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The inclusion of cereal crops with maize and soyabean rotations impacts the morphology and phenology of *Amaranthus tuberculatus*

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Abstract

Simple crop rotations like maize and soyabean grown in succession often coincide with simple weed management tactics, resulting in weed infestations that are highly adapted to the conditions maintained for the selected crops. *Amaranthus tuberculatus* is a prominent weed species which possesses biological characteristics that allow it to persist in maize–soyabean rotations. Spring wheat and oats emerge earlier than *A. tuberculatus* and are grown in narrower rows than maize and soyabean, which may increase their competitive capability. A field study which measured morphological and phenological characteristics of three *A. tuberculatus* cohorts that emerged under fallow, maize, soyabean, spring wheat and oats was conducted to compare their competitive ability. Early emerged *A. tuberculatus* grew similarly in fallow, maize and soyabeans and grew to heights of >150 cm but was significantly reduced in spring wheat and oats, never exceeding 40 cm. Heights of the last emerged cohorts were more influenced by crop canopies never reaching 15 cm in spring wheat and oats compared to >70 cm in fallow. Early emerged *A. tuberculatus* canopy diameter and branches plant^{-1} were reduced by >80% in spring wheat and oats compared to fallow. Flowering was delayed in spring wheat and oats compared to fallow and seed production and biomass were reduced by 99% across cohorts. Growth suppression of *A. tuberculatus*, based on the findings, can be achieved under dense, competitive crop canopies such as in spring wheat and oats and these crops should be considered as additional crops in maize–soyabean rotations.

KEYWORDS

crop rotation diversification, integrated weed management, waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Saur), weed ecology

1 | INTRODUCTION

Crop rotations in the Midwest United States (US) are primarily maize (*Zea mays* L.) and soyabean (*Glycine max* L.) grown in succession or maize-only rotations (Weisberger et al., 2021). Farms in the past included livestock, legumes, small grains and forages grown in extended rotations for nutrient cycling and pest management (Sulc & Tracy, 2007). The advent of synthetic fertilisers and pesticides, coupled with improved mechanisation, reduced the utility of diverse crop rotations (Davis et al., 2012). Current rotations with one or two

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cash crops have increased farm economic growth (Sulc & Tracy, 2007). However, simplified crop rotations are a weed management problem because they often have simple weed management tactics, leading to increased selection pressure on the weed populations (Liebman et al., 2001). This is exemplified within maize and soyabeans rotations which have similar growth phenology with weeds, and growers employ similar weed management tactics (i.e., herbicides). This simplification of weed management tactics causes weed communities to shift, resulting in only a few weed species that are highly adapted to the conditions maintained for the selected crops (Liebman & Dyck, 1993). Weed community shifts were observed in response to the rapid adoption of glyphosate-tolerant crops in the late 1990s, resulting in selected species with natural tolerance or evolved resistance to glyphosate (Owen, 2008). One species that rose to prominence was *Amaranthus tuberculatus* (Moq.) J.D. Saur (waterhemp).

A. tuberculatus is a troublesome weed in the US maize belt (Van Wychen, 2022). Several life-history traits facilitate *A. tuberculatus* to persist in maize and soyabean rotations, including high fecundity even when growing in competition with both crops, allowing for rapid replenishment of the soil seed bank (Hartzler et al., 2004; Nordby & Hartzler, 2004). *A. tuberculatus* possesses extended germination periodicity with cohorts emerging from May through late August in the Midwest US (Nordby & Hartzler, 2004; Werle et al., 2014). The high relative growth rate of *A. tuberculatus* allows for competition within row crops and can reduce maize and soyabean yields by 74% and 56% respectively (Bensch et al., 2003; Steckel & Sprague, 2004b). The dioecious reproductive habit of *A. tuberculatus* results in high genetic diversity, supporting rapid adaptation to management tactics (Costea et al., 2005; Tranel, 2021). Recent Midwest US surveys reported *A. tuberculatus* populations that are multiple herbicide-resistant (MHR) to four, five and six herbicide sites of action (SOA) groups (Schultz et al., 2015; Shergill et al., 2018). Hamberg et al. (2023) reported that over 40% of Iowa *A. tuberculatus* populations had three-way MHR.

Given the propensity of *A. tuberculatus* to evolve resistance to any single control tactic, integrating multiple tactics that combine non-chemical and chemical control tactics should be explored. Non-chemical management tactics for *A. tuberculatus* are now increasingly important and should be included in weed management plans (Liebman et al., 2001). The additive benefits of combining multiple tactics for controlling *A. tuberculatus* within Midwest US maize–soyabean rotations have reduced seed production and biomass by at least 80% (Yadav et al., 2023). In addition, Yadav et al. (2023) concluded that weed control efficacy in the previous crop influenced population densities and control of *A. tuberculatus* in the subsequent year, emphasising the importance of growers considering longer-term weed management and not just for a single season.

Crop competition can significantly impact *A. tuberculatus* population density, biomass, and seed production (Nguyen & Liebman, 2022; Uscanga-Mortera et al., 2007). Furthermore, *A. tuberculatus* emergence timing during the growing season impacts plant biomass, fecundity and phenology (Wu & Owen, 2014). Uscanga-Mortera et al. (2007) reported when *A. tuberculatus* was transplanted after the V5 growth stage in maize and soyabean, dry weight and seed production were reduced by ≥90%. Arsenijevic et al. (2022) found that soyabeans planted in 38 cm rows required fewer days to suppress *A. tuberculatus* growth by 50% compared to soyabeans grown in 76 cm rows. Although maize and soyabean competitiveness on *A. tuberculatus* has been well-studied, few studies have compared *A. tuberculatus* growth within crops that differ in phenology with *A. tuberculatus*. Spring wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.) are cool-season grains planted in late March to mid-April in Iowa and have previously been grown in rotations with soyabean and maize (Sulc & Tracy, 2007). Spring wheat and oats emerge earlier than *A. tuberculatus*, maize and soyabean and are harvested in mid-July compared to September through October for maize or soyabean. In addition, spring wheat and oats are drill-seeded in 19 cm rows while maize and soyabean are typically planted with a 76 cm row spacing in Iowa. Crops planted in narrower row spacings increase ground cover and light interception quickly, thereby potentially reducing weed biomass accumulation (Drews et al., 2009; McLachlan et al., 1993).

The objective of this study was to evaluate how fallow, maize, soyabean, spring wheat and oat canopies affect the morphology and phenology of three *A. tuberculatus* cohorts that emerged at separate timings during the growing season.

2 | MATERIALS AND METHODS

2.1 | Experimental site

Field experiments were conducted in 2022 and 2023 at the Iowa State University Bruner Farm in Boone, IA (42.010° N, 93.736° W). The field site was in a maize–soyabean rotation for more than 10 years and planted with soyabean in 2021. The field was chisel-ploughed before the initiation of the experiments, and fertiliser was applied to the entire experimental area based on the removal rates of the previous crop. Climate data were recorded from a weather station within 5 km of the field site and are summarised in Figure 1.

2.2 | Experimental procedures

The experiment was a factorial arrangement with five canopy types (fallow, maize, soyabean, spring wheat and oat), three *A. tuberculatus* emergence cohorts, and four replications ($N = 5$ canopy types $\times$ 3 cohorts $\times$ 4 replications $=$ 60 plots). Plot dimensions were 3 m $\times$ 10 m with a 1.5 m buffer between treatment plots to reduce edge effects. Planting dates for all crops were normal for the Midwest US given the variations that occur attributable to weather. The experiment was moved to an adjacent location within the same field in 2023. Spring wheat (Commander, Dyna-grow, Loveland, CO, USA) and oats (Defender 265, La Cross Seed, La Cross, WI, USA) were drill-seeded in 19 cm spacing on 30 April 2022 at 2 700 000 and 2 960 000 seeds ha^{-1} respectively. Maize (NK0886-5122, NK

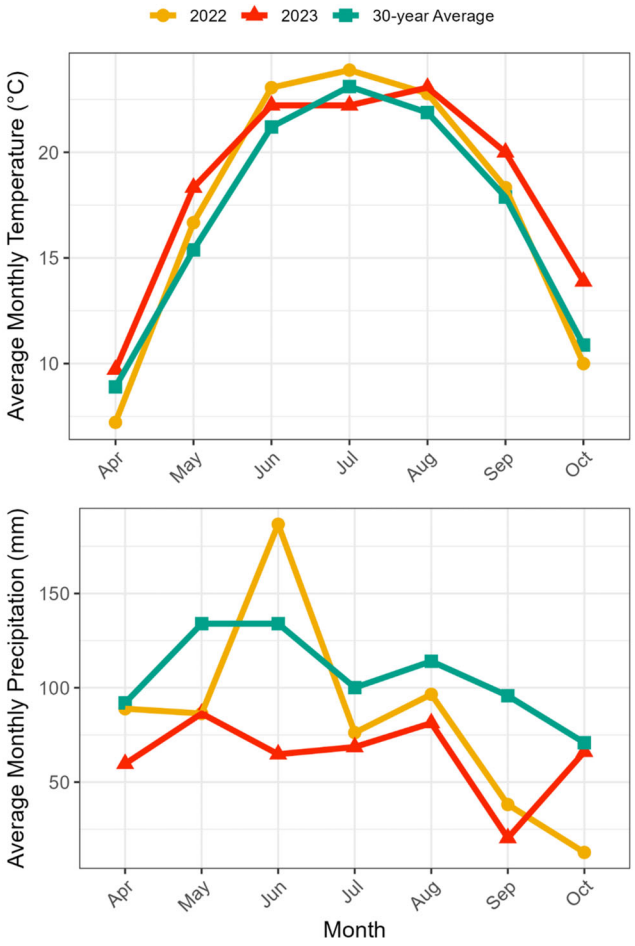


FIGURE 1 Monthly mean air mean temperature and precipitation for the 2022 and 2023 field experiments at Boone, Iowa, USA.

TABLE 1 Crop planting and *Amaranthus tuberculatus* cohort establishment date for 2022 and 2023 experiments at Boone, Iowa, USA.

	Planting date	
Crop	**2022**	**2023**
Maize	16 May	1 May
Soyabeans	16 May	1 May
Spring wheat	30 April	12 April
Oats	30 April	12 April
	Establishment date	
***A. tuberculatus* cohort**	**2022**	**2023**
1	30 May	17 May
2	16 June	23 May
3	4 July	20 June

Syngenta US, Wilmington, DE, USA) and soyabean (S26-E3, NK Syngenta US, Wilmington, DE, USA) were planted in 76 cm rows on 16 May 2022 at 76 000 and 380 000 seeds ha^{-1} respectively. The same varieties, row spacing, and seeding rates were used in 2023; however, planting dates were 12 April for spring wheat and oats and 1 May for maize and soyabean (Table 1). Dry, granular urea fertiliser (46-0-0) was applied to maize, spring wheat and oats for supplemental nitrogen at rates of 28 kg ha^{-1}. Once crops had emerged, stand counts were conducted to confirm that the targeted plant stands had been achieved. The cohort establishment was based upon natural emergence flushes of *A. tuberculatus* seedlings throughout the growing season, with each of the three cohorts being established at 2-week intervals (adapted from Wu & Owen, 2014). *A. tuberculatus* population densities within each flush were counted before cohort establishment each year and averaged 150, 200 and 4 plants m^{-2} for Cohorts 1, 2 and 3 respectively. Cohort establishment consisted of selecting eight *A. tuberculatus* plants that were 0.5 cm tall and at the two-leaf stage. The growth stage and height requirement during selection were to ensure that the *A. tuberculatus* plants had emerged at similar times. Selected plants were spaced at least 0.5 m apart in each plot to minimise intraspecific competition (adapted from Hartzler et al., 2004). Selected plants were marked with plastic zip-ties, and all other vegetation was removed via hand weeding. Cohort 1 was established on 30 May and 17 May in 2022 and 2023 respectively. Cohorts 2 and 3 were established using the above-mentioned methods 2 and 4 weeks after Cohort 1 (Table 1). Harvest for spring wheat and oats occurred on 26 July and 18 July in 2022 and 2023 respectively. Maize and soyabean harvest occurred in mid-October, which was after the completion of all *A. tuberculatus* observations.

2.3 | Data collection

Individual *A. tuberculatus* plant height measurements were conducted weekly for each cohort by measuring the plants from the soil surface to the apical meristem until all plants reached the reproductive stage. Canopy diameter measurements and the number of primary branches were counted at the last height measurement. Canopy diameter was measured by using the main stem as the centre point and measuring the widest plant span (adapted from Uscanga-Mortera et al., 2007), and only primary branches from the main stem were considered when counting branch numbers. Flower initiation and plant sex were recorded for each plant. Cumulative growing degree days (GDD base 10 C) for each plant measurement data were recorded using the following equation:

$$GDD_{10} = \left(\frac{\text{Maximum temperature} + \text{minimum temperature}}{2}\right) - 10. \quad (1)$$

When most seeds had turned black (signifying maturity), plants were excised at the soil surface and placed in 158 L of woven contractor bags (Global Strategies Holding Corp., Chapin, SC, USA) to prevent biomass and seed loss. Plants were then air-dried at room temperature for 1 week and aboveground biomass was recorded. Seed heads were removed from female plants, threshed, and processed through a series of sieves, and an air column (Seedburo® Equipment, Des

Plaines, IL 60018) was used to separate seeds from plant matter. The total seed number for each female *A. tuberculatus* plant was determined by counting the number of seeds within two 0.1 g subsamples and then using the equation (adapted from Jones et al., 2019):

$$T = (S \times 10) \times W, \quad (2)$$

where W is the total seed mass per female plant, S equals the average seed number of the two 0.1 g subsamples and T equals the total number of seeds produced per female *A. tuberculatus* plant.

2.4 | Statistical analysis

All response variables were analysed with R statistical software v. 4.3.2 (R Core Team, 2024) as mixed-effects models using the *lme4* package (Bates et al., 2015). The models for all response variables included canopy type, *A. tuberculatus* cohort, and their interaction as fixed effects and blocks as random effect. The data for canopy diameter, number of branches, plant dry weight and seed production violated the assumptions of normality and homogeneity of variance and were thus log-transformed before analysis (Curran-Everett, 2018). Treatment means were compared using Tukey's honestly significant difference test at $\alpha = 0.05$ using the *emmeans* package (Lenth, 2024). Untransformed means and standard errors are presented for easier interpretation. Plant height data were analysed using nonlinear regression models with the *drc* package in R (Ritz et al., 2015). A three-parameter log-logistic model was fitted to the plant height data using the following equation:

$$y = \frac{d}{1 + \exp[b(\log x - \log e)]}, \quad (3)$$

where y is the *A. tuberculatus* plant height, b is the slope at the inflection point, d is the 'upper limit' and e is the GDD_{10} required to achieve 50% plant height. Due to non-normal data distribution, Kendall's rank correlation coefficient was used to determine correlations between morphological features and seed production using the *cor.test* function in R (Abdi, 2007). Significant differences between years were observed. Therefore, site years are presented separately. The *ggplot2* package was used for all data visualisation (Wickham, 2016).

3 | RESULTS

3.1 | *A. tuberculatus* height

The logistic models show that *A. tuberculatus* plants in Cohort 1 grew similarly in fallow, maize and soyabeans, but growth was severely reduced in spring wheat and oats (Figure 2). In 2022, the GDD required to reach 50 cm (GDD_{50cm}) for Cohort 1 was similar for fallow and maize and less in soyabean, taking 967, 924 and 856 GDD respectively. The GDD_{50cm} for all three was similar in 2023, taking 715, 704 and 727 GDD for fallow, maize and soyabean respectively.

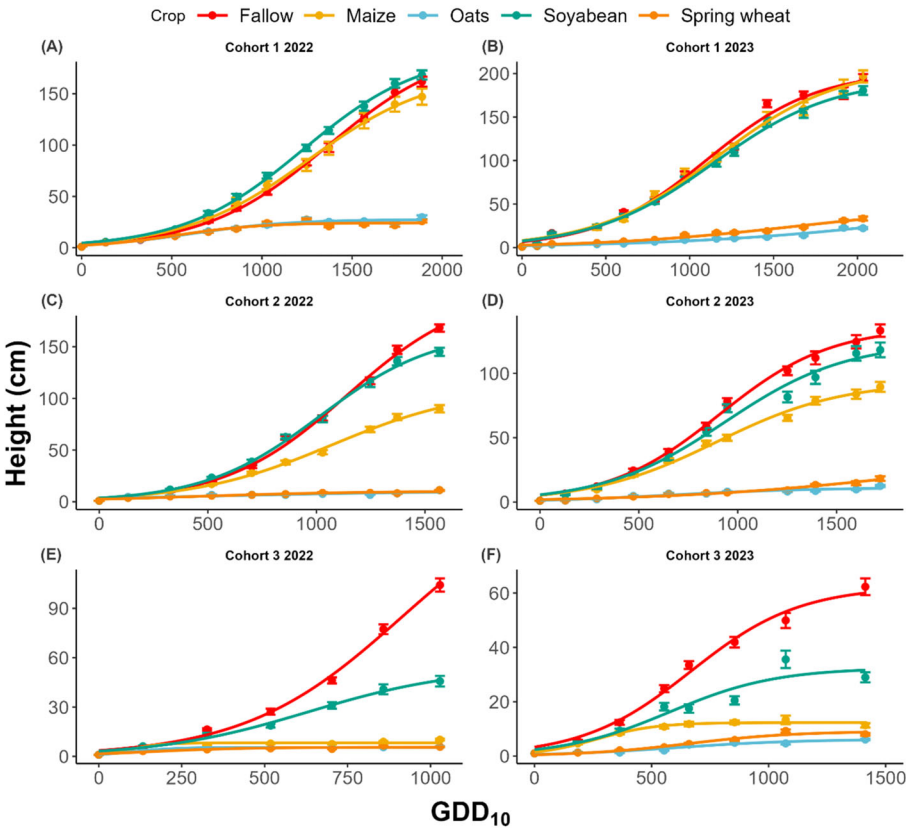


FIGURE 2 Height of *Amaranthus tuberculatus* Cohorts 1 (A and B), 2 (C and D) and 3 (E and F) in fallow, maize, oat, spring wheat and soyabean canopies. Experiments conducted in Boone, Iowa, USA in 2022 (left) and 2023 (right). Points (±SE) represent actual values, whereas lines represent predicted values from a three-parameter log-logistic model.

TABLE 2 Regression parameter estimates for *Amaranthus tuberculatus* height for three cohorts within four crop canopies and fallow in 2022[a].

Canopy	Cohort	Parameter estimates[b] ± SE		
		b	*d*	GDD_{50cm}
Fallow	1	2.37 ± 0.16	251.9 ± 42.5	967.3 ± 74.4
	2	2.65 ± 0.13	227.6 ± 17.7	807.1 ± 23.7
	3	2.08 ± 0.01	138.2 ± 19.2	701.86 ± 494.4
Maize	1	2.18 ± 0.15	211.5 ± 29.6	923.9 ± 68.2
	2	2.04 ± 0.25	143.2 ± 37.3	1009.6 ± 162.8
	3	0.35 ± 0.06	30.1 ± 7.6	–[c]
Soyabean	1	2.30 ± 0.13	223.1 ± 21.3	855.9 ± 39.7
	2	2.61 ± 0.14	169.9 ± 7.7	779.8 ± 15.7
	3	1.46 ± 0.35	99.6 ± 9.9	1097.4 ± 941.6
Oats	1	1.32 ± 0.43	29.9 ± 5.6	–
	2	0.48 ± 0.14	16.9 ± 13.5	–
	3	0.43 ± 0.35	11.4 ± 2.6	–
Spring wheat	1	1.54 ± 0.50	24.7 ± 2.2	–
	2	0.65 ± 0.35	20.2 ± 14.2	–
	3	0.43 ± 0.19	8.3 ± 0.5	–

[a]Cohorts established on 30 May, 16 June and 5 July 2022 respectively.
[b]*b* is the slope at the inflection point, *d* is the upper limit and GDD_{50cm} is the GDD_{10} required for plants to reach 50 cm in height.
[c]Dashes indicate when 50 cm height was never reached.

TABLE 3 Regression parameter estimates for *Amaranthus tuberculatus* height for three cohorts within four crop canopies and fallow in 2023[a].

Canopy	Cohort	Parameter estimates[b] ± SE		
		b	*d*	GDD_{50cm}
Fallow	1	2.08 ± 0.11	213.5 ± 8.5	714.8 ± 14.9
	2	1.87 ± 0.11	154.2 ± 10.1	727.2 ± 32.3
	3	2.08 ± 0.01	70.1 ± 4.8	1032.9 ± 92.2
Maize	1	2.18 ± 0.15	260.2 ± 26.0	703.8 ± 38.7
	2	1.45 ± 0.16	128.1 ± 26.7	935.2 ± 186.2
	3	0.35 ± 0.06	12.4 ± 0.72	–[c]
Soyabean	1	2.61 ± 0.14	228.8 ± 19.4	726.7 ± 32.6
	2	1.63 ± 0.27	162.5 ± 22.5	788.7 ± 84.3
	3	1.46 ± 0.35	32.4 ± 1.5	–
Oats	1	1.28 ± 0.45	36.6 ± 4.5	–
	2	0.95 ± 0.13	18.4 ± 7.0	–
	3	0.32 ± 0.23	5.2 ± 0.68	–
Spring wheat	1	1.25 ± 0.18	38.4 ± 15.7	–
	2	1.27 ± 0.36	22.2 ± 16.5	–
	3	0.37 ± 0.20	8.6 ± 0.97	–

[a]Cohorts established on 17 May, 5 June and 20 June 2023 respectively.
[b]*b* is the slope at the inflection point, *d* is the upper limit and GDD_{50cm} is the GDD_{10} required for plants to reach 50 cm in height.
[c]Dashes indicate when 50 cm height was never reached.

In spring wheat and oats, *A. tuberculatus* plants never reached 50 cm in height in either year (Tables 2 and 3). The Cohort 1 *A. tuberculatus* plants within spring wheat and oats only reached 25 and 30 cm in 2022 and 38 and 37 cm in 2023 respectively.

The influence of crop canopy was greater on later emerging *A. tuberculatus*, cohorts; Cohort 2 plants emerging in maize took 924 and 1033 GDD to reach 50 cm compared to fallow, which were 807 and 727 GDD in 2022 and 2023 respectively. Moreover, in spring

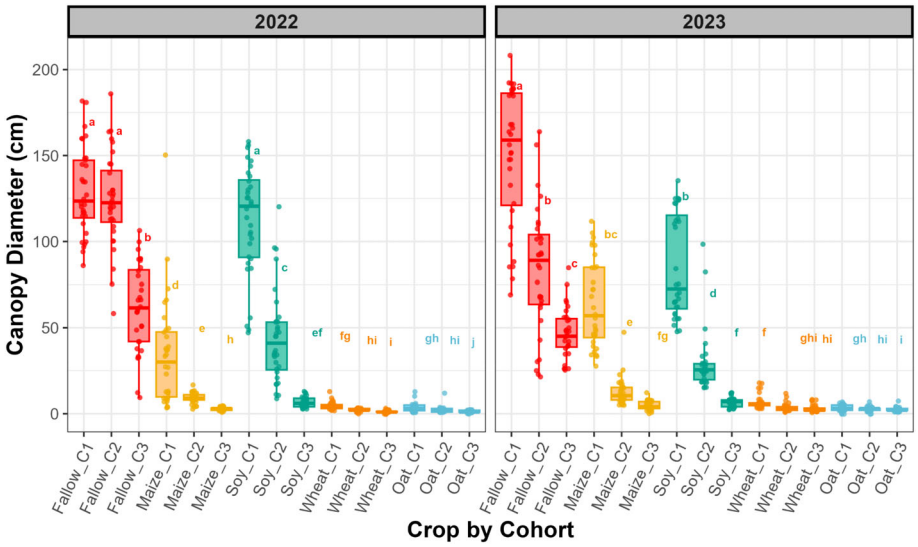


FIGURE 3 Canopy diameter of three *Amaranthus tuberculatus* cohorts (C1, C2 and C3) grown under fallow, maize, soyabean, spring wheat and oat canopies in Boone, Iowa, USA in 2022 (left) and 2023 (right). Cohorts were established on 30 May, 16 June and 5 July in 2022 and on 16 May, 5 June and 20 June in 2023. Boxes, lines, and dots represent the middle 50% of data, minimum and maximum observations within 1.5 the interquartile range, and each individual observation ($N = 32$) respectively. Boxes topped by the same letter(s) are not significantly different; Tukey's honestly significant difference (HSD; $\alpha = 0.05$).

wheat and oats, *A. tuberculatus* plants in Cohort 2 never exceeded 25 cm (Tables 2 and 3). Conversely, soyabean canopy did not affect *A. tuberculatus* height in Cohort 2, with GDD_{50cm} compared to fallow (Tables 2 and 3). As expected across all crop canopies, greater growth suppression was observed for Cohort 3 *A. tuberculatus* plants (Figure 2; Tables 2 and 3). Only *A. tuberculatus* plants in fallow and soyabean reached ≥50 cm in 2022, and only fallow reached ≥50 cm in 2023. Moreover, the maximum heights for Cohort 3 *A. tuberculatus* plants under spring wheat and oat canopies never exceeded 15 cm (Tables 2 and 3).

3.2 | *A. tuberculatus* canopy diameter and branching

The effect of the canopy, cohort and their interaction on the *A. tuberculatus* canopy diameter was significant ($p < 0.001$). The later emerging *A. tuberculatus* plants in Cohorts 2 and 3 had smaller canopies than Cohort 1 plants (Figure 3). *A. tuberculatus* canopy diameter was significantly reduced in maize compared to fallow (Figure 3). The mean canopy diameter of *A. tuberculatus* plants in Cohort 1 was 129 and 150 cm in fallow, compared to 34 and 64 cm in maize in 2022 and 2023 respectively. Similarly, the canopy diameter of *A. tuberculatus* plants was significantly lower in soyabean compared to fallow, except for Cohort 1 in 2022. The canopy diameter for *A. tuberculatus* plants in Cohort 2 in maize was significantly lower than in soyabean (Figure 3). However, in Cohort 3, the results varied, with significant differences observed in 2022 but not in 2023 (Figure 3). Spring wheat and oats severely reduced the canopy diameter of *A. tuberculatus* plants in all three cohorts, and canopies never reached 20 cm (Figure 3).

The effects of canopy, cohort and their interactions on *A. tuberculatus* branching were significant ($p < 0.001$). No differences in the number of branches on *A. tuberculatus* plants were observed for fallow, maize and soyabeans in Cohort 1 (Figure 4). In addition, in 2022, Cohort 2 branching in fallow and soyabean was not significantly different from Cohort 1 fallow, maize and soyabean; however, differences were observed in 2023 (Figure 4). The branch number of *A. tuberculatus* plants in Cohort 3 was reduced in maize and soyabean compared to fallow. Mean branches plant^{-1} in 2022 were 19 in fallow compared to 1 and 6 in maize and soyabean respectively. Moreover, mean branches plant^{-1} in 2023 for fallow were 13 compared to 3 and 4 in maize and soyabean respectively. Branches plant^{-1} were significantly reduced across all cohorts in spring wheat and oat compared to fallow, with means never exceeding 5 for either crop (Figure 4). In addition, in 2023, branches plant^{-1} of *A. tuberculatus* were similar in Cohort 3 maize compared to spring wheat and oats in Cohort 1, which provides further evidence of the suppressive ability of both crops on early emerged *A. tuberculatus*.

3.3 | *A. tuberculatus* biomass, flowering and seed production

The effect of canopy, cohort and their interaction on the dry weight of *A. tuberculatus* was significant ($p < 0.001$). *A. tuberculatus* Cohort 1 plants in fallow were significantly heavier than all other treatments in 2022 and 2023 (Figure 5). In 2022, the mean dry weights of *A. tuberculatus* plants in Cohort 1 were 563, 21 and 156 g in fallow, maize and soyabean respectively. In addition, mean dry weights in Cohort 1 *A. tuberculatus* plants were 423, 28, and 58 g in fallow, maize and soyabean, respectively, in 2023. Significant differences were observed in maize and soyabean for Cohort 1 *A. tuberculatus* plants (Figure 5). Cohort 2 *A. tuberculatus* plants in fallow were significantly heavier than maize and soyabean Cohort 1 plants in 2022. However, in 2023, Cohort 2 fallow plants were heavier than Cohort 1 maize but the same as Cohort 1 soyabean (Figure 5). *A. tuberculatus* plants in Cohort 2 for fallow, maize and soyabean were significantly heavier than Cohort 1 plants in spring wheat and oats (Figure 5). The mean dry weights of *A. tuberculatus* in Cohort 1 were similar in spring wheat

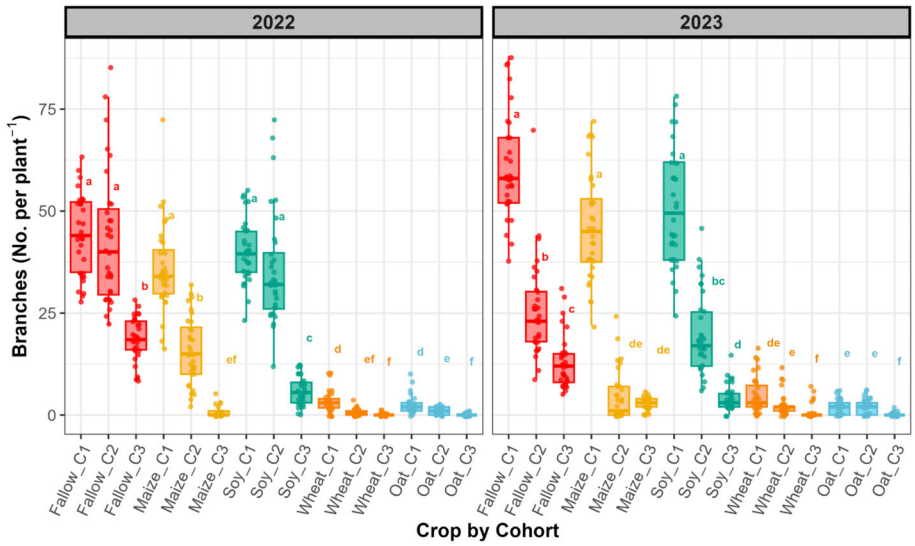


FIGURE 4 Branch numbers of three *Amaranthus tuberculatus* cohorts (C1, C2 and C3) grown under fallow, maize, soyabean, spring wheat and oat canopies in Boone, Iowa, USA in 2022 (left) and 2023 (right). Cohorts were established on 30 May, 16 June and 5 July in 2022 and on 16 May, 5 June and 20 June in 2023. Boxes, lines and dots represent the middle 50% of data, minimum and maximum observations within 1.5 the interquartile range, and each individual observation ($N = 32$) respectively. Boxes topped by the same letter(s) are not significantly different; Tukey's honestly significant difference (HSD; $\alpha = 0.05$).

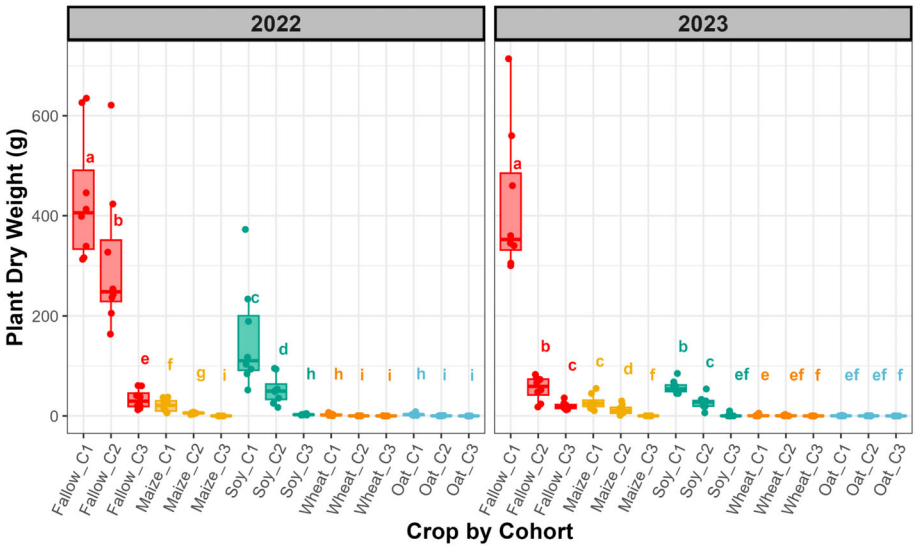


FIGURE 5 Aboveground plant dry weight of three *Amaranthus tuberculatus* cohorts (C1, C2 and C3) grown under fallow, maize, soyabean, spring wheat and oat canopies in Boone, Iowa, USA in 2022 (left) and 2023 (right). Cohorts were established on 30 May, 16 June and 5 July in 2022 and on 16 May, 5 June and 20 June in 2023. Boxes, lines, and dots represent the middle 50% of data, minimum and maximum observations within 1.5 the interquartile range, and each individual observation ($N = 8$) respectively. Boxes topped by the same letter(s) are not significantly different; Tukey's honestly significant difference (HSD; $\alpha = 0.05$).

and oats at <3 and 3 g in 2022 and 1 and 0.4 g in 2023 respectively. *A. tuberculatus* plants in Cohorts 2 and 3 in spring wheat and oats had mean dry weights <1 g for 2022 and 2023, with no statistical differences detected between either in 2022 (Figure 5).

The flowering time for *A. tuberculatus* plants was influenced by cohort, canopy and their interactions ($p < 0.001$). The fastest flowering time was achieved by Cohort 3 *A. tuberculatus* plants in fallow plots and took only 36 and 37 days in 2022 and 2023 respectively. Generally, when comparing cohorts, *A. tuberculatus* plants in Cohort 3 reached the flowering stage in fewer days than Cohorts 1 and 2 within respective crops (Table 4). The influence of canopy on *A. tuberculatus* flowering was varied and inconsistent (Table 4). *A. tuberculatus* Cohort 1 plants in fallow, maize and soyabean reached flowering in a similar number of days in 2022 (Table 4). However, in 2023, plants in Cohort 1 fallow flowered significantly faster than compared to maize and soyabean (Table 4).

One consistent trend within these data was the influence of spring wheat and oat canopies on *A. tuberculatus* flowering. *A. tuberculatus* Cohort 1 plants in spring wheat and oat took significantly longer to reach flowering than all other treatments, taking over ≥70 days in 2022 and 2023. Similarly, *A. tuberculatus* plants in Cohort 2 took 65 and 66 days to flower in spring wheat and oats, respectively, significantly longer than Cohort 2 in fallow plots in 2023. In addition, Cohort 3 *A. tuberculatus* plants in spring wheat and oat never reached 50% flowering (Table 4).

The effects of canopy, cohort and their interactions on *A. tuberculatus* seed production were significant ($p < 0.001$). Cohort 1 *A. tuberculatus* plants in fallow produced significantly more seeds than in maize and soyabean in 2022; however, no statistical differences were detected in 2023 (Table 4). When comparing Cohort 2, plants in fallow plots produced significantly higher seed numbers than in maize, soyabean, spring wheat and oats (Table 3). *A. tuberculatus* Cohort 3 plants in soyabeans produced on average 6858 and 1538 seeds plant^{-1} compared to <50 seeds plant^{-1} in maize, spring wheat, and oats in 2022 and 2023 respectively. Spring wheat and oats significantly reduced *A. tuberculatus* seed production

TABLE 4 Days to flower and seed production of three *Amaranthus tuberculatus* cohorts under fallow, maize, oat, soyabean and spring wheat canopies in Boone, Iowa, USA, in 2022 and 2023.

	2022			2023		
Crop	Cohort[a]	Days to flower[b,c]	Seeds plant^{-1}	Cohort	Days to flower	Seeds plant^{-1}
Fallow	1	63 ± 2.0 b	473 030.4 ± 123 840 a	1	59 ± 2.3 de	167833.2 ± 39 827 a
	2	39 ± 0.4 ef	172 397.6 ± 47 301 ab	2	51 ± 1.1 fg	26663.7 ± 7032 bc
	3	36 ± 0.6 f	36 332.9 ± 8816 bc	3	37 ± 1.9 h	7243.8 ± 2074 def
Maize	1	61 ± 2.3 bc	40 288.9 ± 9698 bc	1	69 ± 1.0 b	32999.0 ± 9628 ab
	2	40 ± 2.7 e	2939.9 ± 695.9 d	2	55 ± 1.5 ef	4345.4 ± 559.5 cd
	3	42 ± 1.8 e	7.8 ± 2.8 f	3	49 ± 2.5 g	16.4 ± 6.9 g
Oat[d]	1	76 ± 1.5 a	411.8 ± 136.4 e	1	77 ± 2.0 a	54.2 ± 53.1 gh
	2	50 ± 0.3 d	150.3 ± 73.1 e	2	66 ± 5.0 bc	0
	3	–	0	3	–	0
Soyabean	1	56 ± 3.5 c	73 052.5 ± 15 953 b	1	63 ± 1.7 bcd	45684.7 ± 13 920 ab
	2	39 ± 0.6 ef	11 620.8 ± 4833 cd	2	62 ± 2.8 cd	3286.9 ± 1326 de
	3	42 ± 1.7 e	6858.9 ± 5520 d	3	54 ± 1.5 efg	1538.2 ± 449.9 ef
Spring wheat	1	70 ± 3.1 a	930.8 ± 364.1 e	1	77 ± 0.5 a	504.2 ± 146.1 f
	2	67 ± 0.9 d	259.9 ± 120.8 e	2	65 ± 2.4 bcd	50.3 ± 50.3 gh
	3	–	0.5 ± 0.4 e	3	–	0

[a]Cohorts 1–3 were established on 30 May, 16 June and 5 July in 2022 and on 16 May, 5 June and 20 June in 2023 respectively.
[b]Days to flower were calculated as the number of days from planting required for 50% of *A. tuberculatus* plants to reach flowering.
[c]Means followed by the same letter(s) within each column are not significantly different; Tukey's honestly significant difference (HSD; $\alpha = 0.05$).
[d]Harvest of oats and spring wheat damaged *A. tuberculatus* plants during the reproductive stage, which may have disrupted flowering.

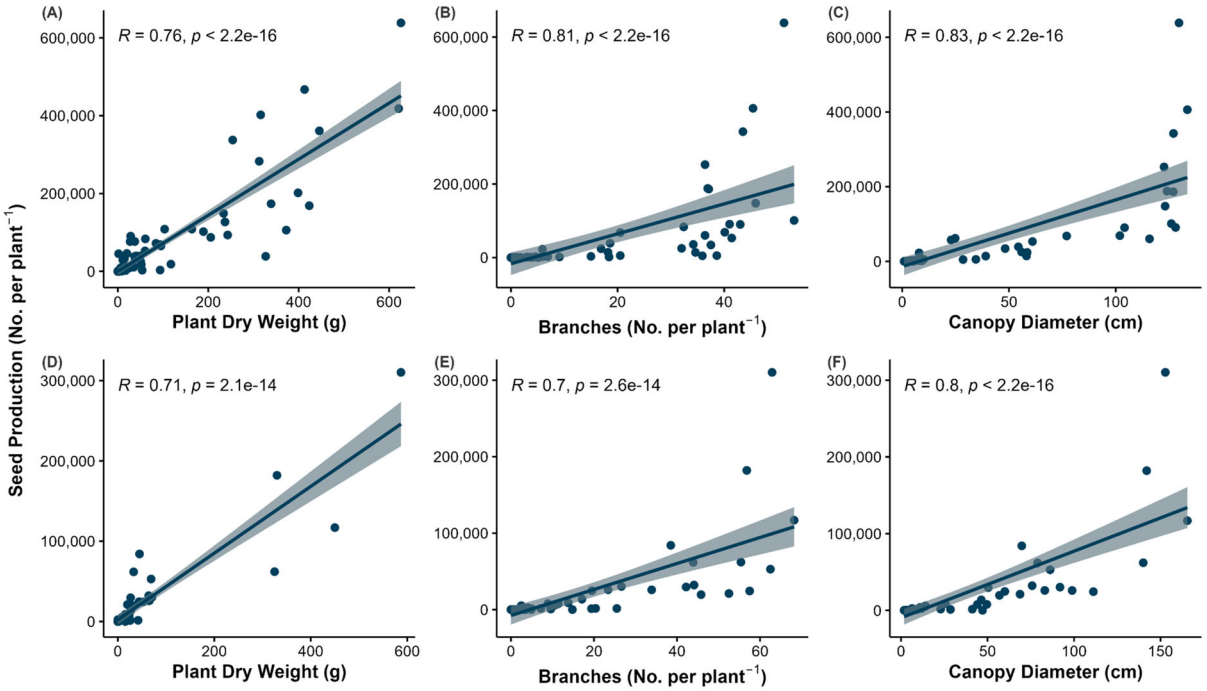


FIGURE 6 Kendall rank correlation of plant dry weight (A and D), branches (B and E), canopy diameter (C and F) and seed production ($N = 60$) of female *Amaranthus tuberculatus* grown under fallow, maize, soyabean, spring wheat and oat canopies in Boone, Iowa, USA in 2022 (top) and 2023 (bottom). Dots represent individual observations whereas lines and bands represent best fit and 95% confidence interval respectively.

across all emergence cohort timings, with averages ranging from 0 to 930 seeds plant^{-1} in 2022 and 2023. When comparing spring wheat and oats to fallow, *A. tuberculatus* seed production plant^{-1} was reduced >99% across all three cohorts (Table 4).

3.4 | Correlation of *A. tuberculatus* morphological features and seed production

Female *A. tuberculatus* dry weight, branches plant^{-1} and canopy diameter plant^{-1} were strongly (≥0.70) correlated with seed production (Figure 6). Dry weight had the weakest correlation, with $R = 0.76$ in 2022 and $R = 0.71$ in 2023. Branches plant^{-1} had a slightly stronger positive correlation than dry weight in 2022 but was similar in 2023 (Figure 6). In addition, canopy diameter had a slightly stronger correlation than branch number and dry weights with R ≥0.80 (Figure 6).

4 | DISCUSSION

The present study revealed that *A. tuberculatus* morphology is influenced by canopy type as well as emergence timing. The heights of *A. tuberculatus* plants that emerged in Cohort 1, were not significantly different when comparing fallow, soyabean and maize. Previous research has shown that when *A. tuberculatus* emerges early in the maize and soyabean growing season and competes season-long, *A. tuberculatus* growth is less impacted by crop competition (Arsenijevic et al., 2022; Uscanga-Mortera et al., 2007). Conversely, the heights *A. tuberculatus* that emerged in Cohort 1 in spring wheat and oats were significantly reduced compared to fallow, which demonstrated the ability of cereal crops to suppress *A. tuberculatus* growth early in the growing season. This is likely due, in part, to earlier crop establishment and rapid canopy closure of spring wheat and oats, which decreased photosynthetically active radiation (PAR) early in the growing season compared to maize, soyabean and fallow (data not shown). *A. tuberculatus* plants that emerged under low PAR conditions had lower biomass accumulation and fecundity compared to those that emerged in higher PAR conditions (Steckel & Sprague, 2004a). The height suppression of subsequent *A. tuberculatus* cohorts was even greater in spring wheat and oat canopy compared to fallow. A similar trend was observed for canopy diameter and branches plant^{-1} of *A. tuberculatus* in spring wheat and oats. *A. tuberculatus* canopy diameter and branches plant^{-1} were significantly reduced across all three cohorts in spring wheat and oats compared to those in fallow, maize and soyabeans. Soyabean canopy had the least influence on *A. tuberculatus* canopy diameter and branches plant^{-1}, where only Cohort 3 was suppressed compared to fallow. This is likely due to Cohort 3 being established just before canopy closure occurred in soyabean, which likely increased shading and reduced PAR availability (data not shown). The reduced canopy diameters and branch numbers observed in the present study are likely due, in part, to *A. tuberculatus* exhibiting a 'shade-avoidance' response, thus allocating resources to elongating the main stem. Previous studies have demonstrated that *Amaranthus* species exhibit shade-avoidance responses when in competition for light, leading to resource partitioning to stem tissue, resulting in greater stem elongation and apical dominance (Brainard et al., 2005; Steckel et al., 2003).

The dry weight of *A. tuberculatus* was influenced by canopy and emergence timing, with later emerging plants having significantly lower dry weights than earlier cohorts. Similar findings have been reported in maize and soyabeans (Arsenijevic et al., 2022; Uscanga-Mortera et al., 2007). Spring wheat and oats reduced *A. tuberculatus* dry weight the most as evidenced by the significant differences observed among all cohorts compared across other canopies. The results observed in the present study corroborate Nguyen & Liebman, (2022), who found that mature *A. tuberculatus* plants were significantly heavier in maize and soyabean than those in oats.

Flowering timing and fecundity were impacted by emergence timing and canopy. *A. tuberculatus* that emerged later in the growing season took fewer days to reach flowering, regardless of the canopy. These findings are consistent with previous research on *A. tuberculatus* phenology; Wu and Owen (2014) reported that late-emerging *A. tuberculatus* plants flowered quicker and had shorter flowering periods compared with early emerged cohorts. A similar response in flowering time has been observed in other *Amaranthus* species, such as *A. retroflexus* and *A. viridis* (Khan et al., 2021). Later emerged cohorts of *A. tuberculatus* likely experienced shortened photoperiods, resulting in rapid flower initiation, which supports that *A. tuberculatus* is a facultative short-day plant with strong flowering plasticity (Wu & Owen, 2014). Results from the present study found that *A. tuberculatus* plants in Cohorts 1 and 2 took significantly more days to flower in spring wheat and oat than in fallow plots. Moreover, *A. tuberculatus* Cohort 3 plants in maize and soyabeans took significantly more days to flower than in fallow plots. Oliveira et al. (2022) reported similar results in a closely related species, finding that late-emerged *Amaranthus palmeri*, cohorts grown under dense maize and soyabean canopies took almost 2 weeks longer to reach flowering than those growing in fallow plots. Plasticity in reproduction timing due to competition can vary by species, with some delaying reproduction when grown under high plant population densities (Weiner, 1988). Plants that emerge later in the growing season under crop canopy are likely to experience higher levels of abiotic and biotic stress than those who emerge before or with the crop. In addition, the harvest timing of spring wheat and oats in Iowa occurs mid to late July, which coincides with the typical flowering timing of *A. tuberculatus*. In the present study, *A. tuberculatus* plants in spring wheat and oat were run over or damaged during harvest, which likely disrupted or delayed flowering further.

Maize, spring wheat and oats reduced *A. tuberculatus* fecundity greater than soyabean when compared across cohorts. Previous findings have reported that seed production of *A. tuberculatus* in maize was lower than in soyabean, even when emerging at similar crop growth stages (Uscanga-Mortera et al., 2007). *A. tuberculatus* plants in spring wheat in oat exhibited drastic reductions in seed production, with Cohort 2 producing <300 seed plant^{-1} and less than 1 seed plant^{-1} in Cohort 3. Although this decrease in *A. tuberculatus*

fecundity further demonstrated the competitive ability of cereals like spring wheat and oats, it is important to consider that seed production of 100–150 seeds plant^{-1} may still allow *A. tuberculatus* to persist and replenish the soil seed bank in subsequent generations (Uscanga-Mortera et al., 2007). Our findings reveal that, although later emerged cohorts may flower faster, the presence of crop canopy can delay the flowering of *A. tuberculatus* and can vary based on crop species. The flowering of Cohort 3 *A. tuberculatus* plants never reached 50% in spring wheat and oats which resulted in nearly 0 seeds plant^{-1}. These findings may suggest that if *A. tuberculatus* control is maintained most of the season, substantial soil seedbank reductions are possible.

The present study revealed a strong positive correlation between morphological features (i.e., dry weight, canopy diameter and branching) of female *A. tuberculatus* and fecundity. Schwartz et al. (2016) also reported a strong positive correlation between *A. tuberculatus* biomass and seed production. Moreover, Mahoney et al. (2021) found that fresh biomass and canopy width were strongly correlated with seed production in *A. palmeri*, with $r = 0.90$ and $r = 0.81$ respectively. To our knowledge, no previous studies have investigated the correlation between branches plant^{-1} and fecundity in *A. tuberculatus*. It was observed that increased branch numbers led to an increased number of inflorescences present on the *A. tuberculatus* plants (data not shown), which likely increased reproductive success. It is well documented in crops such as soybean that branches plant^{-1} is positively correlated with improved seed yield (Carpenter & Board, 1997). Furthermore, a larger canopy likely increased leaf area, thus increasing photosynthesis, allowing for greater resource capture and allocation. Previous research has often only considered plant biomass as a factor when predicting *A. tuberculatus* fecundity (Nguyen & Liebman, 2022). However, these data revealed that additional morphological traits should be considered when predicting *A. tuberculatus* fecundity, but further research is needed. Nevertheless, these data provide evidence that the morphological characteristics of *A. tuberculatus* are strongly correlated with fecundity; therefore, decreased fecundity can likely be achieved if *A. tuberculatus* plants are subjected to environmental conditions that reduce plant dry weight, branching and canopy width.

Based on the findings of this study, reductions in all three of the above-mentioned morphological characteristics can be achieved under dense, competitive crop canopies such as in spring wheat and oats. The inclusion of additional crops that vary in phenology, harvest timing, and weed management practice should be considered in maize–soyabean-dominated rotations of the Midwest US. Clearly, increasing the diversity of crops grown in rotation, particularly those that differ in growth characteristics, can greatly improve the management of *A. tuberculatus* and other spring annual weeds (Liebman & Dyck, 1993). It should be noted that including small grains to increase the crop rotation's diversity does not provide additional herbicide options to control *A. tuberculatus*. Weed management should be considered beyond a single season and include a strategic crop system plan which incorporates multiple weed stressors that vary spatially and temporarily.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the results of this study are available from the corresponding author upon reasonable request.

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