EVALUATION OF HARVEST WEED SEED DESTRUCTION IN U.S. SOYBEAN AND A SURVEY OF WATERHEMP POPULATIONS RESPONSE TO DICAMBA AND GLUFOSINATE

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The undersigned, appointed by the dean of the Graduate School,

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And hereby certify that, in their opinion it is worthy of acceptance.

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LITERATURE REVIEW

Justification

Waterhemp (*Amaranthus tuberculatus* (Moq.) *J. D. Sauer*), has been characterized as the most common and troublesome weed found in soybean (*Glycine max* (L.) Merr.) in the United States (VanWychen 2019). Previous research has shown that waterhemp can reduce soybean yield up to 43% when left uncontrolled (Hager et al. 2002). Herbicide-resistant waterhemp has become a widespread problem in the United States and has currently evolved resistance to seven different herbicide mechanisms of action. In many instances, certain biotypes have evolved resistance to multiple mechanisms of action (Heap 2021). The proliferation of herbicide resistance in waterhemp has created a need for new effective post-emergent control options in soybean. In 2017, agrochemical companies commercialized soybean varieties that can withstand applications of dicamba (3,6-dichloro-2-methoxybenzonic acid) to combat herbicide resistance. Previously, dicamba applications in soybean were restricted due to their inherent sensitivity to this herbicide (Solomon and Bradley 2014; Wax et al. 1969). This quickly became an attractive new option for producers because of its effective control of waterhemp and other difficult-to-control weed species (Johnson et al. 2010; Shergill et al. 2018). Another relatively new and effective option for control of waterhemp in soybean is the use of glufosinate (Aulakh and Jhala 2015). Glufosinate is a non-selective herbicide used for post-emergent control of weeds in glufosinate-resistant crops (Haas 1987). Glufosinate-resistant soybean have been commercially available in the United States since 2009. The newest option for soybean growers is varieties that are resistant to both dicamba and glufosinate. These varieties were commercialized for the 2021 growing season and are part of the continuing effort to introduce technologies that may help to combat herbicide-resistant weeds.

At this time, there have been no documented cases of waterhemp resistance to glufosinate. However, waterhemp has recently evolved resistance to dicamba in Illinois and Tennessee (Bobadilla et al. 2021; Steckel and Foster 2021). Dicamba and glufosinate are two of the most commonly used herbicides for post-emergent control of weeds in U.S. soybean production. Losing these herbicides as effective post-emergence herbicide options for waterhemp would significantly affect soybean producers. Therefore, the objective of this component of research is to evaluate the frequency and distribution of suspected resistance to dicamba and glufosinate in approximately 323 waterhemp populations collected from soybean fields in Missouri, Illinois, Indiana, Ohio, Nebraska, Tennessee, Arkansas and Louisiana.

The increasing problem of herbicide resistance in weeds such as waterhemp has resulted in a greater need for a more integrated approach to weed management, especially in U.S. soybean, cotton (*Gossypium hirsutum* L.), and corn (*Zea mays*) production systems. Harvest weed seed control (HWSC) is a management tool that prevents weed seed from returning to the soil seed bank (Walsh et al. 2016). HWSC systems are widely adopted in Australia to combat the spread of multiple herbicide-resistant weeds such as ryegrass (*Lolium spp.*) (Walsh et al. 2012). Some of the HWSC systems currently used are narrow windrow burning, bale direct system, chaff lining, and impact mills (Shergill et al. 2019). Previous research in Australia has shown that impact mills can be a successful method to reduce weed seed from returning to the soil (Walsh et al. 2012). Impact mill implements are attached to the rear of the combine and use rotating and stationary bars to destroy mature weed seed exiting the combine during grain harvest.

One impact mill commonly used in commercial production in Australia is known as the Seed Terminator™. Independent research has shown the Seed Terminator™ to be an effective HWSC tool in Australian grain production systems (Anonymous 2017). There is currently little to no published research on the ability of impact mills to destroy weed seed in United States soybean production systems. Therefore, the objectives of this component of this research are to: 1) quantify header loss of weed seed during soybean harvest, 2) determine the effectiveness of the Seed Terminator™ at destroying weed seed and reducing weed seedbanks following soybean harvest, and 3) determine the effects of the Seed Terminator™ on combine performance.

Waterhemp

Waterhemp is a very troublesome dioecious broadleaf weed of Midwest cropping systems. It is one of the most troublesome weeds because of its prolific seed production, discontinuous germination, seed persistence, and its dioecious nature which forces it to outcross with nearby waterhemp plants (Franssen et al. 2001; Hartzler et al. 1999; Steckel et al. 2007; Trucco et al. 2005). Under ideal growing conditions, female waterhemp plants possess the ability to produce up to 1,000,000 seeds per plant but generally average 250,000 seeds per plant in a typical environment (Sellars et al. 2003). Waterhemp germinates early in the growing season and can continue to germinate until the fall (Sellers et al. 2003). Early germinating waterhemp plants can reach an average height of 140 cm, but average plant height decreases with delayed emergence (Nordby and Hartzler 2004). Previous research has shown that waterhemp seed can persist in the soil for up to 4 years while maintaining high viability (Buhler & Hartzler, 2001). Waterhemp is dioecious in nature, which leads to high genetic diversity, and can contribute to the speed of selection of herbicide resistant biotypes. (Franssen et al. 2001; Trucco et al. 2005;

Norsworthy et al. 2012). Waterhemp has currently evolved resistance to seven unique herbicide mechanisms of action. These include photosystem II (PSII) inhibitors, acetolactate synthase (ALS) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, enolpyruval shikimate phosphate synthase (EPSPS) inhibitor, hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors, synthetic auxins, and very long chain fatty acid (VLCFA) inhibitors (Heap 2021; Tranel 2021). In most instances, waterhemp biotypes now possess resistance to more than one mechanism of action.

Herbicide Resistance in Waterhemp

The Weed Science Society of America (WSSA) defines herbicide resistance as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA 1998). The use of herbicides is the predominant method of weed management in most grain crops produced in the United States. Weeds respond to the selection pressure imposed on them by repeated exposures to herbicides over time. Herbicides do not create resistance; the resistant biotype exists within the natural population in very low numbers (Vencill et al. 2012). Factors that contribute to the speed of selection of resistance include the frequency of herbicide use, herbicide mechanism of action, biology of the weed species, frequency of resistant biotypes among weed species, and the mechanism responsible for herbicide resistance (Norsworthy et al. 2012). Herbicide resistance can be classified into two types: 1) target site-based resistance, and 2) non-target site-based resistance (Délye et al. 2015). Target-site-based resistance is conferred when the target site gene is altered by gene over expression, gene amplification, or modifications in amino acid sequence (Gaines et al., 2020). Non-target site resistance mechanisms include rapid metabolism, vacuolar sequestration, reduced absorption or translocation, and rapid necrosis. These resistance mechanisms do not involve alterations in the target site of the herbicide (Yuan et al. 2007).

The first case of resistance in waterhemp was reported in 1990 to PSII inhibitors, more specifically atrazine. Target site resistance was the initial mechanism identified. This resistance mechanism was not widespread due to the occurrence of a fitness penalty and the inability of this mechanism to be disseminated by pollen (Anderson et al. 1996). However, non-target-site resistance to atrazine was discovered some 20 years later. This mechanism was attributed to rapid metabolism of the herbicide (Evans et al. 2017). Although target site resistance was discovered much earlier, non-target-target site resistance now accounts for the majority of atrazine resistance in waterhemp.

Documented cases of waterhemp resistance to ALS inhibitors began to surface shortly after the widespread use of these herbicides (Heap 2021). The predominant mechanism of resistance to ALS-inhibiting herbicides is a mutation at the target site in the gene that codes for the ALS enzyme (Patzoldt & Tranel 2007). Since these mutations occur at the target site, they confer a high level of resistance. Waterhemp has also evolved non-target-site resistance to ALS-inhibiting herbicides, most likely due to cytochrome p450-based herbicide metabolism although it remains to be confirmed (Guo et al. 2015; Shergill et al. 2018).

Waterhemp was the first weed to evolve resistance to PPO-inhibiting herbicides. This was accomplished by an amino acid deletion (ΔG210) in the target site (Patzoldt et al. 2006; Shoup et al. 2003). Up to 2018, this was the only known target-site mutation for PPO-inhibitor resistance in waterhemp. Since then, two new mutations have been found in waterhemp that confer resistance at the target site (Nie et al. 2019; Tranel 2021).

In 2004, the first instance of glyphosate-resistant waterhemp was discovered in a soybean field in Missouri that had received consecutive applications of glyphosate for at least six or seven seasons (Legleiter and Bradley 2008). Since then, glyphosate-resistant waterhemp populations have been documented throughout the Midwest (Heap 2021). Currently, waterhemp confers resistance to glyphosate through three different mechanisms; reduced translocation of glyphosate, amplification of the EPSPS gene, and an amino acid substitution at the target site (Bell et al. 2013; Legleiter and Bradley 2008; Lorentz et al. 2014).

Resistance to HPPD-inhibiting herbicides is uncommon among weed species (Heap 2021). Waterhemp resistance to HPPD-inhibiting herbicides was first documented in 2009 in commercial seed corn production fields in both Illinois and Iowa. Commercial seed corn production fields are often not rotated to other crops and HPPD-inhibiting herbicides are used frequently in these fields because of their crop safety (Hausman et al. 2011; McMullan and Green 2011). Lack of crop rotation and repeated use of a single herbicide mechanism of action can contribute to the speed of selection for resistant biotypes (Norsworthy et al. 2012). The resistance mechanism to HPPD-inhibitors in waterhemp was found to be enhanced oxidative metabolism (Ma et al. 2013).

Waterhemp was first discovered to be resistant to the synthetic auxin herbicide 2,4-D in 2009. The resistant biotype was found in a field used for native grass seed production in Nebraska where 2,4-D was applied in sequential seasons, for multiple years (Bernards et al. 2012). Additional 2,4-D resistant biotypes have also been found in more traditional row crop environments in Missouri, Illinois and Nebraska. All of the resistant populations have multiple resistance to other herbicide mechanisms of action as well. The mechanisms responsible for resistance in these biotypes are still not fully understood, but in certain populations, 2,4-D resistance is due to rapid metabolism of the herbicide. (Shergill et al. 2018; Crespo et al. 2017; Evans et al. 2019).

The most recent herbicide group waterhemp has evolved resistance to is the VLCFA inhibitors. This resistance was identified in two Illinois populations that also have multiple resistance to herbicides that act at other sites of action (Strom et al. 2019; Strom et al. 2020). These cases of resistance are unique because the VLCFA-inhibiting herbicides have pre-emergent activity only, whereas all previous cases of resistance in waterhemp were to herbicides that are predominantly used for post-emergent control. The mechanism of resistance that has been characterized thus far in these populations is rapid herbicide detoxification (Strom et al. 2020).

Current Methods of Waterhemp Management in U.S. Soybean

Agrochemical companies have developed soybean varieties that can withstand applications of dicamba and glufosinate to combat the issue of herbicide resistance in waterhemp. Dicamba and glufosinate are two of the most commonly used herbicides for post-emergent control of weeds in soybean in the United States. The loss of glufosinate and dicamba as a result of further resistance evolution in waterhemp would leave most producers with only one effective post-emergent herbicide for the control of this species in soybean - 2,4-D.

Dicamba is a synthetic auxin herbicide that mimics the activity of indole-3-acetic acid, a plant hormone that regulates growth. Dicamba causes an accumulation of ethylene and abscisic acid which inhibits the growth of roots & shoots as well as destroying plant membranes and vascular systems (Grossmann 2010; Kelley and Riechers 2007). The first dicamba formulation was approved for use in the United States in 1967. It was labeled for the control of broadleaf weeds in grass crops and other non-crop areas (EPA 2006). In 2017, Monsanto, now known as Bayer, introduced cotton and soybean varieties that were able to withstand applications of dicamba. This was their solution to the herbicide resistance issue with waterhemp and other weed species. Current dicamba formulations approved for use in dicamba resistant crops are Xtendimax®, Engenia®, and Tavium®. Xtendimax® is labeled for the control of approximately 150 broadleaf weeds in dicamba-resistant cotton and soybean varieties. This includes some of the most common and troublesome weeds, found in the United States such as horseweed (*Erigeron canadensis* L.), common lambsquarters (*Chenopodium album L.*), common ragweed (*Ambrosia artemisiifolia L.*), Palmer amaranth (*Amaranthus palmeri* L.), and waterhemp (Anonymous 2020b; Van Wychen 2019). Before the introduction of dicamba resistant soybean and cotton, there were no documented cases of dicamba resistance in waterhemp. In 2021, two separate cases of dicamba resistance were reported in Illinois and Tennessee (Bobadilla et al. 2021; Steckel and Foster 2021). The adoption of dicamba-resistant crops has led to more frequent dicamba applications and will likely lead to more incidences of dicamba resistance in waterhemp.

Glufosinate inhibits the glutamine synthetase enzyme in the nitrogen assimilation pathway. This inhibition causes a substantial increase in ammonia concentration in the leaves which leads to inhibition of the light reaction in photosynthesis. The inhibition of photosynthesis then leads to the initiation of lipid peroxidation in membranes causing plant death (Hess 2000). Glufosinate is a nonselective post-emergent herbicide that was introduced in 1981 (Burke and Bell 2014). It is most commonly utilized for the control of broadleaf and grass weed species in glufosinate-resistant crops. This includes some of the most common and troublesome weed species found in the U.S. such as foxtail species (*Setaria spp.*), barnyardgrass (*Echinochloa crus-galli*), and waterhemp (Anonymous 2020a; Van Wychen 2019). At this time, Italian ryegrass (*Lolium perenne ssp. multiflorum*) and Palmer amaranth are the only two weed species in the U.S. with confirmed resistance to glufosinate. The two instances of glufosinate resistance in Italian ryegrass were documented in 2010 and 2015, and the mechanisms responsible for resistance are still unknown (Avila-Garcia and Mallory-Smith 2011; Karn et al. 2017). Palmer amaranth was found to be resistant to glufosinate in Arkansas in 2020, and the mechanism of resistance is currently unknown (Barber et al. 2021).

Potential Future Methods of Integrated Waterhemp Management in U.S. Soybean

With the increase of herbicide resistance in weeds and a decline in novel herbicides, there is a need for a more integrated approach to weed management in soybean. Some potential future methods of integrated methods of waterhemp management in soybean include the use electrocution, robotics, biologicals, cover crops and harvest weed seed control techniques. One form of harvest weed seed control is the use of impact mills. Impact mills are implements that are integrated into the rear of the combine to destroy weed seed that exit the combine during grain harvest. This is one way to target weed escapes that survived any earlier season weed management strategies. Weeds that escape earlier management methods are more likely to possess herbicide resistance traits, and if allowed to set seed, would contribute to the development of herbicide resistance in subsequent growing seasons (Jasieniuk et al. 1996; Shergill et al. 2019). Three of the most commonly used impact mill implements are the Integrated Harrington Seed Destructor® (iHSD), Redekop™, and Seed Terminator™. The Seed Terminator™ uses two multi-stage hammer mills that rotate at approximately 2500 rotations per minute (RPM) and are designed to grind, shear, and destroy weed seed (Anonymous 2017). The use of impact mills has been widely adopted in Australia but are not as common in the United States. Previous field research in Australia has shown the use of impact mills to be successful at destroying weed seed exiting the combine. Walsh et al. (2012) found that the Harrington Seed Destructor was able to destroy 93% of wild radish (*Raphanus raphanistrum* L.), 95% of rigid ryegrass (*Lolium rigidum* Gaudin), 99% of wild oat (*Avena fatua* L.), and 99% of brome grass (*Bromus* spp.) seeds during wheat (*Triticum aestivum* L.) harvest. Stationary testing of the iHSD® was also found to be successful in destroying weed seed of species commonly found in soybean production systems in the mid-southern United States. The iHSD® was able to destroy 97.5 to 100% of velvetleaf (*Abutilon theophrasti Medik.*), cocklebur (*Xanthium strumarium* L.), waterhemp, Palmer amaranth, and common lambsquarter seed (Schwartz-Lazaro et al. 2017).

Summary and Objectives

The adoption of glufosinate and dicamba resistant soybean varieties has led to more frequent applications of these herbicides. Increased use of these herbicides can contribute to the speed of selection for resistance in weed species such as waterhemp. The loss of glufosinate and dicamba as effective post-emergent control options for waterhemp would leave most producers with only one effective post-emergent herbicide for the control of this species in soybean. Therefore, the objective of the first component of this research is to evaluate the frequency and distribution of suspected resistance to dicamba and glufosinate in approximately 323 waterhemp populations collected from soybean fields in from Missouri, Illinois, Indiana, Ohio, Nebraska, Tennessee, Arkansas, and Louisiana.

Integrated weed management is necessary for the future of soybean production in the United States. No research has been conducted on the utility of impact mills in U.S. soybean production systems. Therefore, the objectives of the second component of this research are to: 1) quantify header loss of weed seed during soybean harvest, 2) determine the effectiveness of the Seed Terminator™ at destroying weed seed and reducing weed seedbanks following soybean harvest, and 3) determine the effects of the Seed Terminator™ on combine performance.

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CHAPTER II

EVALUATION OF THE SEED TERMINATOR™ IMPLEMENT AS A HARVEST WEED SEED CONTROL TOOL IN SOYBEAN

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ABSTRACT

The distribution of herbicide resistant weeds such as waterhemp (*Amaranthus tuberculatus* (Moq.) *J. D. Sauer*) is has resulted in a greater need for a more integrated approach to weed management, especially in U.S. soybean, cotton, and corn production systems. Previous research has shown harvest weed seed destruction (HWSD) to be an effective method of reducing the amount of weed seed returning to the soil. One form of HWSD is the use of impact mills to destroy weed seed exiting the combine during grain harvest. The Seed Terminator™ is one of the most common commercially available impact mill implements. In 2019 and 2020, we investigated the efficacy and economic value of the Seed Terminator™ impact mill implement in 5 Missouri soybean fields that contained significant waterhemp infestations. Results indicated that 22% to 40% of the available waterhemp seed in the field at harvest drops to the soil surface due to shatter whenever the combine head comes into contact with the waterhemp plant at the time of harvest. Across all locations, an average of 94% of waterhemp seed exiting the Seed Terminator™ was substantially damaged and considered non-viable. Consecutive seasons of use of the Seed Terminator™ on the same field in two of the five locations resulted in a significant reduction of waterhemp in the soil seedbank the spring following harvest. We were also able to determine that engine load increased by 12.5%, fuel consumption was 11.3 liters/hour and 1 liter/ha greater, and there was no statistical difference in productivity when harvesting with a

combine equipped with a Seed Terminator$^{\mathrm{TM}}$ compared to harvest with a conventional combine. Results from this research indicate that the use of impact mills could play a significant role in reducing soil weed seedbanks in soybean production systems in at least the Midwest region of the United States in the future.

INTRODUCTION

Soybean (*Glycine max (L.) Merr*) is Missouri's most economically important crop. In 2021, there were over 5.7 million acres of soybean planted, worth $3.68 billion (MU Extension 2022; USDA,2021). Weed interference poses the most significant threat to soybean yield. Left uncontrolled, weeds can reduce soybean yields in Missouri by 52% and cause a potential loss in value of $1.06 billion annually (Soltani et al. 2017).

Herbicides are the primary method for controlling weeds in U.S. soybean production. However, decades of reliance on herbicides has led to the selection of herbicide-resistant weed species. There are currently 50 herbicide-resistant species found in soybean globally, and waterhemp (*Amaranthus tuberculatus* (Moq.) *J. D. Sauer*) is the most common and troublesome weed species encountered in U.S. soybean production (Heap 2022; VanWychen 2019). Waterhemp has evolved resistance to seven different herbicide mechanisms of action. In many instances, a single population can possess resistance to multiple herbicide mechanisms of action (Heap 2022). In 2018, a Missouri waterhemp population was confirmed with resistance to six different classes of herbicides (Shergill et al. 2018) while in 2019 an Illinois population was documented with seven-way resistance (Strom et al. 2019). There has not been a novel herbicide mechanism of action discovered since the 1980s, which has left growers with few effective herbicide options for weed control in soybean. A more integrated approach to weed management is needed to extend the effectiveness of the current chemistries available. Integrated weed management (IWM) is a sustainable, effective approach that utilizes multiple strategies that consider all available chemical, mechanical, cultural, and biological methods.

Harvest weed seed control (HWSC) is one mechanical method of IWM with the ultimate objective of destroying weed seed at harvest. This approach targets weed escapes that survived earlier-season weed management strategies. Weeds that escape earlier management tactics and remain at harvest are more likely to retain herbicide resistance traits and, if allowed to set seed, would contribute to the development of herbicide resistance in subsequent growing seasons (Jasieniuk et al. 1996; Shergill et al. 2019). Seed that is not destroyed at harvest will return to the soil seedbank and will have the ability to persist for years (Buhler and Hartzler 2001; Burnside et al. 1996). The soil seedbank is a natural storage of weed seed and is influenced by previous cropping systems and environmental conditions (Schwartz et al. 2015).

One method of HWSC is the use of on-combine impact mills that are designed to grind weed seed that exit the combine during grain harvest. Impact mills are integrated into the rear of the combine and use a set of rotating and stationary bars to render weed seed non-viable upon exiting the system. Impact mills were first developed in Australia and have been highly adopted across the region. The most commonly used impact mills on the market today are the Seed Terminator™, Redekop™, and iHSD®. In Australia, impact mills are used during small grain harvest to limit the spread of multiple-resistant weed species like rigid ryegrass (*Lolium rigidum Gaudin*). Walsh et al. (2012) previously reported that the use of impact mills in Australia successfully destroyed 93 to 99% of seed from economically important weed species during commercial grain harvest of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare L.*), and lupin (*Lupinus angustifolius*). Stationary testing of the iHSD® was also found to be successful in destroying 97.5-100% of weed seed from species commonly found in soybean and rice (*Oryza sativa* L.) production systems in the mid-southern United States (Schwartz-Lazaro et al. 2017).

There is little information available on the effectiveness of impact mills used in U.S. soybean production systems. In 2019 and 2020, we evaluated the effectiveness of the Seed Terminator™ impact mill on five weed species commonly encountered in Missouri soybean production. The objectives of this research were to: 1) quantify header loss of weed seed during soybean harvest, 2) determine the effectiveness of the Seed Terminator™ at destroying weed seed and reducing weed seedbanks following soybean harvest, and 3) determine the effects of the Seed Terminator™ on combine performance.

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MATERIALS AND METHODS

Site Description

Field trials were conducted in commercial soybean production fields near Columbia, New Florence, Montgomery City, and Hallsville, Missouri in 2019, and repeated in fields near Columbia, New Florence, and Montgomery City in 2020. Consecutive years of research were conducted within the same fields at the Columbia and New Florence sites; however different fields were used near Montgomery City in 2019 and 2020. Global positioning system (GPS) coordinates were used to record plot boundaries at the Columbia and New Florence sites to conduct consecutive seasons of research. At each site, individual plots were 0.6 ha in size and were arranged in a randomized complete block design with four replications at all sites except for Montgomery City in 2019, where size constraints limited the experiment to three replications.

Harvests of each 0.6-ha plot were conducted with a Case IH Axial-Flow 8250 combine equipped with a 35-foot TerraFlex draper head (Case IH, 700 State St, Racine, WI 53404) and an on-combine Seed Terminator^{TM} impact mill (23 Aldershot Rd, Lonsdale SA 5160, Australia) (Figure 2.5). The two treatments evaluated at each site were harvesting with the Seed Terminator^{TM} engaged or harvesting with the Seed Terminator^{TM} impact mill disengaged as would occur in a conventionally harvested soybean field. The Seed Terminator™ does not have the capability to disengage whenever the combine is harvesting. So, for the conventional harvest treatment, a steel plate was fastened over the Seed Terminator™ to prevent chaff and weed seed from passing through the implement.

Waterhemp was present at all sites at the time of harvest. Waterhemp plant characteristics are listed in Table 2.1, along with harvest dates at each site. In addition to waterhemp, the Montgomery City site contained natural infestations of ivyleaf, pitted, and tall morningglory species [*Ipomoea hederacea Jacq.*; *Ipomoea lacunosa L.*; *Ipomoea purpurea (L.) Roth*], velvetleaf (*Abutilon theophrasti Medik.*), and giant foxtail (*Setaria faberi Herrm.*) in 2019, and the same morningglory species and common lambsquarters (*Chenopodium album L.*) in 2020. The plant characteristics of the weeds evaluated at the Montgomery City sites are listed in Table 2.2.

Pre-Harvest Measurements

To determine the average weed density at the time of harvest six, 1-m^2 counts of each weed species were recorded within each 0.6-ha plot. The average moisture content of each weed species present at the time of harvest was determined by collecting 16 of each of the predominant weed species present at each site. Collections were accomplished by cutting plants at the soil surface, weighing them, and recording each plant's fresh weight. Plants were then placed in a paper bag and dried in a forced-air oven at 37 C for 48 hours. Weights were recorded every 48 h until sample weight no longer decreased between measurements. After the samples were dried entirely, moisture content was determined by the difference in fresh and dry weights. Seedheads of each plant were threshed to establish a baseline of seed per plant at the time of harvest. In 2019, seeds per plant were not recorded at three sites. Estimations for these sites were based on a multi-state study that documented the weed seed retention rate of broadleaf and grass species at soybean maturity (Schwartz-Lazaro et al. 2021a; Schwartz-Lazaro et al. 2021b).

Harvest Sampling

At all sites, the combine was operated under a uniform set of harvest settings and similar speed, regardless of whether the Seed Terminator^{TM} impact mill was engaged while harvesting or not. The reel speed was operated at 9.5 rotations per 15 seconds (38 rotations/minute) and the position of the reel extended over the cutting bar by 14 centimeters. Harvest occurred at approximately 6 km/h.

Header Loss

To measure header loss of weed seed, two metals trays, each measuring 1-m^2 by two centimeters deep, were placed between rows of non-harvested soybean. For collection, the combine was operated at full capacity and normal speed until the header passed over the collection trays. Once the header completely passed over the trays, the combine was abruptly stopped to prevent contamination of chaff that was exiting the rear of the combine. All weed seed and plant material collected in the trays were emptied into paper bags and stored until subsequent processing to determine the amount of weed seed present in each sample. The number of seed in header loss samples was determined by weighing and counting 0.1g, 5.0 g, 0.5

g, 0.5g, and 0.1 g of waterhemp, morningglory spp., velvetleaf, giant foxtail, and common lambsquarters seed, respectively. After seed was weighed and counted, it was then extrapolated to calculate the amount in the entire sample. Three header loss subsamples were taken per 0.6-ha plot at each site.

Threshing Loss

Threshing losses of weed seed were defined as all weed seed that passed through the combine and was expelled from the straw spreader and/or Seed Terminator™ back onto the ground. Threshing losses were determined by placing two, 1-m^2 collection trays behind either side of the rear of the combine as it was actively harvesting at full capacity and at the uniform operating speed. All weed seed and chaff material collected in the trays were emptied into labeled paper bags and stored until subsequent processing for determining the amount of weed seed in the sample. Six threshing loss subsamples were taken per 0.6-ha at each site. Header loss and threshing loss samples represent the total percent of weed seed collected in the field at each site. Percent of non-damaged and damaged seed in each sample was determined by weighing and counting 0.1 g, 5.0 g, 0.5 g, 0.5g, and 0.1 g of waterhemp, morningglory spp., velvetleaf, giant foxtail, and common lambsquarters seed, respectively. After seed was weighed and counted, it was then extrapolated to calculate the amount in the entire sample. Threshing loss samples were also used to compare the amount of non-damaged weed seed returning to the soil between conventional and Seed Terminator™ treatments.

Seed Terminator™ Efficacy

Two insect sweep nets (Flinn Scientific, 770 N Raddant Rd, Batavia, IL 60510) were used to determine the efficacy of the Seed Terminator™ by holding these nets over each exit chute of the implement for approximately 10 seconds as the combine was actively harvesting. All material collected in the nets were emptied into labeled plastic bags and stored until subsequent processing. Six subsamples were collected per 0.6-ha plot for which the Seed Terminator™ was engaged at each site. Seed Terminator efficacy was determined by weighing and counting 0.1 g, 2.0 g, 0.75 g, 0.5g, 0.25g of waterhemp, morningglory spp., velvetleaf, giant foxtail, and common lambsquarters seed, respectively. After the seed was weighed and counted, it was then extrapolated to calculate the percent of damaged seed in the sample.

Combine Performance

The Case IH 8250 combine was equipped with an on-board computer with the ability to record an array of data points while in use. The data points that were utilized to evaluate combine performance in this research were engine load (%), productivity (ha/hr), and fuel consumption (liters/ha and liters/h). Engine load is described as an external restraining torque being applied to the engine. So, whenever an external force acts on the engine, the engine load increases. This data was recorded in separate, 'bulk' areas of the soybean fields in areas outside of the research trials. At each site, the combine harvested approximately 2 ha for comparison of conventional harvesting and harvesting with the Seed Terminator™ engaged. The number of data points subject to analysis was n= 13,481 for both years at all sites. Separate files were made in the on-board computer for each evaluation and were saved for subsequent data analysis.

Post-harvest Sampling

The spring following each harvest soil core samples were collected from conventional and Seed Terminator™ plots to assess for differences in densities of weed seed in the soil seedbank. Sites were separated for soil core analysis due to the variation in starting densities of seed in the soil. Six subsamples (7 cm diameter by 10 cm depth) were collected with a soil auger (W. W. Grainger, Inc., 100 Grainger Pkwy, Lake Forest, IL 60045) per 0.6-ha plot. All samples were stored in labeled plastic bags at -5 C until further analysis. Soil samples were then spread evenly over individual 54 by 27 by 6 cm greenhouse flats (Hummert International, 4500 Earth City Expy, Earth City, MO 63045) previously filled with a commercial potting medium (Pro-Mix BX Mycorrhizae; Premier Tech Horticulture, 127 S 5th St, Quakertown, PA 18951). Flats were maintained in the greenhouse at 30 C with natural light supplemented with metal-halide lamps (600 µmol photon m^{-2} s^{-1}) providing a 14 h photoperiod and were watered as needed. Weed seedling emergence was determined in each flat for three weeks following planting.

Processing Harvest Samples

All header loss, threshing loss, and Seed Terminator™ samples were sifted through a series of sieves, ranging from 8 mm to 0.35 mm (Seedburo Equipment Company, 2293 Mt Prospect Rd, Des Plaines, IL 60018), in order to separate larger pieces of chaff and debris from weed seed in the sample. After each sample was sieved, the sample was passed through an air column (South Dakota Seed Blower, Seedburo Equipment Company, 2293 Mt Prospect Rd, Des Plaines, IL 60018) to remove finer material and obtain a purer sample of weed seed. Samples were then examined for the number of weed seed and the percentage of damaged seed. Seed was classified as damaged if it was less than half the size of a typical seed and/or if 50% of the seed coat was missing (Figure 2.6). Weed seed that meets this criterion is expected to be non-viable (Walsh et al. 2018; Schutt et al. 2020; Davis et al. 2018).

Statistical Analysis

Soil core samples and combine performance were subject to analysis in SAS 9.4 (SAS ® Institute Inc., 100 SAS Campus Dr, Cary, NC 27513) using the PROC GLIMMIX procedure. Means were separated using Fishers Protected Least Significant Difference (LSD) with $P \leq 0.05$. For soil core analysis, sites were considered fixed effects. This is due to the variation in density of the weed seed bank at each site. However, sites were considered random for combine performance. Sites were considered random for combine performance to see the effect over a wider range of environments, rather than site-specific. Seed Terminator™ treatments were considered fixed effects, while year and replication were considered random effects. Year was considered a random effect in the model to make conclusions over a broader range of environments (Blouin et al. 2011; Carmer et al. 1989).

RESULTS AND DISCUSSION

Header and Threshing Loss of Weed Seed

Header loss is usually associated with loss of grain during harvest. In this experiment, header loss is defined as weed seed lost at the combine head due to shatter. Regardless of whether the harvest occurred conventionally or with the Seed Terminator™, if weed seed is lost at the head, the seed will return to the soil seed bank and persist for subsequent growing seasons. Threshing loss in these experiments was defined as any weed seed expelled from the rear of the combine through the straw spreader and/or Seed Terminator™ back to the soil surface. For a conventional soybean harvest, the combination of header and threshing loss measurements helps to illustrate the overall percent of seed lost at the head or deposited from the straw spreader returning to the soil surface.

Waterhemp

Figure 2.1 explains what route waterhemp seed takes to return to the soil seed bank when soybean is harvested with a conventional combine. Across seven site-years, on average 31% of waterhemp seed was lost at the combine head due to shatter. In other words, whether a producer is using conventional harvesting methods, or a harvest weed seed destruction implement, nearly one-third of the waterhemp seed will be lost at the head. The remaining 69% of waterhemp seed passes through the combine and is expelled from the rear, which gives growers an opportunity to target and destroy the seed. The three sites where the highest plant moisture was documented (Columbia 2019, New Florence 2019 and 2020), all had the lowest amount of header loss while the lowest plant moisture content and highest waterhemp seed header loss occurred in Columbia in 2020 (Table 2.1 & Figure 2.1). Based on these observations, harvesting when waterhemp has higher plant moisture content and before complete plant senescence has occurred will increase the number of seed entering the combine.

Other Weed Species

Other weed species evaluated at the Montgomery City sites were morningglory spp., velvetleaf, and giant foxtail in 2019, and morningglory spp. and common lambsquarters in 2020 (Figure 2.2). By far, the highest header loss of weed seed occurred with velvetleaf in 2019 (89%), which may be related to the high density of this weed at this site and low plant moisture content at the time of harvest (Table 2.2). Header loss of morningglory spp. seed was 48% and 58% in 2019 and 2020, respectively, while giant foxtail header loss was 52% in 2019. Header loss of common lambsquarters was 34% in 2020. Of the weed species evaluated in this research, common lambsquarters seed are perhaps most comparable in size to waterhemp, which may explain the similarity in header loss percentage of the two species (Figure 2.1 and 2.2).

Efficacy of the Seed Terminator™

Waterhemp

The Seed Terminator™ was highly effective in destroying the vast majority of weed seed passing through the combine. The implement was able to destroy an average of 94% of waterhemp seed across seven site-years, with damage percentages ranging from 77% to 99% (Table 2.3). These results are consistent with findings from Schwartz-Lazaro (et al. 2017) where the iHSD® was able to destroy 98.4% of waterhemp seed in stationary testing. Only 77% of waterhemp seed at the 2019 Columbia site was determined to be damaged. It is not clear why this percentage was so much lower than the remaining sites as waterhemp plant moisture at the time of harvest was 54%, which was similar to or lower than the plant moisture content of waterhemp at New Florence in 2019 and 2020. Continued testing of the Seed Terminator™ and other impact mill implements will be needed to fully understand their efficacy on waterhemp in other geographies and cropping systems, and to better understand the role of plant moisture content in seed destruction.

Other Weed Species

Of the weed species evaluated in this research, common lambsquarters seed are perhaps next largest in size to waterhemp, and damage to the seed of this species was 97% at the Montgomery City site in 2020 (Table 2.3). The Seed Terminator™ was able to destroy 94% and

99% of morningglory spp. seed in 2019 and 2020, respectively. Schwartz-Lazaro et al. (2017) reported the iHSD® was able to destroy 100% of morningglory spp. seeds in stationary testing. Schwartz-Lazaro et al. (2017) also reported 100% destruction of velvetleaf, but in this research, we only recorded 80% damage to velvetleaf seed at the Montgomery City site in 2019. The average damage percentage for giant foxtail seed was 98% in 2019. Walsh et al. (2018) also reported that the iHSD® was able to successfully destroy 96 to 99% of grass weed seeds commonly found in Australian cropping systems. In stationary testing, Schwartz-Lazaro et al. (2017) found the iHSD® was able to destroy 99-100% of grass species encountered in the mid-southern U.S. rice and soybean cropping systems. Overall, the Seed Terminator™ was able to damage the vast majority of weed seeds commonly encountered in Missouri soybean production systems.

Reduction of Weed Seed Returning to the Soil Seed Bank

Non-Damaged Seed in Threshing Loss Samples

Threshing loss samples allowed us to evaluate everything that is dispensed from the rear of the combine, whether it was from the straw spreader and/or the Seed Terminator™ whenever it was engaged. Overall, the results from these experiments show that not all chaff and weed seed is directed into the Seed Terminator™ whenever it is engaged. A portion of the weed seed is lost and expelled from the straw spreader. Table 2.4 provides a comparison of non-damaged weed seed collected in conventional and Seed Terminator™ threshing samples. Whenever harvest occurred with the Seed Terminator™ engaged, there was a 63 to 97% reduction in the amount of non-damaged waterhemp seed exiting the rear of the combine. When averaged across all sites this equated to an average reduction of 81% less waterhemp seed exiting the combine. When considering the 31% of waterhemp seed that is lost at the combine head due to shattering (Figure 2.1), this equates to a total of 56% less waterhemp seed returning to the soil when harvesting with a Seed Terminator™ compared to harvesting with a conventional combine. The Seed Terminator™ was also able to reduce the amount of non-damaged morningglory spp., velvetleaf, giant foxtail, and common lambsquarters seed exiting the combine and returning to the soil surface by an average of 91%, 97%, 78%, and 94%, respectively (Table 2.4).

Post-harvest Sampling

After one season of use of the Seed Terminator™, there was a reduction in the waterhemp seedbank at the Montgomery City site in 2020, but not at Hallsville in 2020 or Montgomery City in 2021 (Figure 2.3). We were able to evaluate the effectiveness of the Seed Terminator at reducing the weed seedbank in consecutive seasons at the Columbia and New Florence sites. A statistical reduction was not observed in the first year but was observed in the second year at both sites. We speculate that the increase of waterhemp seed in the soil seedbank at the Columbia site in 2021 was due to seed loss and seed shattering that occurred with a November harvest date (Table 2.1). Although it would have been ideal to harvest with the Seed Terminator™ for more than two consecutive seasons, logistical complications prevented this from occurring. Nevertheless, we believe the data thus far indicate that consecutive seasons of harvest with the Seed Terminator™ will result in substantial reductions in the amount of waterhemp seed returning to the soil seedbank. Walsh et al. (2017) also found the use of HWSC systems to be effective at reducing the amount of rigid ryegrass emergence in subsequent growing seasons in Australian cropping systems. Population densities were reduced by an average of 60% using either impact mills, chaff carts, or narrow wind row burning.

Combine Performance

An increase in engine load and fuel consumption was observed at all sites when the Seed Terminator™ was engaged. When averaged over seven site-years, engine load for the Seed Terminator™ was 85.5%, as opposed to 73% with conventional harvesting (Figure 2.4A). Increased fuel consumption was also observed in both liters per hectare and liters per hour whenever the Seed Terminator™ was engaged. Harvesting with the Seed Terminator™ engaged resulted in 1 L/ha and 11.3 L/hr greater fuel consumption than harvesting with a conventional combine (Figure 2.4 B and C). A statistical difference was not observed for productivity where the values for conventional and Seed Terminator™ harvesting were 5.7 ha/hr and 5.9 ha/hr, respectively (Figure 2.4D). Evaluating combine performance between conventional and newly discovered technologies has been done in previous research. Chegini and Mirnezami (2016) evaluated combine performance during wheat harvest with a conventional header and stripper header and found that use of the stripper header resulted in lower fuel consumption, a faster rate of harvest, and higher harvest efficiency. Producers will need to take all of these factors into account when considering the use of any new technologies.

Conclusion

With the evolution of herbicide resistant weed species continuing to grow, harvest weed seed destruction implements will have a utility in U.S. soybean production systems. Limiting the amount of seed lost at the head of the combine will be key for limiting the amount of seed returning to the soil. Our results documented that 22% to 40% of the available waterhemp seed in the field at harvest is lost due to shatter during soybean harvest. The Seed Terminator was able to successfully damage and average of 97%, 98%, 97%, 80%, and 94% of common lambsquarters, giant foxtail, morningglory spp., velvetleaf, and waterhemp seed respectively. Although not all of the weed seed is directed into the Seed Terminator™, some of the chaff and weed seed escapes through the straw spreader. However, whenever the Seed Terminator was engaged, it was able to reduce the amount common lambsquarters, giant foxtail, morningglory spp., velvetleaf, and waterhemp seed exiting the rear of the combine by 94%, 78%, 91%, 97%, and 81%, respectively, compared to conventional harvesting. For 2 of the 5 locations, we were able to evaluate the effects of consecutive seasons of use of the Seed Terminator™ on the same field. Both locations showed a significant reduction of waterhemp in the soil seedbank the spring following harvest. We were also able to determine that engine load was 12.5% higher, fuel consumption was 11.3 liters/hour greater, 1 liter/ha greater and no statitsitcal difference was observed in productivity whenever the Seed Terminator™ was engaged. Using implements like the Seed Terminator™ will aid in limiting the inputs of weed seed returning to the soil seed bank. Along with extending the effectiveness of current chemistries by curbing the selection pressure of herbicide resistant biotypes. Continued research will be needed to evaluate the effectiveness of the seed terminator in other cropping systems and geographies in the United States.

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Table 2.1. Site information and waterhemp plant characteristics at each research site in Missouri in 2019 and 2020.

		Female Waterhemp Plant Characteristics				
Site	Year	Density/m^2	Seeds per Plant	Average Fresh Weight Biomass (g)	Plant Moisture (%)	Harvest Date
Columbia	2019	3.7	121,505	168	54	10/17/2019
	2020	5.3	7,193	54	15	11/5/2020
Hallsville	2019	2.2	175,000[a]	66	26	11/7/2019
Montgomery City	2019	0.5	145,000[a]	-	-	11/19/2019
	2020	0.9	25,299	135	33	11/3/2020
New Florence	2019	5.6	190,000[a]	121	55	10/28/2019
	2020	5.8	8,550	52	73	10/15/2020

[a] Average waterhemp plant produces ~250,000 seeds and loses 6% each week after soybean maturity (Schwartz-Lazaro et al., 2021a).

Table 2.2. Plant characteristics of other weed species at the Montgomery City site in 2019 and 2020.

		Characteristics				
Weed Species	Year	Density/m^2	Seeds per Plant	Average Fresh Weight Biomass (g)	Plant Moisture (%)	Harvest Date
Common Lambsquarters	2020	0.5	86,469	193	93	11/3/2020
Giant Foxtail	2019	20	141[a]	10	20	11/19/2019
Morningglory spp.	2019	-[b]	-	-	-	11/19/2019
	2020	2.3	301	27	11	11/3/2020
Velvetleaf	2019	6.7	1,765[c]	39	10	11/19/2019

[a] Average giant foxtail plant produces ~900 seeds and loses 12.1% each week after soybean maturity (Schwartz-Lazaro et al., 2021b).

[b] Morningglory spp. was present at harvest, but no measurements were recorded in 2019.

[c] Average velvetleaf plant produces ~5,500 seeds and loses 9.7% each week after soybean maturity (Schwartz-Lazaro et al., 2021a).

Table 2.3. Damaged weed seed exiting the Seed Terminator™ at each research site in Missouri in 2019 and 2020.

Site	Weed Species	Year	Damaged weed seed exiting the Seed Terminator™
			--% of Total --
Columbia	Waterhemp	2019	77
		2020	99
Hallsville	Waterhemp	2019	99
Montgomery City	Waterhemp	2019	91
		2020	98
	Morningglory spp.	2019	94
		2020	99
	Velvetleaf	2019	80
	Giant Foxtail	2019	98
	Common Lambsquarters	2020	97
New Florence	Waterhemp	2019	98
		2020	99

Table 2.4. Comparison of non-damaged weed seed deposited from the combine in conventionally harvested and Seed Terminator™ threshing samples.

Site	Year	Weed Species	Harvest Type: Conventional Combine	Harvest Type: Combine with Seed Terminator™	Reduction in Field Deposition of Weed Seed due to Seed Terminator™
			------------ # Waterhemp seed/m^2 ------------		------------ % ------------
Columbia	2019	Waterhemp	10,289	330	97
	2020	Waterhemp	9,450	344	96
Hallsville	2019	Waterhemp	16,897	3,670	78
Montgomery City	2019	Giant Foxtail	215	48	78
		Morningglory spp.	15	2	90
		Velvetleaf	194	6	97
		Waterhemp	128	43	66
	2020	Common Lambsquarters	547	32	94
		Morningglory spp.	50	4	92
		Waterhemp	1,640	48	97
New Florence	2019	Waterhemp	10,190	3,802	63
	2020	Waterhemp	6,179	1,619	74

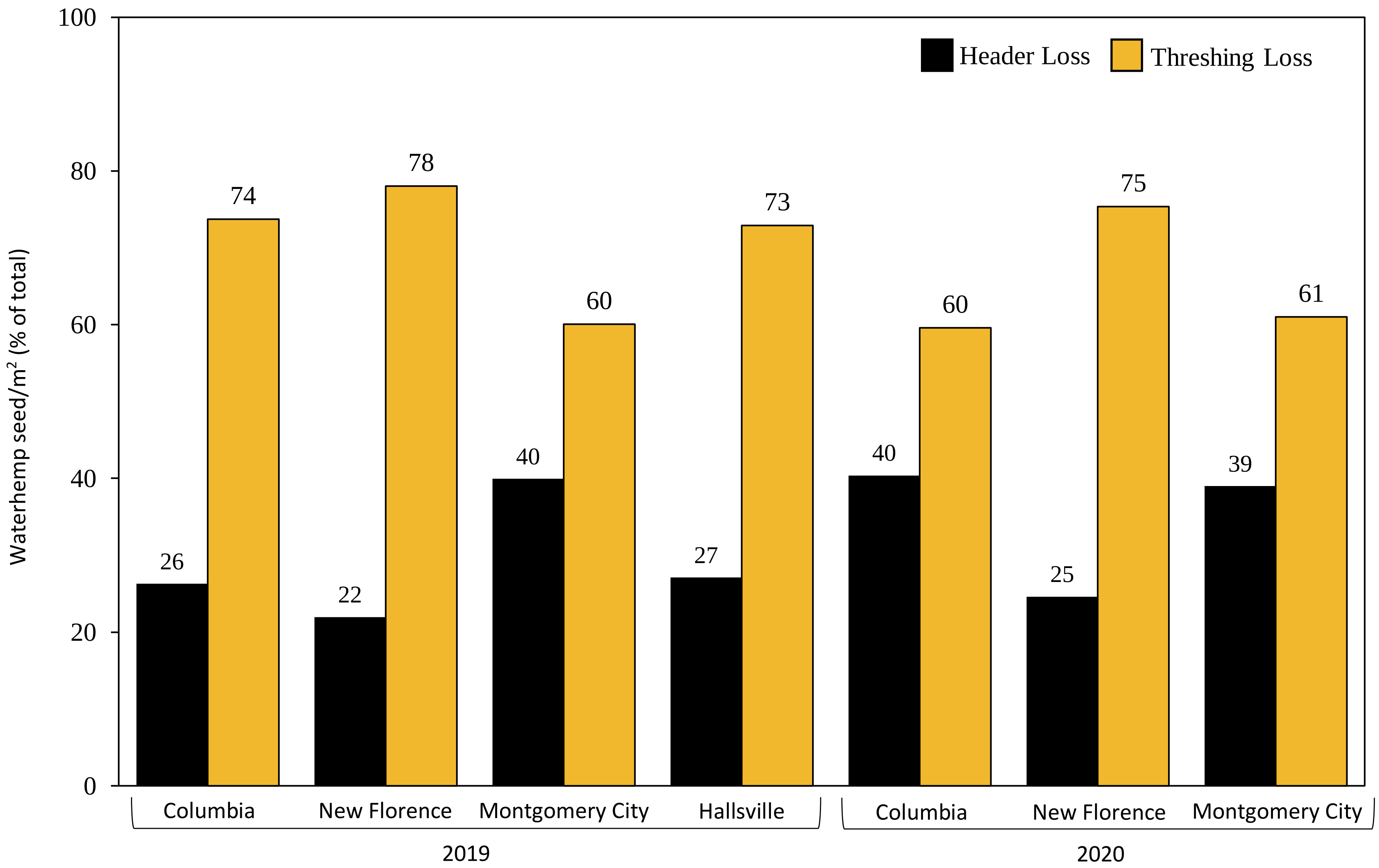

Figure 2.1. Header and threshing loss of waterhemp seed at seven Missouri sites harvested with a conventional combine in 2019 and 2020.

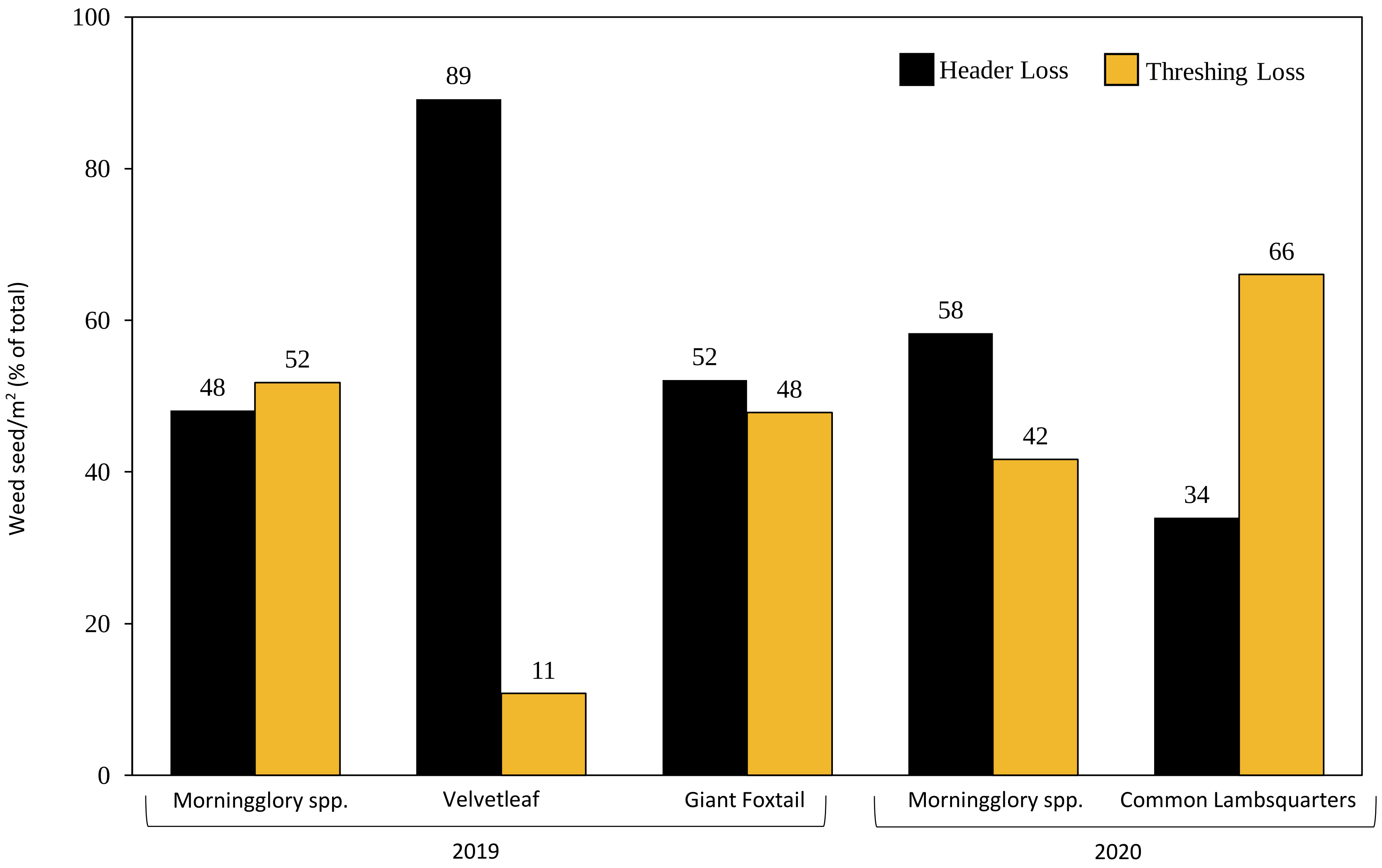

Figure 2.2. Comparison of header and threshing loss of weed seed with a conventional combine at Montgomery City sites in 2019 and 2020.

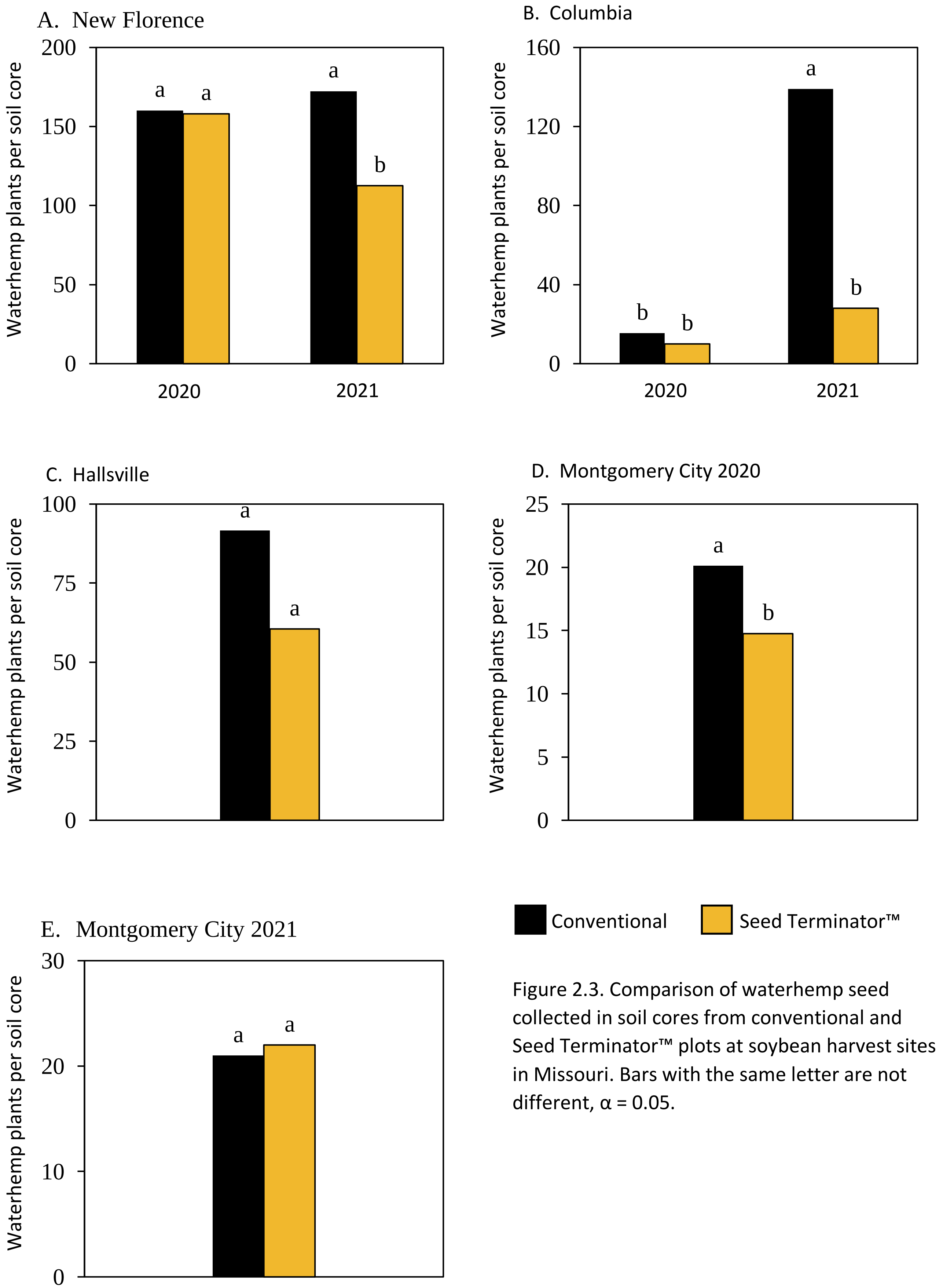

Figure 2.3. Comparison of waterhemp seed collected in soil cores from conventional and Seed Terminator™ plots at soybean harvest sites in Missouri. Bars with the same letter are not different, α = 0.05.

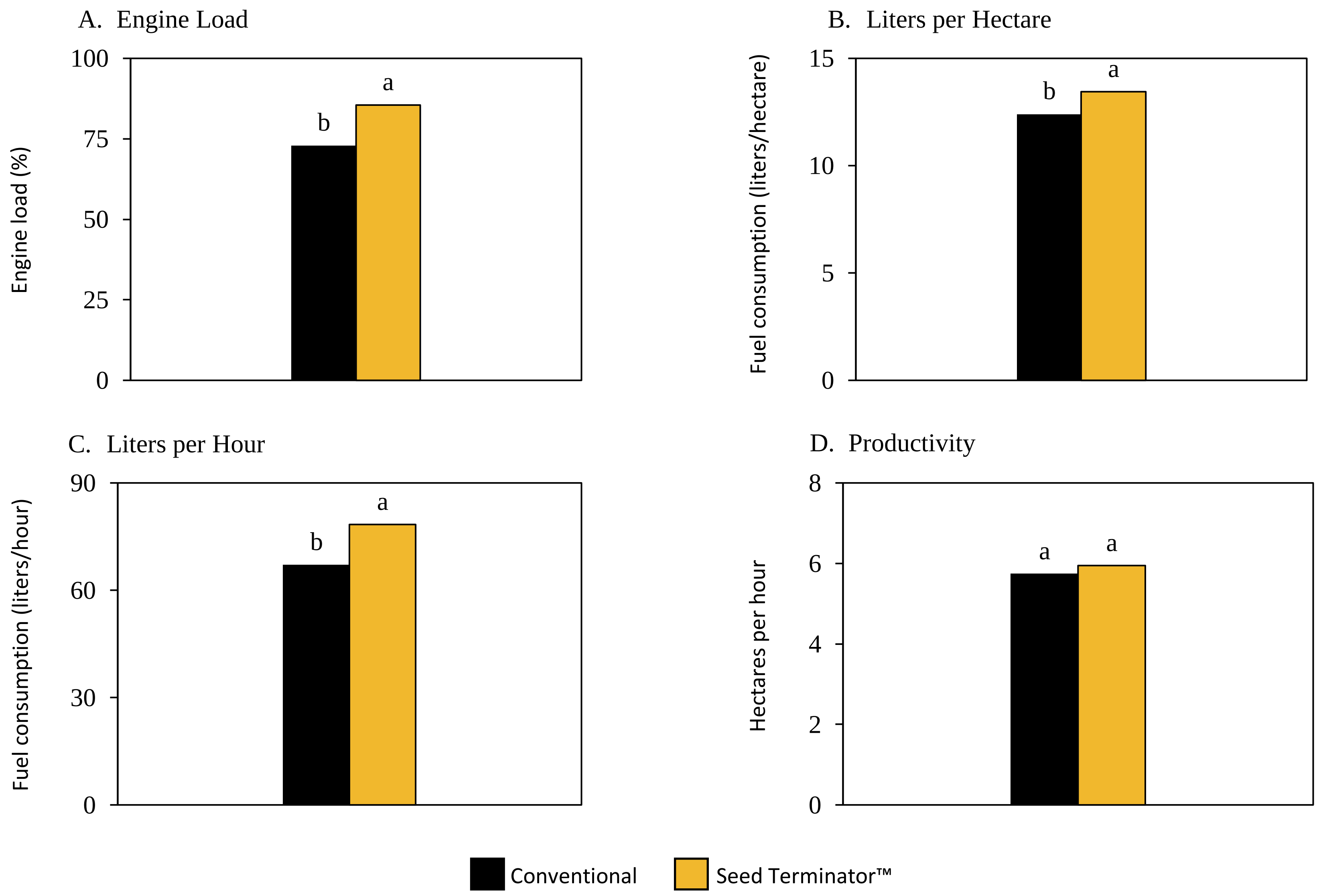

Figure 2.4. Performance of a conventional combine compared to a combine equipped with a Seed Terminator™. Results are combined from 7 site years. Bars with the same letter are not different, α = 0.05.

Figure 2.5. A Case IH 8250, equipped with a Seed Terminator™ implement.

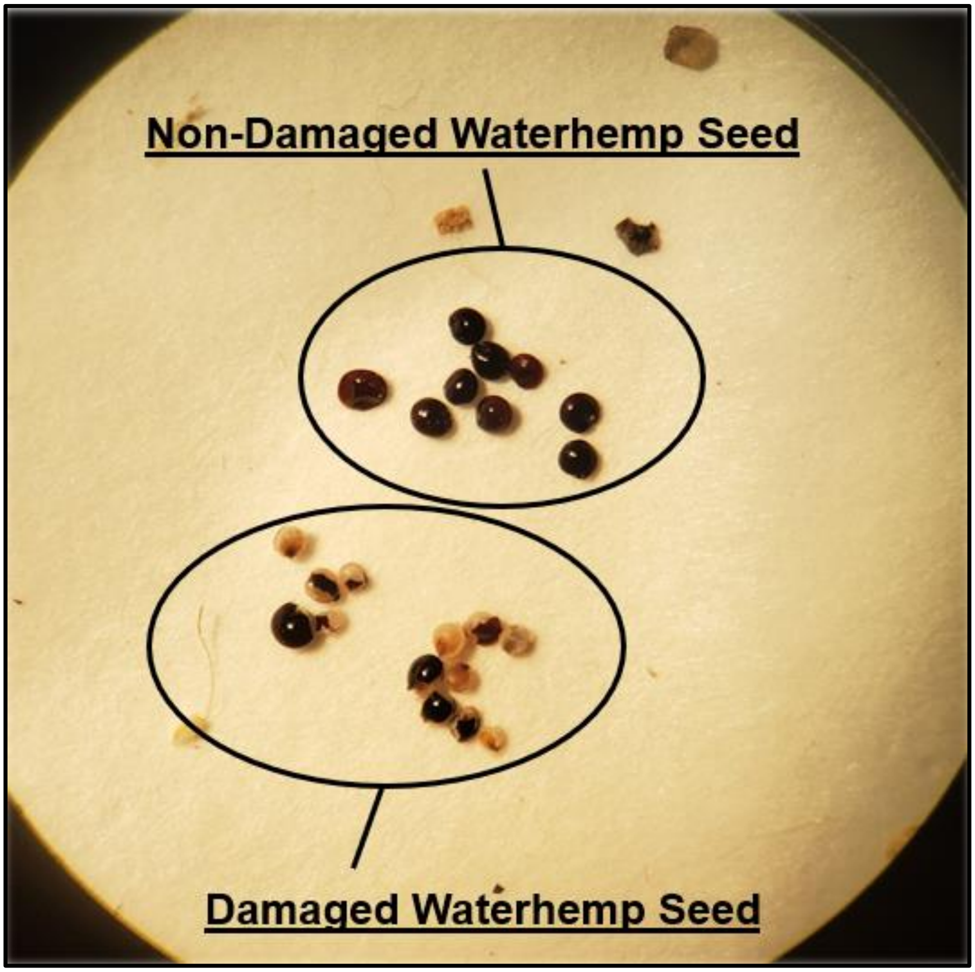

Figure 2.6. Damaged and non-damaged waterhemp seed collected from a Seed Terminator™ threshing loss sample. Waterhemp seed is black to dark red in color and measure 0.8- 1.0mm in diameter.

CHAPTER III

A MULTI-STATE EVALUATION OF THE RESPONSE OF WATERHEMP POPULATIONS FOUND IN SOYBEAN TO DICAMBA AND GLUFOSINATE

Travis Winans, Mandy Bish, and Kevin Bradley

ABSTRACT

Currently, there have been no documented cases of waterhemp with resistance to dicamba or glufosinate. However, there have been instances where a lack of complete control has been reported to either of these herbicides. In 2019, 2020, and 2021 waterhemp (*Amaranthus tuberculatus)* populations collected from soybeans fields in Arkansas, Illinois, Indiana, Louisiana, Missouri, Nebraska, Ohio, and Tennessee were screened to characterize their ability to withstand discriminating doses of dicamba and glufosinate. All waterhemp populations were treated with 560 g ae ha^{-1} and 1120 g ae ha^{-1} of dicamba, and 289 g ai ha^{-1} and 594 g ai ha^{-1} of glufosinate plants reached 8 to 10 cm in height. Visual injury ratings and survival counts were taken 21 days after application. Mean waterhemp survival percentages for the 560 g ae^{-1} dicamba use rate were 30%, 35%, and 11% for 2019, 2020, and 2021 respectively. Survival percentages in response to the 594 g ai ha^{-1} use rate of glufosinate ranged from 12% to 14% for all three years. Results from this survey help us to understand the potential frequency and distribution of waterhemp resistance to dicamba and glufosinate, two of the most common herbicides that are currently being utilized for post-emergence weed control in soybean production in the United States.

INTRODUCTION

Waterhemp (*Amaranthus tuberculatus (Moq.) J. D. Sauer*) is a dioecious summer annual weed native to the United States and has been described as the most common and troublesome species found in soybean (*Glycine max (L.) Merr.*) (USDA 2022; VanWychen 2019). Waterhemp's success can be attributed to its seed dormancy, extended germination throughout the summer months, rapid growth rate, and prolific seed production (Steckel et al. 2007; Hartzler et al. 1999, 2004; Sauer 1957). If left uncontrolled in soybean, waterhemp can cause yield losses between 37 and 44% (Hager et al. 2002; Steckel and Sprague 2004).

Waterhemp favors no-tillage practices because of its shallow seed placement in the soil profile (Felix and Owen 1999). Farmer et al. (2017) determined that deep tillage resulted in a 73% reduction in *Amaranthus* species emergence compared to no-tillage. Prior to the 1990s, tillage was a key component for weed management in U.S. crop production systems. Since then, the adoption of glyphosate-resistant crops and other management factors has led to an increase in no-tillage acres in the U.S. (Young 2006). Only 5% of planted crop acres were produced using conservation practices in 1989, compared to 50% of acres in 2012 and 2015-2017 (CTIC 2022; USDA 2018). The adoption of herbicide-resistant crops and conservation tillage practices has amplified the reliance on herbicides for weed control in soybean (Culpepper et al. 2000; Young 2006). Years of successful, simplified weed management using herbicides alone has led to the selection of herbicide-resistant biotypes. In the United States, waterhemp has evolved resistance to photosystem II (PSII) inhibitors, acetolactate synthase (ALS) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, enolpyruval shikimate phosphate synthase (EPSPS) inhibitor, hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors, synthetic auxins, and very-long-chain fatty acid (VLCFA) inhibitors (Heap 2021). Since waterhemp is dioecious in nature, biotypes can more easily transfer resistant genes from male to female plants via pollen (Liu et al. 2012; Tranel 2021).

In response to the increasing problem of herbicide resistance in waterhemp, agrochemical companies have developed soybean varieties that can withstand applications of dicamba, 2,4-D, and glufosinate. This has led to an increased use of these herbicides because of their effective post-emergent control of waterhemp in soybean (Johnson et al. 2010; Shergill et al. 2018; Aulakh and Jhala 2015; Craigmyle et al. 2013). The latest option for soybean growers is varieties that are resistant to both dicamba and glufosinate. These varieties were commercialized for the 2021 growing season and are part of the continuing effort to introduce technologies that may help to combat herbicide-resistant weeds. At this time, there have been no reported cases of waterhemp resistance to glufosinate. However, dicamba-resistant waterhemp populations were documented in Illinois and Tennessee for the first time in 2021. (Bobadilla et al. 2021; Steckel and Foster 2021). With the increased use of dicamba and glufosinate, it is crucial to understand the potential frequency and distribution of waterhemp populations that may express decreased sensitivity to these herbicides.

Previous herbicide resistance surveys for waterhemp have been conducted in geographies across North America to cite the prevalence of populations with decreased sensitivity to herbicides. In Missouri, surveys of herbicide-resistant waterhemp populations have provided valuable insights regarding the distribution and prevalence of resistance in this species across the state. Rosenbaum and Bradley (2013) determined that 99 out of 144 populations screened from Missouri were resistant to glyphosate (EPSPS). Schultz et al. (2015) confirmed resistance of waterhemp populations collected from Missouri soybean fields to acetolactate synthase (ALS), protoporphyrinogen oxidase (PPO), photosystem II (PSII), and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors. In Illinois, glyphosate resistance was documented in 22 of 80 waterhemp populations collected in 2010 (Chatham et al. 2015). A survey in Ontario, Canada found waterhemp resistance in 82%, 100%, and 76% of populations to glyphosate (EPSPS), imazethapyr (ALS), and atrazine (PSII), respectively (Schryver et al. 2017). These type of surveys for herbicide resistance are conducted to provide producers with information regarding the regional distribution of a particular resistant weed species so that they may adjust management practices accordingly. Therefore, the objective of this research was to evaluate the frequency and distribution of suspected resistance to dicamba and glufosinate in 323 waterhemp populations collected from soybean fields in Missouri, Illinois, Indiana, Ohio, Nebraska, Tennessee, Arkansas, and Louisiana.

MATERIALS AND METHODS

Seedheads from mature female waterhemp plants were collected in 323 soybean fields across eight states in 2018, 2019, and 2020. Cooperating states in the survey were Missouri, Illinois, Indiana, Ohio, Nebraska, Tennessee, Arkansas, and Louisiana. Seedheads were collected approximately two weeks after soybean senescence during September and October each year. Approximately 20 seedheads were collected from each field and combined which constituted a population. Sites for waterhemp collection were primarily determined by scouting soybean fields at harvest for the presence of waterhemp and/or collecting from fields where known herbicide failures occurred. Global positioning system (GPS) coordinates were used to record the location where each population was collected. Following collection, seed were gleaned from waterhemp by threshing seedheads and were sifted through a series of sieves, ranging from 8 mm to 0.35 mm (Seedburo Equipment Company, 2293 Mt Prospect Rd, Des Plaines, IL 60018). After samples were sieved, they were then passed through an air column (South Dakota Seed Blower, Seedburo Equipment Company, 2293 Mt Prospect Rd, Des Plaines, IL 60018) to remove finer material and obtain a purer sample of waterhemp seed. Populations were stored at -5 ºC for approximately four months until screening commenced.

Prior to planting, 0.2 grams of waterhemp seed from each population were weighed and placed into a single 50 mL centrifuge tube (ThermoFisher Scientific, 81 Wyman St, Waltham, MA 02451). For every round of screening, all populations had four 50 mL centrifuge tubes each containing 0.2 grams of seed. Immediately before planting, 25 mL of a 1:1 bleach to water ratio was poured into each tube containing seed. Tubes were put into a rack and placed on a platform shaker (New Brunswick Scientific, 44 Talmadge Rd, Edison NJ 08817) for 15 minutes. The bleach solution was then rinsed out by covering the tube's opening with cheesecloth (GoodCook, 3122 Santa Monica Blvd, Santa Monica, CA 90404) to prevent loss of seed. After rinsing, the tubes were then filled with 40 mL of water. This process was used to improve germination and sterilize the seed (Schultz et al. 2015).

The waterhemp seed and water solution was then evenly distributed across 54 by 27 by 6 cm greenhouse flats (Hummert International, 4500 Earth City Expy, Earth City, MO 63045) with manual single channel pipettes (ThermoFisher Scientific, 81 Wyman St, Waltham, MA 02451). The greenhouse flats were filled with a 1:1 ratio of topsoil and commercial potting medium (Pro-Mix BX Mycorrhizae; Premier Tech Horticulture, 127 S 5th St, Quakertown, PA 18951). Flats were maintained at 30 ºC with natural light supplemented by metal-halide lamps (600 µmol photon m^{-2} s^{-1}), providing a 14-hour photoperiod. Due to limited on-campus greenhouse space and time constraints, hoop house facilities located at Bradford Research Center (38.89°N, -92.20°W) were utilized to conduct one biological replicate of the glufosinate screening in 2020 and two biological replicates of the dicamba screening in 2021. The hoop houses were only utilized for screening waterhemp populations during the months of June through September, as there was no supplemental light or heat provided in these facilities. All waterhemp plants were fertilized one week before applications and watered as needed throughout the course of the experiments.

Applications of dicamba and glufosinate (Table 3.1) were made once waterhemp populations reached 8 to 10 cm in height. Applications were made using a CO_2-pressurized backpack sprayer that delivered 140 L ha^{-1} at 144 kPa. Dicamba applications were made with 11002 induction nozzle tips (TeeJet®, Spraying Systems Co., PO Box 7900, Wheaton, IL 60187). Dicamba was applied at rates of 560 g ae ha^{-1} and 1120 g ae ha^{-1}. Applications of glufosinate were applied at rates of 289 g ai ha^{-1} and 594 g ai ha^{-1} with a CO_2-pressurized backpack sprayer equipped with 8002 flat-fan nozzle tips (TeeJet®). Discriminating doses were used to identify populations that may exhibit a lack of sensitivity to either herbicide. Treatments were arranged in a randomized complete block design with two biological replicates and two technical replicates for each use rate and herbicide.

Ratings of percent visual waterhemp control were made 14, 21, and 28 days after application (DAA) for dicamba and 7, 14, and 21 DAA for glufosinate. Visual estimates of percent control were determined on a 0 to 100% scale, with 0% indicating no phytotoxic effects present and 100% indicating complete plant death. Injury estimates considered chlorosis, necrosis, and growth inhibition. Survival ratings were taken 21 DAA for both herbicide treatments. A waterhemp plant was deemed to have survived the application at 21 DAA if green tissue remained. Percent survival was determined by dividing the number of plants surviving the treatment 21 DAA by the number of plants present at the time of application.

Data was subject to analysis in SAS 9.4 (SAS ® Institute Inc., 100 SAS Campus Dr, Cary, NC 27513) using the PROC GLIMMIX procedure. Means were separated using Fisher's Protected Least Significant Difference (LSD) with $P \leq 0.05$. Herbicides and application rates were considered fixed effects, while year and waterhemp populations were considered random effects. Data from each year was combined and displayed in box and whisker plots using Sigma Plot. (Systat Software Inc., 2107 N 1st St, Ste 360, San Jose, CA 95131).

RESULTS AND DISCUSSION

Dicamba

Waterhemp populations screened in 2021 had consistently higher visual control and lower survival percentages in response to both rates of dicamba when compared to the populations screened in 2019 and 2020 (Figures 3.1 and 3.2). In 2020, mean visual control of the waterhemp populations in response to either dicamba rate was lower than that observed for the populations screened in 2019. For example, the average control of the waterhemp populations in response to the 1120 g ae^{-1} dicamba use rate was 85% in 2019 and 71% in 2020. However, percent survival of the populations in response to the 1120 g ae ha^{-1} use rate was 13% and 12% in 2019 and 2020, respectively. Control percentages statistically separated for populations screened with the 560 g ae ha^{-1} dicamba use rate in 2019, 2020, and 2021 at 64%, 48%,and 95%, respectively. Mean waterhemp survival was similar in response to the 560 g ae ha^{-1} dicamba use rate in 2019 (30%) and 2020 (35%), but much less for populations in 2021 (11%).

Waterhemp populations from Illinois, Indiana, and Missouri were included in all three years of this survey. However, there was no trend towards increasing waterhemp survival from

2019 to 2021 in either of these states, or in Nebraska and Ohio where collections only occurred in two years (Table 3.2). Missouri was the only state that displayed increasing survival percentages from 2019 to 2020 at both use rates. In 2021, states displayed higher visual control ratings and lower survival percentages than in 2019 or 2020, which may be related to the environmental conditions at the time of the experiments. Both biological replicates of the dicamba screenings took place in outdoor hoop houses in 2021 whereas the experiments in 2019 and 2020 were conducted in greenhouses.

Over the three years of this survey, there were 34 counties across five states with populations that contained plants that survived dicamba in all biological and technical replicates (Figure 3.3). Further characterization would be needed to determine if one or more of these populations are resistant to dicamba. Dicamba resistance had not been documented in waterhemp until 2021. The first reported cases were in Champaign county Illinois, and Montgomery county Tennessee (Bobadilla et al. 2021; Steckel and Foster 2021). With the initial release of dicamba-resistant soybean occurring only four years earlier, this could be a direct result of increased use of dicamba for post-emergent weed control. Continued screening efforts will be needed to monitor the status of the efficacy of dicamba for post-emergent control of waterhemp.

Glufosinate

The average visual control of waterhemp following application with glufosinate at 594 g ai ha^{-1} was 89, 91, and 90% for populations collected in 2019, 2020, and 2021, respectively (Figure 3.4). There was more variability in the response of these populations to the 289 g ai ha^{-1} glufosinate use rate, but the average control still ranged from 64 to 78%. Similar results were observed for plant survival; waterhemp populations collected across all three years responded similarly to the 594 g ai ha^{-1} glufosinate use rate with average survival ranging from 12% to 14% (Figure 3.5). However, there was greater variability in survival to the 289 g ai ha^{-1} use rate, with average survival ranging from 27 to 42%.

Missouri, Illinois, and Indiana waterhemp populations responded similarly to the 289 g ai ha^{-1} and 594 g ha^{-1} glufosinate use rates (Table 3.3). Similar results occurred with the two years of waterhemp populations collected in Nebraska and Ohio. Overall, there was no trend towards an increase or decrease in the survival or control of the waterhemp populations across the three years of the study (Table 3.3).

There were 34 counties across four states with populations where survivors were recorded in all biological and technical replicates 21 DAA of glufosinate (Figure 3.6). Further analysis will be necessary to determine if any specific population is resistant to glufosinate. At this point, there have been no waterhemp populations documented with resistance to glufosinate. However, a Palmer amaranth (*Amaranthus palmeri S. Watson*) population in northeast Arkansas was confirmed to be resistant to glufosinate in 2021. This is a result of cotton (*Gossypium hirsutum* L.) producers relying heavily on glufosinate since 2007 (Barber et al. 2021). Similar to dicamba, increased use of glufosinate for post-emergent control in soybean will amplify the selection rate for resistant biotypes. With no clear timeline for the release of herbicides with novel mechanisms of action, growers are left with dicamba, glufosinate, and 2,4-D as the only effective options for post-emergent control of waterhemp in soybean (Duke 2012). Losing these herbicides as effective options would be disadvantageous to soybean producers.

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Table 3.1. Sources of herbicides used in the experiments.

Active Ingredient[a]	Formulation	Trade Name	Manufacturer	Address
Dicamba[b]	2.9 SL	XtendiMax®	Bayer	St. Louis, Missouri
Glufosinate[c]	280 SL	Liberty®	BASF	Raleigh, North Carolina
AMS	3.4 SL	Amsol™	Winfield United	St. Paul, Minnesota
MDS	3.4 SL	Class Act® Ridion®	Winfield United	St. Paul, Minnesota

[a] Abbreviations: AMS, Ammonium sulfate; MDS, Monocarbamide dihydrogen sulfate; SL, soluble (liquid) concentrate.

[b] Treatment contained monocarbamide dihydrogen sulfate.

[c] Treatment contained ammonium sulfate.

Table 3.2. Average response of 323 waterhemp populations collected across eight states and three years to discriminating doses of dicamba.

			Dicamba			
			560 g ae ha^{-1}		1120 g ae ha^{-1}	
State	Populations	Year	Visual Control 21 DAA	Survival 21 DAA	Visual Control 21DAA	Survival 21 DAA
			--	---------- %[a] ----------	--	
Arkansas	1[b]	2021	93 a	12.3 de	96 ab	9 abc
Illinois	16	2019	66 bc	29.4 bc	81 c	18 a
	61	2020	48 f	36.9 a	72 de	11 ab
	39	2021	94 a	10.8 de	93 b	11 ab
Indiana	15	2019	68 bc	29.9 bc	87 bc	11 ab
	19	2020	52 e	32.5 bc	72 de	12 ab
	16	2021	95 a	10.7 de	96 ab	10 ab
Louisiana	6	2019	-	-	94 ab	7 bc
Missouri	26	2019	70 b	28.6 c	89 b	10 ab
	40	2020	46 g	35.2 ab	70 e	13 ab
	27	2021	95 a	10.6 de	96 ab	7 bc
Nebraska	11	2019	69 b	33.1 abc	87 bc	17 a
	8	2021	97 a	6.1 e	99 a	3 c
Ohio	15	2020	55 d	27.9 c	73 d	8 bc
	20	2021	96 a	7.5 e	97 a	6 c
Tennessee	3	2020	62 c	17.4 d	81 c	2 c

[a] Mean values for visual control 21 DAA and survival 21 DAA followed by the same letter are not different ($\alpha = 0.05$)

[b] Values indicate the number of populations screened for the given state in that year.

Table 3.3. Average response of 323 waterhemp populations collected across eight states and three years to discriminating doses of glufosinate.

			Glufosinate			
			289 g ai ha^{-1}		594 g ai ha^{-1}	
State	Populations	Year	Visual Control 21 DAA	Survival 21 DAA	Visual Control 21DAA	Survival 21 DAA
			% [a]			
Arkansas	1[b]	2021	78.3 a-e	22 bc	95 ab	7 a
Illinois	16	2019	81.2 abc	28 b	88 b	15 a
	61	2020	61.1 e	47 a	90 b	15 a
	39	2021	70.9 e	31 b	88 b	17 a
Indiana	15	2019	78.9 a-d	27 b	88 b	11 a
	19	2020	65.6 e	38 b	92 b	12 a
	16	2021	83.3 ab	19 c	91 b	12 a
Louisiana	6	2019	92.3 a	14 c	95 ab	15 a
Missouri	26	2019	74.2 ed	33 b	90 b	10 a
	40	2020	64.5 e	41 b	90 b	13 a
	27	2021	73.0 ed	32 b	91 b	14 a
Nebraska	11	2019	72.9 ed	33 b	94 ab	9 a
	8	2021	72.3 ed	36 b	91 b	13 a
Ohio	15	2020	78.1 b-e	26 b	96 a	8 a
	20	2021	77.1 cde	27 b	88 b	13 a
Tennessee	3	2020	47.9 f	58 a	95 ab	8 a

[a] Mean values for visual control 21 DAA and survival 21 DAA followed by the same letter are not different ($\alpha = 0.05$)

[b] Values indicate the number of populations screened for the given state in that year.

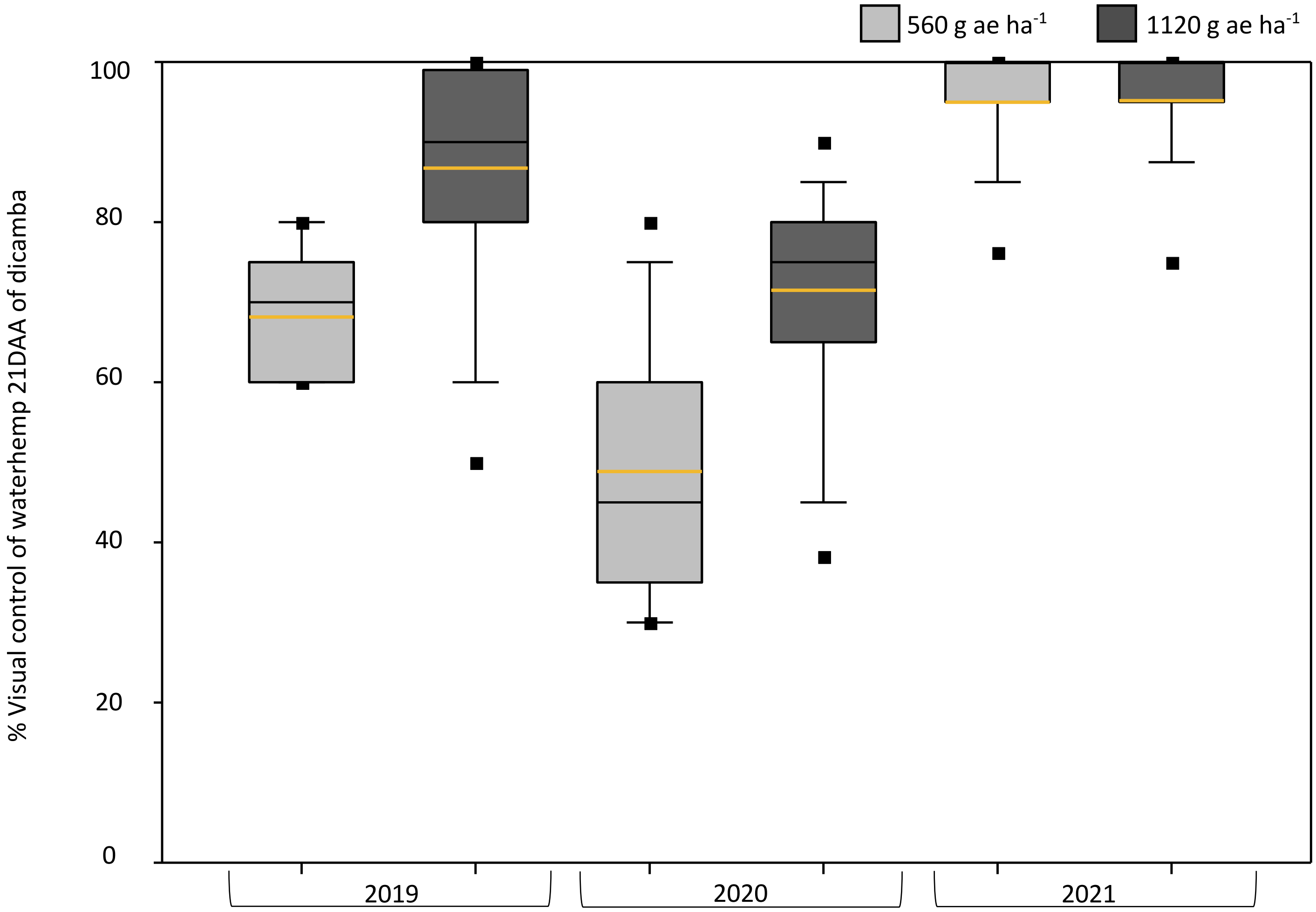

Figure 3.1. Visual control of waterhemp populations 21 days after application with dicamba. Gold lines denote mean visual control; black lines within the box denotes the median. Vertical lines represent the minimum and maximum data points. Black squares denote outliers.

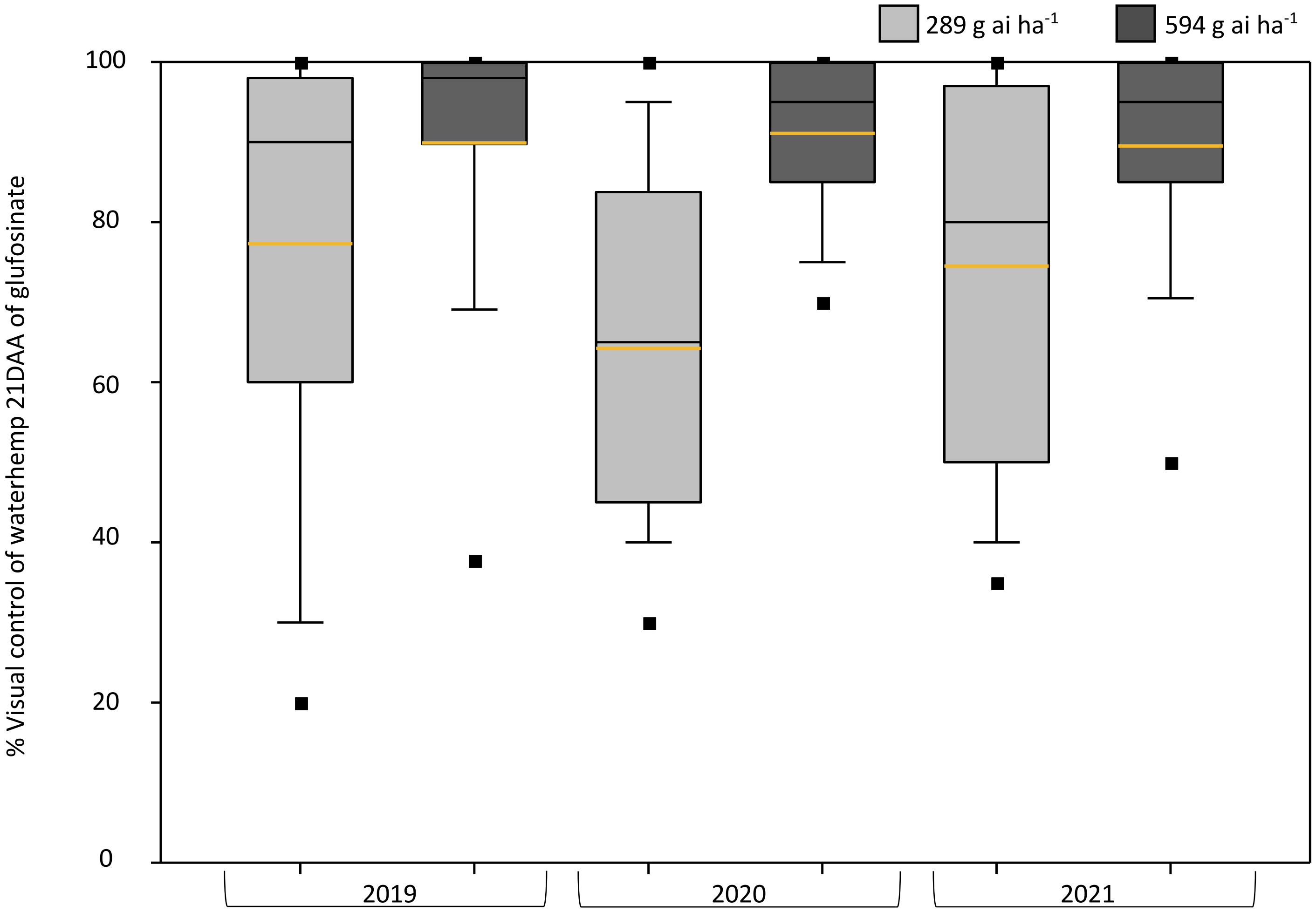

Figure 3.2. Visual control of waterhemp populations 21 days after applications of glufosinate. Gold lines denote mean visual control; black lines within the box denotes the median. Vertical lines represent the minimum and maximum data points. Black squares denote outliers.

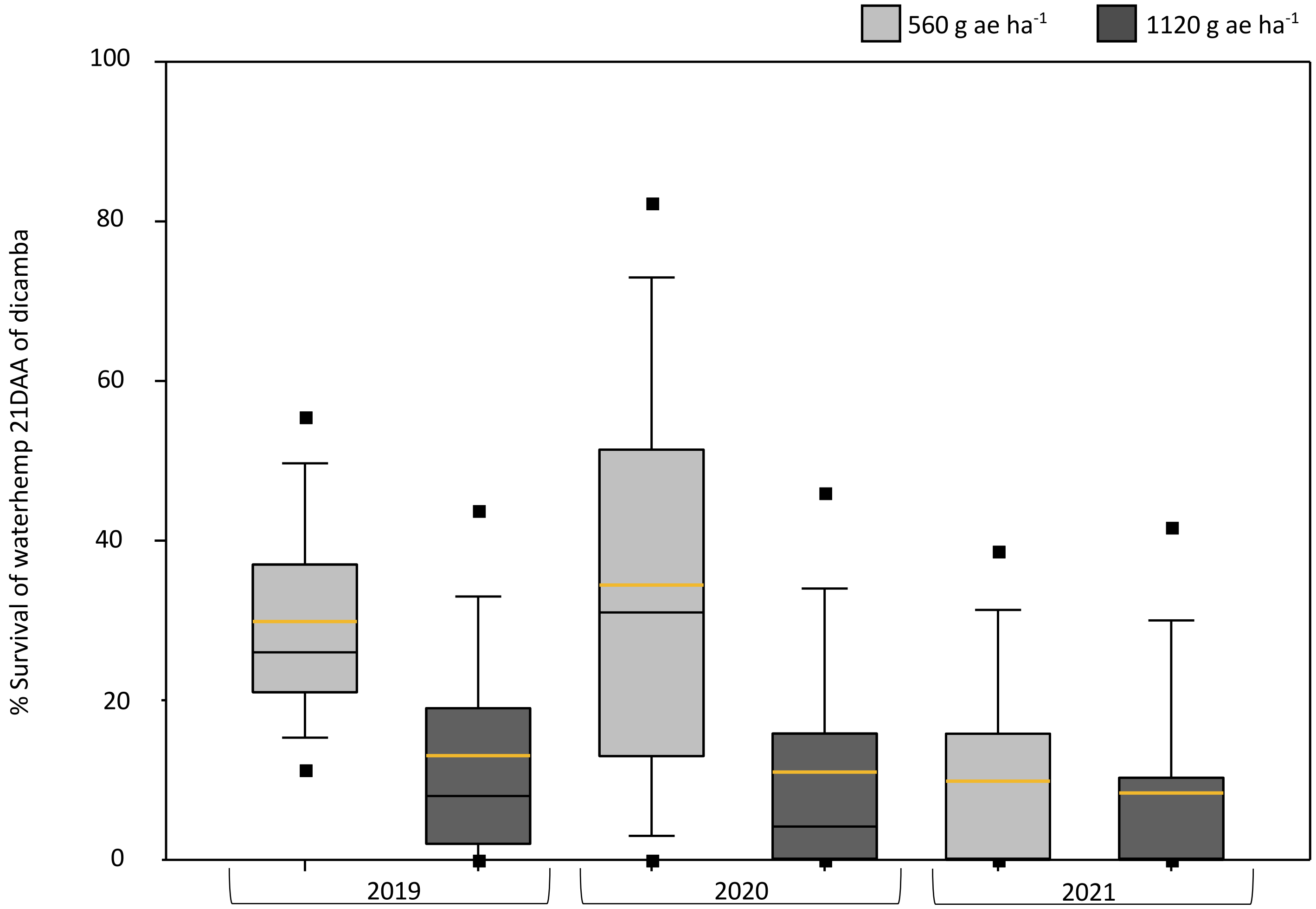

Figure 3.3. Percent survival of waterhemp populations 21 days after applications of dicamba. Gold lines denote mean visual control; black lines within the box denotes the median. Vertical lines represent the minimum and maximum data points. Black squares denote outliers.

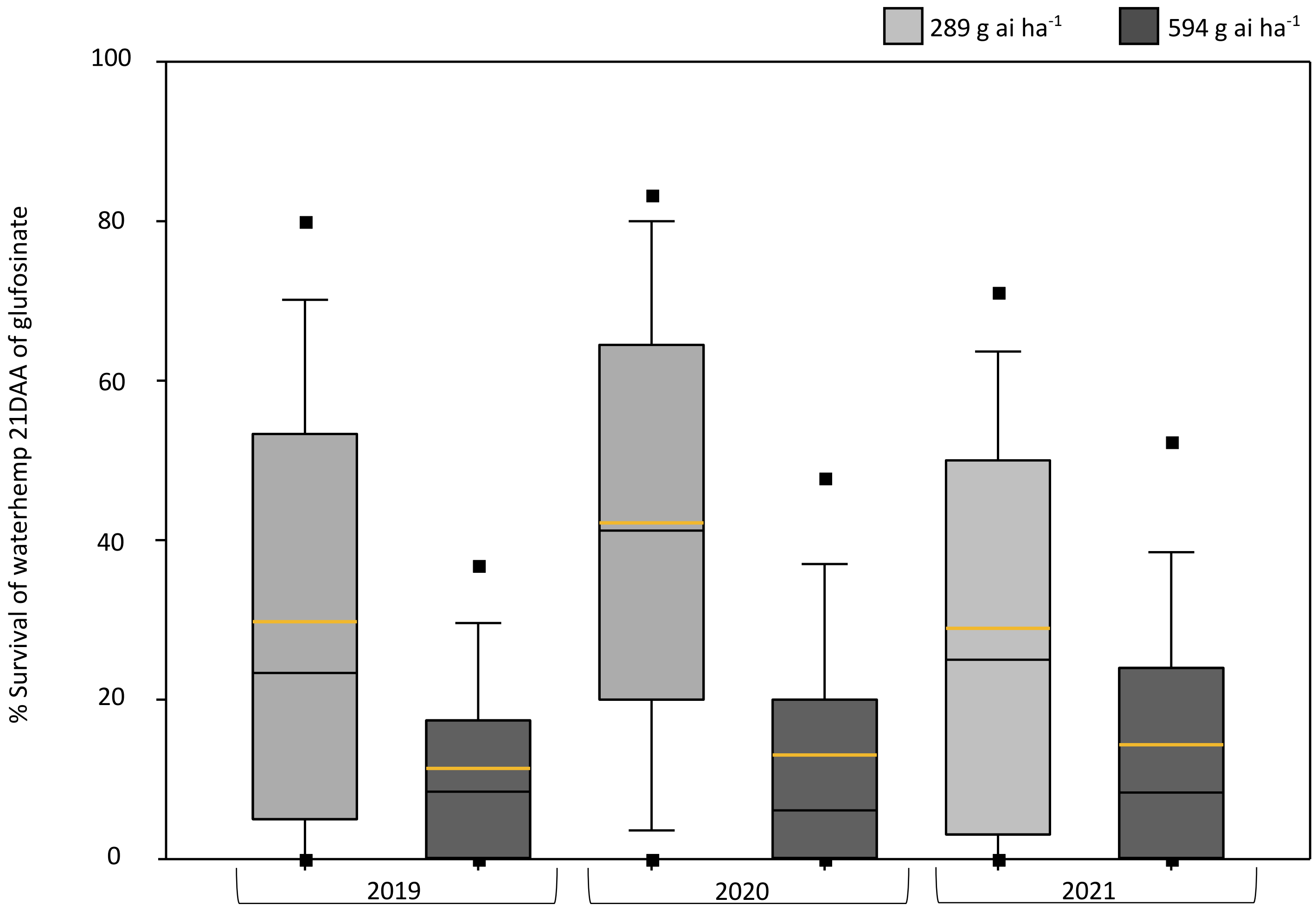

Figure 3.4. Percent survival of waterhemp populations 21 days after applications of glufosinate. Gold lines denote mean visual control; black lines within the box denotes the median. Vertical lines represent the minimum and maximum data points. Black squares denote outliers.

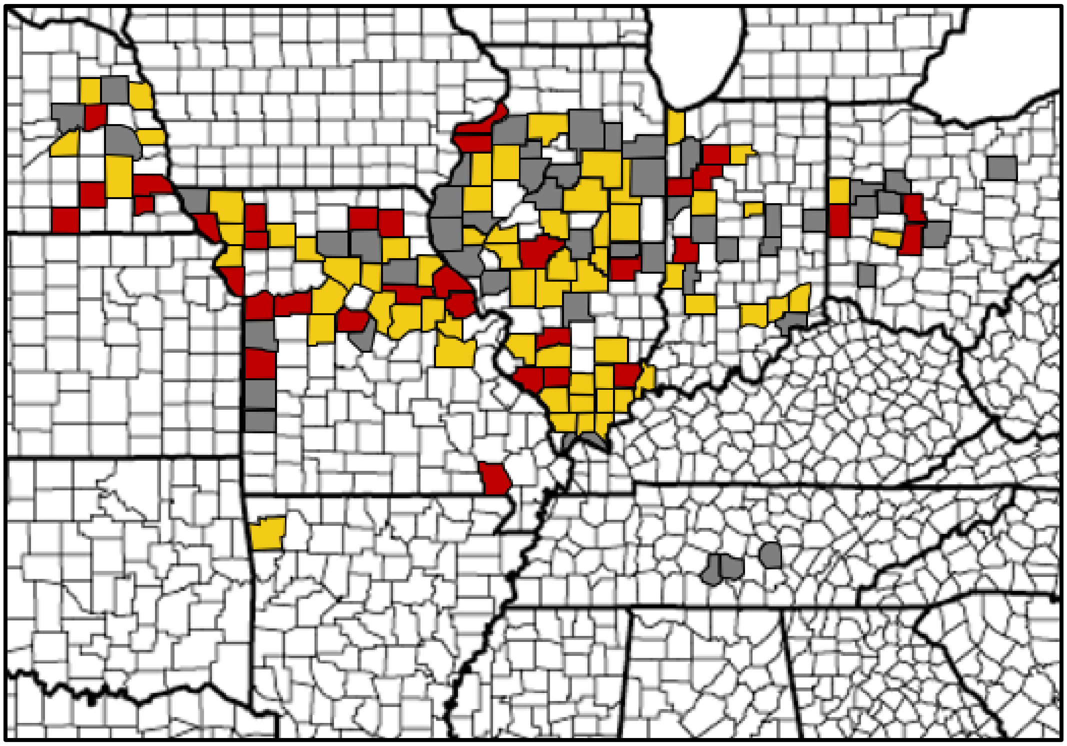

Figure 3.3. Waterhemp population response to dicamba at the county-level. Highlighted counties indicate where one or more populations were collected during the three years of screening. Grey coloring indicates counties with no survivors 21 days after application. Yellow indicates there were survivors in one or more replicates for at least one population. Red indicates survivors were observed in each replicate for at least one population.

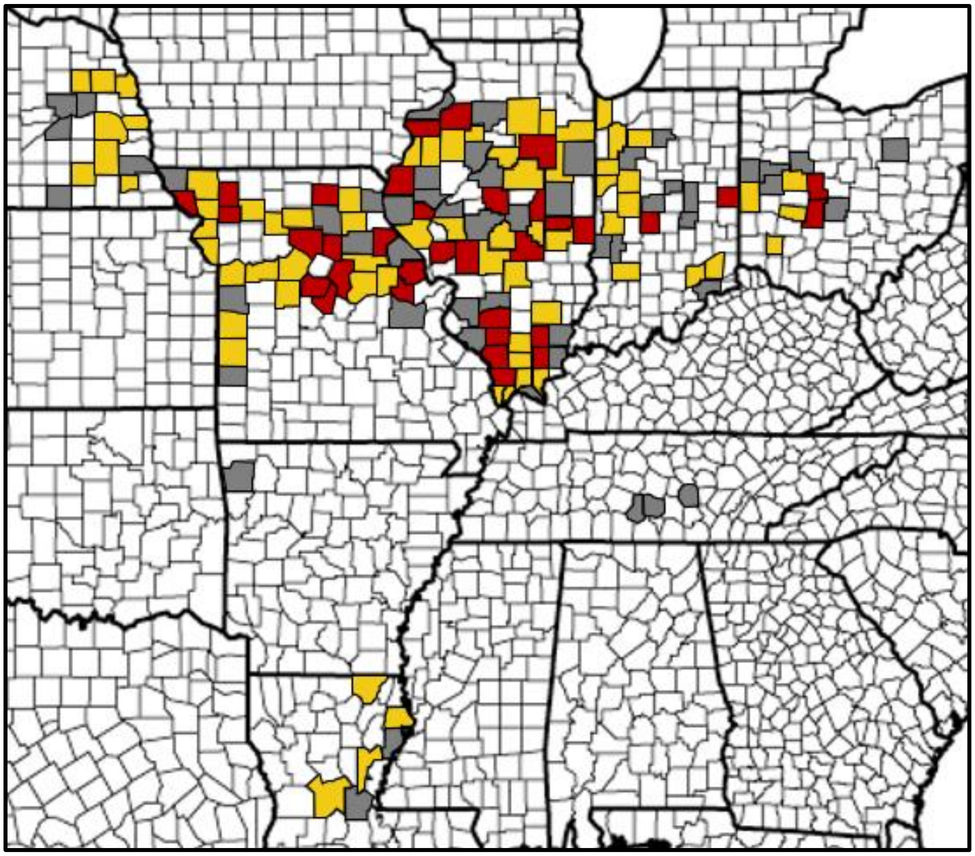

Figure 3.6. Waterhemp population response to glufosinate at the county-level. Highlighted counties indicate where one or more populations were collected during the three years of screening. Grey coloring indicates counties with no survivors 21 days after application. Yellow indicates survivors in one or more replicates for at least one population. Red indicates survivors were observed in each replicate for at least one population.