Short title: Pyridate-based mixtures

Synergistic effect of pyridate-based herbicide mixtures for controlling multiple herbicide-resistant kochia

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Abstract

Multiple herbicide classes resistant (MHCR) kochia poses a serious concern for producers in the Central Great Plains, including western Kansas. Greenhouse and field experiments were conducted at Kansas State University Research and Extension Centers near Hays and Garden City, KS to evaluate pyridate-based postemergence (POST) herbicide mixtures for controlling MHCR kochia. One previously confirmed MHCR population (resistant to atrazine, glyphosate, dicamba, and fluroxypyr) and a susceptible (SUS) kochia population were tested in a greenhouse study. The kochia population at Hays field site was resistant to atrazine, dicamba, and glyphosate while kochia population at Garden City site was resistant to atrazine and glyphosate. Colby's analysis revealed synergistic interactions when pyridate was mixed with atrazine, dicamba, dichlorprop-p, fluroxypyr, glyphosate, or halauxifen/fluroxypyr and resulted in ≥94% control and shoot dry biomass reduction of MHCR kochia in a greenhouse study. Similarly, synergistic interactions were observed for MHCR kochia control in fallow field studies at both sites when pyridate was mixed with glyphosate or atrazine. Kochia control was increased from 26% to 90% with the application of glyphosate + pyridate and from 28% to 95% with atrazine + pyridate at both sites as compared to separate applications of glyphosate or atrazine. This is the first report for such a strong synergistic effect for both glyphosate and atrazine mixtures with pyridate on a weed resistant to both. All other pyridate-based herbicide mixtures showed an additive interaction and resulted in better control of MHCR kochia (87 to 100%) as compared to their individual applications (23 to 92%) across both sites except 2,4-D. These results suggest that pyridate can play a crucial role in various POST herbicide mixtures for effective control of MHCR kochia.

Nomenclature: Atrazine; dicamba; dichlorprop-p; glyphosate; halauxifen/fluroxypyr; kochia, *Bassia scoparia* (L.) A. J. Scott; pyridate

Keywords: *Bassia scoparia* (L.) A. J. Scott; dicamba; glyphosate; pyridate; U.S. Great Plains

Introduction

Kochia is a C$_4$ summer annual broadleaf weed in the Chenopodiaceae family and is a major challenge to crop production in the U.S. Great Plains (Kumar et al. 2019a). Kochia has been reported as the second most troublesome weed in alfalfa (*Medicago sativa* L.), canola (*Brassica napus* L.), and spring cereals (Van Wychen 2022). Kochia is an early emerging summer annual weed, appearing early in the spring with an extended period of emergence (from mid-February through mid-June) (Dille et al. 2017; Friesen et al. 2009; Kumar et al. 2018). Kochia is tolerant to abiotic stresses (heat, drought, and salinity), a prolific seed producer (one plant can produce >100,000 seeds) and exhibits a wind-mediated tumble mechanism of seed dispersal (Christoffoleti et al. 1997; Friesen et al. 2009; Kumar et al. 2019a). Season-long interference of kochia can reduce grain yield by 68% in corn (*Zea mays* L.), 62% in sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*], 46% in sugarbeet (*Beta vulgaris* L.), and 23% in sunflower (*Helianthus annuus* L.) (Geddes and Sharpe 2022). Similarly, kochia at densities of 240 to 520 plants m^{-2} has been reported to reduce spring wheat (*Triticum aestivum* L.) grain yield by 60% (Friesen et al. 1991). Kochia plants escaping postemergence (POST) herbicide applications in the spring or late-emerging cohorts can also hinder mechanical wheat harvest (Kumar and Jha 2015a; Torbiak et al. 2021). In addition to many crop situations, kochia infestation is also highly problematic during fallow periods of the crop rotations (winter wheat-fallow or winter wheat-summer crop-fallow) (Kumar et al. 2019a).

Kochia populations resistant to five different herbicide sites of action (SOA) (WSSA Groups 2, 4, 5, 9, and 14) have been documented (Heap 2024; Kumar et al. 2019a; Sharpe et al. 2023; Westra et al. 2019). The widespread evolution of glyphosate- and acetolactate synthase (ALS) inhibitors-resistant kochia populations have been reported across the U.S. Great Plains (Godar et al. 2015; Kumar et al. 2019a). With the frequent use of dicamba and fluroxypyr, resistance to these herbicides has also become more prevalent (Heap 2024; Kumar et al. 2019b; Westra et al. 2019). Furthermore, a few kochia populations with resistance to four herbicide SOA, including inhibitors of photosystem (PS) II (Group 5), ALS (Group 2), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Group 9), and synthetic auxins (Group 4) have also been reported from Kansas (Heap 2024). This rapid evolution of multiple herbicide classes resistant (MHCR) kochia populations poses a serious management challenge by limiting the effectiveness of commonly used POST herbicides.

Pyridate, introduced in 1976, is a contact, POST herbicide that belongs to the photosystem (PS) II inhibitor mode of action (WSSA Group 6). It controls both grass and broadleaf weeds by inhibiting electron transfer due to blocking amino acid histidine 215 at the A site of PS II (Székács 2021). Tonks and Westra (1997) have previously reported 16 to 60% control of sulfonylurea-resistant kochia with pyridate alone. Wicks et al. (1994) reported 89% control of atrazine-resistant kochia with pyridate alone at 21 days after treatment (DAT). The application of two or more herbicide SOA as a mixture is generally recommended to mitigate/delay the evolution of herbicide resistance in weeds as a component of integrated weed management (Beckie and Reboud 2009; Green 1991). For example, Wicks et al. (1993) reported >95% control of atrazine-resistant kochia with pyridate + flurochloridone at 21 to 30 DAT. Nonetheless, limited research exists regarding effectiveness of pyridate-based herbicide mixtures to control MHCR kochia. Therefore, the main objective of this study was to evaluate pyridate-based POST herbicide mixtures for controlling MHCR kochia in western KS.

Materials and Methods

Greenhouse Study

Greenhouse experiments were conducted at Kansas State University Agricultural Research Center (KSU-ARC) near Hays, KS in the spring of 2022 and repeated in the fall of 2022. A previously confirmed MHCR kochia population and a susceptible population (SUS) were used. The MHCR kochia population was originally collected from Garden City, KS, and was confirmed resistant to atrazine, glyphosate, dicamba, and fluroxypyr (Kumar et al. 2019b; 2020) and the SUS population was collected from a pasture field at KSU-ARC in 2020 and was confirmed susceptible to glyphosate, dicamba, atrazine, and fluroxypyr (Kumar et al. 2019b). Seeds for both MHCR and SUS kochia populations were separately sown in plastic trays (54- by 28- by 10-cm) containing commercial potting mixture (Miracle-Gro® Moisture Control® Potting Mix, Miracle-Gro Lawn Products, Scottslawn Road, Marysville, OH). The temperature in the greenhouse was maintained at 25/23 ± 3 C day/night and a 16/8 h (day/night) photoperiod was supplemented with metal-halide lamps (560 μmol m^{-2} s^{-1}). When kochia seedlings from each population reached 2 to 3 cm tall, they were transplanted into 10- by 10-cm squared plastic pots (1 seedling per pot) containing the same potting mixture as mentioned above. Experiments were conducted in a randomized complete block design with 12 replications (one plant per pot = one

replication). Kochia seedlings from both populations were watered once daily to avoid moisture stress. Actively growing young kochia seedlings (6 to 9 cm tall) from each population were treated with selected POST treatments according to the herbicides label guidelines (Table 1) using a stationary cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing, Hollandale, MN), equipped with an even flat-fan nozzle tip (TeeJet 8001EXR, Spraying System, Wheaton, IL) calibrated to deliver 132 L ha^{-1} of spray solution at 241 kPa. Percent visual control ratings were recorded at 7, 21, and 28 DAT. Ratings were based on herbicide injury symptoms such as chlorosis, epinasty (curling, twisting, and cupping), and necrosis of kochia seedlings on a scale of 0% (no control) to 100% (complete control). At 28 DAT, each plant was clipped at the soil surface, placed in a paper bag and dried at 65 C for 4 days to obtain shoot dry biomass. The data for shoot dry biomass was used to calculate the percent reduction of shoot dry biomass using equation 1:

$$\text{Shoot dry biomass reduction } (\%) = \left[\frac{BC - BT}{BC}\right] X\ 100 \qquad \text{Eqn. 1}$$

where *BC* is the average shoot dry biomass from the nontreated check treatment and *BT* is the shoot dry biomass from a treated pot. Price estimates for herbicides were obtained from the average prices listed in the K-State Chemical Weed Control Guide for 2022 and 2023 (Lancaster et al. 2022, 2023).

Field Experiments

Field experiments were conducted in fallow fields at KSU-ARC near Hays, KS and at Kansas State University Southwest Research and Extension Center (KSU-SWREC) near Garden City, KS in 2023 (sorghum stubble at Hays and wheat stubble in Garden City). Soil type at KSU-ARC site was Roxbury silt loam with a pH of 7.4 and 2.1% organic matter while the soil type at KSU-SWREC site was Ulysses silt loam with a pH of 8.0 and 1.4% organic matter. The field site at KSU-ARC had a history of the kochia population surviving field-use rate of atrazine (1120 g ha^{-1}), dicamba (560 g ha^{-1}), and glyphosate (1260 g ha^{-1}) (personal observations). Similarly, the kochia population at KSU-SWREC site had previously survived POST applications of atrazine and glyphosate (personal communication with Dr. Randall Currie). At both sites, the same treatments as the greenhouse experiment (Table 1) were laid out in a randomized complete block design with four replications. Plot size was 3 m wide by 9 m long at both sites. Data for daily maximum and minimum air temperature and precipitation at both field sites during the study

period were obtained from nearby Kansas Mesonet (https://mesonet.k-state.edu) weather stations. For KSU-ARC site, the weather station (38.8495°N, 99.3446°W) was located approximately 500 m away from the study site and for KSU-SWREC, the weather station was approximately 700 m away from the study site (37.997°N, 100.815°W). Weather data from both sites are presented in Figures 1 and 2. All treatments (Table 1) were applied at their recommended field-use rates on young actively growing kochia seedlings (5- to 9-cm tall) using a CO_2 pressurized backpack sprayer equipped with four flat-fan nozzles (TeeJet 8001XR, Spraying Systems Co., P.O. Box 7900, Wheaton, IL), calibrated to deliver 132 L ha^{-1} of spray solution at 276 kPa. Treatments were applied on May 23 at KSU-ARC and on May 29 at KSU-SWREC. Data for percent visual control of kochia was recorded at 7, 14, and 28 DAT on a scale of 0 to 100%, where 0% indicates no control and 100% indicates complete control as compared to nontreated check. Kochia density was recorded at 28 DAT using a 1-m^2 quadrat placed at the center of each plot and the kochia plants were manually clipped at the soil level and shoot dry biomass was determined after oven-drying the samples at 65 C for 4 days. Similar to the greenhouse study, kochia density and aboveground shoot dry biomass data were converted to a percent reduction compared to nontreated.

Statistical Analyses

All the data collected from greenhouse and field studies were subjected to ANOVA using PROC MIXED procedure in SAS 9.3 (SAS Institute, Inc., SAS Campus Drive, Cary, NC). The data from nontreated plots were not included in the analyses. Data were checked for the ANOVA assumptions using the PROC UNIVARIATE procedure in SAS. Data on percent visual control, percent reduction in density, and percent reduction in shoot dry biomass were arcsine square root transformed before analysis to improve the homogeneity of variance and normality of the residuals; however, back-transformed data were presented with mean separation based on the transformed data. Treatments, experimental runs, kochia populations, and their interactions were considered fixed effects for greenhouse experiments, whereas sites, treatments, and their interactions were considered fixed effects for field experiments. The replication and all interactions involving replication were considered as random effects for both greenhouse and field experiments. Data from both runs from greenhouse experiments were pooled because of non-significant experimental run-by-herbicide treatment interaction (P = 0.36). Data from KSU-

ARC and KSU-SWREC sites were analyzed separately because of significant treatment-by-site interaction (P <0.0001). Treatment means were separated using Fisher's protected LSD test (P < 0.05).

Expected values with herbicide mixtures for percent kochia control and percent reduction in shoot dry biomass for greenhouse study and for percent kochia control, percent reduction in density, and percent reduction in shoot dry biomass for field studies were calculated using Colby's analysis with equation (Eqn. 2) to determine the interaction of tested herbicides (Colby 1967):

$$E = (X + Y) - \frac{(XY)}{100} \qquad \text{Eqn. 2}$$

where E is the expected values of the response variable to the application of herbicides A + B in a mixture, and X and Y are the respective observed values of the response variables to the individual application of herbicide A and B, respectively. The expected and observed percent kochia control, percent reduction in density, and percent reduction in shoot dry biomass were compared using t-tests to determine if those mean values differed. When the observed mean for the mixture was less than the expected mean, the interaction was considered antagonistic. If the observed mean was greater than the expected mean, the interaction was considered synergistic. The interaction of herbicide mixture was considered additive when expected and observed means were not different according to t-test P < 0.05 (Colby 1967).

Results and Discussion

Greenhouse Study

Percent visual control. Both MHCR and SUS kochia populations showed differential responses with a significant interaction between populations and herbicide treatments (P <0.0001). All pyridate-based herbicide mixtures, except 2,4-D + pyridate, resulted in >90% control of MHCR kochia and all other herbicides (without pyridate) resulted in less than 50% control of MHCR kochia population at 14 and 28 DAT, except for bromoxynil/pyrasulfotole (92% at 28 DAT) (Table 2). The increased control from 28 to 79% at 14 DAT with 2,4-D + pyridate compared to 2,4-D alone was not enough to alleviate it to an acceptable level. Application of atrazine, halauxifen/fluroxypyr, glyphosate, fluroxypyr, or dicamba provided 11 to 49% control of MHCR kochia population at 28 DAT, however, the control increased to 98 to 100% after adding pyridate with these herbicides. The addition of pyridate increased the cost of the mixtures by $27 ha^{-1}

(Table 1). Colby's analysis further revealed synergistic interactions for MHCR kochia control when pyridate was mixed with atrazine, dichlorprop-p, halauxifen/fluroxypyr, glyphosate, fluroxypyr, or dicamba (Table 2). These results indicate the benefit of adding pyridate with these POST herbicides to control MHCR kochia. Application of bromoxynil/pyrasulfotole alone or with pyridate resulted in 92 to 100% control of MHCR kochia population. It is important to note that no synergism was observed with pyridate + bromoxynil/pyrasulfotole because the expected control of this combination was already very high (96%). All pyridate-based herbicide mixtures (except 2,4-D + pyridate) provided >95% control of SUS kochia population at 14 and 28 DAT (Table 2). Bromoxynil/pyrasulfotole and atrazine resulted in 97 to 100% control of SUS kochia population at 28 DAT followed by dicamba (91%), fluroxypyr (88%), glyphosate (88%), and halauxifen/fluroxypyr (85%). Previous studies have also reported effective kochia control (85 to 100%) with bromoxynil + pyrasulfotole (Kumar and Jha 2015b; Sbatella et al. 2019). Application of pyridate alone provided 52 to 72% control of both MHCR and SUS populations at 28 DAT. Tonks and Westra (1997) have also previously reported 16 to 60% control of sulfonylurea-resistant kochia with pyridate alone. Kousta et al. (2024) reported 53% control of common lambsquaters (*Chenopodium album* L) with pyridate alone. Wicks et al. (1993) reported >95 control of atrazine-resistant kochia with pyridate + flurochloridone at 21 to 30 DAT. The application of 2,4-D provided only 12 to 30% control for both MHCR and SUS populations at 28 DAT. Several previous studies have also reported poor kochia control with 2,4-D alone (Friesen et al. 1993; Nandula and Manthey 2002; Tonks and Westra 1997).

Percent reduction in shoot dry biomass. Consistent with percent visual control, all pyridate-based herbicide mixtures provided 94 to 100% reduction in shoot dry biomass of MHCR kochia, except 2,4-D + pyridate (Table 2). The MHCR population was confirmed resistant to atrazine, glyphosate, dicamba, and fluroxypyr (Kumar et al. 2019b; 2020). Application of 2,4-D and glyphosate provided the least shoot dry biomass reduction (14 to 17%) of MHCR kochia. Percent reduction in shoot dry biomass changed from 17 to 99% with glyphosate + pyridate, 33 to 99% with fluroxypyr + pyridate, and 45 to 99% with dicamba + pyridate compared to glyphosate, fluroxypyr, or dicamba alone, respectively. Greater observed percent reduction of shoot dry biomass for MHCR kochia as compared to the expected values for dicamba + pyridate, glyphosate + pyridate, halauxifen/fluroxypyr + pyridate, fluroxypyr + pyridate, dichlorprop-p +

pyridate, and atrazine + pyridate indicated the synergistic interactions of pyridate-based mixtures (Table 2). Consistent with percent control ratings, all herbicide treatments except dichlorprop-p, pyridate, 2,4-D, and 2,4-D + pyridate resulted in >90% reduction in shoot dry biomass of the SUS population. Atrazine, bromoxynil/pyrasulfotole, and all pyridate-based herbicide mixtures resulted in 97 to 100% shoot dry biomass reduction of SUS population except for 2,4-D + pyridate. Both kochia control and shoot dry biomass reduction were strongly correlated with correlation coefficients of 0.9427 and 0.9809 for MHCR and SUS populations, respectively (data not shown). These results are consistent with previous researchers, who have reported excellent levels of reduction in shoot dry biomass (83 to 99%) of glyphosate-resistant kochia with bromoxynil/pyrasulfotole and bromoxynil/MCPA (Burton et al. 2014; Kumar et al. 2014). Dichlorprop-p + pyridate provided 94 to 98% reduction in shoot dry biomass for both MHCR and SUS kochia populations. In our previous research, we have also observed 87 to 89% shoot dry biomass reduction of MHCR kochia population with dicamba + halauxifen/fluroxypyr + dichlorprop-p (Dhanda et al. 2023). In that same study, synergistic interactions were also observed when dicamba was mixed with dichlorprop-p, 2,4-D, dichlorprop-p + 2,4-D, and halauxifen/fluroxypyr + 2,4-D for shoot dry biomass reductions (86 to 92%) of MHCR kochia (Dhanda et al. 2023).

Field Experiments

Daily mean air temperatures during the study period (end of May through end of June) at KSU-ARC ranged from 14 to 24 C with a total precipitation of 111 mm at KSU-ARC site (Figure 1). Daily mean air temperatures at KSU-SWREC were similar and ranged from 14 to 29 C during the study period (end of May through June). However, the KSU-SWREC site had relatively more precipitation of 156 mm during the study period (Figure 2). At the KSU-ARC site, only kochia was the dominant weed whereas, at the KSU-SWREC site, kochia and Palmer amaranth (*Amaranthus palmeri* S. Watson) were the two dominant weeds.

At the KSU-ARC site, all pyridate-based herbicide mixtures provided ≥85% control of MHCR kochia population at 14 and 28 DAT, except 2,4-D + pyridate (Table 3). The lower control with 2,4-D + pyridate might be because of low efficacy of 2,4-D for kochia control as reported by several previous studies (Friesen et al. 1993; Nandula and Manthey 2002; Tonks and Westra 1997). The relatively lower kochia control with pyridate-based herbicide mixtures in

field conditions as compared to greenhouse might be due to the dry field conditions (Figure 1) and higher density of kochia in the field (165 plants m^{-2}) forming a thick mat, which likely reduced the herbicide coverage. Complete plant coverage for contact herbicides like pyridate is important to achieve effective control (Butts et al. 2021). Mixing pyridate with either bromoxynil/pyrasulfotole, fluroxypyr, or atrazine provided 88 to 90% visual control at 14 DAT and 91 to 94% at 28 DAT (Table 3). These same treatments reduced kochia density by 96 to 98% compared to the nontreated weedy check at 28 DAT. Similarly, two-way mixtures increased percent visual kochia control from 28 to 88% with glyphosate + pyridate, 33 to 91% with atrazine + pyridate, and 48 to 89% with dicamba + pyridate as compared to glyphosate, atrazine, or dicamba alone, respectively (Table 3). Synergistic interactions were observed when pyridate was mixed with glyphosate, atrazine, or dicamba which resulted in greater percent visual control with mixtures than their standalone applications (Table 3). Wicks et al. (1993) also reported 56 to 84% control of atrazine-resistant kochia with cyanazine + atrazine + pyridate at 21 to 30 DAT as compared to cyanazine + atrazine (0 to 7% control). In that same study, higher control of atrazine-resistant kochia was reported with pyridate + flurochloridone (98 to 100%) as compared to flurochloridone alone (70 to 96%). In our current study, synergistic interactions were also observed for density reduction when pyridate was mixed with atrazine, dicamba, dichlorprop-p, fluroxypyr, glyphosate, or halauxifen/fluroxypyr (Table 3). Consistent with percent control, all pyridate-based mixtures, except 2,4-D + pyridate provided ≥90% shoot dry biomass reduction. The application of 2,4-D + pyridate provided 63% kochia control and 65% shoot dry biomass reduction at 28 DAT while 2,4-D alone provided the least kochia control (14%) and shoot dry biomass reduction (23%) (Table 3). Mixing of pyridate with glyphosate, atrazine, or dicamba resulted in ≥90% shoot dry biomass reduction as compared to glyphosate, atrazine, or dicamba alone treatments (39 to 53% reduction). Colby's analysis for shoot dry biomass reduction revealed a synergistic interaction between glyphosate and pyridate (Table 3). The correlation coefficient for kochia control and shoot dry biomass reduction was 0.888 and it was 0.9301 for kochia control and density reduction (data not shown). The high correlation coefficient values suggest that all response variables were consistent in describing herbicide efficacy. For all other pyridate-based mixtures, no significant differences were reported between observed values for shoot dry biomass reduction and expected values indicating additive effects of mixing pyridate for better control of MHCR kochia as compared to not adding pyridate in the mixtures. These

results are consistent with Wicks et al. (1993), who previously reported greater reduction in shoot dry biomass of atrazine-resistant kochia with pyridate + flurochloridone (93%) as compared to flurochloridone alone (76%).

At the KSU-SWREC site, percent visual control and percent reduction of shoot dry biomass of MHCR kochia were relatively greater compared to KSU-ARC site. In general, kochia density was less at KSU-SWREC (13 plants m^{-2}) as compared to KSU-ARC (165 plants m^{-2}), which likely improved herbicide coverage at KSW-SWREC compared to the dense mat of kochia at KSU-ARC (Table 4). Also, more precipitation at KSU-SWREC (156 mm) than KSU-ARC (111 mm) may have resulted in actively growing kochia at time of herbicide application (Figures 1 and 2). All pyridate-based mixtures provided ≥85% control at 14 DAT and >90% at 28 DAT, except 2,4-D + pyridate. Bromoxynil/pyrasulfotole alone or with pyridate resulted in 92 to 100% control and 81 to 98% reduction in kochia density at 28 DAT compared to nontreated (Table 4). Furthermore, mixing pyridate with atrazine, fluroxypyr, halauxifen/fluroxypyr, and dicamba resulted in 94 to 98% kochia control at 28 DAT. These treatments reduced kochia density by 85 to 97% compared to nontreated (Table 4). Colby's analysis further revealed synergistic interactions between pyridate and atrazine as well as pyridate and glyphosate for kochia control (Table 4). Consistent with percent control, bromoxynil/pyrasulfotole and all pyridate-based mixtures, except 2,4-D + pyridate provided ≥90% shoot dry biomass reduction. Based on Colby's analysis, synergistic interactions for kochia density reduction and shoot dry biomass reduction were observed when pyridate was mixed with atrazine or glyphosate. The shoot dry biomass reduction increased from 18% to 99% with atrazine + pyridate and 15% to 90% with glyphosate + pyridate compared to atrazine or glyphosate alone. Similarly, kochia density reduction was greater (85 to 96%) when pyridate was mixed with atrazine or glyphosate as compared to their individual application (23%) without pyridate. The least reduction in kochia density (18 to 23%) and shoot dry biomass reduction (15 to 23%) was obtained with the 2,4-D, glyphosate, and atrazine alone treatments (Table 4). The correlation coefficient between kochia control and shoot dry biomass reduction was 0.9733 (data not shown). Similarly, the correlation coefficient between kochia control and density reduction was 0.9456 (data not shown). These high correlation coefficient values suggest that all response variables consistently represented herbicide efficacy. These results indicate that pyridate can play an important role as partner with other herbicides for effective control of MHCR kochia. These findings are consistent with Seidel

and Russell (1990), who previously reported greater control (91 to 97%) of lanceleaf sage (*Salvia reflexa* L. Hornem.) and turnipweed (*Rapistrum rugosum* L. All) when pyridate was mixed with metribuzin as compared to pyridate alone (59 to 78% control).

Practical Implications

With the widespread evolution and spread of MHCR weed populations, the use of herbicide mixtures containing multiple SOA could play a crucial role in managing these MHCR weed populations and to further delay/mitigate evolution of herbicide resistance (Norsworthy et al. 2012). Results from the current study suggest that mixing of pyridate with atrazine, dicamba, dichlorprop-p, fluroxypyr, glyphosate, or halauxifen/fluroxypyr had synergistic or additive interactions and resulted in greater control of MHCR kochia than without adding pyridate in mixtures. These pyridate-based mixtures can effectively control MHCR kochia in fallow fields or burndown scenarios (prior to crop planting or after crop harvest) with careful considerations of the rotational crops to be grown. However, it is important to note that these pyridate-based mixtures incur higher costs, ranging from \$33 to \$60 ha^{-1} compared to their standalone applications, ranging from \$6 to \$33 ha^{-1} (Table 1). At present, according to pyridate label (Anonymous 2024), it is labeled to apply in field corn, seed corn, popcorn, chickpea (*Cicer arietinum* L.), lentils (*Lens culinaris* Medik.) and mint (*Mentha arvensis* L). It is critical to note that overreliance on these pyridate-based mixtures should be avoided to prevent further evolution and spread of MHCR kochia populations. Along with these pyridate-based mixtures, growers should also integrate other weed control tactics in their crop rotations, including the use of effective pre-emergence herbicides, competitive crop rotations, narrow crop row spacing, cover crops, occasional tillage, harvest weed seed control technologies (chaff lining, impact mills), and precision spray technologies for controlling the seed bank of MHCR kochia. Future studies will investigate the potential biochemical interactions to understand the possible underlying mechanism(s) of synergistic interactions among pyridate-based mixtures for MHCR kochia control.

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Table 1. List of herbicides alone or in mixtures with pyridate tested for controlling multiple herbicide classes resistant kochia under greenhouse and field experiments.

Herbicide(s)[1]	WSSA group[2]	Trade name	Rate	Cost of herbicide(s)	Manufacturer	Adjuvant[3,4]
			g ai or ae ha^{-1}	US\$ ha^{-1}		
2,4-D	4_1	Weedone LV4	538	8	Nufarm	-
Atrazine	5_1	AAtrex 4L	560	6	Syngenta	-
Bromoxynil/pyrasulfotole	$6_1/27$	Huskie	(230 + 41)	33	Bayer Crop Science	NIS
Dicamba	4_5	Clarity	560	31	BASF	NIS
Dichlorprop-p	4_1	Duplosan	560	14	Nufarm	NIS
Fluroxypyr	4_4	Starane Ultra	157	27	Corteva Agriscience	-
Glyphosate	9	Roundup PowerMax	1260	31	Bayer Crop Science	AMS
Halauxifen/fluroxypyr	$4_3/4_4$	Pixxaro	(5 + 123)	17	Corteva Agriscience	-
Pyridate	6_2	Tough 5EC	350	27	Belchim Crop Protection	NIS
2,4-D + pyridate	$4_1 + 6_2$	Weedone LV4 + Tough 5EC	538 + 350	35	Nufarm and Belchim Crop Protection	NIS
Atrazine + pyridate	$5_1 + 6_2$	AAtrex 4L + Tough 5EC	560 + 350	33	Syngenta and Belchim Crop Protection	NIS
Bromoxynil/pyrasulfotole + pyridate	$6_1/27_1 + 6_2$	Huskie + Tough 5EC	(230 + 41) + 350	60	Bayer Crop Science and Belchim Crop Protection	NIS
Dicamba + pyridate	$4_5 + 6_2$	Clarity + Tough 5EC	560 + 350	58	BASF and Belchim Crop Protection	NIS
Dichlorprop-p + pyridate	$4_1 + 6_2$	Duplosan + Tough 5EC	560 + 350	41	Nufarm and Belchim Crop Protection	NIS
Fluroxypyr + pyridate	$4_4 + 6_2$	Starane Ultra + Tough 5EC	157 + 350	54	Corteva Agriscience and Belchim Crop Protection	NIS
Glyphosate + pyridate	$9 + 6_2$	Roundup PowerMax + Tough 5EC	1260 + 350	58	Bayer Crop Science and Belchim Crop Protection	AMS
Halauxifen/fluroxypyr + pyridate	$4_3/4_4 + 6_2$	Pixxaro + Tough 5EC	(5 + 123) + 350	44	Corteva Agriscience and Belchim Crop Protection	NIS

[1]A slash refers to a mixture product of two herbicides

[2]WSSA Groups: 4 – auxin mimics: 4_1 – phenoxycarboxylates, 4_3 – 6-arylpicolinates, 4_4 – pyridyloxycarboxylates, 4_5 – benzoates; 5 – D1 Serine 264 binders: 5_1 – Triazines; 6 – D1 Histidine 215 binders: 6_1 – nitriles, 6_2 – phenyl-pyridazines; 9 – inhibition of enolpyruvyl shikimate phosphate synthase; 27 – inhibition of hydroxyphenyl pyruvate dioxygenase: 27_1 – pyrazoles (Source HRAC, Global Herbicide Classification Lookup | Herbicide Resistance Action Committee – hracglobal.com)

[3]Abbreviations: AMS, ammonium sulphate; NIS, nonionic surfactant

[4]Adjuvant rates: AMS (2% w/v); NIS, 0.25% v/v

Table 2. Percent control at 14 and 28 days after treatment (DAT) and shoot dry biomass reduction of multiple herbicide classes resistant (MHCR)[*] and susceptible (SUS) kochia populations in the greenhouse experiment.[1,2]

	MHCR					SUS				
	Weed control			Shoot dry biomass reduction		Weed control			Shoot dry biomass reduction	
	14 DAT	28 DAT		28 DAT		14 DAT	28 DAT		28 DAT	
Treatments	Observed	Observed	Expected	Observed	Expected	Observed	Observed	Expected	Observed	Expected
--- % ---										
2,4-D	28 g	12 f	-	14 e	-	36 h	30 d	-	37 f	-
Atrazine	51 f	49 de	-	55 bc	-	97 ab	97 a	-	94 b	-
Bromoxynil/pyrasulfotole	89 cd	92 b	-	96 a	-	100 a	100 a	-	99 a	-
Dicamba	50 f	42 e	-	45 cd	-	85 de	91 b	-	92 b	-
Dichlorprop-p	39 f	45 de	-	41 cd	-	66 g	70 c	-	78 e	-
Fluroxypyr	40 f	42 e	-	33 d	-	84 ef	88 b	-	92 b	-
Glyphosate	10 h	11 f	-	17 e	-	90 cd	88 b	-	90 bc	-
Halauxifen/fluroxypyr	66 e	45 de	-	48 cd	-	84 ef	85 b	-	90 bc	-
Pyridate	48 f	52 d	-	53 bc	-	69 g	72 c	-	81 de	-
2,4-D + pyridate	79 d	67 c	58	65 b	60	86 de	83 b	81	85 cd	88
Atrazine + pyridate	100 a	100 a	77*	100 a	79*	100 a	100 a	99	100 a	99
Bromoxynil/pyrasulfotole + pyridate	100 a	100 a	96	100 a	98	100 a	100 a	100	100 a	100
Dicamba + pyridate	94 bc	98 a	72*	99 a	75*	94 bc	99 a	99	99 a	99
Dichlorprop-p + pyridate	93 bc	94 ab	74*	94 a	70*	96 ab	97 a	91	98 a	96
Fluroxypyr + pyridate	97 a	98 a	72*	99 a	69*	98 a	99 a	96	99 a	99
Glyphosate + pyridate	98 a	99 a	57*	99 a	65*	98 ab	100 a	97	100 a	98
Halauxifen/fluroxypyr + pyridate	96 ab	100 a	71*	100 a	78*	97 ab	100 a	95	100 a	97

[1]Means followed by the same letter within a column are not different according to Fisher's protected LSD at $P < 0.05$.

[2]Asterisks indicate that observed and expected values were different as determined by t-test ($P < 0.05$), indicating synergistic interaction of herbicides applied in mixtures based on Colby's equation (Eqn. 2)

[*]Kochia population confirmed resistant to atrazine, glyphosate, dicamba, and fluroxypyr

Table 3. Average percent control at 14 and 28 days after treatment (DAT), density reduction, and shoot dry biomass reduction of atrazine-, dicamba-, and glyphosate-resistant kochia in a field study during 2023 at Kansas State University Agricultural Research Center (KSU-ARC) near Hays, KS.[1,2,3]

Treatments	Weed control					Density reduction				Shoot dry biomass reduction		
	14 DAT		28 DAT			28 DAT				28 DAT		
	Observed		Observed		Expected	Observed			Expected	Observed		Expected
	--- % ---											
Nontreated	-		-		-	0		(165)	-	-		-
2,4-D	10	f	14	i	-	31	j	(114)	-	23	h	-
Atrazine	22	f	33	gh	-	41	hi	(97)	-	47	fg	-
Bromoxynil/pyrasulfotole	74	bc	81	bc	-	81	c	(31)	-	85	bc	-
Dicamba	41	e	48	fg	-	53	fg	(77)	-	53	fg	-
Dichlorprop-p	53	de	61	def	-	55	ef	(74)	-	65	de	-
Fluroxypyr	39	e	69	cd	-	65	d	(59)	-	74	cd	-
Glyphosate	19	f	28	h	-	37	ij	(105)	-	39	g	-
Halauxifen/fluroxypyr	45	e	75	cd	-	63	de	(62)	-	71	d	-
Pyridate	46	e	51	ef	-	46	gh	(89)	-	58	ef	-
2,4-D + pyridate	70	cd	63	de	57	71	d	(48)	63	65	de	68
Atrazine + pyridate	88	a	91	a	66*	97	a	(6)	65*	91	ab	78
Bromoxynil/pyrasulfotole + pyridate	90	a	94	a	91	98	a	(4)	90	95	a	94
Dicamba + pyridate	84	abc	89	ab	74*	90	ab	(16)	79*	90	ab	79
Dichlorprop-p + pyridate	85	ab	87	ab	82	91	ab	(15)	78*	90	ab	85
Fluroxypyr + pyridate	87	a	92	a	85	96	a	(7)	81*	90	ab	89
Glyphosate + pyridate	85	ab	88	ab	65*	86	bc	(23)	66*	91	ab	74*
Halauxifen/fluroxypyr + pyridate	81	bc	90	ab	88	95	a	(9)	80*	91	ab	88

[1]Means followed by the same letter within a column are not different according to Fisher's protected LSD at P < 0.05.

[2]Asterisks indicate that observed and expected values were different as determined by t-test (P < 0.05), indicating synergistic interaction of herbicides applied in mixtures based on Colby's equation (Eqn. 2)

[3]Values in the parenthesis are observed kochia density (plants m^{-2})

Table 4. Average percent control at 14 and 28 days after treatment (DAT), density reduction, and shoot dry biomass reduction of atrazine- and glyphosate-resistant kochia in a field study during 2023 at Kansas State University Southwest Research and Extension Center (KSU-SWREC) near Garden City, KS.[1,2,3]

Treatments	Weed control			Density reduction		Shoot dry biomass reduction	
	14 DAT	28 DAT		28 DAT		28 DAT	
	Observed	Observed	Expected	Observed	Expected	Observed	Expected
	--- % ---						
Nontreated	-	-	-	0 (13)	-	-	-
2,4-D	33 i	24 g	-	18 f (11)	-	23 e	-
Atrazine	23 i	23 g	-	23 f (10)	-	18 e	-
Bromoxynil/pyrasulfotole	89 cd	92 bc	-	81 bc (3)	-	91 abc	-
Dicamba	70 gh	78 de	-	65 d (5)	-	75 cd	-
Dichlorprop-p	69 gh	75 e	-	52 e (6)	-	71 cd	-
Fluroxypyr	75 fg	86 cd	-	70 cd (4)	-	88 bc	-
Glyphosate	25 i	24 g	-	23 f (10)	-	15 e	-
Halauxifen/fluroxypyr	84 de	85 cd	-	70 cd (4)	-	81 bcd	-
Pyridate	60 h	66 f	-	63 de (5)	-	62 d	-
2,4-D + pyridate	80 ef	77 de	76	70 cd (4)	70	75 cd	73
Atrazine + pyridate	96 a	98 a	65*	96 a (1)	77*	99 a	73*
Bromoxynil/pyrasulfotole + pyridate	98 a	100 a	99	98 a (0)	93	99 a	96
Dicamba + pyridate	90 bc	94 ab	95	85 ab (2)	83	92 abc	92
Dichlorprop-p + pyridate	85 de	91 bc	91	85 ab (2)	84	91 abc	93
Fluroxypyr + pyridate	94 ab	96 ab	94	97 a (0)	88	96 ab	95
Glyphosate + pyridate	87 de	92 bc	80*	85 ab (2)	70*	90 abc	69*
Halauxifen/fluroxypyr + pyridate	95 a	95 ab	96	92 ab (1)	85	94 ab	96

[1]Means followed by the same letter within a column are not different according to Fisher's protected LSD at P < 0.05.

[2]Asterisks indicate that observed and expected values were different as determined by t-test (P < 0.05), indicating synergistic interaction of herbicides applied in mixtures based on Colby's equation (Eqn. 2)

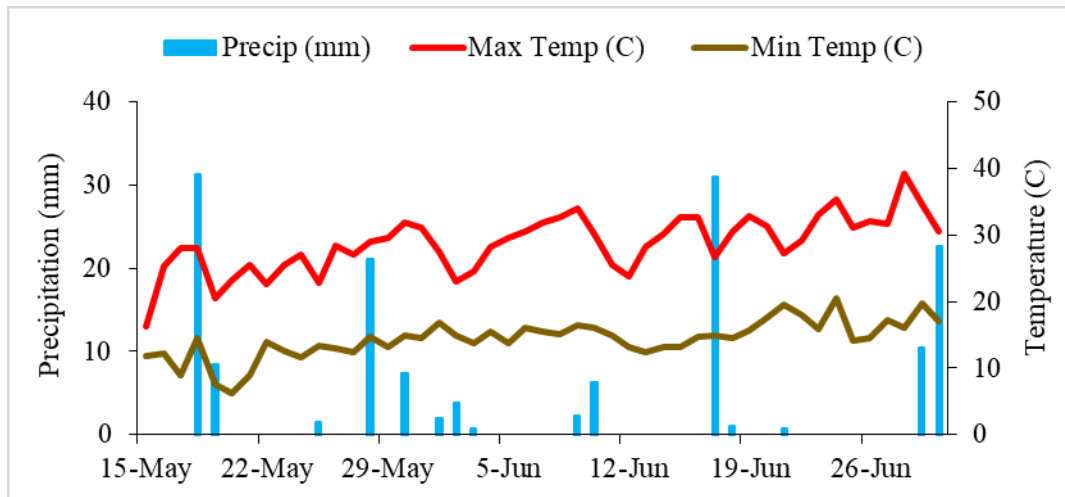[3]Values in the parenthesis are observed kochia density (plants m^{-2})

Figure 1. Daily minimum and maximum air temperature (C) and precipitation (mm) during the growing season at Kansas State University Agricultural Research Center (KSU-ARC) near Hays, KS.
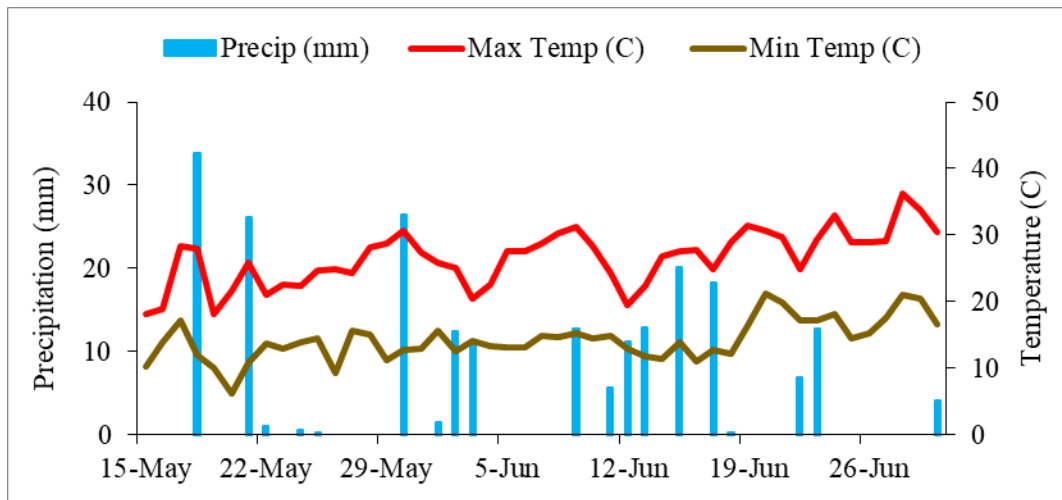
Figure 2. Daily minimum and maximum air temperature (C) and precipitation (mm) during the growing season at Kansas State University Southwest Research and Extension Center (KSU-SWREC) near Garden City, KS.