can be improved by reducing the row spacing. Reducing the soybean row spacing helps the crop in achieving canopy closure quicker with a greater light interception (Board et al. 1992; Steckel and Sprague 2004). This crop-induced shading reduces weed dry matter accumulation (McLachlan et al. 1993), therefore improving the efficacy of other weed

control tactics. Biomass and density of late emerging *A. tuberculatus* plants were reduced by >40% in 19-cm-wide rows of soybean compared to 76-cm-wide rows (Steckel and Sprague 2004). Young et al. (2001) reported an improved weed control by reducing the soybean row spacing. In that study, a POST application of glyphosate at 630 g ha<sup>-1</sup> provided >90% control of *A. tuberculatus* in 19-cm-wide rows compared to <90% control in 76-cm-wide rows. Also, narrow-row soybean delays the critical time for weed removal (CTWR). CTWR occurred at third-trifoliolate stage of soybean in 19-cm-wide rows compared to first-trifoliolate stage in 76-cm-wide rows (Knezevic et al. 2003). In addition, narrow-row soybean can produce higher grain yields than wide-row soybean due to increased duration of light interception and total dry matter accumulation (Board et al. 1990).

Late-season escapes due to extended emergence periodicity, phenological plasticity, and control failures are the most common drivers for seedbank inputs of *A. tuberculatus*. Although late-emerged/escaped seedlings of *A. tuberculatus* rarely cause crop yield losses (Nordby and Hartzler 2004), they can still produce significant amount of seeds (Steckel et al. 2003). Therefore, minimizing seed production (especially, in a weed species like *A. tuberculatus* where herbicide-resistant (HR) populations are norms in the production fields) should be a high priority to slow the evolution of herbicide resistance (Neve et al. 2011) and reduce burden on management practices in succeeding crops (Cousens 1987; Hartzler and Roth 1993). Harvest Weed Seed Control (HWSC) methods such as weed seed destruction at harvest have been widely adopted to combat multiple herbicide resistance in Australian small-grain production systems. Walsh et al. (2012) reported >90% destruction of annual/rigid ryegrass (*Lolium rigidum* Gaudin) seeds contained in wheat (*Triticum aestivum* L) chaff using a stationary cage mill. This concept

has evolved over time and led to the commercial production of weed seed destruction units such as Integrated Harrington Seed Destructor, Seed Terminator, and Redekop™ Seed Control Unit.

The non-chemical control tactics (reviewed above) are not likely to provide an acceptable level of *A. tuberculatus* control when used alone. In addition, due to a high level of genetic variation among populations (Waselkov and Olsen 2014), this weed species can evolve resistance to any single control tactic heavily relied on. However, their cumulative effect would be higher than the individual effect, thereby delaying the resistance evolution (Anderson 2003; Liebman and Gallandt 1997). In addition, efficacy of these non-chemical tactics in a year/crop will most likely be influenced by the efficacy of weed control method used in the previous year/crop. Several researchers have reported improved efficacy of chemical or mechanical tactics in fields with a low compared to a high initial weed density (Buhler et al. 1992; Dieleman et al. 1999; Hartzler and Roth 1993; Khedir and Roeth 1981; Winkle et al. 1981). For example, premix of metolachlor and atrazine provided 95% control of giant foxtail (*Setaria faberi* Hermm.) in plots that had 100% control in the previous year compared with 75% control in plots that had 70% control in the previous year (Hartzler and Roth 1993). This highlights the importance of effectively managing weed seedbanks to prevent future infestations and improving the efficacy of weed control tactics in subsequent years.

Despite huge ecological significance of initial weed seedbank on the success of weed control practices (Buhler 1999a, 1999b; Mirsky et al. 2013), surprisingly, there is currently no published field study which has quantified the effect of prior year's weed control programs on the efficacy of current year's control tactics in managing *A. tuberculatus*. Information on the interaction of cereal rye cover crop and soybean row spacing on control of *A. tuberculatus* is also lacking. Therefore, the objectives of this research were to: (1) determine the impact of weed

control programs used in prior year's corn on the efficacy of cereal rye cover crop and soybean row spacing in managing *A. tuberculatus* in the following soybean crop, (2) evaluate the combined effect of cereal rye cover crop and soybean row spacing on the density, biomass, and seed production of *A. tuberculatus* in soybean, (3) evaluate the efficacy of weed seed destruction at-harvest to prevent seedbank replenishments from *A. tuberculatus* escapes/survivors in soybean.

## **Materials and Methods**

### **Experimental Site**

Field experiments were conducted during 2019 and 2020 at two sites; the Iowa State University Curtiss Farm in Ames, Iowa (42°01'N, 93°67'W) and Bruner Farm in Boone, Iowa (42°01'N, 93°74'W). The soil at Ames site was a mixture of Canisteo (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) and Clarion sandy clay loam (fine-loamy, mixed, superactive, mesic Typic Hapludolls) with 4.4% organic matter and a pH of 7.4. The soil at Bruner site was a mixture of Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Canisteo silty clay loam with 4.0% organic matter, and a pH of 6.9. Fields at both sites had been under soybean (2018)- corn (2017) rotation in the previous two years. In year 2018 and planted with soybean year prior to initiating experiments. Experimental sites had a known infestation of ALS-inhibitor and glyphosate-resistant (GR) *A. tuberculatus*. Records of average air temperatures and total precipitation during 2019 to 2020 growing seasons are summarized in Table 1.

### **Experimental Design**

This study spanned over two years, with corn grown in 2019 and soybean in 2020. A randomized strip-strip plot design was used with four replications. The study design included 12 treatments resulting from three factors. First factor consisted three levels of *A. tuberculatus*

control (30, 90, and 100%) in corn. Three different herbicide programs were used to achieve three levels of *A. tuberculatus* control. Second factor consisted cereal rye cover crop vs. no cover crop established following corn harvest. The third factor consisted wide rows of soybean (76-cm) vs. narrow rows of soybean (38-cm). The first factor was assigned to the main strips (9 m wide by 30 m long). The second factor was assigned to the sub strips (15 m wide by 27 m long) across the main strips. And, third factor was assigned to the sub-sub strips (4.5 m wide by 15 m long) along the main strips. Also, 1.5- and 12-m-wide alleyways between the main strips and replications, respectively were kept to allow field operations. An additional treatment (objective 3) was applied to the 100%-control-strip of corn in fall 2020 at the time of soybean harvest.

### **Field Operations**

Fields were tilled with chisel plow before start of the experiments in spring 2019. At Ames, a glyphosate- and glufosinate-resistant corn (Var. PO589AM; Pioneer<sup>®</sup>, Johnston, IA 50131) was planted on June 3, 2019. At Boone site, a glyphosate- and glufosinate-resistant corn (Var. PO157AM) was planted on June 4, 2019. Corn was planted in 76-cm-wide rows at 79,074 seeds ha<sup>-1</sup> using a John Deere 7100 MaxEmerge<sup>™</sup> planter (One John Deere Place Moline, IL 61265). The 30% control level of first factor was achieved by spraying one preemergent (PRE)-followed by (fb) one postemergent (POST)-herbicide (Table 2). The 90% control level was achieved by spraying two PRE- fb two POST-herbicides (Table 2). All the PRE herbicides were applied on the day of corn planting using an ATV-mounted CO<sub>2</sub>-pressurized boom sprayer equipped with Turbo TeeJet<sup>®</sup> Induction nozzles (TTI 110015VS; Spraying Systems Co., Wheaton, IL 60187). The sprayer was calibrated to deliver 140 L ha<sup>-1</sup> at 241 kPa. All the POST herbicides were applied at V5 to V6 growth stage of corn using a tractor-mounted compressed-



air boom sprayer equipped with TT 11002VS nozzles. The sprayer was calibrated to deliver 187 L ha<sup>-1</sup> at 207 kPa.

In current herbicide-led crop production practices of the Midwest US, it is rare to achieve 100% control of weed populations on a large scale (Bagavathiannan and Norsworthy 2012). Therefore, the 100% control level of first factor was, originally designed based on the assumptions that any seedbank inputs from late-season escapes in 90%-control-strip would be prevented by hand-weeding. Although there were some escapes in 90%-control-strip, they did not produce any seeds (Table 3). Therefore, the 100% control level (no seed input) was achieved without hand-weeding. However, to create a weed escape scenario, at the time of corn harvest, about 9000 seeds m<sup>-2</sup> (equivalent to 10% of the seeds produced in 30%-control-strip) were harvested from *A. tuberculatus* plants growing in adjacent-nonexperimental area and spread uniformly into the 90%-control-strip.

Corn was harvested on October 7, 2019 at Ames site and October 9, 2019 at Boone using a plot combine (John Deere 9450). Cereal rye cover crop was drill-seeded on October 17, 2019 at both sites in sub strips across the main strips. Cereal rye was seeded in corn stubble with a no-till drill (Marliss Industries, Jonesboro, AR 72401) in 19-cm-wide rows at 67 kg ha<sup>-1</sup>. The following spring, a glyphosate-, glufosinate-, and 2,4-D-resistant soybean (Var. S20-E3; NK<sup>®</sup> Seeds, Syngenta, Greensboro, NC 27419) was planted on May 22, 2020 at both sites. Soybean was planted in standing cereal rye (“planting green”) in 38- and 76-cm-wide rows at 322,470 seeds ha<sup>-1</sup> using a John Deere 7100 MaxEmerge™ planter. Soybean in 38- and 76-cm-wide row was planted in sub-sub strips along the main strips. On the same day, a broadcast application of glyphosate (1,261 g ae ha<sup>-1</sup>) plus S-metolachlor (1,788 g ai ha<sup>-1</sup>) was applied across the entire experimental area to terminate the cereal rye (at anthesis growth-stage) and to provide early-

season weed control. The herbicides were applied using a tractor-mounted compressed-air boom sprayer equipped with TT 11002VS nozzles and calibrated to deliver 187 L ha<sup>-1</sup> at 207 kPa. No other herbicide application was made to the plots.

Soybean from each sub-sub-plot in 30%- and 90%-control-strips was harvested on October 1, 2020 using the plot combine. Whereas, to prevent seedbank additions by escapes/survivors (objective 3), soybean from sub-plots in 100%-control-strip was harvested with a commercial combine (John Deere S680) equipped with Redekop™ Seed Control Unit/weed seed destructor (Redekop Manufacturing, RR#4 Hwy#16 West Saskatoon, SK Canada S7K 3J7).

### **Data Collection**

Effect of corn herbicide programs on *A. tuberculatus* in 2019 was assessed by measuring percent visual control, plant density, and seed production. Percent visual control was assessed on a scale of 0 to 100 (where, 0 means no control; 100 means complete control) at 4 wk after PRE (WAPRE) or 4 wk after planting (WAP) and 2 wk after POST (WAPOST) or 6 WAP. Plant density was counted from four 1-m<sup>2</sup> quadrats per main strip at 4 WAPRE, 2 WAPOST, and 9 WAPOST or 13 WAP. No new seedling emergence occurred after 9 WAPOST or 13 WAP. Seed production was quantified by carefully harvesting seed-producing-plants from four 1-m<sup>2</sup> quadrats in each main strip at the time of corn harvesting. Harvested plants were placed in DelNet pollination bags (DelStar Technologies Inc., Middletown, DE 19709) and air-dried for one wk. Seeds were threshed by rubbing the inflorescences between the hands, cleaned with hand-held sieves and with a seed blower (Seedburo® Equipment Company, Des Plaines, IL 60018), and weighed. Average seed weight was determined by weighing four sub-samples of 1000 seeds. Then, seeds m<sup>-2</sup> were calculated by dividing total sample weight with average seed weight. Once

finished, the seed samples were then returned to the sampling-quadrats before planting cereal rye cover crop.

The following spring, cereal rye cover crop growth was assessed by measuring plant-height of 10 random plants and collecting aboveground dry-biomass in two 1-linear-m rows from each main strip on the day of termination. Biomass samples from each strip were combined together, oven-dried at 60 C for 5 d, and weighed. Effect of cereal rye on soybean emergence was assessed by counting number of plants in four 1-linear-m rows from each sub-sub plot at 3 wk after planting (WAP). Effect of prior year's herbicide program, cereal rye, and soybean row spacing on *A. tuberculatus* growth and development in soybean was assessed by measuring seedling emergence, aboveground biomass, and seed production. Seedling emergence was counted from two 0.25-m<sup>2</sup> quadrats in each sub-sub-plot at two-week interval until no further emergence. A seedling considered emerged at full expansion of both cotyledons. Aboveground biomass was collected from two 0.25-m<sup>2</sup> quadrats at the time of soybean canopy-closure and harvest. Biomass samples from each sub-sub plot were combined together, oven-dried at 60 C for 5 d, and weighed. Seed production of *A. tuberculatus* in each sub-sub-plot of soybean was quantified using similar process described above for corn, except two 0.25-m<sup>2</sup> quadrats were harvested instead of four 1-m<sup>2</sup> quadrats. Soybean grain yields from each sub-sub-plot in 30%- and 90%-control-strips were recorded and adjusted to 13% moisture content. No grain yield was measured in 100%-control-strip due to insufficient plot length for a commercial combine.

Effect of Redekop™ Seed Control Unit/ weed seed destructor on the physical destruction of *A. tuberculatus* seeds was assessed by collecting threshed residue from back of the combine in four aluminum pans (38 cm by 24 cm) in each sub-plot. Pans were placed on the ground once the combine-header had passed, but before passing the threshed residue. Pans were kept there until

combine completed the whole pass. Pans were spaced at 2 m apart and put in a zig-zag pattern in each sub-plot. Pan samples from each sub-plot were combined together and cleaned (using process described above for corn). Small amount of physical-damage to weed seeds can drastically reduce their viability in the following seasons (Davis et al. 2008; Shergill et al. 2020). Therefore, seeds from each sample were visually inspected and divided into two groups: intact or slightly abraded seeds were considered potentially germinable, whereas highly abraded, ground, or sliced seeds were considered potentially non-germinable. Since it was difficult to accurately separate finely ground *A. tuberculatus* seeds from the finely ground soybean chaff, only seeds falling in the first category were used for further calculations. Number of seeds in the first category were counted using the 1000-seed-weight method described above for corn. Efficacy of Redekop™ weed seed destructor in preventing soil seedbank replenishment of *A. tuberculatus* was calculated using the following equation:

$$E = \frac{C-S}{C} \times 100$$

Where, E is the percent efficacy of weed seed destructor. And, C and S are the number of intact seeds in 100%-control-strip at sub-plot levels before and after using the weed seed destructor, respectively.

### **Statistical Analysis**

Normality of residuals and homogeneity of variance were tested using Shapiro-Wilk test and Levene's test, respectively and using diagnostic residual plots (Kozak and Piepho 2018). *Amaranthus tuberculatus* percent control, density, seed production, aboveground biomass, cereal rye plant height and aboveground biomass, and soybean grain yield were analyzed using PROC MIXED in SAS 9.4 software (SAS Institute Inc., Cary, NC 27513). For all response variables, experimental site and replication were considered random effects, whereas herbicide, cover crop,

soybean row spacing, and interaction effects were considered fixed effects in the model.

Appropriate degrees of freedom in the model were obtained by Satterthwaite approximation method (Satterthwaite 1946). Estimated means were compared using Tukey test at a significance level of  $\alpha = 0.05$ . Since a rescue treatment (objective 3) was imposed on the 100%-control-strip at sub-plot levels, new means consisting number of potential germinable seeds in each treatment were created. These new means were compared using the same method used for original seed production means.

## Results and Discussion

Site by treatment interactions for the response variables were not significant of the two years of the study (corn-soybean rotation); therefore, data from both sites were pooled. Monthly mean air temperatures during 2019 (corn) and 2020 (soybean) growing seasons at both sites ranged from 15 to 25 C (Table 1). Cumulative precipitation during the study period was 551 mm in 2019 and 346 mm in 2020 (Table 1).

Average plant height and aboveground dry-biomass of cereal rye across the treatments ranged from 97 to 99 cm and 5,080 to 5,440 kg ha<sup>-1</sup>, respectively. The amounts of biomass accumulated in this study are within the range of cereal rye cover crop biomass required to achieve consistent weed suppression (Bunchek et al. 2020; Mirsky et al. 2012; Wallace et al. 2017).

### *Amaranthus tuberculatus* Control in Corn

In corn (2019), S-metolachlor PRE provided 20% control of *A. tuberculatus* at 4 wk after PRE (WAPRE), whereas saflufenacil + pyroxasulfone PRE provided 80% control (Table 3). Similarly, *A. tuberculatus* density in S-metolachlor PRE plots averaged 250 plants m<sup>-2</sup> compared to 20 plants m<sup>-2</sup> in saflufenacil + pyroxasulfone PRE plots. A POST application of glyphosate in plots treated with S-metolachlor PRE further reduced *A. tuberculatus* density to 40 plants m<sup>-2</sup> at

2 WAPOST; however, visible control remained about 35% due to presence of GR *A. tuberculatus* plants in the field. In contrast, a POST application of glufosinate + S-metolachlor in plots treated with saflufenacil + pyroxasulfone PRE reduced *A. tuberculatus* density to 2 plants m<sup>-2</sup> and increased visible control to  $\geq 97\%$ . Furthermore, *A. tuberculatus* density in those plots did not increase significantly over the remaining growing season and none of the plants produced seeds. Whereas, in the S-metolachlor PRE fb glyphosate POST treatment, *A. tuberculatus* density increased to 70 plants m<sup>-2</sup> at 9 WAPOST, a 60% increase in density from 2 WAPOST. These plants produced up to 93,300 seeds m<sup>-2</sup> at the time of corn harvest.

S-metolachlor is one of the most commonly used PRE herbicides for *A. tuberculatus* control in corn, specifically in combination with atrazine (Sarangi and Jhala 2018). However, S-metolachlor alone may not provide satisfactory control of HR *A. tuberculatus* populations. Hausman et al. (2013) reported <20% control of 4-hydroxyphenylpyruvate dioxygenase (HPPD)-resistant *A. tuberculatus* at 4 WAPRE with S-metolachlor at 1,600 g ai ha<sup>-1</sup>. In addition, *A. tuberculatus* population resistant to S-metolachlor herbicide (very-long-chain fatty-acid inhibitor) has been reported (Strom et al. 2019). Therefore, PRE herbicides with multiple sites of action (SOA) should be used in a PRE fb POST (two-pass) program to achieve effective control of HR *A. tuberculatus*. For example, PRE application of pyroxasulfone 560 g ai ha<sup>-1</sup> alone provided only 63% control of HPPD-resistant *A. tuberculatus* at 6 WAPRE, whereas pyroxasulfone + saflufenacil + atrazine at 149 + 75 + 560 g ai ha<sup>-1</sup> provided 97% control (Oliveira et al. 2017).

### ***Amaranthus tuberculatus* Control in Soybean**

Density, biomass accumulation, and seed production of *A. tuberculatus* in soybean were influenced by percent control and end-season weed seed inputs during previous year's corn

(Table 4 and 5). Similarly, presence of cereal rye cover crop and/or reduced row spacing (38- vs. 76-cm-wide rows) of soybean influenced *A. tuberculatus* density, biomass accumulation, and seed production. There was a significant interaction between prior year's percent control and cereal rye cover crop for *A. tuberculatus* density, biomass accumulation, and seed production in soybean (Table 4). Similarly, interaction between prior year's percent control and soybean row spacing for *A. tuberculatus* density and biomass accumulation at 9 wk after planting (WAP) was significant. The three-way interaction between prior year's percent control, cover crop, and soybean row spacing was significant only for *A. tuberculatus* biomass accumulation and seed production at the time of soybean harvest. Since two-way and three-way interactions were significant, treatment means for each response variable were compared at sub-sub plot level (Table 5). Diversity of weed control tactics was lowest in the treatment which included herbicides from two sites of action in 2019 corn, received no cover crop in 2020 soybean, and soybean was planted in 76-cm-wide rows. This treatment produced had the highest density, aboveground biomass, and seed production of *A. tuberculatus*, and therefore, it was considered the least effective control treatment for comparison.

### ***Amaranthus tuberculatus* Density in Soybean**

All the three weed control tactics tested reduced the density of *A. tuberculatus* in 2020 soybean at 3 WAP and 9 WAP (Table 4 and 5). In the least effective control treatment, *A. tuberculatus* density averaged 380 plants m<sup>-2</sup> at 9 WAP in soybean (2020), 130% higher than the treatments where control in the previous year's corn (2019) was high level ( $\geq 90\%$ ). In the absence of high level control in 2019 corn and cover crop, planting soybean in 38-cm-wide rows instead of 76-cm-wide rows reduced *A. tuberculatus* density by 14% at 9 WAP. Similarly, in the absence of high level control in 2019 corn and narrow row planting, presence of the cereal rye

cover crop reduced *A. tuberculatus* density by 23% at 9 WAP (Table 5; Figure 1). Overall, inclusion of a single control tactic, either high level control in 2019 corn, a cereal cover crop, or narrow-row soybean, reduced *A. tuberculatus* density by at least 13% at 9 WAP compared with the least effective control treatment. Similarly, inclusion of either of those two control tactics reduced *A. tuberculatus* density by at least 40% at 9 WAP. Furthermore, combination of all three tactics reduced *A. tuberculatus* density by at least 77% at 9 WAP. Lowest density of *A. tuberculatus* in soybean occurred in plots with no seed inputs during previous year's corn (100% control), received cereal rye cover crop, and soybean was planted in narrow rows (38-cm-wide). This most diverse treatment reduced *A. tuberculatus* density by 85% compared with the least effective control treatment (Table 5).

Previous studies have shown that level of weed control achieved in the prior year can influence the weed control efficacy in the current year. For example, Hartzler and Roth (1993) reported that a premix of S-metolachlor and atrazine provided 88% control of smooth pigweed (*Amaranthus hybridus* L.) in plots that had received 100% control in the previous year compared to only 66% control in plots that had received 70% control in the previous year. Cereal rye cover crop or reduced row spacing of soybean has been effective in reducing *A. tuberculatus* density in soybean. For instance, in a field study conducted over eight site-years, cereal rye cover crop alone reduced late-season *A. tuberculatus* density by 40% compared to no cover crop treatment (Cornelius and Bradley 2017). Similarly, *A. tuberculatus* density was reduced by 30% when soybean was planted in 19- vs. 76-cm-wide rows (Steckel and Sprague 2004).

### ***Amaranthus tuberculatus* Biomass in Soybean**

Aboveground biomass accumulation of *A. tuberculatus* in soybean at 9 WAP and at the time of soybean harvest was reduced by all the three weed control tactics tested (Table 4 and 5).



In the least effective control treatment, *A. tuberculatus* accumulated 250 g m<sup>-2</sup> biomass at 9 WAP, a 60% increase from the treatments where percent control in 2019 corn was  $\geq 90$  (Table 5). In the absence of high level control during previous year and cover crop, planting soybean in 38-cm-wide rows instead of 76-cm-wide rows reduced *A. tuberculatus* biomass by 60 and 30% at 9 WAP and at the time of soybean harvest, respectively. Steckel and Sprague (2004) reported that planting soybean in narrow rows (19- vs. 76-cm-wide) reduced late-season *A. tuberculatus* biomass by >75%. Similarly, in the absence of high level control during previous year and soybean planted in narrow rows, presence of cover crop reduced *A. tuberculatus* biomass by >50% at 9 WAP (Table 5; Figure 1). Consistent with our results, William et al. (1998) reported that cereal rye cover crop reduced *A. tuberculatus* and *A. retroflexus* canopy volume by about 70% in soybean at 3 WAP.

Overall, inclusion of a single control tactic, either a high level control during previous year, a cover crop, or a planting of soybean in narrow (38-cm-wide) rows, reduced *A. tuberculatus* biomass by at least 24% at the time of soybean harvest compared with the least effective control treatment. Similarly, inclusion of either of two control tactics reduced *A. tuberculatus* biomass by at least 40% at the time of soybean harvest. Furthermore, combination of all three tactics reduced *A. tuberculatus* biomass by at least 80% at the time of soybean harvest. Lowest biomass accumulation of *A. tuberculatus* occurred in the treatment with no weed seed inputs during previous year's corn (100% control), received cereal rye cover crop, and soybean was planted in 38-cm-wide rows. This most diverse treatment reduced *A. tuberculatus* biomass by 95% at 9 WAP and by 80% at the time of soybean harvest, compared with the least effective control treatment (Table 5).

### ***Amaranthus tuberculatus* Seed Production in Soybean**

Similar to density and biomass, *A. tuberculatus* seed production at the time of soybean harvest was affected by all three weed control tactics tested (Table 4 and 5). In the least effective weed control treatment, *A. tuberculatus* produced 184,000 seeds m<sup>-2</sup>, a 30% increase from the treatments where percent control in 2019 corn was  $\geq 90$ . In the absence of high level control during previous year and cereal rye cover crop, soybean planted in 38-cm-wide rows instead of 76-cm-wide rows reduced *A. tuberculatus* seed production by 42% (Table 5; Figure 2). In a previous research, planting soybean in a reduced row spacing (19- vs. 76-cm-wide) decreased *A. tuberculatus* seed production by >30% (Steckel and Sprague 2004). Similarly, in the absence of high level control during previous year and narrow-row spacing in soybean, cereal rye cover crop alone reduced *A. tuberculatus* seed production by 83% (Table 5; Figure 2). These results are consistent with the previous findings by William et al. (1998) where the presence of a cereal rye cover crop reduced *A. tuberculatus* and *A. retroflexus* plant fitness by >80% in soybean. However, in plots with the highest level control during previous year (100%), presence of the cereal rye cover crop reduced seed production of *A. tuberculatus* by only 38%. This is likely due to a high biomass accumulation by *A. tuberculatus* plants in the plots at the time of soybean harvest (Table 5). Use of a single effective control tactic, either high level control in 2019 corn, a cereal cover crop, or narrow-row soybean reduced *A. tuberculatus* seed production by at least 23% compared with the least effective control treatment. Use of either of two control tactics reduced *A. tuberculatus* seed production by at least 58%; whereas, combination of all three tactics (most diverse) reduced *A. tuberculatus* seed production by at least 85%. The two non-chemical tactics, namely cereal rye cover crop and narrow row soybean were effective in

reducing the weed seed production by 85% regardless of the herbicide program used in previous year's corn (Table 5; Figure 2).

### ***Amaranthus tuberculatus* Seed Destruction at Soybean Harvest**

The Redekop™ Seed Control Unit (weed seed destructor) at soybean harvest was very effective in reducing the number of potentially germinable *A. tuberculatus* seeds entering the soil seedbank (Figure 2). Since weed seed destructor was attached to a commercial combine with a 9-m-wide header, sub-sub plots comprising narrow- and wide-row soybean within a sub-plot were harvested together. Therefore, efficacy of weed seed destructor was averaged across the soybean row spacing, and was evaluated only in the presence or absence of the cereal rye cover crop. In the absence of the cover crop, number of potentially germinable *A. tuberculatus* seeds added to the soil seedbank averaged 11,000 seeds m<sup>-2</sup>, 89% lower than when seed destructor was not used (Figure 2). Similarly, in cover crop plots, harvest weed seed destruction reduced the number of potentially germinable *A. tuberculatus* seeds added to the soil seedbank by 88% compared to when the seed destructor was not used. Although there is no published research from the U.S. on the efficacy of seed destructors in a combine-fitted system in soybean fields, these results support previous research conducted on stationary impact mills. For example, Shergill et al. (2020) reported that a stationary impact mill physically destroyed 96% of *A. tuberculatus* seeds that passed through the mill along with the soybean chaff. Schwartz-Lazaro et al. (2017) also reported a 98% physical destruction of *A. tuberculatus* seeds when passed through a stationary impact mill along with a rice chaff.

### **Soybean Grain Yield**

Presence of cereal rye cover crop (terminated at soybean planting) did not affect soybean stand count (Figure 1) or soybean grain yield (Table 5). Previous research has reported that

cereal rye cover crop provided weed suppression without reducing soybean grain yield (Bish et al. 2021; Bunchek et al. 2020; Hodgskiss et al. 2021). Among all factors tested, only soybean row spacing affected soybean grain yield ( $p = 0.03$ ). Planting soybean in 38-cm vs. 76-cm-wide rows increased soybean grain yield by 18% (Table 5). This was partly attributed to the reduced weed interference (Table 5) and increased light interception by the soybean canopy in narrow rows. For instance, soybean planted in narrow (38-cm-wide) rows achieved canopy closure by 9 WAP, approximately two wk earlier than the soybean planted in wide rows (data not shown). Previous studies suggest that reducing the soybean row spacing shortens the time required to achieve canopy closure, thereby enhancing light interception and weed suppression (Board et al. 1990; Légère and Schreiber 1989; Steckel and Sprague 2004).

### **Management Implications**

The results from this research indicate that diverse weed control tactics (“many little hammers”) are required to manage HR waterhemp seed banks in corn-soybean rotations. Late-season weed survivors/escapes may not affect crop yields negatively, but their seed inputs can augment the seedbank, which makes the seedbank more persistent and imposes a high strain on the weed control practices in the subsequent crop in the rotation (Cousens 1987; Dieleman et al. 1999; Hartzler and Roth 1993). This is more important when combating HR weed populations as a high seedbank density will put more burden on subsequent herbicides and accelerate the evolution of MHR weed populations (Neve et al. 2011). Corn and soybean canopy affect *A. tuberculatus* density, growth and seed production differently (Uscanga-Mortera et al. 2007) and may require different management practices. Therefore, control tactics targeting weed seed inputs such as an aggressive herbicide program (multiple sites of action) in corn and a weed seed destructor in soybean can be used to prevent seedbank additions of *A. tuberculatus* populations.

*Amaranthus tuberculatus* has an extended emergence period (Hartzler et al. 1999) and a single control tactic is not simply enough (Tranel 2020). The results of this research indicate that multi-tactics strategies targeting at multiple, life-stages of weed are required to effectively manage this species. This was accomplished by utilizing an effective multiple sites of action herbicide program, cereal rye cover crop, planting soybean in narrow rows, and a harvest weed seed control (weed seed destructor) strategy in the corn-soybean rotation. Cereal rye cover crop provided early-season *A. tuberculatus* control, which was mostly through physical suppression (Teasdale and Mohler 1993). Allelopathy might have played a role in early-season weed suppression (Teasdale et al. 2012), although not quantified in this study. Planting soybean in a reduced row spacing was also a complementary strategy to control late-emerging *A. tuberculatus* cohorts by shading late-emerging seedlings under the soybean canopy (Steckel and Sprague 2004). This was most likely attributed to a reductions in R:FR ratio under the soybean canopy in narrow vs. wide rows, also documented in *A. palmeri* (Jha and Norsworthy 2009). Nonetheless, weed seed inputs by late-season escapes are common in large-production fields despite use of excellent herbicide programs (Bagavathiannan and Norsworthy 2012). In a conventional system with a commercial combine, those weed seeds are also harvested with the crop, separated from the grains along with the crop chaff, and spread back to the field. Weed seed destructor attached to a commercial combine in this study destroyed *A. tuberculatus* seeds in the chaff with ~90% efficacy at the time of soybean harvest.

Finally, none of the control tactics tested provided satisfactory levels of *A. tuberculatus* control when used alone. However, their cumulative effect provided excellent (>95%) control of *A. tuberculatus*. This was most likely achieved by reducing the burden on subsequent control tactics. For example, a high weed seedbank density can decrease the quantity of soil-applied

herbicides absorbed by individual plants (Hoffman and Lavy 1978; Winkle et al. 1981), resulting in a reduced efficacy and a greater burden on a subsequent postemergence herbicide program. Similarly, a cereal rye cover crop alone would not provide a complete control of *A. tuberculatus*, but can potentially increase herbicide efficacy by reducing plant density and biomass of individuals (Myers et al. 2005; Wallace et al. 2019). Furthermore, combinations of these multi-tactics reduced *A. tuberculatus* density and size of plants surviving until crop harvest. This is also important as the high amount of green plant material entering the combine at soybean harvest can reduce the functionality of the HWSC method (personal observation). In conclusion, this study shows that how “many little hammers” spanned over two cropping seasons in a corn-soybean rotation can be used to successfully manage *A. tuberculatus* populations resistant to the “big hammer”, herbicide. Future research is needed to determine how these ecological tactics, namely cereal rye cover crop and narrow-row soybean can affect *A. tuberculatus* percent seed retention at the time of soybean harvest, a crucial factor for the success of HWSC methods.

### **Acknowledgments**

We thank the Department of Agronomy at Iowa State University and the Iowa Soybean Association for providing funding to support this research. We also appreciate the technical assistance provided by Damian Franzenburg, Itthiphonh Macvilay, James Lee, Trey Woodall, Avery Bennett, Edward Dearden, Ryan Hamberg, Alexis Meadows, and Austin Schleich in conducting the multi-site field studies.

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**Table 2.1.** Average air temperatures and total precipitation during 2019 and 2020 growing seasons at the Iowa State University Research Farms near Ames, IA and Boone, IA.

	Average temperature		Precipitation	
	2019	2020	2019	2020
	C		mm	
May	15	15	202	127
June	22	24	90	44
July	24	25	105	69
August	21	23	43	32
September	21	17	111	74
Total	—	—	551	346

**Table 2.2.** Herbicide programs for waterhemp (*Amaranthus tuberculatus*) control in corn (corn-soybean rotation) in 2019 at the Iowa State University Research Farms near Ames, IA and Boone, IA.<sup>a, b</sup>

Control program	Herbicide(s)	Rate	Timing	Trade name	Manufacturer
		g ai or ae ha <sup>-1</sup>			
30%-control	S-metolachlor fb glyphosate	1,788 fb 1,261	PRE fb POST	Dual II Magnum® fb Roundup PowerMAX®	Syngenta Crop Prot., Greensboro, NC 27419; Bayer Crop Science, St. Louis, MO 63167
90%-control <sup>c</sup>	Saflufenacil + pyroxasulfone fb glufosinate + S-metolachlor	50 + 91 fb 656 + 1,539	PRE fb POST	Sharpen® + Zidua® fb Liberty® + Dual II Magnum®	BASF Corp., Research Triangle Park, NC 27709; BASF Corp., Research Triangle Park, NC 27709; BASF Corp., Research Triangle Park, NC 27709; Syngenta Crop Prot., Greensboro, NC 27419
100%-control	Saflufenacil + pyroxasulfone fb glufosinate + S-metolachlor	50 + 91 fb 656 + 1,539	PRE fb POST	Sharpen® + Zidua® fb Liberty® + Dual II Magnum®	BASF Corp., Research Triangle Park, NC 27709; BASF Corp., Research Triangle Park, NC 27709; BASF Corp., Research Triangle Park, NC 27709; Syngenta Crop Prot., Greensboro, NC 27419
<sup>a</sup> Abbreviation: fb, followed by. <sup>b</sup> PRE herbicides were applied at corn planting. POST herbicides were applied at V5 to V6 growth stage of corn. <sup>c</sup> 90%-control represents late-season weed seed inputs by the survivors/escapes. This was achieved by manually spreading <i>A. tuberculatus</i> seeds (equivalent to 10% of the seeds produced in 30%-control) in to the plots at the time of corn harvest.					

**Table 2.3.** Waterhemp (*Amaranthus tuberculatus*) control, density, biomass, and seed production with different herbicide programs in corn (corn-soybean rotation) in 2019 at the Iowa State University Research Farms near Ames, IA and Boone, IA.<sup>a-c</sup>

Herbicide programs	Rate	Timing	Control				Density						Seed production
			4 WAPRE		2 WAPOST		4 WAPRE		2 WAPOST		9 WAPOST		At corn harvest
	g ai or ae ha <sup>-1</sup>		%				No. plants m <sup>-2</sup>						No. m <sup>-2</sup>
S-metolachlor fb glyphosate	1,788 fb 1,261	PRE fb POST	18	b	35	b	253	a	43	a	69	a	93,300
Saflufenacil + pyroxasulfone fb glufosinate + S-metolachlor	50 + 91 fb 656 + 1,539	PRE fb POST	81	a	97	a	20	b	2	b	5	b	0
Saflufenacil + pyroxasulfone fb glufosinate + S-metolachlor	50 + 91 fb 656 + 1,539	PRE fb POST	84	a	98	a	21	b	2	b	2	b	0

<sup>a</sup>Abbreviations: fb, followed by; WAPRE, wk after PRE; WAPOST, wk after POST  
<sup>b</sup>PRE herbicides were applied at corn planting. POST herbicides were applied at V5 to V6 growth stage of corn.  
<sup>c</sup>Treatment means within a column with same letters are not significantly different (Tukey test,  $\alpha = 0.05$ ).

**Table 2.4.** Test of fixed effects on waterhemp (*Amaranthus tuberculatus*) density, biomass, seed production, and grain yield in soybean (corn-soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA and Boone, IA.<sup>a</sup>

Fixed effects	<i>A. tuberculatus</i>					
	Density		Aboveground biomass		Seed production	Soybean grain yield
	3 WAP	9 WAP	9 WAP	At soybean harvest	At soybean harvest	
<i>p</i> -value						
Prior year's control <sup>b</sup>	<0.001	<0.001	<0.001	0.024	<0.001	0.237
Cover crop <sup>c</sup>	0.002	0.004	<0.001	<0.001	<0.001	0.931
Row spacing <sup>d</sup>	<0.001	<0.001	<0.001	<0.001	<0.001	0.029
Prior year's control × Cover crop <sup>e</sup>	<0.001	<0.001	<0.001	0.002	<0.001	0.24
Prior year's control × Row spacing <sup>e</sup>	<0.001	<0.001	<0.001	<0.398	<0.877	0.397
Cover crop × Row spacing <sup>e</sup>	0.015	0.082	0.006	0.075	<0.001	0.331
Prior year's control × Cover crop × Row spacing <sup>e</sup>	0.512	0.53	0.355	0.006	<0.001	0.921

<sup>a</sup>Abbreviations: WAP, wk after (soybean) planting.  
<sup>b</sup>First factor (three levels of weed control in previous year's corn [2019]: 30%, 90%, and 100%).  
<sup>c</sup>Second factor (cereal rye [*Secale cereale*] cover crop vs. no cover crop in soybean in 2020).  
<sup>d</sup>Third factor (76-cm-wide vs. 38-cm-wide rows of soybean in 2020).  
<sup>e</sup>Interaction effects between the factors.

**Table 2.5.** Effect of corn weed control programs, cereal rye (*Secale cereale*) cover crop, and soybean row spacing on waterhemp (*Amaranthus tuberculatus*) density, biomass, and seed production in soybean (corn-soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA and Boone, IA.<sup>a, b</sup>

<i>A. tuberculatus</i> control in corn	Cereal rye cover crop in soybean	Soybean row spacing	Density				Aboveground biomass				Seed production		Soybean grain yield <sup>c</sup>	
			3 WAP		9 WAP		9 WAP		At soybean harvest		At soybean harvest		At soybean harvest	
			No. plants m <sup>-2</sup>				g m <sup>-2</sup>				No. m <sup>-2</sup>		kg ha <sup>-1</sup>	
30%-control	No cover crop	76-cm rows	320	a	378	a	248	a	429	a	184,000	a	3,280	bc
		38-cm rows	288	b	327	b	97	c	295	bc	106,000	c	3,790	ab
	Cover crop	76-cm rows	238	c	292	c	122	c	157	e	32,000	g	3,280	bc
		38-cm rows	179	d	224	d	23	ef	81	f	26,000	gh	4,200	a
90%-control	No cover crop	76-cm rows	124	e	162	e	158	b	324	b	140,000	b	3,420	abc
		38-cm rows	121	e	154	e	97	c	194	de	55,000	ef	3,640	abc
	Cover crop	76-cm rows	80	f	113	f	45	de	96	f	36,000	fg	3,010	c
		38-cm rows	62	g	85	gh	17	ef	81	f	27,000	gh	3,570	abc
100%-control	No cover crop	76-cm rows	46	gh	106	fg	106	c	302	bc	120,000	bc	—	
		38-cm rows	46	gh	93	fgh	50	de	252	cd	77,000	d	—	
	Cover crop	76-cm rows	40	gh	68	hi	55	d	190	de	74,000	de	—	
		38-cm rows	31	i	54	i	11	f	80	f	25,000	gh	—	

<sup>a</sup>Abbreviations: WAP, wk after (soybean) planting.

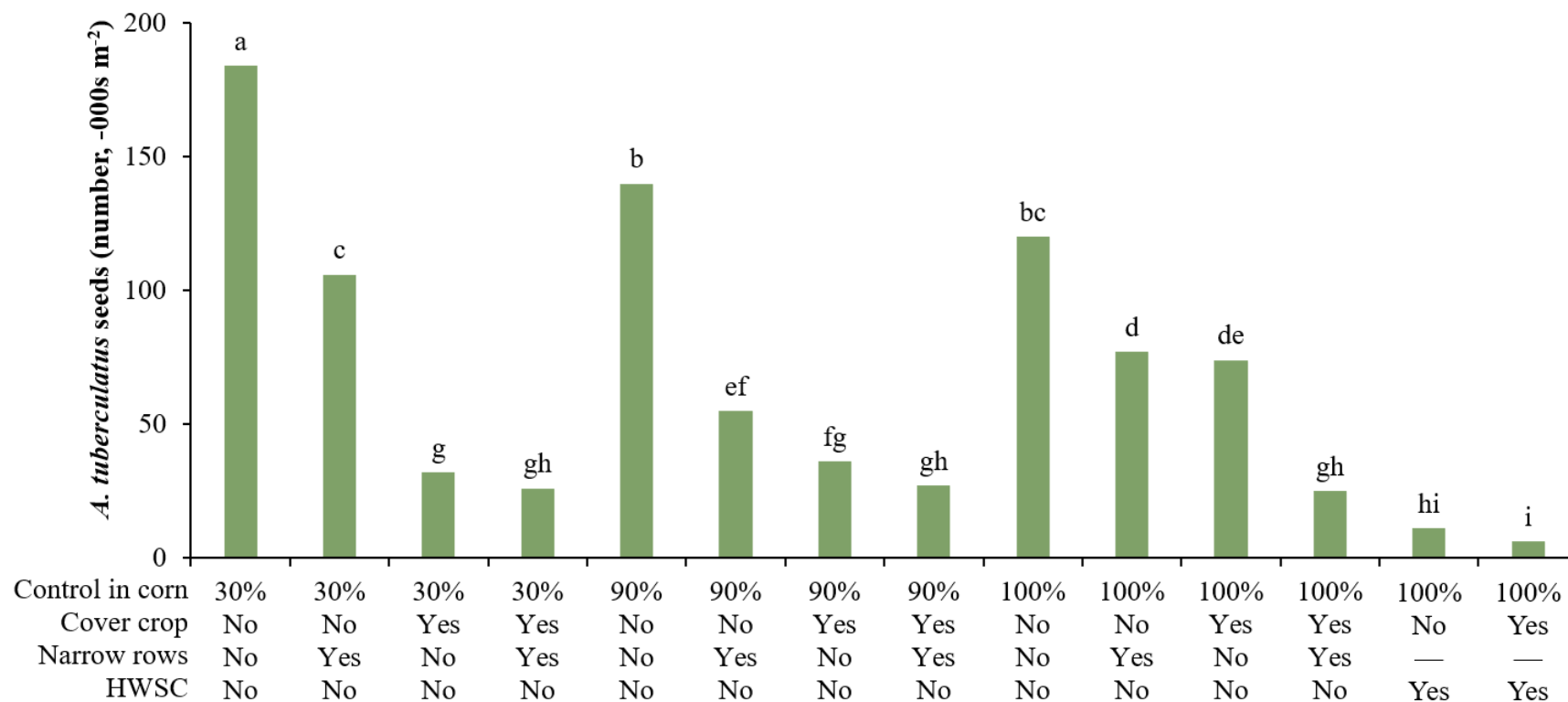
<sup>b</sup>Treatment means within a column with same letter(s) are not significantly different (Tukey test,  $\alpha = 0.05$ ).

<sup>c</sup>Dashes indicate that no data were collected for the treatments.





**Figure 2.1.** Effect of cereal rye (*Secale cereale*) cover crop on emergence and growth of waterhemp (*Amaranthus tuberculatus*) in soybean (corn-soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA and Boone, IA. No cereal rye (left) and cereal rye cover crop (right).



**Diversity of *A. tuberculatus* management practices in soybean**

**Figure 2.2.** Impact of multi-tactic weed management practices on waterhemp (*Amaranthus tuberculatus*) seed additions to the soil seedbank in soybean (corn-soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA and Boone, IA. The first row on x-axis indicates percent *A. tuberculatus* control in 2019 corn. The second row on x-axis indicates the presence (Yes) or absence (No) of a cereal rye (*Secale cereale*) cover crop in soybean in 2020. Similarly, third row indicates presence of 38-cm-wide rows of soybean (narrow rows; Yes) vs. 76-cm-wide rows of soybean (wide rows, No). Dashes indicate that data were averaged across the soybean row spacing. The fourth row indicates presence (Yes) or absence (No) of a weed seed destructor (a Harvest Weed Seed Control method) at the time of soybean harvest. Bars (indicating means) with same letter(s) are not significantly different (Tukey test,  $\alpha = 0.05$ ).

**CHAPTER 3. EFFECT OF WATER POTENTIAL AND TEMPERATURE ON THE GERMINATION CHARACTERISTICS OF KOCHIA (*BASSIA SCOPARIA*) ACCESSIONS ACROSS THE NORTHERN GREAT PLAINS OF U.S.**

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Modified from a manuscript to be submitted to *Weed Science*

**Abstract**

Development of integrated weed management (IWM) strategies requires knowledge of weed emergence timing and duration, which are primarily regulated by moisture and thermal requirements for seed germination. Laboratory experiments were conducted in fall 2017 to 2018 to quantify moisture and thermal requirements for germination of 44 kochia (*Bassia scoparia* [L.] A.J. Scott) accessions under controlled conditions. *Bassia scoparia* accessions were collected in fall of 2016 from crop fields of northern (Huntley, MT; Powell, WY) and southern (Lingle, WY; Scottsbluff, NE) regions in the northern Great Plains of US. Ten water potentials from 0 to -2.1 MPa and eight constant temperatures from 4 to 26 C were evaluated. Moisture requirements for germination of *B. scoparia* accessions did not differ between north and south region. At no water stress (0 MPa), all the *B. scoparia* accessions had >98% germination and achieved 50% of the maximum germination ( $t_{50}$ ) in <1 day. At -1.6 MPa, a water stress higher than permanent wilting point for most plants, 25% seeds of all *B. scoparia* accessions still germinated. It required a water stress of -0.85 MPa and -1.9 MPa to reduce *B. scoparia* germination by 10 and 90%, respectively. In the temperature experiment, all tested *B. scoparia* accessions had >88% germination. Germination rate was the highest at temperatures between 14 to 26 C for all the accessions and did not differ among accessions from north and south region.

At this range, all the accessions had a  $t_{50}$  of <1 day. However, at 4 C, *B. scoparia* accessions from north region had higher germination rate and cumulative germination than accessions from south region. Overall, these results indicate that rapid germination of *B. scoparia* at a wide range of moisture and temperatures can possibly be exploited using methods such as stale seedbed approach prior to crop planting.

### **Introduction**

*Kochia* (*Bassia scoparia* [L.] A.J. Scott) is a summer annual, broadleaf weed in Amaranthaceae family (formerly Chenopodiaceae), and native to central-eastern Europe and western Asia (Georgia 1914; Whitson et al. 1991). *Bassia scoparia* is the most problematic weed in arid and semiarid regions of the North American Great Plains (Kumar et al. 2019). Several unique biological characteristics such as low temperature and soil moisture requirements for germination, significant outcrossing, high genetic variation, high seed production, tumble mechanism of seed dispersal, and short seed longevity helped this species in becoming the most problematic weed of the U.S. Great Plains (Gressel and Segel 1978; Kumar et al. 2019). Low temperature and soil moisture requirements for germination are the most important characteristics which allow *B. scoparia* to easily compete with spring-planted crops and other weed species of the region.

*Bassia scoparia* is often the first species to emerge in the spring due to the lack of seed dormancy and low-temperature requirements for germination (Dyer et al. 1993; Schwinghamer and Van Acker 2008). *Bassia scoparia* seeds are either non-dormant or exhibit very little (<5%) dormancy (Dyer et al. 1993), therefore mature seeds germinate as soon as the germination requirements are met. Although it has low temperature requirements for germination, *B. scoparia* seeds can germinate over a wide range of temperatures from 3.5 to 40 C (Eberlein and Fore

1984). Also, alternate vs. constant temperature regimes do not affect *B. scoparia*'s temperature requirements for germination.

*Bassia scoparia* can germinate at soil moisture levels where other species fail to germinate (Everitt et al. 1983) or even certain herbicides do not activate (Sebastian et al. 2017). This makes *B. scoparia* even more competitive in dry environments. Although soils are sufficiently moist in spring, moisture can become a limiting factor for the germination and subsequent emergence, and growth of crop or weeds later in the season. Therefore, early emergence in the spring enables *B. scoparia* to acquire limited soil moisture in the arid regions and provides a competition advantages over crops and other weed species (Dyer et al. 1993). Furthermore, *B. scoparia* is a highly water use efficient plant as it has a C<sub>4</sub> photosynthetic pathway (Chu and Sanderson 2008). Since *B. scoparia* can germinate at soil moisture levels lower than the moisture required for soil-applied preemergence (PRE) herbicides activation (Sebastian et al. 2017), many PRE herbicides do not provide consistent control of this species in arid regions.

Competitive dominance of weeds in crops is largely determined by their relative time of emergence (Cousens et al. 1987), which is primarily regulated by soil temperature and water potential (Bradford 2002). In addition, timing of weed control practices and weed emergence should match to obtain the full potential of those weed control practices (Ogg and Dawson 1984). Therefore, improved knowledge of temperature and moisture requirements for *B. scoparia* seed germination is required to precisely predict its timing and duration of emergence in a given region. Variable germination requirements and emergence patterns for *B. scoparia* have been reported in different geographical regions (Anderson and Nielsen 1996; Dille et al. 2017; Kumar et al. 2018a; Schwinghamer and Van Acker 2008). For instance, Dille et al. (2017)

reported that *B. scoparia* populations from Kansas required 690 growing-degree days (GDD) to achieve 90% emergence, compared to only 230 GDD by Nebraska and Wyoming populations. Similarly, it required 1500 GDD to achieve 90% emergence in a tilled environment compared to 2400 GDD in no-till environment. In that same study, it was also reported that majority of *B. scoparia* populations emerged before May and little emerged after June. In contrast, Anderson and Nielsen (1996), and Schwinghamer and Van Acker (2008) reported that *B. scoparia* populations exhibit an extended period of emergence as late as mid-August. Kumar et al. (2018a) also observed differential emergence pattern of *B. scoparia* populations collected from the U.S. Great Plains in no-till dryland environment of Montana, further suggesting the presence of different emergence “biotypes” among field populations of *B. scoparia*. These differences in germination requirements or emergence patterns are not unusual as there is a substantial amount of genetic/phenotypic variations present among *B. scoparia* populations (Bell et al. 1972; Mengistu and Messersmith 2002). Also, cropping systems and crop production practices can also influence the life-history traits of a weed species (Cousens and Mortimer 1995; Sbatella and Wilson 2010).

Although some studies have been conducted to quantify *B. scoparia*'s thermal requirements for germination (Kumar and Jha 2017; Kumar et al. 2018b), it is unclear whether observed differences in *B. scoparia* emergence patterns across the geographical sites are due to differential thermal requirements or differential moisture requirements among the populations. Additionally, information on germination requirements collected across a wide geographical area can help in developing robust models to predict weed emergence patterns precisely (Myers et al. 2004). Therefore, the objectives of this study were 1) to quantify the temperature and moisture requirements of *B. scoparia* accessions collected from 44 cropland field locations across the

northern Great Plains of U.S. (Montana, Wyoming, and Nebraska), and 2) to compare thermal or moisture requirements for germination of *B. scoparia* accessions between northern and southern parts of the three-state region.

## **Materials and Methods**

### **Seed Collection**

Fully matured seeds of *B. scoparia* plants growing in crop fields were collected in fall of 2016 from four sites representing three states of the northern Great Plains of U.S. These field sites included Huntley in Montana, Powell and Lingle in Wyoming, and Scottsbluff in Nebraska (Figure 1). Each site included 11 different field locations (approx. 10 km apart from each other). Therefore, total number of *B. scoparia* seed samples were 44 and considered as 44 separate accessions. To quantify the effect of geographical regions on the germination requirements, accessions were divided in two groups, north and south region. Based on the locations of the sites in the region, Huntley and Powell sites were included in the north region, whereas Lingle and Scottsbluff sites were included in the south region. All collected *B. scoparia* seed samples were dried for 4 wk at room temperature, hand threshed, and then cleaned using small mesh-sieves. Cleaned seed samples were stored in paper bags at 4 C until used for the germination experiments.

### **Water Potential Experiment**

Laboratory experiments were conducted at the Montana State University Southern Agricultural Research Center (MSU SARC), Huntley, MT in fall 2017 to quantify water potential requirements for germination of *B. scoparia* accessions. Ten water potential ( $\Psi$ ) treatments ranging from 0 to -2.1 MPa were created by using polyethylene glycol (PEG 8000; Fisher Scientific Company, One Reagent Lane, Fair Lawn, NJ 07410) using the methods described by Michel (1983). Ten  $\Psi$  treatments included, 0, -0.1, -0.3, -0.5, -0.7, -0.9, -1.2, -1.6, -

1.8, and -2.1 MPa. Each treatment was replicated three times. For each experimental unit, 50 seeds from each accession were counted and placed between two layers of filter papers (Whatman<sup>®</sup> Grade 2, Sigma-Aldrich, Inc., St. Louis, MO 68178) in a 10-cm-diameter petri dish (Fisher Scientific Company). Each petri dish was moistened with 7 ml of PEG solution, except 0  $\Psi$  treatment which was watered with 7 ml of distilled water. Petri dishes were sealed with a thermoplastic wrapper (Parafilm<sup>™</sup> M; Fisher Scientific Company) to prevent water loss through evaporation. Since light is not required for *B. scoparia* seed germination (Everitt et al. 1983), petri dishes were placed in dark in growth chambers (VWR<sup>®</sup> Signature<sup>™</sup>; VWR, 100 Matsonford Road, Radnor, PA 19087) set to a constant temperature of 20 C. The 20 C temperature was selected as PEG solution was prepared for this temperature and it is optimum temperature for the germination of *B. scoparia* seeds (Eberlein and Fore 1984; Everitt et al. 1983; Kumar and Jha 2017). Inside the growth chambers, petri dishes were arranged in a randomized complete block design.

### **Temperature Experiment**

Temperature requirements for germination of *B. scoparia* accessions were quantified in the laboratory experiments conducted at the MSU SARC, Huntley, MT in fall 2018. Eight constant temperature treatments ranging from 4 to 26 C were used. Eight temperature treatments included, 4, 8, 11.5, 14.5, 18, 21, 24, and 26 C. Temperature treatments were created by assigning each treatment a separate growth chamber (VWR<sup>®</sup> Signature<sup>™</sup>) set at the required temperature. Petri dishes were prepared and maintained similarly as explained for the water potential experiment. Each petri dish was moistened with 7 ml of distilled water.



## Data Collection and Statistical Analysis

*Bassia scoparia* seed germination was observed on daily basis for two wk. Germinated seeds were counted and removed from the petri dishes at each observation time. A seed was considered germinated when the tip of protruding radicle uncoiled (Dyer et al. 1993; Young et al. 1981). *Bassia scoparia* germination data from each experiment were analyzed in the statistical environment R (R Core Team 2019) using an R extension package “drc” (Ritz et al. 2019). First, data from each observation period were arranged to an event-time format (Ritz et al. 2013). Then, a three-parameter log-logistic model was fitted using the following equation (Equation 1; Ritz et al. 2013) to extract the parameter estimates, cumulative germination curves, and standard errors:

$$F(t) = \frac{d}{1 + \exp\{b[\log(t) - \log(t_{50})]\}} \quad [1]$$

Where  $F(t)$  denotes the proportion of seeds germinated between time 0 (start of the experiment) and time  $t$ . Parameter  $d$  denotes the upper limit (maximum germination occurred at the end of observation period). Parameter  $t_{50}$  denotes the time taken by 50% of the total seeds (upper limit,  $d$ ) in the test to germinate. Lastly, parameter  $b$  denotes the slope of germination curve at time  $t_{50}$ . In the water potential experiment, overall seed germination from all the accessions decreased to <30% at  $\Psi$  of -1.6 and -1.8 MPa, and ceased completely at -2.1 MPa. The lower germination proportions at -1.6 and -1.8 MPa did not allow model fit and parameter estimations. Therefore, only seven  $\Psi$  treatments ranging from 0, to -1.2 MPa were used to fit the model and generate germination curves. However, in the temperature experiment, *B. scoparia* seeds germinated in high proportions at all the temperature levels, and therefore all of the eight treatments were used to fit the model and generate germination curves. Accuracy of model fit was tested using the lack of fit test in “drc” package (Knezevic et al. 2007).

Additionally, a second three-parameter log-logistic model was fitted using the following equation (Equation 2; Knezevic et al. 2007) to quantify germination response of *B. scoparia* accession to the increasing levels of water stress (lower water potential):

$$y = \frac{d}{1 + \exp\{b[\log x - \log e]\}} \quad [2]$$

Where  $y$  denotes the percent reduction in germination (relative to no water stress;  $\Psi$  of 0 MPa) and  $x$  denotes the level of water stress or water potential. Parameter  $d$  denotes the upper limit. Parameter  $e$  denotes the  $\Psi_{50}$  (water stress required to reduce the germination by 50%). And, parameter  $b$  denotes the relative slope around  $\Psi_{50}$ . Values of  $\Psi_{10}$  and  $\Psi_{90}$  were calculated using the ED function of the “drc” package.

To compare northern *B. scoparia* accessions to southern accessions for the germination requirements, a two-step procedure described by Jensen et al. (2017) was used. In the first step, parameters of interest—  $t_{50}$  and duration of germination ( $t_{95}-t_5$ ) —were obtained for each accession using the equation 1. Then, in the second step, these parameters were analyzed using a mixed-effects model in the lmer function of “lme4” package of *R* (Bates et al. 2015). In the model, accessions were considered as random effects, whereas treatments and regions were considered as fixed effects. Results were visualized in graphs using “ggplot2” package.

## Results and Discussion

### Effect of Water Potential

Response of *B. scoparia* accessions from four sites across the three-state region to the different levels of water potential ( $\Psi$ ) or water stress over time is presented in Table 1 and Figure 2. Accessions from all the four sites germinated in high proportions (>60%) at  $\Psi$  of -1.2 MPa or higher. At no water stress (0 MPa), almost all of the *B. scoparia* seeds (>98%) germinated from each site. This is not surprising as *B. scoparia* exhibits rapid and high levels of germination when

optimum conditions are met. Dyer et al. (1993) and Thompson et al. (1994) have previously reported >95% germination of *B. scoparia* in <3 days at no water stress. At  $\Psi$  of -0.9 MPa or higher, all the *B. scoparia* accessions achieved 50% of the maximum germination in two days. Similarly, at  $\Psi$  of -1.2 MPa, accessions took four to eight days to achieve the 50% germination; where Powell accessions took the shortest time (4 days) and Scottsbluff took the longest time (8 days). Also, the Powell accessions achieved 15% higher germination than Scottsbluff accessions at  $\Psi$  of -1.2 MPa.

Parameter estimates for the effect of water potential ( $\Psi$ ) or water stress on the maximum germination of *B. scoparia* accessions from each of the four sites are presented in Table 2 and Figure 3. A proportion of *B. scoparia* seeds from each site was able to germinate at the range of water potentials from 0 (no stress) to -1.8 MPa (high level of stress). However, increases in the water stress (more negative  $\Psi$ ) significantly reduced *B. scoparia* germination rate (Figure 2) and total germination (Figure 3) for all the accessions. Total germination of *B. scoparia* accessions from all the sites started declining rapidly at  $\Psi$  of -1.5 MPa and declined to <30% at -1.8 MPa (Figure 3). It required  $\Psi$  of -0.85 MPa or lower to reduce total germination of *B. scoparia* accessions by 10%. These results are in accordance with Everitt et al. (1983), who previously reported that *B. scoparia* germination did not decline until osmotic potential of the solution reached -0.8 MPa. In the current experiment, it required  $\Psi$  of -1.9 MPa or lower to reduce *B. scoparia* germination by 90%. Among the sites, Scottsbluff accessions were more sensitive to the water stress ( $\Psi_{50}$  of -1.30 MPa) than the Powell accessions ( $\Psi_{50}$  of -1.45 MPa). However, no differences were observed between these two sites for  $\Psi_{90}$  values.

Results of the mixed-effects analysis to compare germination patterns of *B. scoparia* accessions across the north-south transect in response to  $\Psi$  are presented in Figure 4. Time to

achieve 50% of the maximum germination increased with decreasing  $\Psi$ , but did not differ between *B. scoparia* accessions from north to south region. Similarly, the duration of germination ( $t_{95}-t_5$ ) increased with decreasing  $\Psi$ , but no differences were observed between *B. scoparia* accessions from north and south region. On an average, accessions from north and south region completed germination in 25 days at  $\Psi$  of -1.2 MPa. Effects of individual accessions within a region had more variation for the rate and duration of germination than between the regions. Mengistu and Messersmith (2002) previously reported higher levels of genetic diversity within a *B. scoparia* population than between the populations. Our results indicate that differential emergence patterns of *B. scoparia* accessions previously observed between the regions (Anderson and Nielsen 1996; Dille et al. 2017; Kumar et al. 2018a; Schwinghamer and Van Acker 2008) may not be explained by differential moisture requirements of *B. scoparia* accessions. Instead, it is possible that these differences are the results of differential temperature requirements among *B. scoparia* populations (Kumar et al. 2018b) or population shifts led by intensive selection pressure of local cropping practices (Beckie et al. 2018; Sbatella and Wilson 2010; Sebastian et al. 2017).

### **Effect of Temperature**

Effect of temperature on the germination characteristics of *B. scoparia* accessions from four sites across the three-state region is presented in Table 3 and Figure 5. Accessions from the four sites germinated in high proportions (>88%) across the temperature range tested. Germination rate ( $1/t_{50}$ ) was lowest at 4 C for all the accessions. However, they achieved 50% of the maximum germination in 3 days at this temperature. This indicates that temperatures above 4 C are not likely to have a large effect on the germination rate and total germination of *B. scoparia* seeds. At 14 C or above, all the accessions achieved 50% germination in <1 d.

Therefore, germination rate was highest at temperatures between 14 to 26 C for all the accessions, indicating a wide-range of optimum temperature for *B. scoparia*. At this temperature range, all the accessions had a total germination of >90%. Overall, Powell accessions had significantly higher total germination (>98%) than accessions from other sites. Dyer et al. (1993) reported >99% total germination by *B. scoparia* at 17 C in a 2-d period.

Results of the mixed-effects analysis to compare germination patterns of *B. scoparia* accessions across the north-south transect in response to temperature are presented in Figure 6. Time taken to achieve 50% of the maximum germination by *B. scoparia* accessions decreased with increasing temperature, but did not differ between accessions from north to south region at temperatures of 8 C or above. Similarly, the duration of germination ( $t_{95}-t_5$ ) decreased slightly with increasing temperature above 4 C, but did not differ between accessions from north to south region. Regardless of the temperature treatments, *B. scoparia* accessions from both the regions completed their germination process in <10 d. This indicates that *B. scoparia* seeds can rapidly accumulate heat units required for germination. It has been reported that *B. scoparia* can initiate emergence immediately when minimum soil-temperature rises above 3 C (Nussbaum et al. 1985). Therefore, temperature may not be a limiting factor for the germination duration of *B. scoparia* once it rises above 4 C in a region (Everitt et al. 1983).

Regional differences in emergence patterns of *B. scoparia* populations are likely to arise at low temperatures. For example, at 4 C, accessions from north region took five hours less to achieve 50% germination than accessions from south region (Table 4; Figure 7). Although this is not a large difference, it is likely to increase with further reductions in temperature and requires further research. Dille et al. (2017) previously reported that naturally occurring *B. scoparia* populations in Wyoming and Nebraska required 80 GDD less to initiate emergence than

populations in Kansas. These differences in thermal requirements among *B. scoparia* populations could be attributed to differential seed physiology as a result of cropping practices. For example, Dyer et al. (1993) found that rapid germination in ALS-resistant *B. scoparia* accessions compared to the susceptible accessions was due to the presence of high levels of branched-chain amino acids in resistant-accessions, which was caused by the mutations conferring resistance. In contrast, Beckie et al. (2018) reported that two out of three glyphosate-resistant *B. scoparia* populations (collected from different regions) showed delayed and lower germination than susceptible populations, indicating an avoidance mechanism rather than a pleiotropic effect of the resistance. Similarly, Sbatella and Wilson (2010) reported that continuous use of isoxaflutole in an experimental plot in corn (*Zea mays* L.) resulted in delayed *B. scoparia* emergence compared to the adjacent fields which were never exposed to isoxaflutole. Both populations showed similar sensitivity to isoxaflutole, however *B. scoparia* seeds from the experimental plot showed a high level of dormancy compared to the seeds from adjacent fields, which accounted for the delayed emergence. These results indicate that *B. scoparia* is likely to adapt to the cropping practices and exhibit differential emergence patterns.

### **Management Implications**

The results of these experiments indicate a wide optimum-range of temperatures and moisture levels for *B. scoparia* germination. *Bassia scoparia* ability to germinate in high proportions in a short period of time at low temperatures reinforces its competitive advantage over other weed species. For example, *B. scoparia* achieved 80% emergence at the time (Schwinghamer and Van Acker 2008) when other weed species common to the northern Great Plains started emerging (Bullied et al. 2003). In general, the range of optimum temperature and moisture was consistent across the geographical regions. However, at low temperatures, *B.*

*scoparia* from south region had lower germination than *B. scoparia* from north region, indicating need of site-specific management practices.

In water potential experiment, one fourth of *B. scoparia* seeds germinated at moisture levels lower than the permanent wilting point (-1.6 MPa) of most plants. This indicates that *B. scoparia* can germinate at  $\Psi$  levels where most weeds or crop species are not able to germinate (Guillemain et al. 2013; Hoveland and Buchanan 1973). For example, downy brome (*Bromus tectorum* L.), a problematic weed in the US Great Plains (Stougaard et al. 2004; Thill et al. 1984), did not germinate when  $\Psi$  dropped below -1.5 MPa (Thill et al. 1979). Similarly, wheat (*Triticum aestivum* L.), a major crop in the region, did not germinate at  $\Psi$  of -1.5 MPa (Singh et al. 2013). *Bassia scoparia* ability to germinate at such low moisture conditions reduces interspecific competition (Bullied et al. 2003) and sensitivity to soil-applied herbicides (Sebastian et al. 2017), making this species highly likely to thrive in dry-sites (Teasdale et al. 2003). As a result, it can lead to competitive displacement of other weed species (Pickett and Bazzaz 1976) and/or *B. scoparia* population shifts (Sebastian et al. 2017). Therefore, *B. scoparia* can become even more difficult to manage in future with the trends of frequent droughts in the northern Great Plains of US (Wienhold et al. 2018).

One of the practical ways to deplete soil seedbanks of a troublesome weed species is to identify and manipulate the environmental factors which control their germination and emergence (Schonbeck and Eglely 1980). Models have been used to predict weed emergence in a specific region or across the regions. However, these models often rely solely on GDD to predict weed emergence (Myers et al. 2004). Use of hydrothermal time, which includes both soil temperature and moisture parameters can improve the accuracy of emergence prediction (Bradford 2002; Forcella 1998; King and Oliver 1994). Therefore, parameter values generated

from this study can be used to develop *B. scoparia* emergence model and predict its emergence patterns across the three-state region using historical climate data. Knowing the time of *B. scoparia* emergence in a particular geographical site can be used to modify its cropping practices. For example, seedbanks of early-emerging populations can be exhausted using a stale seedbed approach prior to the planting of main crop. Similarly, cohorts of late emerging populations can be suppressed using competitive crops planted at early dates. Moreover, germination characteristics quantified in this study can be used in secondary models to evaluate risks of different cropping systems over a wide geographical area (Colbach et al. 2006; Gardarin et al. 2010). In conclusion, these experiments show that *B. scoparia* possesses unique germination characteristics, which should be exploited using ecologically-based multi-tactic strategies to control this species.

### Acknowledgments

We thank the Montana State University Southern Agricultural Research Center, Huntley, MT for providing resources and support to the graduate student to conduct this study. We also thank the USDA National Institute of Food and Agriculture for providing funding to conduct this research (project accession no. 1010972).

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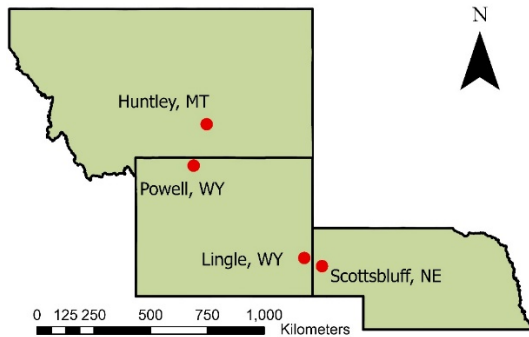


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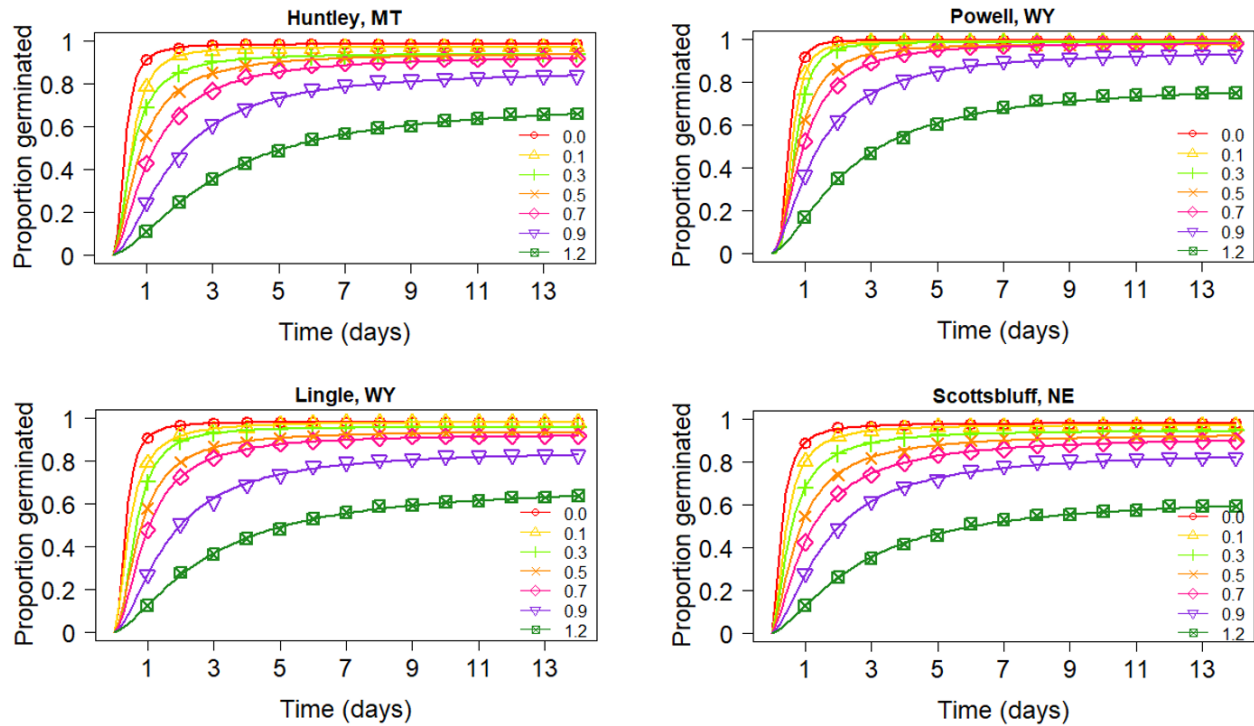
## Figures and Tables



**Figure 3.1.** Geographical map of four sites used to collect 44 *Bassia scoparia* accessions across the three-state region in the northern Great Plains of US.

**Table 3.1.** Estimated parameter values using the log-logistic model (Equation 1) to quantify the effect of water potential on the germination characteristics of *Bassia scoparia* accessions (collected from four sites) at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2017.

Water potential (MPa)	Parameter estimates ( $\pm$ SE)*					
	<i>b</i>	<i>t</i> <sub>50</sub>	<i>d</i>	<i>b</i>	<i>t</i> <sub>50</sub>	<i>d</i>
	Huntley, MT			Powell, WY		
0	-1.06 (0.45)	0.11 (0.11)	99 (1.4)	-2.45 (1.12)	0.37 (0.18)	100 (0.1)
-0.1	-1.27 (0.33)	0.35 (0.13)	97 (2.0)	-2.16 (0.65)	0.46 (0.13)	100 (0.1)
-0.3	-0.94 (0.21)	0.41 (0.14)	94 (2.8)	-2.23 (0.52)	0.61 (0.11)	99 (1.0)
-0.5	-1.14 (0.19)	0.78 (0.17)	94 (3.0)	-1.71 (0.31)	0.73 (0.12)	98 (1.3)
-0.7	-1.19 (0.17)	1.23 (0.22)	94 (3.4)	-1.74 (0.26)	0.94 (0.13)	98 (1.7)
-0.9	-1.14 (0.15)	2.36 (0.42)	87 (4.7)	-1.33 (0.18)	1.45 (0.23)	95 (3.3)
-1.2	-0.99 (0.13)	6.15 (1.29)	72 (7.1)	-1.03 (0.13)	3.84 (0.75)	79 (5.9)
	Lingle, WY			Scottsbluff, NE		
0	-0.93 (0.40)	0.08 (0.08)	98 (1.5)	-0.90 (0.35)	0.10 (0.10)	98 (1.6)
-0.1	-1.32 (0.34)	0.36 (0.12)	98 (1.5)	-1.08 (0.29)	0.27 (0.12)	97 (1.9)
-0.3	-1.23 (0.27)	0.48 (0.12)	96 (2.3)	-0.98 (0.21)	0.45 (0.14)	95 (2.7)
-0.5	-1.11 (0.20)	0.71 (0.13)	94 (2.9)	-1.01 (0.17)	0.79 (0.19)	93 (3.3)
-0.7	-1.15 (0.18)	1.00 (0.19)	92 (3.2)	-1.06 (0.16)	1.24 (0.25)	92 (3.9)
-0.9	-1.05 (0.14)	2.20 (0.42)	85 (4.7)	-1.01 (0.14)	2.24 (0.45)	85 (4.9)
-1.2	-0.90 (0.12)	6.39 (1.48)	69 (7.0)	-0.81 (0.12)	7.53 (1.98)	64 (6.9)
Parameter <i>b</i> is the relative slope around <i>t</i> <sub>50</sub> . Parameter <i>t</i> <sub>50</sub> is the time (days) taken to achieve 50% of the maximum germination. And, parameter <i>d</i> is the maximum germination (%) at the end of the observation period. *Values in parentheses represent standard errors of the means.						

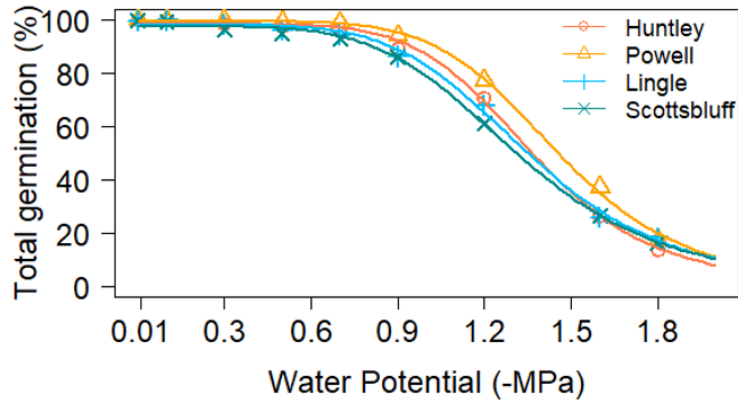


**Figure 3.2.** Germination response of *Bassia scoparia* accessions from four sites across the three-state region to different levels of water stress. Study was conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2017. Each curve represents germination response (cumulative proportion) at a given water potential (-MPa) over time (d). Curves were generated using a three-parameter log-logistic model (Equation 1). Symbols on the curves are the observed means of 11 accessions.

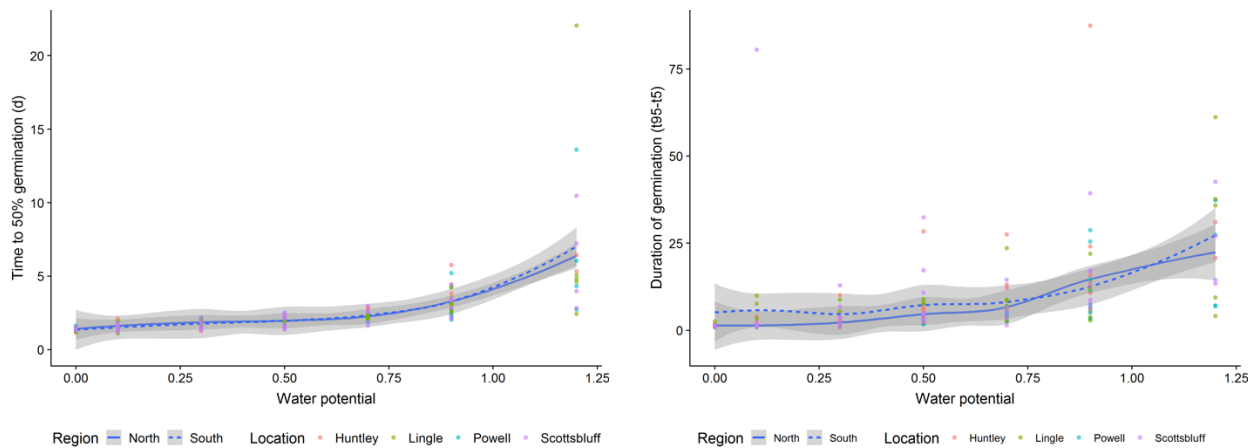
**Table 3.2.** Estimated parameter values using the log-logistic model (Equation 2) to quantify the effect of water potential on the maximum germination of *Bassia scoparia* accessions (collected from four sites) at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2017.

Site	Parameter estimates ( $\pm$ SE)*				
	$b$	$\Psi_{10}$	$\Psi_{50}$	$\Psi_{90}$	$d$
Huntley, MT	6.46 (0.47)	0.98 (0.03)	1.37 (0.02)	1.93 (0.04)	98 (1.09)
Powell, WY	6.54 (0.48)	0.91 (0.03)	1.46 (0.02)	2.03 (0.05)	99 (1.06)
Lingle, WY	5.50 (0.40)	1.04 (0.03)	1.36 (0.02)	2.04 (0.05)	98 (1.14)
Scottsbluff, NE	5.16 (0.36)	0.86 (0.03)	1.32 (0.02)	2.03 (0.06)	97 (1.16)

Parameter  $b$  is the relative slope around  $\Psi_{50}$ .  
Parameter  $\Psi_{50}$  is the water potential/stress (-MPa) required to reduce the germination by 50%.  
Similarly,  $\Psi_{10}$  and  $\Psi_{90}$  are the water potential/stress (-MPa) required to reduce the germination by 10 and 90%, respectively.  
And, parameter  $d$  is the maximum germination (%) at the end of the observation period.  
\*Values in parentheses represent standard errors of the means.



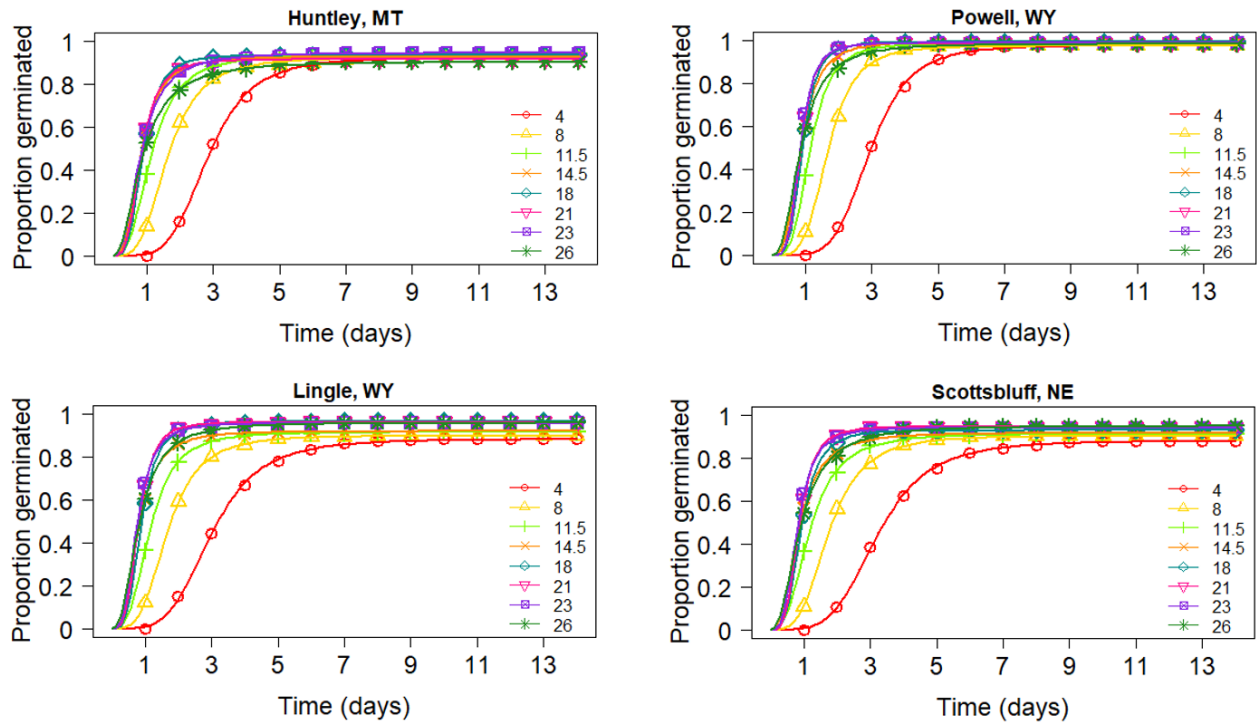
**Figure 3.3.** Effect of water potential on the maximum germination of *Bassia scoparia* accessions from four sites across the three-state region. Study was conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2017. Each curve represents percent maximum germination over a range of water potentials. Curves were generated using a three-parameter log-logistic model (Equation 2). Symbols on the curves are the observed means of 11 accessions.



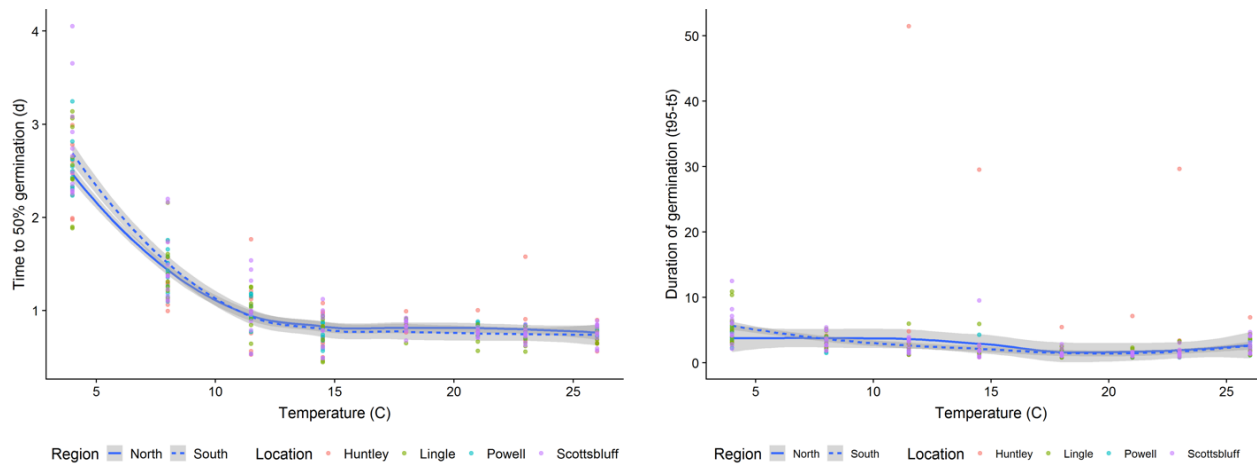
**Figure 3.4.** Effect of water potential (-MPa) on the germination rate (on left) and the germination duration (on right) of *Bassia scoparia* accessions from four sites across the three-state region. Study was conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2017. Response lines were fitted with a mixed-effects model. Solid blue line represents response of accessions from northern region (Huntley and Powell), whereas dashed blue line represents response of accessions from southern region (Lingle and Scottsbluff). Colored-round symbols along the lines represent parameter values of individual accessions. Shaded-gray band along the line represents 95% confidence interval.

**Table 3.3.** Estimated parameter values using the log-logistic model (Equation 1) to quantify the effect of temperature on the germination characteristics of *Bassia scoparia* accessions (collected from four sites) at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2018.

Temperature (C)	Parameter estimates ( $\pm$ SE)*					
	<i>b</i>	<i>t</i> <sub>50</sub>	<i>d</i>	<i>b</i>	<i>t</i> <sub>50</sub>	<i>d</i>
	Huntley, MT			Powell, WY		
4	-4.42 (0.49)	2.85 (0.14)	92 (3.1)	-4.99 (0.53)	2.96 (0.14)	98 (1.7)
8	-3.41 (0.40)	1.64 (0.11)	92 (3.1)	-4.24 (0.48)	1.67 (0.13)	98 (1.8)
12	-2.83 (0.40)	1.14 (0.09)	94 (2.7)	-3.76 (0.51)	1.15 (0.09)	99 (1.2)
15	-2.85 (0.58)	0.81 (0.08)	93 (3.0)	-4.20 (0.60)	0.87 (0.07)	99 (0.1)
18	-3.60 (0.72)	0.88 (0.07)	94 (2.8)	-4.25 (0.85)	0.92 (0.07)	99 (0.1)
21	-3.33 (0.71)	0.83 (0.07)	92 (3.2)	-4.25 (0.96)	0.86 (0.06)	99 (1.1)
23	-2.25 (0.48)	0.84 (0.09)	95 (2.6)	-4.15 (0.95)	0.85 (0.06)	99 (1.1)
26	-2.15 (0.40)	0.86 (0.10)	90 (3.4)	-2.57 (0.46)	0.86 (0.09)	98 (1.4)
	Lingle, WY			Scottsbluff, NE		
4	-4.17 (0.47)	2.99 (0.16)	88 (3.7)	-4.22 (0.47)	3.21 (0.17)	88 (3.7)
8	-3.58 (0.42)	1.67 (0.10)	90 (3.5)	-3.49 (0.40)	1.75 (0.11)	90 (3.4)
12	-3.10 (0.44)	1.15 (0.08)	92 (3.1)	-2.79 (0.39)	1.17 (0.10)	92 (3.2)
15	-2.82 (0.59)	0.79 (0.09)	97 (3.1)	-2.57 (0.49)	0.83 (0.09)	92 (3.2)
18	-4.68 (0.73)	0.89 (0.06)	97 (2.1)	-3.56 (0.66)	0.92 (0.06)	93 (2.9)
21	-4.67 (0.86)	0.80 (0.07)	96 (2.2)	-3.79 (0.81)	0.86 (0.06)	94 (2.6)
23	-4.21 (0.75)	0.76 (0.08)	96 (2.3)	-3.34 (0.73)	0.80 (0.07)	94 (2.7)
26	-2.49 (0.48)	0.81 (0.09)	96 (2.3)	-2.32 (0.41)	0.89 (0.10)	95 (2.5)
Parameter <i>b</i> is the relative slope around <i>t</i> <sub>50</sub> . Parameter <i>t</i> <sub>50</sub> is the time (days) taken to achieve 50% of the maximum germination. And, parameter <i>d</i> is the maximum germination (%) at the end of the observation period. *Values in parentheses represent standard errors of the means.						



**Figure 3.5.** Germination response of *Bassia scoparia* accessions from four sites across the three-state region to different temperature treatments. Study was conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2018. Each curve represents germination response (cumulative proportion) at a given temperature (C) over time (d). Curves were generated using a three-parameter log-logistic model (Equation 1). Symbols on the curves are the observed means of 11 accessions.



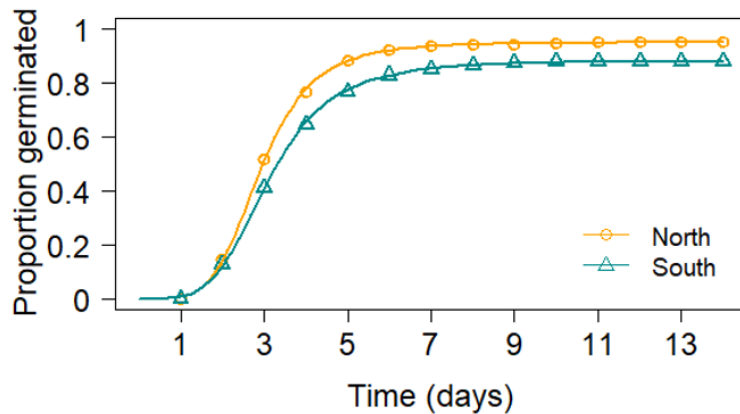
**Figure 3.6.** Effect of temperature (C) on the germination rate (on left) and the germination duration (on right) of *Bassia scoparia* accessions from four sites across the three-state region. Study was conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2018. Response lines were fitted with a mixed-effects model. Solid blue line represents response of accessions from northern region (Huntley and Powell), whereas dashed blue line represents response of accessions from southern region (Lingle and Scottsbluff). Colored-round symbols along the lines represent parameter values of individual accessions. Shaded-gray band along the line represents 95% confidence interval.



**Table 3.4.** Estimated parameter values using the log-logistic model (Equation 1) to quantify the effect of low temperature (4 C) on the rate and total germination of *Bassia scoparia* accessions across the North-South transect from a study conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2018.

Region**	Parameter estimates ( $\pm$ SE)*				
	$b$	$t_{10}$	$t_{50}$	$t_{90}$	$d$
North	-4.19 (0.11)	1.82 (0.03)	2.91 (0.03)	4.64 (0.07)	95 (0.53)
South	-4.19 (0.10)	1.84 (0.03)	3.11 (0.03)	5.26 (0.09)	88 (0.82)

Parameter  $b$  is the relative slope around  $t_{50}$ .  
 Parameter  $t_{50}$  is the time (days) taken to achieve 50% of the maximum germination. Similarly,  $t_{10}$  and  $t_{90}$  are the time (days) taken to achieve 10 and 90% of the maximum germination, respectively.  
 And, parameter  $d$  is the maximum germination (%) at the end of the observation period.  
 \*Values in parentheses represent standard errors of the means.  
 \*\*Huntley and Powell sites were included in the North region, whereas Lingle and Scottsbluff sites were included in the south region.



**Figure 3.7.** Effect of low temperature (4 C) on the rate and total germination of *Bassia scoparia* accessions from north (Huntley and Powell) and south (Lingle and Scottsbluff) region. Study was conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT in fall 2018. Each curve represents germination response over time (d). Curves were generated using a three-parameter log-logistic model (Equation 1). Symbols on the curves are the observed means of 22 accessions.

## CHAPTER 4. EFFECT OF WINTER WHEAT COVER CROP AND IRRIGATION ON THE EMERGENCE PATTERNS OF KOCHIA (*BASSIA SCOPARIA*) ACROSS THE NORTHERN GREAT PLAINS OF U.S.

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Modified from a manuscript to be submitted to *Weed Science*

### Abstract

Development of multiple herbicide-resistant (HR) kochia (*Bassia scoparia* [L.] A.J. Scott) across the northern U.S. Great Plains is a serious concern. The problem is even more serious in sugar beet (*Beta vulgaris* L.) based crop rotations due to widespread occurrence of glyphosate and ALS-resistant *B. scoparia*. Since these two herbicide chemistries are the only viable options for *B. scoparia* control in sugar beet, there is an immediate need for ecologically-based strategies to manage HR *B. scoparia*. Therefore, field experiments were conducted during 2017 to 2019 to quantify the effect of winter wheat cover crop and irrigation on *B. scoparia* emergence at two sites, Huntley, Montana and Scottsbluff, Nebraska. Winter wheat cover crop and irrigation treatments did not influence *B. scoparia* emergence at Huntley. This was mainly due to insufficient canopy cover by winter wheat during the emergence period of *B. scoparia*. A majority of *B. scoparia* emerged before the first irrigation was applied. However, cover crop and irrigation influenced *B. scoparia* emergence at Scottsbluff. Winter wheat cover crop did not reduce cumulative emergence of *B. scoparia*, but delayed the initiation and rate of emergence compared to the fallow (no cover crop) treatment. Irrigation prompted earlier and faster emergence of *B. scoparia* at Scottsbluff. It required at least two irrigations to stimulate *B. scoparia* emergence in cover crop plots compared with only one irrigation in the fallow. Hence,

winter wheat as a cover crop is not likely to be beneficial in suppressing *B. scoparia* emergence in this region. Instead, *B. scoparia* emergence can effectively be stimulated by irrigations during early spring and then controlled with either tillage or non-selective herbicides (stale seedbed approach). This would potentially reduce *B. scoparia* cohorts emerging in late-planted crops such as dry bean (*Phaseolus vulgaris* L.) grown in rotation with sugar beet in the Northern Great Plains region.

### Introduction

Kochia (*Bassia scoparia* [L.] A.J. Scott) is the most problematic weeds across the arid and semiarid regions of northern Great Plains of the United States. It has developed resistance to four different groups of herbicides including acetolactate synthase (ALS) inhibitors, photosystem II (PS II) inhibitors, glyphosate, and synthetic auxins (Heap 2021). *Bassia scoparia* populations resistant to ALS and PS II inhibitors, ALS inhibitors and glyphosate, or glyphosate and synthetic auxins have been reported in several U.S. states. Most importantly, the presence of *B. scoparia* populations resistant to ALS-inhibitors and glyphosate (two most widely used herbicides in sugar beet [*Beta vulgaris* L.]) has become a norm in production fields across the Northern Great Plains (Kumar et al. 2019). Therefore, integrated weed management (IWM) strategies beyond herbicides are urgently needed to manage this weed, specifically in sugar beet, which is highly vulnerable to *B. scoparia* competition (Mesbah et al. 1994) and has very few viable herbicide options to control this weed (Sbatella et al. 2019). Weed seedbanks are the primary source of weed infestations in arable fields. Non-chemical weed management strategies such as cover crops and/or stale seedbed approach can be used to either suppress or exhaust *B. scoparia* seedbanks.

Cover crops have been used in several cropping systems across the U.S. and proven to be effective in suppressing weeds (Teasdale et al. 2007). However, the use of cover crops for weed

management is very limited in the Northern U.S. Great Plains (Carr et al. 2012). Excessive water use, poor overwinter survival, and insufficient biomass accumulation by many cover crop species are the key challenges. Winter wheat (*Triticum aestivum* L.) is the major crop in this region, which is often grown in wheat-fallow rotations to conserve limited soil moisture (Nielsen and Calderón 2011). Due to its winter hardiness (Fowler 2012), winter wheat has the potential to be used as a cover crop to suppress weeds emerging early in the spring such as *B. scoparia*. However, soil moisture and temperature deviations due to the presence of a cover crop can greatly influence weed emergence patterns, including *B. scoparia* (Teasdale and Mohler 1993; Wicks et al. 2004; Yang et al. 2021). Therefore, it requires careful considerations before implementation of such strategies.

Stale seedbed has been used to limit weed seedling emerging in the main crop by exhausting seedbanks prior to planting the cash crop. This is achieved primarily by stimulating weed seed emergence using shallow tillage, irrigation, or delayed planting (Boyd et al. 2006; Johnson and Mullinix 2000). Emerged weed flushes then can be killed by shallow tillage, flaming, or nonselective herbicides (Caldwell et al. 2001). Delayed planting may not be feasible in northern temperate regions due to short growing seasons. Therefore, efforts should be focused on stimulating weed seedling emergence in early-spring.

*Bassia scoparia* is often the first species to emerge in the spring of northern U.S. Great Plains due to lack of seed dormancy (Dyer et al. 1993; Schwingamer and Van Acker 2008). Despite having low temperature requirements for germination, *B. scoparia* seeds can germinate over a wide range of temperatures from 3.5 to 40 C (Eberlein and Fore 1984). Although the majority of *B. scoparia* emerges before May (Dille et al. 2017; Schwingamer and Van Acker 2008), a significant proportion can still emerge up until mid-August (Anderson and Nielsen

1996). In the later study, those late-emerging cohorts were associated with rainfall events. Therefore, it is possible that the emergence of late cohorts can be stimulated early in the spring using one or more irrigation events.

Dry beans (*Phaseolus vulgaris* L.) are often recommended in rotation with sugar beet as a strategy to manage HR *B. scoparia* (Kumar et al. 2019; Mosqueda et al. 2020; Sbatella et al. 2019). This is because dry beans are well adapted to this region and planted much later than sugar beet (Miller 2002), thereby offering greater opportunities (herbicides and/or tillage) to control the germinable fraction of the weed seed bank prior to planting dry beans. Currently there is no study that evaluates ecological IWM strategies to deplete *B. scoparia* seed banks prior to late-planted crops. In addition, information regarding the effect of winter wheat as a cover crop on *B. scoparia* emergence patterns in the Great Plains region is lacking. Therefore, the objectives of this study were 1) to quantify the potential of using winter wheat as a cover crop to suppress *B. scoparia* emergence, and 2) to evaluate the effectiveness of spring irrigations in stimulating *B. scoparia* emergence.

## Materials and Methods

### Experimental Sites

Field experiments were conducted during 2017 to 2019 at two sites (Figure 1): the Montana State University Southern Agricultural Research Center, Huntley, Montana (45°92'N, 108°25'W) and the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, Nebraska (41°89'N, 103°68'W). Field experiments were repeated over time (2017-2018 and 2018-2019) at the Huntley but not at the Scottsbluff (2017-2018). The soil at Huntley site was Lohmiller silty clay (fine, smectitic, calcareous, mesic Torrertic Ustifluvents) with 1.6% organic matter and a pH of 8.1. The soil at Scottsbluff site was Glenberg sandy loam (Ustic Torrfluvents) with 0.9% organic matter and a pH of 8.0. Fields at the Huntley site were

maintained under a wheat-fallow rotation for two years prior to the initiation of the study. Field at Scottsbluff site was under wet corn (early harvest for cattle feed) in 2017 and grain corn in 2016. Fields at both sites were maintained relatively weed-free, with no known infestation of *B. scoparia*. Before cover crop planting in fall, fields were prepared by one ploughing and one roller harrowing. Records of average air temperature and total precipitation during 2018 and 2019 growing seasons and the past 30 yr are summarized in Table 1.

### **Experimental Design**

A two-factor strip-split plot design was used with four replications. The main strip factor consisted of winter wheat cover crop vs. fallow (no cover crop). Five irrigation treatments were assigned to the split plots across the main strip. Irrigation treatments included no, one, two, three, and four irrigations. Irrigation treatments were spaced at about 2-wk interval. Each strip was 7.3 m wide and 27.5 m long, whereas each split plot was 5.5 m wide and 7.3 m long. A 9 m wide alleyway was left between the irrigation treatments to prevent water drifting from one plot to another.

### **Field Operations**

In spring 2017, seed-mixtures of 11 *B. scoparia* accessions, representative of each site (see section *Seed Collection* in the chapter III) was grown on the edges of the fields to be used for the experiments. In fall 2017, fully-ripened seeds were harvested, threshed and cleaned. A known-number of seeds at each site was used to uniformly infest each split-split plot. About 1000 and 500 seeds m<sup>-2</sup> were used at Huntley and Scottsbluff sites, respectively. Seeds required for each plot were counted by 1000-seed weight basis, put in separate coin envelopes, and then hand-spread to the individual plots.

Winter wheat was drill-seeded in 19-cm wide rows in the cover crop strips. The dates of cover crop planting are listed in Table 2. At Huntley, winter wheat var. Yellowstone was drill seeded at a rate of 67 kg ha<sup>-1</sup>. At Scottsbluff, winter wheat var. Brawl CL Plus was seeded at 112 kg ha<sup>-1</sup>. Cover crop was terminated in the following spring 1 d prior to last irrigation event using glyphosate (1,261 g ae ha<sup>-1</sup>). Glyphosate was applied using a CO<sub>2</sub>-pressurized backpack sprayer equipped with TT 11002VS nozzles and calibrated to deliver 187 L ha<sup>-1</sup> at 276 kPa. Irrigation treatments were applied using a Lateral Move Irrigation System (Reinke Manufacturing Co., 1040 Road 5300, Deshler, Nebraska 68340) calibrated to deliver 25 mm of water. To insure the desired-amount of irrigation water applied, eight rain gauges (two replication<sup>-1</sup>) were installed on the field plots (at 40-cm height) in a ‘W’ pattern. Dates for the irrigation treatments are listed in Table 2. Ground water was used at the Scottsbluff site to irrigate the plots, whereas surface canal water was used at the Huntley site.

### **Data Collection**

*Bassia scoparia* emergence was assessed by counting seedlings at 2-wk intervals at the Huntley site and 1-wk interval at the Scottsbluff site. The typical emergence period for *B. scoparia* in the Great Plains region is from March through June (Becker 1978; Dille et al. 2017; Schwinghamer and Van Acker 2008). Therefore, plots were scouted weekly starting March 15 of 2018 and 2019 to record the initiation of emergence. In the current studies, *B. scoparia* started emerging around the first wk of April at all site-yr. Two permanent 0.25-m<sup>2</sup> quadrats in each sub-sub plot were established. Seedlings were counted and removed from the quadrats at each observation period until no further emergence occurred for two wk in any of the plots.

## Statistical Analysis

Data for seedling emergence at a biweekly interval were analyzed using PROC MIXED in SAS 9.4 software (SAS Institute Inc., Cary, NC 27513). Normality of residuals and homogeneity of variance were tested using Shapiro-Wilk test and Levene's test, respectively, and using diagnostic residual plots (Kozak and Piepho 2018). Experimental site, cover crop, irrigation frequency, and interaction effects were considered fixed effects in the model. Appropriate degrees of freedom in the model were obtained by Satterthwaite approximation method (Satterthwaite 1946). Estimated means were compared using Tukey test at a significance level of  $\alpha = 0.05$ .

Response of *B. scoparia* emergence to cover crop and irrigation treatments over time was analyzed in the statistical programming language *R* (R Core Team 2019) using a *R* extension package “drc” (Ritz et al. 2019). First, data from each observation period were arranged in an event-time format (Ritz et al. 2013). Then, a three-parameter log-logistic model was fitted using the following equation (Equation 1; Ritz et al. 2013):

$$F(t) = \frac{d}{1 + \exp\{b[\log(t) - \log(t_{50})]\}} [1]$$

Where  $F(t)$  denotes the proportion of seeds emerged between time 0 (start of the emergence period) and time  $t$ . Parameter  $d$  denotes the upper limit (maximum emergence occurred in each growing season). Parameter  $t_{50}$  denotes the time (day of year [DOY]) to achieve 50% of the maximum emergence (relative to the upper limit,  $d$ ). Lastly, parameter  $b$  denotes the slope of emergence curves around  $t_{50}$ .

Additionally, time to achieve 10 and 90% of the maximum emergence,  $t_{10}$  and  $t_{90}$ , respectively were calculated using the ED function as described by Knezevic et al. (2007).



## Results and Discussion

Year by treatment interaction for *B. scoparia* emergence was not significant at the Huntley site. Therefore, data from both years were pooled. However, the site by treatment interaction was significant; therefore, data for each site are presented separately. In addition, the interaction between cover crop and irrigation was significant for the Scottsbluff site.

The biweekly average air temperature was slightly lower at the Huntley site compared to the Scottsbluff site during the *B. scoparia* emergence period, specifically in early-season (Table 1). For example, the average temperature at Huntley was 2 to 3 C lower than Scottsbluff until the second wk of April. However, the average temperature did not differ much between the sites on later dates. In contrast, the amount of precipitation received at Huntley was much higher than Scottsbluff. For example, in March and April months of 2018, Huntley received 120 mm precipitation, whereas Scottsbluff received only 50 mm during this period. Overall, Scottsbluff received only 240 mm, whereas Huntley received 310 mm of precipitation in 2018. In 2019, Huntley received 220 mm precipitation, whereas Scottsbluff received 160 mm.

### Field Study at Huntley

#### *Effect of Cover Crop*

Winter wheat cover crop did not affect *B. scoparia* seedling emergence at Huntley site (Table 3). On April 15, *B. scoparia* seedling density ranged from 90 to 120 plants m<sup>-2</sup> across the cover crop treatments (Table 4). Under low temperatures during early spring (March 1 through April 1) in the Northern Great Plains, winter wheat exhibits slow tillering-growth (Wicks et al. 2004). The insufficient ground cover by winter wheat during early emergence period of *B. scoparia* most likely contributed to the lack of the cover crop effect. Similar results have been reported in previous studies on the effect of winter wheat cover crop on *B. scoparia* (Anderson et al. 2007; Wicks and Smika 1990). Additionally, *B. scoparia* germination is not affected by light

or canopy induced shading (Everitt et al. 1983). Nyamusamba (2014) previously reported that the presence of a crop (wheat) did not influence *B. scoparia* density, although it greatly reduced the weed biomass.

Regardless of presence or absence of the cover crop, *B. scoparia* started emerging in the first wk of April through end of May in both years (Table 5, Figure 2). However, a few seedlings (<1% of cumulative emergence) still emerged in June. The cumulative emergence ranged between 62 to 72% during the season. Fifty and 90% of the cumulative emergence occurred by the first and last wk of May, respectively. These results indicate that *B. scoparia* has an early and rapid emergence characteristic in Northern Great Plains of the U.S. These results are in accordance with the previous findings (Dille et al. 2017; Kumar et al. 2018; Schwinghamer and Van Acker 2008) that fewer growing degree days were required for *B. scoparia* populations from northern compared to southern latitudes of the Great Plains.

#### *Effect of Irrigation*

Similar to the cover crop treatment, irrigation treatments did not influence *B. scoparia* seedling emergence at Huntley site (Table 3). This was mainly due to the early, rapid emergence of *B. scoparia*. About 90% of the in-season cumulative emergence occurred (Table 5) even before the first irrigation event at this site (Table 2). A very few seedlings (<1% of cumulative emergence) emerged after May 30 regardless of the irrigation events (data not shown). These results indicate that in Northern Great Plains, *B. scoparia* may receive enough moisture through winter snow or spring rainfall (Table 1) for early, rapid emergence, and therefore, irrigation applied after the month of May are less likely to stimulate emergence. However, irrigation events during early spring (April to mid-May) may stimulate *B. scoparia* emergence, specifically when soil moisture is limited which needs further investigation.

## Field Study at Scottsbluff

### *Effect of Cover Crop*

Unlike Huntley site, the winter wheat cover crop influenced *B. scoparia* seedling emergence at Scottsbluff site. The interaction of cover crop by irrigation was also significant (Table 3). In the absence of irrigation, *B. scoparia* density was significantly lower in cover crop plots compared to fallow (no cover crop) plots on April 15 (Table 6). Similarly, with one irrigation, *B. scoparia* density was significantly lower in cover crop compared to fallow plots until May 30, the period with a limited rainfall (Table 1). However, the differences were not significant after May 30, which might be attributed to a high rainfall event at the end of May (Table 1). Moreover, in the absence or presence of one irrigation, cover crop delayed the initiation of *B. scoparia* emergence ( $t_{10}$ ) by 10 d (Table 7; Figure 3). Similarly, in those plots, cover crop increased the time to achieve 50% ( $t_{50}$ ) and 90% ( $t_{90}$ ) of cumulative emergence by more than five and eight wk, respectively.

These results indicate that the lower density and a delayed emergence of *B. scoparia* in cover crop vs. fallow plots was most likely not due to the physical suppression by the cover crop. Instead, it was attributed to a low moisture availability for *B. scoparia* seedling emergence in the presence of the cover crop. Previous researchers (Anderson et al. 2007; Wicks et al. 2004) reported that winter wheat may not produce sufficient biomass or canopy architecture (in early-spring) to physically suppress early-emerging weeds such as *B. scoparia* under a limited soil moisture condition. Similarly, the cover crop did not affect *B. scoparia* density at the Huntley site even when sufficient moisture was present for emergence (Table 4). Additionally, *B. scoparia* density was higher in cover crop plots compared to the fallow plots when three or more irrigations were applied. Another possible reason for lower density and delayed emergence of *B.*

*scoparia* in cover crop plots during early-spring was due to low temperatures near soil-surface in cover crop plots compared to fallow plots. Winter cover crops tend to increase the soil-surface temperature in winter periods, but consistently decrease it during early-spring periods (Teasdale and Mohler 1993; Yang et al. 2021). This may have delayed the optimum temperature required for rapid emergence of *B. scoparia* (Williams et al. 1998).

#### *Effect of Irrigation*

Similar to winter wheat cover crop, irrigation treatment had a significant effect on the seedling emergence of *B. scoparia* at Scottsbluff site (Table 3). The interaction of cover crop by irrigation was also significant. The effect of irrigation on stimulating *B. scoparia* emergence during the early spring was more effective in fallow compared to cover crop plots. For example, the first irrigation event (applied on April 11) stimulated *B. scoparia* emergence drastically in fallow plots. However, additional irrigation events did not improve emergence over time. This was most likely due to fulfillment of moisture requirement for emergence through one irrigation in the early-spring (Table 1).

The first irrigation event did not improve *B. scoparia* emergence in cover crop plots (Table 6). This was probably due to two main reasons. First, one irrigation was not sufficient to fulfill the moisture deficiency caused by the cover crop water use. Secondly, the soil surface temperature was still below the optimum temperature required for *B. scoparia* emergence. It is common to have limited soil moisture in spring across the northern Great Plains of U.S. (Carr et al. 2012; Padbury et al. 2002; Wienhold et al 2018). Additionally, an increase from one to two irrigation(s) reduced the time to achieve 50% ( $t_{50}$ ) and 90% ( $t_{90}$ ) of cumulative emergence by two and four wk, respectively (Table 7; Figure 3). An irrigation beyond two irrigation treatments did

not further improve *B. scoparia* emergence. There was also co-occurrence of rainfall with late-irrigation events (Table 1 and 2).

### Management Implications

The results of this research indicate that *B. scoparia* emergence patterns are site-specific and varied between the northern and southern states of the U.S. Great Plains. Furthermore, the response of *B. scoparia* to ecologically based management strategies such as cover crop and stale seedbed would differ between the two sites. Soil moisture is an important factor in determining the success of winter wheat cover crop for *B. scoparia* management in the semi-arid Great Plains region. In the absence of cover crop and irrigation, *B. scoparia* populations at Scottsbluff began emerging one wk earlier than at Huntley because of higher temperatures at the Scottsbluff in early-spring (Table 1). Similarly, *B. scoparia* at Scottsbluff achieved 50 and 90% of the maximum emergence by April 16 and May 4, respectively; whereas, *B. scoparia* at Huntley achieved 50 and 90% of the maximum emergence by May 1 and May 25, respectively. *Bassia scoparia* emergence was sparse after May 30 at Huntley, but a relatively higher percentage of weed seedlings emerged after May 30 at Scottsbluff (Table 6). The peak emergence period of *B. scoparia* was two to three wk earlier in Nebraska compared to Montana. However, late emergence flushes in Nebraska can reduce the utility of a diverse crop rotation strategy that relies on late-planted crops to exhaust the weed seed bank prior to crop emergence (Anderson 1994, 2000). This may not be a concern in Montana since a majority of *B. scoparia* would emerge before planting of crops such as dry beans, which is often planted during the last wk of May to the first wk of June (Miller et al. 2002).

Response of *B. scoparia* to the cover crop and irrigation treatments at the Scottsbluff site indicate that a winter wheat cover crop can delay the emergence of *B. scoparia* and can potentially increase density of *B. scoparia* in-crop. In addition, at least two irrigations were

required to stimulate *B. scoparia* emergence in cover crop plots compared with only one irrigation in fallow plots. Therefore, strategies such as winter wheat cover crop are not likely to be useful in the semi-arid regions of the northern U.S. Great Plains in managing HR *B. scoparia*. Instead, *B. scoparia* emergence can successfully be stimulated using irrigations in the early spring and then controlled with either tillage or non-selective herbicides. This stale seedbed strategy can help in exhausting *B. scoparia* seedbank prior to late-planted crop such as dry bean grown in rotation with sugar beet (planted in mid-April). However, securing surface water for irrigation in the early-spring can potentially be a challenge because the surface/canal water for irrigation is regulated by local irrigation districts in this Great Plains region. The results of the research suggest that growers should apply the first irrigation as soon as it is available during the early spring to best utilize the stale seedbed IWM approach to manage *B. scoparia* seedbanks.

### **Acknowledgments**

We thank the Montana State University Southern Agricultural Research Center, Huntley, MT for providing resources and support to the graduate student to conduct this study. We also thank the USDA National Institute of Food and Agriculture for providing funding to conduct this research (project accession no. 1010972).

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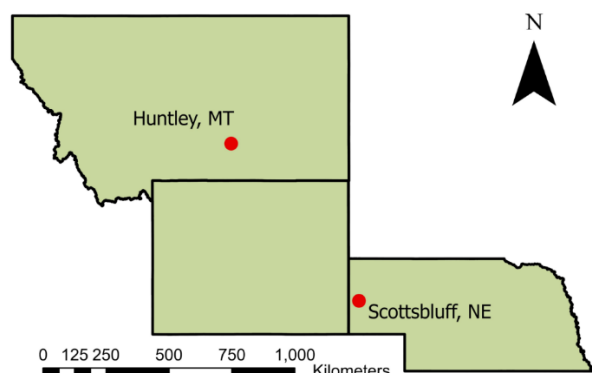
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## Figure and Tables



**Figure 4.1.** Geographical locations of two sites used to quantify the effect of winter wheat cover crop and irrigation frequency on the emergence patterns of *Bassia scoparia* in field studies in 2018 and 2019.

**Table 4.1.** Average air temperatures and total precipitation during 2018 and 2019 growing seasons at the Montana State University Southern Agricultural Research Center, Huntley, MT and the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, NE.<sup>a, b</sup>

	Huntley, MT						Scottsbluff, NE					
	Average temperature			Precipitation			Average temperature			Precipitation		
	2018	2019	30-yr	2018	2019	30-yr	2018	2019	30-yr	2018	2019	30-yr
	C			mm			C			mm		
March 1 to 15	-4	-10	3	7	10	21	4	-6	4	15	0	27
March 16 to 31	2	4		21	2		5	5		7	0	
April 1 to 15	2	7	8	45	30	42	4	7	8	19	0	50
April 16 to 30	11	10		50	18		9	10		8	13	
May 1 to 15	14	10	13	13	5	65	13	10	14	36	6	71
May 16 to 31	17	11		103	89		18	10		106	100	
June 1 to 15	18	18	17	26	41	53	23	18	20	1	13	65
June 16 to 30	18	17		46	21		20	19		45	28	
Season total	—	—	—	311	216	181	—	—	—	237	160	53

<sup>a</sup>2018 and 2019 data were recorded on the local weather stations located at each site's farm.  
<sup>b</sup>30-yr average data for both the sites were obtained online from the High Plains RCC CLIMOD website; available at <http://climod.unl.edu/>.

**Table 4.2.** Dates of field operations conducted during 2017 to 2019 growing seasons at the Montana State University Southern Agricultural Research Center, Huntley, MT and the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, NE.

Field operation	Huntley, MT			Scottsbluff, NE*	
	2017	2018	2019	2017	2018
Cover crop planting	October 18	October 8	—	October 15	—
First irrigation	—	May 27	June 4	—	April 11
Second irrigation	—	June 11	June 12	—	April 25
Third irrigation	—	June 18	June 18	—	May 9
Fourth irrigation**	—	June 27	June 25	—	May 23
*Field study at Scottsbluff site was not repeated over time.					

**Table 4.3.** Test of fixed effects on *Bassia scoparia* seedling emergence during 2018 and 2019 field studies conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT and the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, NE.

Fixed effects	Huntly, MT				Scottsbluff, NE					
	April 15	April 30	May 15	May 30	April 15	April 30	May 15	May 30	June 15	June 30
	<i>p</i> -value									
Cover crop <sup>a</sup>	0.28	0.06	0.21	0.22	<0.01	0.12	0.98	0.32	0.22	0.15
Irrigation <sup>b</sup>	0.42	0.06	0.20	0.19	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cover crop × Irrigation <sup>c</sup>	0.06	0.26	0.44	0.43	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
<sup>a</sup> First factor (winter wheat cover crop vs. fallow). <sup>b</sup> Second factor (irrigation frequencies; no, one, two, three, and four irrigations). <sup>c</sup> Interaction effects between the factors.										

**Table 4.4.** Effect of winter wheat cover crop and irrigation frequency on *Bassia scoparia* seedling emergence averaged over 2018 and 2019 field studies conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT.<sup>a, b</sup>

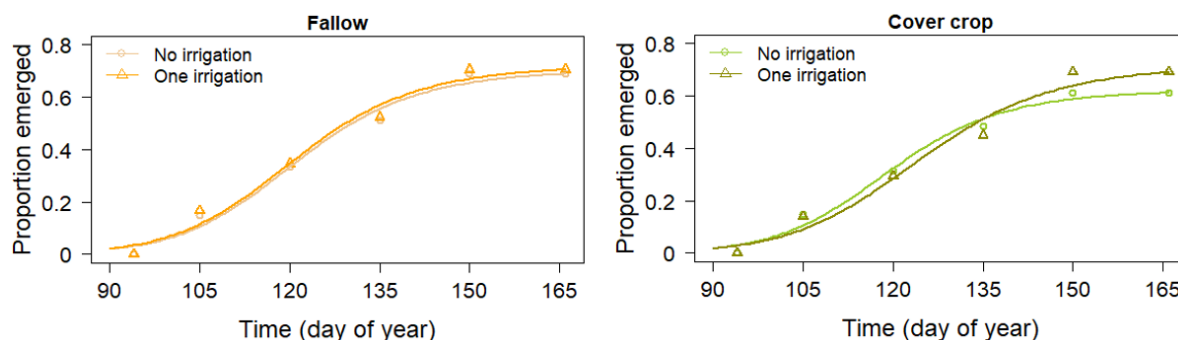
Cover crop	Irrigation	April 15		April 30		May 15		May 30	
		Seedlings m <sup>-2</sup>							
Fallow	No	87	a	219	a	300	a	302	a
	One	140	a	275	a	343	a	346	a
Winter wheat	No	117	a	273	a	343	a	344	a
	One	82	a	297	a	363	a	365	a

<sup>a</sup>*Bassia scoparia* seedling numbers are the cumulative emergence occurred up until each calendar date.  
<sup>b</sup>Treatment means within a column with same letter(s) are not significantly different (Tukey test,  $\alpha = 0.05$ ).

**Table 4.5.** Estimated parameter values using the log-logistic model (Equation 1) for the effect of winter wheat cover crop and irrigation frequency on *Bassia scoparia* seedling emergence averaged over 2018 and 2019 field studies conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT.

Cover crop	Irrigations	Parameter estimates ( $\pm$ SE)*				
		<i>b</i>	<i>t</i> <sub>10</sub>	<i>t</i> <sub>50</sub>	<i>t</i> <sub>90</sub>	<i>d</i>
Fallow	No	-12.2 (0.3)	101 (0.6)	121 (0.5)	145 (1.0)	70 (1.1)
	One	-11.9 (0.4)	100 (0.7)	121 (0.6)	145 (1.1)	72 (1.2)
Winter wheat	No	-12.5 (0.4)	100 (0.8)	119 (0.7)	142 (1.2)	62 (1.4)
	One	-11.3 (0.4)	103 (0.8)	125 (0.7)	151 (1.4)	72 (1.4)

Parameter *b* is the relative slope around *t*<sub>50</sub>.  
 Parameter *t*<sub>50</sub> is the time (day of year) to achieve 50% of the maximum emergence. Similarly, *t*<sub>10</sub> and *t*<sub>90</sub> are the time (day of year) to achieve 10 and 90% of the maximum emergence, respectively.  
 And, parameter *d* is the maximum emergence (%) at the end of the end of observation period.  
 \*Values in parentheses represent standard errors of the means.



**Figure 4.2.** Emergence response of *Bassia scoparia* to winter wheat cover crop and irrigation frequency over time (day of year) averaged over 2018 and 2019 field studies conducted at the Montana State University Southern Agricultural Research Center, Huntley, MT. Curves were generated using a three-parameter log-logistic model (Equation 1). Symbols on the curves are the observed means of 4 replicates.

**Table 4.6.** Effect of winter wheat cover crop and irrigation frequency on *Bassia scoparia* seedling emergence in 2018 field study conducted at the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, NE.<sup>a, b</sup>

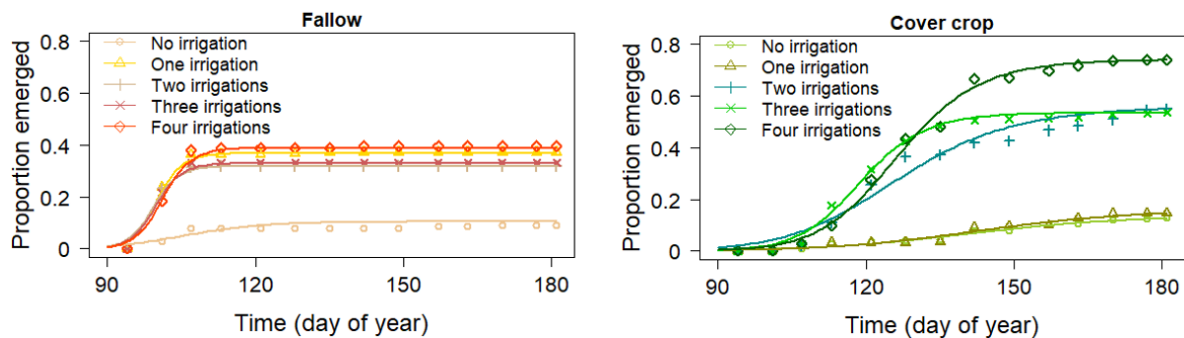
Cover crop	Irrigation	Seedlings m <sup>-2</sup>											
		April 15		April 30		May 15		May 30		June 15		June 30	
Fallow	No	36	b	36	c	36	b	36	cd	44	e	47	e
	One	180	a	182	ab	182	a	183	b	183	bcd	183	cd
	Two	156	a	160	ab	157	a	157	bc	157	cd	157	cd
	Three	162	a	163	ab	163	a	163	bc	163	bcd	163	cd
	Four	194	a	200	a	205	a	205	b	205	bcd	205	bc
Winter wheat	No	4	c	21	c	36	b	66	cd	87	de	102	de
	One	12	bc	20	c	35	b	75	cd	99	de	111	de
	Two	11	bc	132	b	205	a	241	b	277	ab	313	ab
	Three	15	bc	161	ab	238	a	256	ab	256	bc	264	bc
	Four	13	bc	133	b	228	a	317	a	343	a	349	a

<sup>a</sup>*Bassia scoparia* seedling numbers are the cumulative emergence occurred up until each calendar date.  
<sup>b</sup>Treatment means within a column with same letter(s) are not significantly different (Tukey test,  $\alpha = 0.05$ ).

**Table 4.7.** Estimated parameter values using the log-logistic model (Equation 1) for the effect of winter wheat cover crop and irrigation frequency on *Bassia scoparia* seedling emergence in 2018 field study conducted at the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, NE.

Cover crop	Irrigations	Parameter estimates ( $\pm$ SE)*				
		$b$	$t_{10}$	$t_{50}$	$t_{90}$	$d$
Fallow	No	-13.7 (1.2)	91 (1.3)	106 (1.2)	124 (2.6)	12 (0.1)
	One	-38.2 (1.6)	94 (0.3)	100 (0.2)	106 (0.3)	37 (0.1)
	Two	-38.8 (1.7)	94 (0.3)	99 (0.8)	105 (0.3)	32 (0.1)
	Three	-38.4 (1.7)	94 (0.3)	100 (0.2)	106 (0.3)	33 (0.1)
	Four	-35.5 (1.4)	95 (0.3)	101 (0.2)	108 (0.4)	39 (0.1)
Winter wheat	No	-8.8 (0.7)	111 (2.3)	143 (2.6)	183 (5.9)	14 (0.1)
	One	-9.1 (0.7)	114 (2.1)	145 (2.4)	184 (5.6)	17 (0.1)
	Two	-11.0 (0.4)	104 (0.8)	127 (0.8)	155 (1.6)	56 (0.1)
	Three	-18.5 (0.6)	106 (0.5)	119 (0.4)	134 (0.7)	54 (0.1)
	Four	-15.6 (0.4)	110 (0.5)	127 (0.4)	146 (0.8)	74 (0.1)

Parameter  $b$  is the relative slope around  $t_{50}$ .  
 Parameter  $t_{50}$  is the time (day of year) to achieve 50% of the maximum emergence. Similarly,  $t_{10}$  and  $t_{90}$  are the time (day of year) to achieve 10 and 90% of the maximum emergence, respectively. And, parameter  $d$  is the maximum emergence (%) at the end of the end of observation period.  
 \*Values in parentheses represent standard errors of the means.



**Figure 4.3.** Emergence response of *Bassia scoparia* to winter wheat cover crop and irrigation frequency over time (day of year) in 2018 field study conducted at the University of Nebraska Panhandle Research & Extension Center, Scottsbluff, NE. Curves were generated using a three-parameter log-logistic model (Equation 1). Symbols on the curves are the observed means of 4 replicates.

## CHAPTER 5. GENERAL CONCLUSION

A sole use of therapeutic approach to kill a pest is the biggest culprit for the “wicked evolution” of pesticide resistance. Herbicides have been used to control weeds for past several decades, but are far behind to eradicate weeds from cropping systems. This is partly due to our negligence to include ecologically based multi-tactic strategies to manage weeds. This has collectively threatened sustainability of major cropping systems of the United States and around the world. Knowledge of weed biology and ecology is a prerequisite for developing effective integrated weed management (IWM) strategies.

This research quantified germination requirements of *B. scoparia* and the effect of cover crop and irrigation on emergence patterns across the U.S. Great Plains. Additionally, ecologically based multi-tactic strategies such as weed seedbank prevention, cover crops, and crop competitiveness were tested to manage HR *A. tuberculatus* in a corn-soybean rotation.

Results from this research indicate that ecologically based strategies such as cover crops, stale seedbeds, weed seedbank prevention, and crop competitiveness can effectively manage HR weed populations. However, implementation of these ecologically based strategies requires careful considerations. For example, a cereal rye cover crop reduced *A. tuberculatus* cumulative emergence by >50%. In contrast, winter wheat cover crop did not reduce *B. scoparia* cumulative emergence. Winter wheat cover crop delayed *B. scoparia* emergence, implying that more seedlings would emerge in the cash crop.

In the Great Plains, a stale seedbed approach has a higher potential in Nebraska than Montana region to stimulate *B. scoparia* emergence in the early spring and reduce weed emergence in the late-planted crops. Planting soybean in narrow rows (38-cm-wide) reduced *A. tuberculatus* seedling emergence in the crop. Additionally, strategies such as herbicides from

multiple, effective sites of action, and weed seed destruction at harvest can be used to prevent weed seed inputs to the soil seedbank in corn-soybean rotations.

Although germination requirements did not differ at optimum temperatures (14 to 26 C), at 4 C, *B. scoparia* accessions from north region had higher germination rate and cumulative germination than accessions from south region. Therefore, future research should quantify the differential germination characteristics among *B. scoparia* accessions at low temperatures (<4 C). Future research is also needed to determine how cereal rye cover crop and narrow-row soybean can affect *A. tuberculatus* percent seed retention and seed viability at the time of soybean harvest, crucial factors for the success of harvest weed seed control methods.