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Managing herbicide-resistant waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) seedbanks by integrating several management tactics

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Abstract

Herbicide-resistant waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) continues to threaten the productivity of many US crops. Thus, integrating non-herbicidal tactics with herbicides to reduce weed seedbanks is gaining popularity. A field study was conducted near Logan, IA from fall 2021 to fall 2022 to evaluate the impacts of cereal rye (*Secale cereale* L.) cover crop, herbicides, and harvest weed seed destruction on the waterhemp seedbank inputs. The cereal rye cover crop reduced waterhemp seed production by 90% compared to treatments without the cereal rye cover crop, while herbicides did not significantly affect waterhemp seed production. Furthermore, 96% of waterhemp seeds that entered the combine at harvest were destroyed and seed destruction effectiveness was not affected by cereal rye cover crop, herbicide program, or combine speed. Thus, cover crops and weed seed destruction are effective tactics to minimize waterhemp seedbank inputs. Waterhemp seed retention and combine header losses of waterhemp seeds were evaluated at harvest. Waterhemp seed retention was not affected by the cereal rye cover crop or herbicide programs. Only 30% of waterhemp seeds remained on the plant at harvest; the likely causes of seed shattering were late harvest and windy conditions prior to harvest. Waterhemp seed shattering from the combine header averaged 2.6% and was not affected by cover crop, herbicide program, or combine speed. These results support the inclusion of non-herbicidal control tactics to reduce herbicide-resistant waterhemp seedbanks and suggest the need for future research to optimize weed seed destruction.

1 | INTRODUCTION

Waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) is an important weed in Midwestern US soybean (*Glycine max* L.) production (Van Wychen, 2019) due in part to a rapid and competitive growth habit and can reduce soybean yields by 44% (Horak & Loughin, 2000; Steckel & Sprague, 2004). Waterhemp produces prolific quantities

Abbreviations: ALS, acetolactate synthase; CRCC, cereal rye cover crop; CTS, combine travel speed; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; HP, herbicide program; HP1, herbicide program 1; HP2, herbicide program 2; HPPD, 4-hydroxphenylpyruvate dioxygenase; HR, herbicide-resistant; HWSC, harvest weed seed control; IWM, integrated weed management; PPO, protoporphyrinogen oxidase; PSII, photosystem II; WSD, weed seed destruction.

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of seed and rapidly evolved herbicide-resistant (HR) traits because of dioecious reproduction (Hartzler et al., 2004; Heap, 2014). Waterhemp has evolved resistance to acetolactate synthase (ALS) inhibitor herbicides, synthetic auxins, photosystem II (PSII) inhibitors, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), protoporphyrinogen oxidase (PPO) inhibitors, very long chain fatty acid synthesis, and 4-hydroxphenylpyruvate dioxygenase (HPPD) inhibiting herbicides (Heap, 2023). Waterhemp with herbicide resistance to ALS, PSII, and EPSPS inhibitor herbicides is widespread in Iowa, while resistance to PPO and HPPD inhibitor herbicides is growing (Hamberg et al., 2021). Minimizing weed seedbank inputs is important to prevent future HR waterhemp populations (Norsworthy et al., 2016; Walsh et al., 2013).

The primary source of annual weeds in a crop field is the weed seedbank (Cavers, 1983). Weed seedbanks vary greatly by species and population density, and resultant weed infestations reduce crop yields (Buhler et al., 1997). Seedbanks can be reduced using tactics that reduce weed seed production and seed germinability (Gallandt, 2006; Schweizer & Zimdahl, 1984). Gallandt (2006) and Schweizer and Zimdahl (1984) concluded cover crop residues and herbicides delayed weed emergence, reduced weed establishment, and weed seedbank inputs. Understanding the effects of weed management on weed seedbank dynamics is important to improve long-term weed control.

Cover crops and herbicide use to control weeds is well documented (Harker & O'Donovan, 2013; Teasdale et al., 2007; Werle et al., 2017). A yellow sweetclover (*Melilotus officinalis* L.) cover crop suppressed emergence of kochia (*Bassia scoparia* [L.] A. J. Scott), Russian thistle (*Salsola tragus* L.), dandelion (*Taraxacum officinale* F. H. Wigg), perennial sowthistle (*Sonchus arvensis* L.), and field pennycress (*Thlaspi arvense* L.) by 97% (Reddy & Koger, 2004). Cereal rye cover crops (CRCC) reduced weed biomass by 99% (Hayden et al., 2012).

Herbicides are a cost effective, simple, and convenient tactic for weed control (Kim et al., 2002; Powles et al., 1996). Used together, cover crops and herbicides can dramatically reduce weed seedbank inputs (Brennan & Smith, 2005; Norsworthy et al., 2016; Teasdale et al., 2007). Conversely, weed control failures due to unfavorable environmental conditions, poor crop management, or evolved herbicide resistance in weeds can reduce the utility of these methods (Owen, 2016; Teasdale, 1996).

Harvest weed seed control (HWSC) is an additional tactic for weed seedbank management (Walsh et al., 2017). Crop combines can collect 85%–95% of harvested weed seeds and move them into the crop chaff during harvest (Green et al., 2020; Walsh et al., 2012). HWSC methods process the weed seedbearing chaff in narrow-windrow burning, chaff lining, chaff carts, bale-direct, or weed seed destruction (WSD) (Walsh et al., 2017). WSD pulverizes weed seeds via high impact cage mills included with the crop combine (Walsh et al., 2012). Weed seed destructors can damage a reported 90% of weed seeds depending on the weed species; damaged weed seeds are not viable (Schwartz-Lazaro et al., 2017; Walsh et al., 2012).

Core Ideas

- Managing herbicide-resistant waterhemp seedbank inputs with several agronomic practices was studied.
- Reducing waterhemp seed production with cereal rye cover crop residue was observed.
- The impact of agronomic factors such as cereal rye cover crop and herbicide program on waterhemp seed retention was determined.
- The impact of several agronomic factors and combine travel speed on waterhemp seed loss at the combine header was assessed.
- Damage effectiveness of weed seed destructor on waterhemp seed was evaluated.

Factors that limit WSD effectiveness include poor weed seed retention on the plant at crop harvest, weed seed losses from the combine header during harvest, and weed seed losses to the clean grain fraction (Green et al., 2020; Meadows et al., 2021; Schwartz et al., 2016). A high proportion of weed seed must be retained on the plant at harvest to maximize WSD effectiveness (Walsh et al., 2018). Schwartz et al. (2016) confirmed 98% of waterhemp seed production was retained on plants at soybean maturity, indicating most weed seeds likely enter the combine during harvest. However, several factors cause seed retention to vary.

Header losses can drastically reduce the number of weed seeds that enter the combine. Header losses occur when the combine header reel and sickle bar shake the plant, thus dispersing seeds to the ground rather than into the combine. Waterhemp seed header losses range from 9% to 40% (Meadows et al., 2021; Winans et al., 2021).

An element that may affect weed seed header loss is combine travel speed (CTS). Contrasting results have been published describing the impact of CTS on header losses of weed seeds, indicating more research should evaluate the relationship of combine speed and weed seed header losses (de Menezes et al., 2018; Jalali & Abdi, 2014; Wilkins et al., 1996).

The concept of integrated weed management (IWM) is "a holistic approach to weed management that integrates different methods of weed control to provide the crop with an advantage over weeds" (Harker & O'Donovan, 2013).

Researchers have investigated IWM for decades (Chikowo et al., 2009; Shaw, 1982; Swanton & Weise, 1991). Buhler (2002) describes the two main elements of IWM as, "(1) the use of multiple control tactics and (2) the integration of pest biology knowledge into the management system." Individual tactics such as cover crops, herbicides, and WSD have limitations and overreliance on a single tactic may cause weed control failures. However, incorporating cover crops, herbicides, and WSD in a soybean production system represents a novel and robust IWM approach to manage waterhemp seedbank dynamics.

The objectives of this research were to quantify the impact of CRCC and herbicide programs (HP) on waterhemp seed production and seed retention, to quantify the impact of a CRCC, HP, and CTS on header loss of waterhemp seeds, and to evaluate the impact of a CRCC, HP, and CTS on waterhemp seed destruction.

2 | MATERIALS AND METHODS

2.1 | Experimental site

Field experiments were conducted from fall 2021 to fall 2022 near Logan, IA on a commercial crop field (41°63′N, 95°81′W). The three soil types were a Dockery-Quiver silt loam (fine-silty, mixed, superactive, nonacid, mesic Mollic Fluvaquent), a Napier silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludoll), and a Colo silty clay loam (fine-silty, mixed, superactive, mesic Cumulic Endoaquoll) with 2.1%, 3.5%, and 6% organic matter, respectively (Soil Survey Staff, n.d.). The previous crops were no-till corn (*Zea mays* L.) in 2020 and no-till soybean in 2021. The site has glyphosate-resistant waterhemp (Larry Buss, personal communication, August 25, 2021). Temperature at the experimental site was identical to the 5-year average, but 2022 precipitation was 27% less than the 5-year average (Supplemental Table S1) (Iowa Environmental Mesonet Staff, 2023).

2.2 | Experiment design

The experiment design was a randomized split-split plot with three replications and included 12 treatments derived from three factors. The whole plot factor compared CRCC use. The split plot factor compared two HP. Herbicide program 1 (HP1) was a preemergence application of glyphosate (1261 g ai ha^{-1}), dicamba (558 g ae ha^{-1}), and pyroxasulfone (183 g ai ha^{-1}). Herbicide program 2 (HP2) was a preemergence application of glyphosate (1261 g ai ha^{-1}), dicamba (558 g ae ha^{-1}), and a premix of flumioxazin (70 g ai ha^{-1}) and pyroxasulfone (89 g ai ha^{-1}) followed by a postemergence application of lactofen (175 g ai ha^{-1}) and methylated seed oil (1.75 L ha^{-1}). The split-split plot factor was WSD at two CTS (2.4 kmph and 4.0 kmph) plus a control, where plots were harvested at 4.0 kmph without WSD. The whole plots were 70 m by 46 m. The split plots were 32 m by 46 m and the split-split plots were 10.67 m by 46 m. A 6 m alley was established between split plots to avoid the effects of the herbicide treatments.

2.3 | Field operations

Soybeans were harvested on October 1, 2021 using a commercial combine (John Deere S670, Deere and Company). Cereal rye (var. Hazlot) was drill-seeded into soybean stubble on October 6, 2021 at a population density of 67 kg ha^{-1} in 19 cm rows. Soybeans (var. P28A51X) with resistance to glyphosate and dicamba at a population density of 333,450 seeds ha^{-1} in 38 cm rows were planted on May 21, 2022 into a live CRCC.

Preemergence herbicide treatments were applied on May 23, 2022 to terminate the CRCC at anthesis. The postemergence herbicide was applied on June 29, 2022. Herbicides were applied with a tractor mounted sprayer calibrated to apply 140 L ha^{-1} at 207 kPa. Preemergence herbicide applications used 110015TTI nozzles (Spraying Systems Co.), while the postemergence application used 110015TT nozzles. Soybean was harvested on October, 13 2022 using a commercial combine (John Deere S670, Deere and Company) equipped with a Redekop SCU (Seed control unit, Redekop Manufacturing) to provide WSD.

2.4 | Data collection

Prior to termination, 24 0.5 m^2 samples of CRCC biomass were arbitrarily selected and collected per whole plot. The effect of CRCC residue and HP on waterhemp fecundity was evaluated in fall 2022. Waterhemp seeds were collected on September 13, 2022 from an arbitrarily selected area that was 1.14 m wide by 5 m in each split-split plot. All waterhemp seedheads were carefully harvested, bagged, and air-dried for 10 days. Seeds were then hand threshed, sieved, and cleaned with an air column separator (Seedburo Equipment Company). After cleaning, composite seed sample weights were recorded and 0.1 g subsamples of waterhemp seeds were counted. Total seeds per sample was determined using Equation (1) as follows:

$$Y = X \times \frac{Z}{0.1\ \text{g}} \quad (1)$$

where Y was waterhemp seeds per sample, X was sample weight, Z was seed count, and 0.1 g was subsample weight.

Overall seed production per m^2 was determined using the following equation:

$$P = \frac{Y}{5.715\ m^2} \quad (2)$$

where P was waterhemp seed production per m^2, Y was seeds per sample, and 5.715 m^2 was area of harvested seed production.

Walsh and Powles (2014) suggested weeds should retain at least >75% of seeds at harvest for effective WSD. San Martín et al. (2021) speculated that agronomic practices can influence seed retention on grass weed species. Therefore, our study evaluated if agronomic practices such as CRCC and HP affected waterhemp seed retention. One female plant was arbitrarily selected per split-split plot on August 24, 2022 to evaluate waterhemp seed retention, plants were bagged (White Noseeum Mosquito Netting, Online Fabric Store), and bags were collected on October 12, 2022. Seeds remaining on the plant were considered retained, while seeds from the bag were considered shattered. Sample processing was identical to the process described for seed production. Seed retention was calculated using Equation (3) as follows:

$$R = \frac{r}{r + s} * 100 \quad (3)$$

where R was percent waterhemp seed retention, s was number of shattered seeds, and r was number of retained seeds.

Evaluation of CRCC, HP, and CTS impact on combine header losses of waterhemp seeds occurred at harvest. Pairings of female plants were arbitrarily selected for this evaluation. The plants within these pairs were referred to as "comparable" plants and "header" plants for the remainder of the paper. Comparable plants were collected the day prior to harvest and provided the estimated seeds per plant, while header plants quantified waterhemp seed shatter from combine header during the harvest process. One or two female plants were selected as header plants based on two criteria modified from Uscanga-Mortera et al. (2007). The first criterion required plants to be within 10% height and 25% diameter of the comparable plants. Criterion two required that female plants occur within the outer 2 m of each split-split plot to allow constant CTS during sample collection. Prior to harvest, header plants had two 68 cm by 104 cm seed trays placed at their base. Seed trays were collected immediately after the combine header passed to prevent chaff from contaminating the sample. Samples were cleaned with hand sieves and an air column separator prior to counting. Header losses were quantified using Equation (4) as follows:

$$H = \frac{L}{C} \times 100 \quad (4)$$

where H was percent header loss, L was waterhemp seeds counted from trays, and C was comparable plant seed counts.

The final determination was the effect of CRCC, HP, and CTS on waterhemp seed destruction effectiveness. Seed destruction effectiveness was assessed using chaff collected during harvest. As the combine traveled across plots, eight 68 cm by 104 cm trays were placed in a zig-zag pattern under the weed seed destructor and combine chopper to collect the chaff that contained both intact and damaged waterhemp seeds. Samples were cleaned using hand sieves and a vibratory separator as modified from Gregg and Billups (2010). Collected seeds were then hand counted and visually evaluated to determine if they were intact or damaged. Seed destruction effectiveness was evaluated using Equation (5) as follows:

$$E = \frac{c - t}{c} * 100 \quad (5)$$

where E was percent effectiveness, c was intact seeds from control plots, and t was intact seeds from treated plots.

2.5 | Statistical analysis

Waterhemp seed production, seed retention, header loss, and seed destruction data were analyzed using PROC GLIMMIX in SAS 9.4 software (SAS Institute Inc.). CRCC, HP, CTS, and their interactions were considered fixed effects in the analysis. Replication and interactions with the fixed effects were considered random effects. Significant fixed effects were further evaluated using Tukey's honestly significant difference test ($\alpha = 0.05$). Results of fixed effects are listed in Table 1.

3 | RESULTS AND DISCUSSION

3.1 | Waterhemp seed production

CRCC biomass prior to termination ranged from 7000 to 8850 kg ha^{-1}. CRCC biomass greater than 5000 kg ha^{-1} suppressed summer annual weed population densities and weed biomass (Bunchek et al., 2020; Mirsky et al., 2012). Our results for waterhemp seed production ranged from 1120 to 332,310 seeds m^{-2} and were significantly affected by CRCC (Table 1). Plots without CRCC averaged 116,360 waterhemp seeds m^{-2} while CRCC plots averaged 11,790 seeds m^{-2}, a 90% reduction in seed production which was consistent with Yadav et al. (2023) who reported that a CRCC biomass of 5000 kg ha^{-1} reduced waterhemp seed production by 83%. Previous studies investigated the impact of live cover crops rather than cover crop residues on weed seed production, and these studies similarly concluded cover crops reduced weed seed production (Brainard & Bellinder, 2004; Brainard et al., 2011; Brennan & Smith, 2005).

TABLE 1 Fixed effects for cereal rye cover crop, herbicide program, and combine speed and interactions on waterhemp seed production, seed retention, header losses, and seed destruction.

Source of variation	*df*	Seed production	Seed retention	Header loss	Seed destruction
		p value[a]			
CRCC	1	0.013[b]	0.424	0.4592	0.249
HP	1	0.773	0.360	0.9898	0.065
CRCC * HP	1	0.544	0.121	0.3097	0.475
CTS	1	–	–	0.1339	0.890
CRCC * CTS	1	–	–	0.7265	0.281
HP * CTS	1	–	–	0.3154	0.296
CRCC * HP * CTS	1	–	–	0.1966	0.386

Abbreviations: CRCC, cereal rye cover crop; CTS, combine travel speed; HP, herbicide program.

[a]Values reported as *p* values from logarithmic transformation dataset.

[b]Significant at the 0.05 level of probability.

Waterhemp seed production was not affected by HP (Table 1). Waterhemp seed production was 56,620 and 71,530 seeds m^{-2} for HP1 and HP2, respectively. Our results contradicted those by Legleiter et al. (2009), who reported significant differences between HP for waterhemp seed production. Legleiter et al. (2009) used metolachlor applied preemergence which was more effective for waterhemp control than the herbicide used in our study. Our HP applied PPO inhibiting herbicides at preemergence and postemergence timings, which is not a best management practice for herbicidal weed management and less effective than metolachlor (Norsworthy et al., 2012). Norsworthy et al. (2012) suggested rotating herbicide sites of action for optimal weed control and minimizing selection for herbicide resistance. We considered applying glufosinate allowing the rotation of sites of action at postemergence applications, but glufosinate-tolerant soybeans were not available.

3.2 | Waterhemp seed retention

Waterhemp seed retention was not affected by CRCC or HP (Table 1). Waterhemp seed retention ranged from 4% to 60% and averaged 30% in our study. Schwartz-Lazaro et al. (2021) and Walsh and Powles (2014) stated >75% of seed retention at harvest was crucial to maximize the effectiveness of WSD equipment. We speculate that the low waterhemp seed retention in our study was attributable to environmental factors. Windstorms impacted seed retention on common lambsquarters (*Chenopodium album* L.), green foxtail (*Setaria viridis* (L.) Beauv.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), redroot pigweed (*Amaranthus retroflexus* L.), wild mustard (*Sinapis arvensis* L.), and wild proso millet (*Panicum miliaceum* L.) (Forcella et al., 1996). Prior to harvest, the experiment experienced several windstorms with wind speeds >10 m s^{-1} (Weather Underground Staff, n.d.). Moreover, the relatively late harvest likely reduced waterhemp seed retention. Previous reports from Iowa reported waterhemp seed retention ranged from 49% to 78% and varied by harvest date (Bennett, 2021; Meadows et al., 2021).

3.3 | Effect of combine header on waterhemp seed shatter

Waterhemp seed shatter due to the combine header was not affected by CRCC, HP, or CTS (Table 1). Waterhemp seed shatter ranged from 0.1% to 16% and averaged 2.6% in our study. Previously reported waterhemp seed shatter from a combine header ranged from 9% to 40% (Meadows et al., 2021; Winans et al., 2021), and indicated waterhemp seed shatter during harvest can vary greatly.

3.4 | Effectiveness of weed seed destruction

Waterhemp seed destruction effectiveness was not affected by CRCC, HP, or CTS (Table 1). To our knowledge, the impacts of CRCC, HP, and CTS on WSD have not been previously evaluated. Other studies evaluated the impact of cage mill revolutions per minute, type of crop chaff, crop chaff quantity, and chaff moisture levels on WSD effectiveness (Schwartz-Lazaro et al., 2017; Walsh et al., 2012). Our data provided evidence that CTS, in addition to CRCC and HP, did not impact WSD effectiveness.

WSD effectiveness was determined by comparing intact waterhemp seeds collected from threshed residue in the control plots to the intact waterhemp seeds in the operational weed seed destructor. There was a significant difference ($p < 0.001$) of intact waterhemp seeds collected when the destructor was off versus operational. No differences ($p = 0.987$) were observed in WSD effectiveness for CTS. The percentage of waterhemp seeds damaged was 96% and

TABLE 2 Effect of combine speed on waterhemp seed destruction.

Speed (kmph)[a]	Intact seeds[b]
Control	35,169a
2.4	1395b
4.0	1188b

Note: Treatment means within a column with the same letters are not significantly different (Tukey honestly significant difference, $\alpha = 0.05$). Tukey HSD test was performed post logarithmic data transformation.

[a]Seed destructor was off in the control treatment. Seed destructor was on in 2.4 kmph and 4.0 kmph treatments.

[b]Values in table are presented as non-transformed data.

96.6% at 2.4 kmph and 4.0 kmph, respectively (Table 2). Shergill et al. (2020) reported 96.2% waterhemp seed damage, and Schwartz-Lazaro et al. (2017) reported 98.4% waterhemp seed damage from weed seed destructors. However, these studies were conducted with a stationary impact mill, while our study was conducted with an impact mill integrated into the combine.

4 | CONCLUSION

Our results indicated a diverse suite of weed control tactics reduced waterhemp seedbank inputs and thus improved future crop production. Future weed management programs should emphasize reducing weed seedbanks to improve weed management practices and crop yield (Gallandt, 2006; Norsworthy et al., 2012; Schwartz-Lazaro & Copes, 2019). CRCC reduced waterhemp seed production and the weed seed destructor reduced the number of viable seeds, demonstrating these methods are effective at reducing waterhemp seedbank inputs.

One primary research objective was to identify agronomic practices that impacted WSD. Agronomic practices such as CRCC and HP did not impact waterhemp seed retention, header losses of waterhemp seeds, or WSD effectiveness. Therefore, all three tactics can be incorporated into an IWM program without adverse effects. WSD effectiveness depends on waterhemp seed retention (Walsh & Powles, 2014). In our study, waterhemp seed retention was 30% at harvest and Walsh and Powles (2014) suggested that >75% seed retention for several grass and broadleaf weeds at harvest maximized WSD effectiveness. The relatively low waterhemp seed retention in our study was likely attributable to windstorms and a late harvest date (Forcella et al., 1996; Meadows et al., 2021). We suggest prioritizing harvest of weedy fields first and prior to windstorms to mitigate the problem of waterhemp seed retention at harvest. If waterhemp seed retention is low, WSD may not be worthwhile in reducing seed returns to the soil seedbank. Waterhemp seed losses from the combine header were not affected by CRCC, HP, or CTS. However, further research is needed to determine if higher waterhemp population density, harvest date or more effective HPs may impact the utility of WSD in soybean production.

Sustainable weed management strategies should prioritize the long-term impact of reduced weed seedbanks. Incorporating CRCC, HP, and WSD in an IWM program are important options for Midwestern U.S. soybean producers who are battling HR waterhemp.

AUTHOR CONTRIBUTIONS

Austin H. Schleich: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Mark A. Licht**: Project administratio; writing—original draft. **Micheal D. K. Owen**: Writing—review and editing. **Ramawatar Yadav**: Conceptualization; methodology; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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