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Multi-tactic strategies to manage herbicide-resistant waterhemp (*Amaranthus tuberculatus*) in corn–soybean rotations of the U.S. Midwest

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Research Article

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Abstract

Field experiments were conducted over 2 yr (2019 to 2020) at two locations in Iowa to evaluate multi-tactic strategies for managing multiple herbicide–resistant (MHR) waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] in a corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] rotation. The effect of three herbicide programs on *A. tuberculatus* control was tested in corn (2019). The effects of the prior year's corn weed control, a cereal rye (*Secale cereale* L.) cover crop, and soybean row spacing (38-cm vs. 76-cm wide) on *A. tuberculatus* density, biomass, and seed production were tested in soybean (2020). A herbicide program used in corn with two sites of action provided only 35% control of MHR *A. tuberculatus* compared with ≥97% control by a herbicide program with three sites of action. In soybean, adequate control of *A. tuberculatus* (≥90%) in the prior year's corn crop and use of a cover crop or narrow rows reduced *A. tuberculatus* density by more than 60% at 3 and 9 wk after planting (WAP) compared with inadequate control (30%) in the prior year's corn and no cover crop. Cover crop and narrow-row soybean reduced *A. tuberculatus* density by 44% at 3 WAP compared with no cover crop and wide-row soybean. Inclusion of a single control tactic, adequate control (≥90%) with multiple herbicides in the prior year's corn, use of a cover crop, or narrow-row soybean reduced *A. tuberculatus* biomass and seed production at soybean harvest by at least 24% compared with inadequate control (30%) in the prior year's corn, no cover crop, and wide-row soybean. The combination of all three control tactics reduced *A. tuberculatus* biomass and seed production at soybean harvest by at least 80%. In conclusion, diverse control tactics targeting *A. tuberculatus* at multiple life-cycle stages can make substantial contributions to the management of MHR populations.

Introduction

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

Amaranthus tuberculatus has developed resistance to herbicides from seven site of action groups, including synthetic auxins, and inhibitors of 5-enolpyruvylshikimate-3-phosphate synthase (glyphosate), acetolactate synthase (ALS), photosystem II, protoporphyrinogen oxidase, 4-hydroxyphenylpyruvate dioxygenase, and very-long-chain fatty-acid synthesis (Tranel 2021). Additionally, widespread occurrence of multiple herbicide–resistant (MHR) *A. tuberculatus* populations across the midwestern U.S. states (Heap 2022) has seriously limited soybean growers' ability to use postemergence herbicides for weed control (Sarangi et al. 2019). Because *A. tuberculatus* has a high propensity to evolve resistance against any single control tactic (Tranel 2021), herbicide rotation, a commonly recommended strategy to combat herbicide resistance, will not work for this species (Wu et al. 2018). Therefore, ecologically based, multi-tactic strategies are needed for a sustainable management of *A. tuberculatus*.

Cover crops have been beneficial in preventing soil erosion by providing surface residue (Mohler and Teasdale 1993), trapping residual nitrate that otherwise would leach out in drainage water (Kaspar et al. 2012), improving soil organic matter and physical properties (Moore et al. 2014), and suppressing weeds (Teasdale et al. 2007). Cereal rye (*Secale cereale* L.) is the most widely used cover crop in the U.S. Midwest due to its winter hardiness, ease of establishment, and high biomass accumulation (Snapp and Surapur 2018; Teasdale 1996). Cereal rye suppresses weeds primarily through physically impeding emergence and growth (Teasdale and Mohler 1993) and also by inhibiting seed germination through release of allelochemicals (Teasdale et al. 2012). The level of weed suppression gained by use of a cereal rye cover crop has been shown to be directly correlated with cover crop biomass at the time of termination and weed emergence patterns (Mirsky et al. 2011, 2013). Cornelius and Bradley (2017) reported that a cereal rye cover crop reduced *A. tuberculatus* emergence by more than 35% when terminated 2 wk before soybean planting.

Crop competitiveness is an important factor in determining the outcomes of crop–weed interference and can be improved by reducing the row spacing. Reducing soybean row spacing helps the crop in achieving canopy closure faster with greater light interception (Board et al. 1992; Steckel and Sprague 2004). This crop-induced shading can reduce weed dry matter accumulation (McLachlan et al. 1993), thereby improving the efficacy of other weed control tactics. Biomass and density of late-emerging *A. tuberculatus* plants were reduced by more than 40% in 19-cm-wide rows of soybean compared with 76-cm-wide rows (Steckel and Sprague 2004). Young et al. (2001) reported improvement in weed control by reducing soybean row spacing. Postemergence application of glyphosate at 630 g ha^{-1} provided more than 90% control of *A. tuberculatus* in 19-cm-wide rows compared with less than 90% control in 76-cm-wide rows. Narrow-row soybean also can delay the critical time for weed removal (CTWR). CTWR occurred at the third-trifoliate stage of soybean in 19-cm-wide rows compared with the first-trifoliate stage in 76-cm wide rows (Knezevic et al. 2003). In addition, narrow-row soybean can produce higher grain yields than wide-row soybean due to increased duration of light interception and total dry matter accumulation (Board et al. 1990).

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

Despite the ecological significance of initial weed seedbank population densities on the success of weed control practices (Buhler 1999a, 1999b; Mirsky et al. 2013), there are currently limited published field studies that have quantified the effect of the prior year's weed control programs on the efficacy of managing *A. tuberculatus* in the subsequent year. Information on the interaction of cereal rye cover crop and soybean row spacing on control of *A. tuberculatus* is also lacking. Therefore, the objective of this research was to determine the impact of a prior year's corn weed control, cereal rye cover crop, and soybean row spacing on *A. tuberculatus* density, biomass, and seed production in the subsequent soybean crop.

Table 1. Average air temperatures and total precipitation during 2019 and 2020 growing seasons at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

	Average temperature			Precipitation		
	2019	2020	30-yr avg	2019	2020	30-yr avg
		C			mm	
May	15	15	16	202	127	134
June	22	24	21	90	44	141
July	24	25	23	105	69	119
August	21	23	22	43	32	119
September	21	17	18	111	74	96
Total	—	—	—	551	346	609

[a]Temperature and precipitation data were obtained online from the Iowa State University's Iowa Environmental Mesonet website: https://mesonet.agron.iastate.edu/agweather.

Materials and Methods

Experimental Site

Field experiments were conducted during 2019 and 2020 at two sites: the Iowa State University Curtiss Farm in Ames, IA (42.005°N, 93.671°W) and the university's Bruner Farm in Boone, IA (42.010°N, 93.736°W). The soil at the Ames site was a mixture of Canisteo (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) and Clarion sandy clay loam (fine-loamy, mixed, superactive, mesic Typic Hapludolls) with 4.4% organic matter and a pH of 7.4. The soil at the Bruner site was a mixture of Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and Canisteo silty clay loam with 4.0% organic matter and a pH of 6.9. Fields at both sites had been under a corn (2017)–soybean (2018) rotation in the previous 2 yr. Experimental sites had a known infestation of MHR *A. tuberculatus* that was resistant to ALS inhibitors and glyphosate. Records of average air temperatures and total precipitation during 2019 and 2020 growing seasons are summarized in Table 1.

Experimental Design

This study was conducted over 2 yr, with corn grown in 2019 and soybean in 2020 at each of the two sites. A randomized strip-strip-plot design (Figure 1) was used with four replications at each site. The study design included 12 treatments resulting from three factors. The first factor consisted of three levels of *A. tuberculatus* control (30%, 90%, and 100%) in corn. Three different herbicide programs were used to achieve the three levels of *A. tuberculatus* control. The second factor consisted of use of a cereal rye cover crop established following corn harvest versus no cover crop. The third factor consisted of wide-row (76-cm) versus narrow-row (38-cm) soybean spacing. The first factor was assigned to the main strips (9.1-m wide by 30.5-m long). The second factor was assigned to the substrips (9.1-m wide by 15.2-m long) across the main strips, while the third factor was assigned to the sub-substrips (4.6-m wide by 15.2-m long) along the main strips. In addition, 1.5- and 12-m-wide alleyways between main strips and replications, respectively, were maintained to allow field operations.

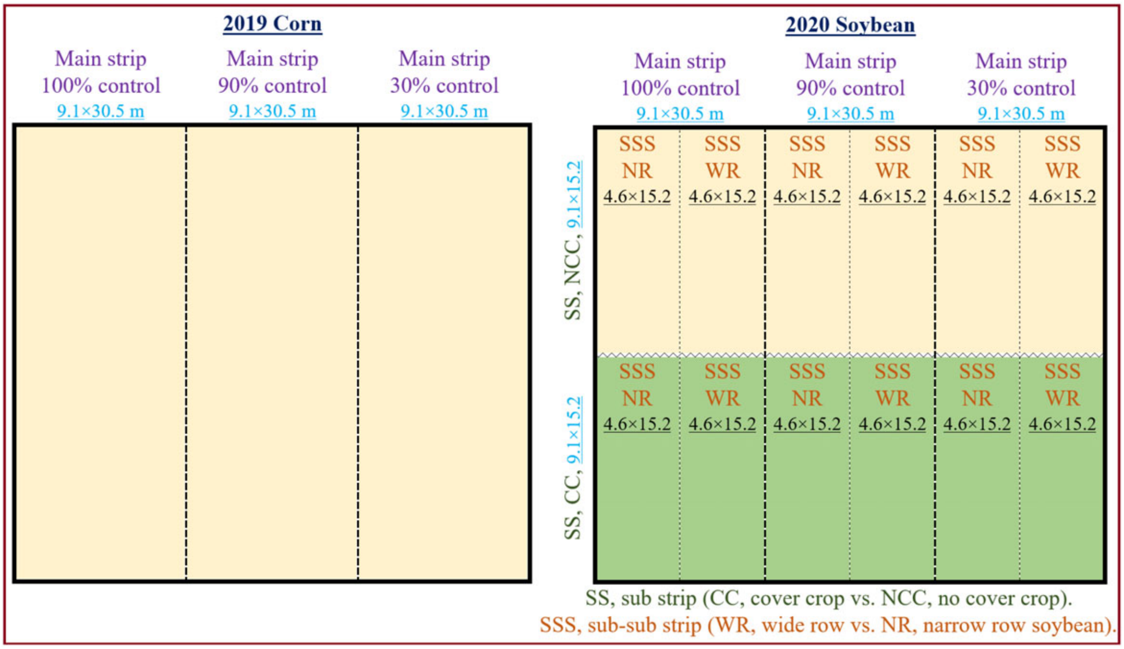

Figure 1. Plot layout of the field experiments conducted over 2 yr (2019 to 2020) in a corn–soybean rotation at the Iowa State University Research Farms near Ames, IA, and Boone, IA.

Field Operations

Fields were tilled with a chisel plow before initiation of experiments in spring 2019. At Ames, a glyphosate- and glufosinate-resistant corn (PO589AM, Pioneer®, Johnston, IA 50131) was planted on June 3, 2019. At Boone, a glyphosate- and glufosinate-resistant corn (PO157AM, Pioneer®) was planted on June 4, 2019. Corn was planted in 76-cm-wide rows at 79,074 seeds ha^{-1} using a John Deere 7100 MaxEmerge™ planter (John Deere, Moline, IL 61265). The 30% control level of the main strip was achieved by applying a preemergence herbicide with one site of action followed by (fb) a postemergence herbicide (Table 2). The 90% control level was achieved by applying preemergence herbicides with two sites of action fb postemergence herbicides with two sites of action (Table 2). All preemergence herbicides were applied on the day of corn planting using an ATV-mounted CO_2-pressurized boom sprayer equipped with Turbo TeeJet® Induction nozzles (TTI 110015VS, Spraying Systems, Wheaton, IL 60187). The sprayer was calibrated to deliver 140 L ha^{-1} at 241 kPa. All postemergence herbicides were applied at the V5 to V6 growth stage of corn using a tractor-mounted compressed-air boom sprayer equipped with TT 11002VS nozzles. The sprayer was calibrated to deliver 187 L ha^{-1} at 207 kPa.

In current herbicide-led crop production practices of the U.S. Midwest, it is rare to achieve 100% control of weed populations on a large scale. Therefore, the 100% control level of the main strip factor was designed based on the assumption that any seedbank inputs from late-season weed escapes in the 90% control strips in corn would be prevented by hand weeding. Although there were some escapes in the 90% control strips, they did not produce any seed (Table 3). Therefore, the 100% control level (no seed input) was achieved without hand weeding. However, to create a weed escape scenario, about 9,000 seeds m^{-2} (equivalent to 10% of the seeds produced in the 30% control strip) were harvested from *A. tuberculatus* plants growing in an adjacent nonexperimental area and spread uniformly in the 90% control strip at the time of corn harvest.

Corn was harvested on October 7, 2019, at Ames and October 9, 2019, at Boone using a plot combine (John Deere 9450). A cereal rye cover crop (‘Elbon’) was drill seeded on October 17, 2019, at both sites in substrips across the main strips. Cereal rye was seeded into corn stubble with a no-till drill (Marliss Industries, Jonesboro, AR 72401) in 19-cm-wide rows at 67 kg ha^{-1}. The following spring, a glyphosate-, glufosinate-, and 2,4-D-resistant soybean (S20-E3, NK® Seeds, Syngenta, Greensboro, NC 27419) was planted on May 22, 2020, at both sites. Soybean was planted in standing cereal rye (“planting green”) in 38- and 76-cm-wide rows at 322,470 seeds ha^{-1} using a John Deere 7100 MaxEmerge™ planter. Soybean in 38- and 76-cm-wide rows was planted in sub-substrips along the main strips. On the same day, a broadcast application of glyphosate (1,261 g ae ha^{-1}) plus *S*-metolachlor (1,788 g ai ha^{-1}) was applied across the entire experimental area (including the no cover crop strips) to terminate the cereal rye (at the anthesis growth stage) and to provide early-season weed control. The herbicides were applied using a tractor-mounted compressed-air boom sprayer equipped with TT 11002VS nozzles and calibrated to deliver 187 L ha^{-1} at 207 kPa. No other herbicide application was made to the strips.

Soybean from each sub-substrip in the 30% and 90% control strips was harvested on October 1, 2020, using a plot combine. Soybean from the 100% control strips was harvested with a commercial combine (John Deere S680) at the substrip level for a different set of objectives (not included in this study). Therefore, data on soybean grain yield from 100% control strips are not included in this study.

Data Collection

The effect of corn herbicide programs on *A. tuberculatus* in 2019 was assessed by measuring percent visible control, plant density,

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Table 2. Herbicide programs for *Amaranthus tuberculatus* control in corn (corn–soybean rotation) in 2019 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

Control program	Herbicide(s)	Rate	Timing[b]	Trade name	Manufacturer
		—g ai or ae ha^{-1}—			
30% control	*S*-metolachlor	1,788	PRE	Dual II Magnum®	Syngenta Crop Protection, Greensboro, NC 27419;
	fb	fb	fb	fb	
	glyphosate	1,261	POST	Roundup PowerMax®	Bayer Crop Science, St Louis, MO 63167
90% control[c]	Saflufenacil + pyroxasulfone	50 + 91	PRE	Sharpen® + Zidua®	BASF Corp., Research Triangle Park, NC 27709;
	fb	fb	fb	fb	BASF Corp., Research Triangle Park, NC 27709;
	glufosinate + *S*-metolachlor	656 + 1,539	POST	Liberty® + Dual II Magnum®	BASF Corp., Research Triangle Park, NC 27709; Syngenta Crop Protection, Greensboro, NC 27419
100% control	Saflufenacil + pyroxasulfone	50 + 91	PRE	Sharpen® + Zidua®	BASF Corp., Research Triangle Park, NC 27709;
	fb	fb	fb	fb	BASF Corp., Research Triangle Park, NC 27709;
	glufosinate + *S*-metolachlor	656 + 1,539	POST	Liberty® + Dual II Magnum®	BASF Corp., Research Triangle Park, NC 27709; Syngenta Crop Protection, Greensboro, NC 27419

[a]Abbreviations: fb, followed by; POST, postemergence; PRE, preemergence.
[b]PRE herbicides were applied at corn planting. POST herbicides were applied at V5 to V6 growth stage of corn.
[c]The 90% control represents late-season weed seed inputs by the survivors/escapes. This was achieved by manually spreading *A. tuberculatus* seeds (equivalent to 10% of the seeds produced in the 30% control strip) uniformly in the plots at the time of corn harvest.

Table 3. *Amaranthus tuberculatus* visible control, density, biomass, and seed production with different herbicide programs in corn (corn–soybean rotation) in 2019 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

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

[a]Abbreviations: fb, followed by; POST, postemergence; PRE, preemergence; WAPOST, weeks after POST; WAPRE, weeks after PRE.
[b]PRE herbicides were applied at corn planting. POST herbicides were applied at V5 to V6 growth stage of corn.
[c]Treatment means within a column with same letter are not significantly different (Tukey test, α = 0.05).

and seed production. Percent visible control was assessed on a scale of 0 to 100 (where, 0 means no control; 100 means complete control) at 4 wk after preemergence (WAPRE) and 2 wk after postemergence (WAPOST) herbicide applications. Plant density was counted from four 1-m^2 quadrats per main strip at 4 WAPRE, 2 WAPOST, and 9 WAPOST. No new seedling emergence occurred after 9 WAPOST. *Amaranthus tuberculatus* seed production was quantified by harvesting female plants from four 1-m^2 quadrats in each main strip at the time of corn harvest. Harvested plants were placed in DelNet pollination bags (DelStar Technologies, Middletown, DE 19709) and air-dried for 1 wk. Seeds were threshed by rubbing the inflorescences between the hands and then cleaned with handheld sieves and a seed blower (Seedburo® Equipment, Des Plaines, IL 60018). An average seed weight was determined by weighing four subsamples of 1,000 seeds. Then, seeds per square meter were calculated by dividing the total sample weight by the average seed weight. Seed samples were then returned to the sampling quadrats in the field before cereal rye cover crop planting.

The cereal rye cover crop growth was assessed in the spring of 2020 by measuring plant height of 10 randomly selected plants and collecting aboveground biomass in two linear 1-m rows from each main strip on the day of termination. Biomass samples from each strip were combined, oven-dried at 60 C for 5 d, and weighed. The effect of the cereal rye cover crop on soybean emergence was assessed by counting the number of plants in four linear 1-m rows from each sub-substrip at 3 wk after planting (WAP). The effects of the prior year's corn weed control, cereal rye, and soybean row spacing on *A. tuberculatus* growth and development were assessed by measuring plant density, aboveground biomass, and seed production in soybean (2020) at each study site. *Amaranthus tuberculatus* density was counted from two 0.25-m^2 quadrats in each sub-substrip at 3 and 9 WAP. Aboveground weed biomass was collected from two 0.25-m^2 quadrats at the time of soybean

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canopy closure and at harvest. Biomass samples from each sub-substrip were combined, oven-dried at 60 C for 5 d, and weighed. Seed production of *A. tuberculatus* in each sub-substrip in soybean was quantified using a process similar to that described earlier for corn, except two 0.25-m^2 quadrats were harvested instead of four 1-m^2 quadrats. Soybean grain yields from each sub-substrip in 30% and 90% control strips were recorded and adjusted to 13% moisture content.

Statistical Analysis

Normality of residuals and homogeneity of variance were assessed using a Shapiro-Wilk test and Levene's test, respectively, and diagnostic residual plots (Kozak and Piepho 2018). *Amaranthus tuberculatus* percent control, density, seed production, aboveground biomass, cereal rye plant height and aboveground biomass, and soybean grain yield were analyzed using PROC MIXED in SAS v. 9.4 software (SAS Institute, Cary, NC 27513). For all response variables, experimental site and replication were considered random effects, whereas prior year's weed control, cover crop, soybean row spacing, and their interactions were considered fixed effects in the model. Appropriate degrees of freedom in the model were obtained by the Satterthwaite approximation method (Satterthwaite 1946). Estimated means were compared using the Tukey test at a significance level of $\alpha = 0.05$.

Results and Discussion

Site by treatment interactions for the response variables were not significant; therefore, data from both sites were pooled. Monthly mean air temperatures during 2019 (corn) and 2020 (soybean) growing seasons at both sites were close to the historical monthly means (Table 1). Cumulative precipitation during the typical emergence period of *A. tuberculatus* (May to July; Hartzler et al. 1999) in 2019 was close to the historical means of this period. In contrast, cumulative precipitation during the typical emergence period of *A. tuberculatus* in 2020 was 39% lower compared with the historical means of this period (Table 1).

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

Amaranthus tuberculatus Control in Corn

In corn (2019), *S*-metolachlor preemergence provided 18% control of *A. tuberculatus* at 4 WAPRE, whereas saflufenacil + pyroxasulfone preemergence provided ≥81% control (Table 3). Similarly, *A. tuberculatus* density in *S*-metolachlor preemergence plots averaged 253 plants m^{-2} compared with 20 plants m^{-2} in saflufenacil + pyroxasulfone preemergence plots. A postemergence application of glyphosate in plots treated with *S*-metolachlor preemergence further reduced *A. tuberculatus* density to 43 plants m^{-2} at 2 WAPOST; however, visible control did not exceed 35% due to the presence of glyphosate-resistant *A. tuberculatus* population in the field. In contrast, a postemergence application of glufosinate + *S*-metolachlor in plots treated with saflufenacil + pyroxasulfone preemergence reduced *A. tuberculatus* density to 2 plants m^{-2} and increased visible control to ≥97%. Furthermore, *A. tuberculatus* density in those plots did not increase over the remaining growing season, and none of the plants produced seeds. In contrast, in the *S*-metolachlor preemergence fb glyphosate postemergence treatment, *A. tuberculatus* density increased to 69 plants m^{-2} at 9 WAPOST, a 60% increase in density compared with the density at 2 WAPOST. These plants produced up to 93,300 seeds m^{-2} at the time of corn harvest.

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

Amaranthus tuberculatus Control in Soybean

Density, biomass accumulation, and seed production of *A. tuberculatus* in soybean (2020) were influenced by the level of *A. tuberculatus* control in the prior year's corn crop in the corn–soybean rotation (Table 4). Similarly, the presence of cereal rye cover crop and/or row spacing (38-cm- vs. 76-cm-wide rows) of soybean influenced *A. tuberculatus* density, biomass accumulation, and seed production. Cereal rye cover crop or soybean row spacing had a significant two-way interaction with prior year's corn weed control for *A. tuberculatus* density at 3 and 9 WAP and biomass accumulation at 9 WAP. A two-way interaction between cereal rye cover crop and soybean row spacing was significant for *A. tuberculatus* density at 3 WAP and biomass accumulation at 9 WAP. The three-way interaction between prior year's corn weed control, cereal rye cover crop, and soybean row spacing was significant only for *A. tuberculatus* biomass accumulation and seed production at soybean harvest. Treatment means for two-way interactions were compared at the subplot level (Tables 5–7), whereas treatment means for the three-way interactions were compared at the sub-subplot level (Table 8).

Amaranthus tuberculatus Density in Soybean

All three weed control tactics tested (prior year's corn weed control, cereal rye cover crop, and soybean row spacing) reduced the density of *A. tuberculatus* in soybean at 3 and 9 WAP in 2020 (Tables 5–7). Among the treatments tested, *A. tuberculatus* density at 3 and 9 WAP was highest in the treatment that had inadequate *A. tuberculatus* control (30%) in the prior year's corn and no cover crop or wide-row soybean in the following year (Tables 5 and 6). Adequate control (≥90%) in the prior year's corn and presence of the cover crop reduced *A. tuberculatus* density by more than 70% at 3 and 9 WAP compared with inadequate control (30%) in the prior year's corn and no cover crop (Table 5). Similarly, adequate control (≥90%) in the prior year's corn and narrow-row soybean reduced *A. tuberculatus* density by more than 60% at 3 and 9 WAP compared with inadequate control (30%) in the prior year's corn and wide-row soybean (Table 6). Presence of the cover crop and narrow-row soybean reduced *A. tuberculatus* density by 44% at

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Table 4. Significance of fixed effects on *Amaranthus tuberculatus* density, biomass, seed production, and grain yield in soybean (corn–soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

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

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

Table 5. Effect of prior year's corn weed control and cereal rye cover crop on *Amaranthus tuberculatus* density and biomass in soybean (corn–soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

		Density[b]		Above ground biomass[b]
A. tuberculatus control in corn	Cereal rye cover crop in soybean	3 WAP	9 WAP	9 WAP
		—no. plants m^{-2}—		—g m^{-2}—
30% control	No cover crop	304 a	352 a	172 a
	Cover crop	209 b	258 b	73 c
90% control	No cover crop	122 c	158 c	127 b
	Cover crop	71 d	99 d	31 d
100% control	No cover crop	46 e	99 d	78 c
	Cover crop	35 e	61 e	33 d

[a]Abbreviation: WAP, weeks after (soybean) planting.
[b]Treatment means are the estimated values from PROC MIXED. Treatment means within a column with same letter are not significantly different (Tukey test, α = 0.05).

Table 6. Effect of prior year's corn weed control and soybean row spacing on *Amaranthus tuberculatus* density and biomass in soybean (corn–soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

		Density[b]		Above ground biomass[b]
A. tuberculatus control in corn	Soybean row spacing	3 WAP	9 WAP	9 WAP
		—no. plants m^{-2}—		—g m^{-2}—
30% control	76-cm rows	279 a	335 a	185 a
	38-cm rows	233 b	275 b	60 cd
90% control	76-cm rows	102 c	137 c	102 b
	38-cm rows	91 c	120 d	57 d
100% control	76-cm rows	43 d	87 e	80 bc
	38-cm rows	38 d	73 e	31 e

[a]Abbreviation: WAP, weeks after (soybean) planting.
[b]Treatment means are the estimated values from PROC MIXED. Treatment means within a column with same letter(s) are not significantly different (Tukey test, α = 0.05).

3 WAP compared with no cover crop and wide-row soybean (Table 7). The two-way interaction between cereal rye cover crop and soybean row spacing was not significant for *A. tuberculatus* density at 9 WAP (Table 4).

Table 7. Effect of cereal rye cover crop and soybean row spacing on *Amaranthus tuberculatus* density and biomass in soybean (corn–soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.[a]

		Density[b]	Above ground biomass[b]
Cereal rye cover crop in soybean	Soybean row spacing	3 WAP[c]	9 WAP
		—no. plants m^{-2}—	—g m^{-2}—
No cover crop	76-cm rows	163 a	171 a
	38-cm rows	151 a	81 b
Cover crop	76-cm rows	119 b	74 b
	38-cm rows	91 c	17 c

[a]Abbreviation: WAP, weeks after (soybean) planting.
[b]Treatment means are the estimated values from PROC MIXED. Treatment means within a column with same letter are not significantly different (Tukey test, α = 0.05).
[c]The *A. tuberculatus* density at 9 WAP is not shown in the table, as cover crop by soybean row spacing interaction was not significant.

Previous studies have shown that the level of weed control achieved in the prior year/crop can influence the weed control efficacy in the subsequent year/crop. For example, Hartzler and Roth (1993) reported that a premix of *S*-metolachlor and atrazine provided 88% control of smooth pigweed (*Amaranthus hybridus* L.) in plots that had received 100% control in the prior year compared with only 66% control in plots that had received 70% control in the prior year. Previous studies have shown that a cereal rye cover crop or reduced row spacing of soybean were effective in reducing *A. tuberculatus* density in soybean. In a field study conducted over 8 site-years, a cereal rye cover crop alone reduced late-season *A. tuberculatus* density by 40% compared with a no cover crop treatment (Cornelius and Bradley 2017). Similarly, *A. tuberculatus* density was reduced by 30% when soybean was planted in 19-cm- versus 76-cm-wide rows (Steckel and Sprague 2004).

Amaranthus tuberculatus Biomass in Soybean

Aboveground biomass accumulation of *A. tuberculatus* in soybean at 9 WAP and at the time of soybean harvest was reduced by all three weed control tactics tested (prior year's corn weed control, cereal rye cover crop, and soybean row spacing) (Tables 5–7). Among the treatments tested, highest biomass accumulation of *A. tuberculatus* at 9 WAP occurred in the treatments that had

Table 8. Effect of prior year's corn weed control, cereal rye cover crop, and soybean row spacing on *Amaranthus tuberculatus* biomass and seed production at soybean harvest (corn–soybean rotation) in 2020 at the Iowa State University Research Farms near Ames, IA, and Boone, IA.

A. *tuberculatus* control in corn	Cereal rye cover crop in soybean	Soybean row spacing	Above ground biomass[a]	Seed production[a]
			—g m^{-2}—	—no. m^{-2}—
30% control	No cover crop	76-cm rows	429 a	184,000 a
		38-cm rows	295 bc	106,000 c
	Cover crop	76-cm rows	157 e	32,000 g
		38-cm rows	81 f	26,000 g
90% control	No cover crop	76-cm rows	324 b	140,000 b
		38-cm rows	194 de	55,000 ef
	Cover crop	76-cm rows	96 f	36,000 fg
		38-cm rows	81 f	27,000 g
100% control	No cover crop	76-cm rows	302 bc	120,000 bc
		38-cm rows	252 cd	77,000 d
	Cover crop	76-cm rows	190 de	74,000 de
		38-cm rows	80 f	25,000 g

[a]Treatment means are the estimated values from PROC MIXED. Treatment means within a column with same letter(s) are not significantly different (Tukey test, $\alpha = 0.05$).

inadequate *A. tuberculatus* control (30%) in the prior year's corn and no cover crop or wide-row soybean in the following year (Tables 5 and 6). Adequate control (≥90%) in prior year's corn and presence of cover crop reduced *A. tuberculatus* biomass accumulation by more than 80% at 9 WAP compared with inadequate control (30%) in prior year's corn and no cover crop (Table 5). A combination of 90% control in the prior year's corn and narrow-row soybean reduced *A. tuberculatus* biomass by 69% at 9 WAP compared with inadequate control (30%) in the prior year's corn and wide-row soybean (Table 6). In contrast, a combination of 100% control in the prior year's corn and narrow-row soybean reduced *A. tuberculatus* biomass by 83% at 9 WAP compared with inadequate control (30%) in the prior year's corn and wide-row soybean. Presence of the cover crop and narrow-row soybean reduced *A. tuberculatus* biomass accumulation by 90% at 9 WAP compared with no cover crop and wide-row soybean (Table 7). Consistent with our results, Steckel and Sprague (2004) reported that planting soybean in narrow rows (19-cm vs. 76-cm wide) reduced late-season *A. tuberculatus* biomass by more than 75%. Williams et al. (1998) reported that a cereal rye cover crop reduced *A. tuberculatus* and redroot pigweed (*Amaranthus retroflexus* L.) canopy volume by 70% in soybean at 3 WAP.

Overall, inclusion of a single control tactic, adequate control (≥90%) with multiple herbicides in the prior year's corn, use of a rye cover crop, or narrow-row soybean, reduced *A. tuberculatus* biomass accumulation by at least 25% at the time of soybean harvest compared with inadequate control (30%) in the prior year's corn, no cover crop, and wide-row soybean (Table 8). Inclusion of any combination of two of the three control tactics reduced *A. tuberculatus* biomass accumulation by at least 40% at the time of soybean harvest, but the combination of all three control tactics reduced *A. tuberculatus* biomass accumulation by at least 80% at the time of soybean harvest.

Amaranthus tuberculatus Seed Production in Soybean

Similar to density and biomass, *A. tuberculatus* seed production at the time of soybean harvest was affected by all three weed control tactics tested (prior year's weed control, cereal rye cover crop, and soybean row spacing) (Table 8). Inadequate *A. tuberculatus* control (30%) in the prior year's corn, no cover crop, and wide-row soybean resulted in the production of the largest number of seeds (184,000 seeds m^{-2}) among the treatments tested. In the treatment that had inadequate *A. tuberculatus* control (30%) in the prior year's corn and no cover crop, narrow-row soybean reduced *A. tuberculatus* seed production by 42%. In a previous study, planting soybean in a reduced row spacing (19-cm vs. 76-cm wide) decreased *A. tuberculatus* seed production by more than 30% (Steckel and Sprague 2004). In the present study, the treatment that had inadequate *A. tuberculatus* control (30%) in the prior year's corn and wide-row soybean, the cover crop alone reduced *A. tuberculatus* seed production by 83%. These results are consistent with previous findings by Williams et al. (1998) wherein the presence of a cereal rye cover crop reduced *A. tuberculatus* and *A. retroflexus* fecundity by more than 80% in soybean. Our results also indicated that in the treatment that had 100% *A. tuberculatus* control in the prior year's corn and wide-row soybean, presence of the cover crop reduced *A. tuberculatus* seed production by only 38%. This is likely due to a high biomass accumulation by *A. tuberculatus* plants in the treatment at the time of soybean harvest (Table 8).

Overall, inclusion of a single control tactic, adequate control (≥90%) in the prior year's corn, use of a cover crop, or narrow-row soybean, reduced *A. tuberculatus* seed production by at least 24% compared with inadequate control (30%) in the prior year's corn, no cover crop, and wide-row soybean. Combined use of the two nonchemical control tactics, namely cereal rye cover crop and narrow-row soybean, was effective in reducing *A. tuberculatus* seed production by 85%, regardless of the weed control level in the prior year's corn.

Soybean Grain Yield

Planting soybean into a cereal rye cover crop did not affect soybean stand count (data not shown) or soybean grain yield (Table 4). Previous research also reported that a cereal rye cover crop provided weed suppression without reducing soybean grain yield (Bish et al. 2021; Bunchek et al. 2020; Hodgskiss et al. 2021). Among all factors tested, only soybean row spacing affected soybean grain yield ($P = 0.03$). Narrow-row soybean produced 14% higher grain yield (3,800 kg ha^{-1}) than wide-row soybean (3,250 kg ha^{-1}). This was likely attributable to increased light interception by the soybean canopy in narrow rows compared with wide rows. For instance, soybean planted in narrow rows achieved canopy closure by 9 WAP, approximately 2 wk earlier than the soybean planted in wide rows (data not shown). Previous studies suggest that reducing the soybean row spacing shortens the time required to achieve canopy closure, thereby enhancing light interception and weed suppression (Board et al. 1990; Légère and Schreiber 1989; Steckel and Sprague 2004).

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Management Implications

Results from the present study indicate that combinations of diverse weed control tactics ("many little hammers") are required to manage MHR *A. tuberculatus* seedbanks in corn–soybean rotations. Late-season weed survivors/escapes may not reduce crop yields, but seed inputs can augment the weed seedbank, which makes the seedbank more persistent and imposes a high burden on weed control practices in the subsequent crop(s) in the rotation (Cousens 1987; Dieleman et al. 1999; Hartzler and Roth 1993). This is more important when combating HR weed populations, as a high weed seedbank density will put more burden on subsequent herbicides and accelerate the evolution of MHR weed populations (Neve et al. 2011). Corn and soybean canopy development affect *A. tuberculatus* density, growth, and seed production differently (Uscanga-Mortera et al. 2007) and may require different management practices. Therefore, control tactics targeting weed seed production, such as an aggressive herbicide program employing multiple sites of action in corn and a cereal rye cover crop or narrow-row soybean, can be used to reduce seedbank additions of HR *A. tuberculatus* populations.

Amaranthus tuberculatus has an extended emergence period (Hartzler et al. 1999), and it is likely that a single control tactic will not provide adequate weed control (Tranel 2021). Therefore, multi-tactic strategies targeting *A. tuberculatus* at multiple life-cycle stages are desirable at a cropping-system level. For instance, adequate *A. tuberculatus* control achieved by utilizing a multiple sites of action herbicide program in prior year's corn reduced plant density in the following year's soybean. A cereal rye cover crop provided early-season *A. tuberculatus* control in soybean, mostly through physical suppression (Teasdale and Mohler 1993). Allelopathy might also have played a role in early-season weed suppression (Teasdale et al. 2012), although it was not quantified in the present study. Planting soybean in a reduced row spacing was also a complementary strategy to control late-emerging *A. tuberculatus* cohorts through early crop canopy closure shading (Steckel and Sprague 2004).

Finally, none of the control tactics tested provided satisfactory levels of *A. tuberculatus* control when used alone. However, the cumulative effect of combining adequate control in prior year's corn, a cereal rye cover crop, and narrow-row soybean provided more than 80% control of *A. tuberculatus*. It might also have reduced the burden on subsequent control tactics. For example, a high weed seedbank density can decrease the quantity of soil-applied herbicides absorbed by individual plants (Hoffman and Lavy 1978; Winkle et al. 1981), resulting in reduced efficacy and a greater burden on a subsequent postemergence herbicide(s). Similarly, a cereal rye cover crop alone would not provide effective control of *A. tuberculatus*, but could potentially increase herbicide efficacy by reducing weed density and biomass at the time of postemergence application (Myers et al. 2005; Wallace et al. 2019). In conclusion, our study shows that "many little hammers" across 2 yr in a corn–soybean rotation can be used to suppress *A. tuberculatus* populations that are resistant to the "big hammer"—herbicide. It should be noted that weed control tactics evaluated in the present study were focused on controlling *A. tuberculatus* during the early season. Therefore, additional control tactics such as harvest weed seed control should be used to reduce *A. tuberculatus* seeds returning to seedbank from the escapes/survivors. Future research is needed to determine how these ecological tactics, namely cereal rye cover crop and narrow-row soybean, can affect *A. tuberculatus* percent seed retention at the time of soybean harvest, a crucial factor for the success of harvest weed seed control methods.

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