This is a “preproof” accepted article for Weed Science. This version may be subject to change in the production process, *and does not include access to supplementary material*.
DOI: 10.1017/wet.2025.24

Short title: PPO Herbicides & Amaranth Sex

Does exposure to PPO-inhibiting herbicides alter the male-to-female sex ratio of Palmer amaranth?

Mafia M. Rumpa[1], Sirwan Babaei[2], Ronald F. Krausz[3], David J. Gibson[4], Eric J Miller[5], and Karla L. Gage[6]

[1] Former Graduate Student, Southern Illinois University, Carbondale, Illinois, USA; [2] Assistant Professor (ORCID 0000-0001-5084-2140), Department of Crop Production and Genetics, University of Kurdistan, Kurdistan, Iran / Visiting Scientist, School of Agricultural Sciences, Southern Illinois University, Carbondale, Illinois, USA; [3] Former Research Station Manager, School of Agricultural Sciences, Southern Illinois University, Carbondale, Illinois, USA; [4] Professor, School of Biological Sciences, Southern Illinois University, Carbondale, Illinois, 62901-6509 USA (ORCID 0000-0002-0308-7506); [5] Associate Scientist, School of Agricultural Sciences, Southern Illinois University, Carbondale, Illinois, USA,[6] Associate Professor (ORCID 0000-0002-6235-8804), School of Agricultural Sciences / School of Biological Sciences, Southern Illinois University, Carbondale, Illinois, USA.

Author for correspondence: Karla L. Gage, Associate Professor, School of Agricultural Sciences / School of Biological Sciences, Southern Illinois University, Carbondale, 62901-6509, Illinois, USA, (E-mail: kgage@siu.edu)

Abstract

Palmer amaranth, a competitive weed in cotton and soybeans, poses challenges due to its rapid growth, high fertility, and herbicide resistance. Effective management strategies targeting sex ratios could reduce seed production by female plants. Protoporphyrinogen oxidase (PPO-) inhibiting herbicides play a role in the evolving resistance of *Amaranthus* spp. in the Midwestern US. These herbicides may also affect the male-to-female ratio of Palmer amaranth. A two-year field experiment (2015 and 2016) was conducted in a soybean field in Collinsville, Illinois, evaluating various preemergence and postemergence PPO-inhibiting herbicide treatments. Untreated Palmer amaranth populations exhibited a bias towards females. Pre-emergence application of sulfentrazone and flumioxazin effectively reduced Palmer amaranth density (1.66 plants m^{-2}) throughout the season, while post-emergence applications of fomesafen and lactofen provided limited control (27 and 31 plants m^{-2}). Early-season mortality was high (96 %) among Palmer amaranth seedlings, especially with pyroxasulfone+fluthiacet-methyl treatment. Fomesafen increased female biomass (28.8 %) while reducing male biomass compared to non-treated control. In 2015, pyroxasulfone+fluthiacet-methyl and acetochlor altered the male-to-female sex ratio compared to the non-treated control, with pyroxasulfone+fluthiacet-methyl reducing the proportion of females (-0.11 M:F) and acetochlor slightly increasing the proportion of males (0.03 M:F), though not different from a 1:1 ratio. In 2016, pendimethalin and flumioxazin-2 resulted in a strong female-biased sex ratio, with an almost exclusively female population. In both years, the non-treated control plots (-0.58 and -0.55 M:F) maintained a naturally female-biased sex ratio, deviating significantly from a 1:1 ratio. These findings suggest that specific herbicide treatments can alter the sex ratio. . Understanding sex determination in Palmer amaranth holds promise for developing more effective control strategies in the future.

Nomenclature: flumioxazin; sulfentrazone; Palmer amaranth, *Amaranthus palmeri* S. Watson; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

Keywords: dioecy, herbicide resistance, sex ratio, PPO inhibitors, weed control.

Introduction

The control of Palmer amaranth is challenging due to its rapid growth and germination throughout the growing season, high fecundity, and ability to capture more resources than neighboring plants (Keeley et al. 1987; Norsworthy et al. 2012). This species can substantially increase the soil seed bank in one growing season by producing 200,000 to 600,000 seeds female plant^{-1} under ideal conditions and without competition (Keeley et al. 1987; Steckel 2007). Palmer amaranth is one of the most competitive weeds in agricultural cropping systems and can reduce yield by 78 to 91%, respectively, at densities of 9 plants m^{-2} for crops like soybean and corn (Guo and Al-Khatib 2003; Massinga et al. 2003).

Amaranthus spp., such as Palmer amaranth and waterhemp [*Amarnathus tuberculatus* (Moq.) J. D. Sauer], are known for the ability to evolve rapidly in response to environmental conditions, in part because of the characteristic of dioecy (having distinct male and female plants) where gene flow is required between plants for reproduction. One of the most well-known examples is the rapid selection of herbicide resistance. Overreliance on a single mode of action in the past decade, i.e. glyphosate, has led to high selection pressure, increased the rate of resistance, and altered the effectiveness and success of weed management programs (Riggins and Tranel 2012). Palmer amaranth has developed resistance to multiple herbicide site of action (SOA) groups, including acetolactate synthase-inhibitors (SOA 2) and 5-enolpyruvylshikimate-3-phosphate synthase-inhibitor (SOA 9) which are very common in US cropping systems, microtubule-inhibitors (SOA 3), auxin mimics (SOA 4), photosystem II-inhibitors - serine 264 binders (SOA 5), photosystem II-inhibitors - histidine 215 binders (SOA 6), protoporphyrinogen oxidase-inhibitors (SOA 14), hydroxyphenylpyruvate dioxygenase-inhibitors (SOA 27), glutamine synthetase-inhibitor (SOA 10), and very long-chain fatty acid synthesis-inhibitors (SOA 15), which remains limited (Heap 2023).

Consequently, preemergence (PRE) application of soil residual herbicides, along with integrated weed management tactics, are becoming increasingly important, where herbicide application has limited postemergence (POST) options, especially for row crops like soybean and cotton (*Gossypium hirsutum* L.) (Lorestani et al. 2022; Norsworthy et al. 2012). Additionally, dioecious *Amaranthus* spp. may respond to environmental influences through an altered sex ratio. Populations of waterhemp expressed male bias when exposed to composted swine manure (Liebman et al. 2004). Changes in *A. rudis* sex ratios have been linked to soil moisture and air chemistry, as well as fertility, where populations in Illinois were female-biased, but Ontario populations expressed a 1:1 male-to-female sex ratio (Costea et al., 2005; Lemen, 1980).

Dioecious plant populations often deviate from the anticipated 1:1 primary sex ratio due to variations in parental investment (Fisher and Bennett 2011). As Palmer amaranth has evolved herbicide resistance to 10 sites of action (Heap 2023), fewer practical tools are left to control this species in the field. The evolution of herbicide resistance to other herbicides led to increased use of PPO-inhibiting herbicides for controlling Palmer amaranth (Sosnoskie et al. 2012) which has subsequently also resulted in the evolution of resistance to PPO-inhibiting herbicides. Understanding the effects of management on the population sex ratio could lead to the development of methods for controlling the density of seed-producing females in the future, and previous research has shown that PPO-inhibiting herbicides can influence the sex ratio of Palmer amaranth populations under controlled greenhouse conditions (Rumpa et al. 2019). The objectives of this study are to provide a field-based assessment of two objectives: first, to assess the male-to-female sex ratio of Palmer amaranth populations after exposure to PPO-inhibiting herbicides as well as other commonly used herbicides (in 2015 and 2016), and second, to investigate the PPO-inhibiting herbicides effect on the growth characteristics of male and female plants (in 2016).

Materials and Methods

Location and experimental procedure

The field experiment was conducted throughout the growing seasons of 2015 and 2016 in Collinsville, Illinois (38.69°N, 90.02°W) on soil classified as an Orion silt loam (coarse-silty, mixed, super active, mesic Aquic Udifluvents) with 2.5 % organic matter content, CEC of 14, and pH 7.1. The selection of experimental sites was based on two key criteria: Palmer amaranth populations with confirmed glyphosate resistance and fields with a history of corn cultivation in a tilled system during the year immediately preceding the experiment. Corn was chosen as a requirement due to its typically limited reliance on PPO-inhibiting herbicides compared to soybeans, reducing the likelihood of pre-existing resistance to this herbicide class. The experiment employed a completely randomized block design with three replicates to examine the impact of 12 PRE and two POST herbicides (Table 1) on the variation among sex ratios. The

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

experiment was conducted in soybean (variety P 37T09L) planted at 345,333 seeds ha^{-1} with a row spacing of 76 cm on May 19, 2015, and May 22, 2016, and the plots used had dimensions of 3 × 10 m.

Herbicide application

Herbicide applications based on the recommended rate were performed using a CO_2 backpack sprayer equipped with a 2 m boom and XR8002 flat fan nozzles spaced 50 cm apart, delivering 140 L ha^{-1} at 30 PSI. Twelve PRE and two POST herbicide treatments were applied on May 19, 2015, and June 12, 2015, and May 22, 2016, and June 13, 2016, respectively, when Palmer amaranth plants were between 8 and 12 cm tall (Table 1). The target height was 8 to 10 cm tall, but applications were delayed in 2015 due to rainfall.

Following the PRE and POST herbicide applications in 2016, the plots were monitored at two-week intervals at 15, 29, 44, 57, 71, and 85 days after treatment (DAT). During each visit, 20 newly emerged plants were randomly selected and marked with toothpicks, and flags were positioned to identify them without causing harm. The marked plants were checked for mortality, and any toothpicks and flags from dead plants were removed. Surviving plants were then identified by attaching a loose plastic tag to their stems. The tagging process was designed to easily distinguish any additional individuals that emerged alongside the marked plant. Distinctive colored plastic tags were employed to differentiate cohorts during each sampling of the plots. The initial set of tagged plants was labeled as cohort-1, followed by the second set after a two-week interval, referred to as cohort-2, and finally, the third and last set was designated as cohort-3.

During the initial visit in 2016, two selected locations within each treated and non-treated plot were designated for placing 0.5 m^2 quadrats. Wire flags were permanently attached to the corners of each quadrat to ensure their identification throughout the experiment. The plant count within each quadrat was recorded during each sampling event. Only end of season measures were collected in 2015. Once the seeds on the plants reached maturity (early October close to soybean maturity), data collection in 2015 and 2016 involved randomly selecting one m^2 area within each plot. Performance assessment included counting the total number of male and female plants within the quadrats and measuring the dry weights of male and female biomass.

Statistical analysis

The visible injury ratings (2016 only) were subjected to analysis using a one-way ANOVA using SAS software v. 9.4, employing a rating scale where 0 represented no injury and 100 indicated plant death. For density analysis following herbicide application (2016 only), a 3-way repeated mixed model was utilized. Similarly, a 3-way mixed model analysis was employed for biomass analysis (2015 and 2016), analyzed separately by year. Deviations from the evolutionary expected 1:1 sex ratio (0.5:0.5 proportions) and from the 1:1.38 – 1:22 male:female sex ratio (0.58 and 0.55 proportions of females) observed in non-treated control plots in 2015 and 2016, respectively, was tested using separate paired t-tests on the proportion of males-to-females in each plot and analyzed separately by year. Survivorship data were collected in 2016 and were assessed using a Cox proportional hazard test in SAS 9.4 (Proc phreg), using the non-treated control as the baseline for comparison. A preliminary proportional hazard test indicated a significant deviation of the observed residual from randomly generated processes ($p<0.05$), necessitating the use of a non-proportional model. To address this, separate Cox proportional hazard models were fitted for each cohort, resulting in an appropriate non-proportional model as suggested by Fox (2000).

Results and Discussion

Herbicide Efficacy

The efficacy of herbicides on Palmer amaranth was influenced by the timing of application in 2016 (Table 2). Preemergence application of herbicides resulted in 95% to 97% control of Palmer amaranth at 15 days after treatment (DAT), except for pyroxasulfone, which exhibited a slightly lower control rate of 92% (Table 2). There was no observable crop injury. At the 29 DAT, all PRE applications demonstrated 80% to 95% control of Palmer amaranth. Sulfentrazone-3 demonstrated the greatest efficacy when applied at a rate of 340 g ai ha^{-1}. All PRE herbicides provided 40% to 77% control of Palmer amaranth at 56 DAT, with sulfentrazone-3 exhibiting the greatest efficacy at 77%. Within 7 days after the POST (DA-POST) applications, all herbicides provided 90% control of Palmer amaranth. However, herbicide efficacy decreased to 20% for all treatments 15 DA-POST application (Table 2). Conversely, POST-herbicides resulted in only 33% control of Palmer amaranth after 35 DA-POST (Table 2).

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Following 2015 data collection, this study population of Palmer amaranth was identified as resistant to glyphosate and PPO-inhibiting herbicides. The site history does not suggest significant selection pressure for PPO-inhibiting herbicide resistance (Meyer et al. 2015; Tranel et al. 2011), implying that the resistant biotype may have been introduced by migratory birds traveling along the Mississippi Flyway route or through contaminated equipment. A random selection of 16 plants from the site was sent for quantitative PCR tests in early 2016, and the result showed three plants (18.8%) were heterozygous for PPO-inhibitor-resistance i.e. possessing the glycine 210 deletion (ΔG210) (Heap 2023).

The application of PRE herbicides demonstrated effective control of Palmer amaranth, even in a population with some level of PPO-inhibitor resistance. This aligns with studies indicating that soil-applied herbicides are more effective on resistant populations because they act on seeds or seedlings at early growth stages when resistance mechanisms are less active (Busi et al. 2020; Norsworthy et al. 2012; Vencill et al. 2012). In contrast, POST herbicides exhibited greater variability and demonstrated inadequate control efficacy, and the efficacy of POST applications of lactofen and fomesafen may have been impacted by PPO-inhibitor resistance at the site. Therefore, growers need to place greater importance on developing robust weed management programs, operating as if PPO-inhibitor or other increasing resistances in Palmer amaranth are already present on site to avoid control failures (Jenkins et al. 2017).

These results endorse implementing best management practices in weed control, including the strategic use of soil residual herbicides and careful selection of appropriate herbicides and, ideally, the inclusion of additional integrated weed management tactics when possible, to achieve acceptable levels of Palmer amaranth control (Goncalves et al. 2019; Reinhardt et al. 2022; Sweat et al. 1998; Ward et al. 2013).

Density

3-way repeated measures mixed model analysis ($P < 0.05$) was conducted to assess the impact of herbicides on Palmer amaranth density in 2016. The results revealed significant effects of both PRE and POST herbicide treatments across six consecutive periods (15, 29, 44, 57, 71, 85 DAT) on Palmer amaranth density ($F_{70, 150} = 7.23$, $p < 0.0001$). In the absence of herbicide treatment (non-treated control), Palmer amaranth density reached its highest level at 594.5 ± 89.1 plants m-

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

^{2} during the initial sampling period, gradually decreasing to 107.0 ± 18.7 plants m^{-2} by the final sampling period.

The density of Palmer amaranth varied among the plots with PRE herbicide applied. The highest density was observed in plots treated with pendimethalin and pyroxasulfone, (32.5 ± 24.3 and 33.3 ± 14.8 plants m^{-2}, respectively), during the first sampling period. On the other hand, plots treated with sulfentrazone-3 and flumioxazin-3 exhibited the lowest density (0.8 ± 0.8 and 2.5 ± 1.4 plants m^{-2}, respectively). By the last sampling period, the density ranged from 2 to 8 plants m^{-2} in all the PRE treated plots, with the lowest density observed in plots treated with sulfentrazone-3 (1.66 ± 1.66 plants m^{-2}) and flumioxazin-3 (1.66 ± 0.83 plants m^{-2}) (Figure 1a, Table 3). The density observed in the POST herbicide treatments, specifically fomesafen and lactofen, was 27.0 ± 6.2 and 31.0 ± 3.6 plants m^{-2}, respectively. These values were 4 to 15 times higher compared to all the PRE-treated plots at the last sampling period (Figure 1b and Table 3).

A significant occurrence of self-thinning was evident in the plots, leading to the elimination of smaller plants and the suppression of growth in other individuals, especially in high-density areas. The self-thinning phenomenon was particularly noticeable in the non-treated control plots. This observation could be attributed to the escalating temperatures experienced throughout the summer season, wherein plants growing in dense populations under high temperatures are likely to face moisture limitations (Bazzaz and McConnaughay 1992). Palmer amaranth density was notably lower in plots treated with PRE herbicides compared to the non-treated plots. Among all the PRE treatments, plots treated with pendimethalin and pyroxasulfone exhibited the highest plant density both at 15 and 85 DAT. Pendimethalin is not associated with optimal control of Palmer amaranth, with previous studies indicating control between 44 and 82% 20 days after PRE application (Whitaker et al. 2011). This higher density in pendimethalin and pyroxasulfone-treated plots could also be due to their relatively short persistence in the soil, lasting approximately 44 days for pendimethalin and 16-26 days for pyroxasulfone, which are approximate half-life ranges reported (Shaner 2014). It is important to note that methods for determining half-life values may vary across sources, potentially influencing these estimates. In contrast, sulfentrazone-treated plots displayed good control efficacy and had the lowest density of Palmer amaranth. This reduced density in sulfentrazone-treated plots may be attributed to the herbicide's longer persistence in the soil, lasting approximately 121-302 days (Shaner 2014). The extended persistence of sulfentrazone likely contributed to its effective control of Palmer amaranth, leading to a lower plant density in these plots (Shaner 2014). While half-life values provide a general indication of herbicide persistence, additional factors such as soil type, environmental conditions, and application timing may also influence field outcomes.

Survivorship

Survivorship curves describe how the proportion of survivors in a population changes over time. There are three main types: Type I: High survival throughout life, with a significant drop in older age (e.g., humans); Type II: Constant mortality rate throughout life (e.g., some plants, birds and small mammals); Type III: High mortality early in life, but those who survive tend to live longer (e.g., many plants and fish) (Lonsdale 1988). These curves reflect different life-history strategies. For most of the plants, a Type III pattern is common, with high early mortality and fewer survivors reaching maturity. Understanding this helps to interpret survival patterns and better manage weed populations in response to environmental stressors, such as herbicide treatments (Gage et al. 2015; Lonsdale 1988).

Survivorship of Palmer amaranth following the application of PRE and POST herbicides in 2016 was examined using a non-proportional Cox hazard regression model for each cohort. The analysis revealed a significant result for cohort-1 (Wald Chi-Square = 28.8, $p = 0.004$ at $df = 12$), indicating that the hazard ratios differed significantly from the non-treated control. However, for cohorts 2 and 3, the test results were not significant (both $p > 0.05$). Upon analyzing the Maximum Likelihood Estimates for cohort-1, it was found that the hazard ratios were significantly different from the non-treated control only for pendimethalin. This result indicates that in plots treated with pendimethalin, the plants had an increased likelihood of mortality compared to the non-treated control (Tables 4 and 5). No other treatments exhibited significant differences from the non-treated control, although the hazard ratios for several treatments differed from those of pendimethalin and flumioxazin-2 (Table 4).

The survivorship curves for cohort-1 and cohort-2 exhibited a type-III pattern, except for the pyroxasulfone+fluthiacet-methyl in cohort-2. Type-III curves indicated high mortality rates and a rapid decline in the number of seedlings during the initial weeks after emergence, followed by a decreasing mortality rate over time (Figures 2a and 2b). Notably, no survivors were observed in the sulfentrazone-2 and sulfentrazone-3 for cohort-2 by August 1st (Figure 2b). In contrast, the survivorship curves for cohort-3 displayed a type-II pattern, indicating a constant mortality risk

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

throughout the lifetime. By August 1st, no survivors were found among cohort-3 plants, except in the acetochlor, lactofen, and fomesafen treatments (Figure 2c).

At all the sites, both the treated plots and the non-treated control plots displayed survivorship curves categorized as type II and type III. Type II curves imply a consistent mortality risk throughout the lifetime, while type III curves exhibit high mortality rates during the early stages of emergence, followed by a decrease in mortality rates as time progresses. The presence of both types of survivorship curves suggests variations in the survival patterns of the plant population in different plots, with some plots experiencing consistent mortality risks over time (type II) and others witnessing higher early mortality rates that diminish over time (type III). These findings provide notable information into the dynamic nature of survivorship and mortality risks in the Palmer amaranth populations across different treatment plots. Indeed, type II survivorship curves are often associated with perennial herbaceous species, exhibiting distinct seasonal oscillations of mortality rates and an increased risk of mortality during growth and reproductive stages. These curves reflect the inherent challenges that perennial herbaceous plants face, especially during phases of active growth and reproduction, where mortality rates may fluctuate due to environmental factors, competition, and resource availability. As a result, the type II survivorship pattern highlights the dynamic and complex nature of survival in such plant populations over their life cycle, providing meaningful findings into the strategies and vulnerabilities of these species in their natural environments (Schwartz and Gibson 2014). Meanwhile, type III survivorship curves, like those observed in other annual species, are characterized by high mortality rates during the juvenile stage, leading to a rapid decrease in the number of seedlings. This pattern highlights the challenges faced by annual species during their early life stages, where they are particularly vulnerable to various environmental stressors, competition, and resource limitations. The type III survivorship pattern indicates that only a small proportion of the initial seedlings survive to reach adulthood, emphasizing the intense selection pressures and the importance of successful seedling establishment for annual species to maintain their population sizes. This type of survivorship curve is a common feature in many annual plant species and plays a crucial role in shaping the dynamics of their populations in their respective habitats (Klemow and Raynal 1983). Many survivors were identified in the first cohorts, where individuals that germinated early were generally able to complete their life cycle. These observations allowed for an estimation of pre-reproductive mortality, like that seen in other herbaceous species (Hawthorn and Cavers 1976; Thomas and Dale 1975). While the specific reason for mortality was not recorded, several factors may have contributed to the high mortality of individuals in different plots. These factors include herbicide persistence in the soil, herbivory, shading, competition from neighboring plants, or drought. Each of these factors could have played a role in influencing the survival rates of individuals in the studied plots, emphasizing the complexity of ecological interactions and their impact on plant mortality in diverse environments (Schwartz et al. 2016). An extreme drought condition was observed during the early growing season in 2016, and this could have contributed to the mortality of the species in Collinsville, Illinois. The severe drought stress experienced by the plants during their initial growth phase may have hampered their ability to establish and thrive, leading to higher mortality rates in the population (Figure 3).

Biomass

In 2015, a significant interaction effect between PRE and POST herbicide applications and sex was observed for Palmer amaranth biomass at maturity ($F_{12, 40} = 2.32$, $p < 0.0234$) (Table 6). The mean biomass values for both female and male plants indicated that those treated with s-metolachlor exhibited greater biomass compared to other treated populations, although the difference was not statistically significant. Conversely, male plants treated with pyroxasulfone had lower biomass compared to other treated plants (Figure 4a).

In 2016, a significant interaction effect between PRE and POST herbicide applications and sex was observed for Palmer amaranth biomass at maturity ($F_{9, 22} = 2.95$, $p < 0.0186$) (Table 6). The mean comparison of biomass indicated that female populations treated with lactofen exhibited greater biomass compared to lactofen-treated males, as well as fomesafen-treated males and females, saflufenacil-treated males, flumioxazin-1-treated males, and pyroxasulfone-treated males. However, the male plants treated with lactofen had the lowest biomass and did not show a significant difference from the biomass of s-metolachlor-treated females, fomesafen-treated males, pendimethalin-treated females, sulfentrazone-2-treated females, sulfentrazone-1-treated females, and flumioxazin-3-treated females (Figure 4b).

Among all the treatments, the POST applications of lactofen and fomesafen, and the PRE application of flumioxazin were specifically compared for their effects on female and male biomass. These treatments resulted in higher female biomass compared to male biomass, which

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

aligned with the pattern observed in the non-treated control plots. This comparison highlights the differential effects of these herbicide treatments on biomass allocation between sexes. This difference in biomass allocation is a common phenomenon in dioecious species, where females tend to invest more resources in the production of reproductive parts and seeds, leading to size dimorphism between male and female plants. However, PPO-inhibitor resistance was documented in Palmer amaranth from the Collinsville, Illinois study site (Heap 2023; Jenkins et al. 2017). Confirmation of resistance in the plants was carried out through quantitative PCR on 16 randomly selected plants from plots treated with lactofen or fomesafen. The results revealed that 3 out of 16 plants were confirmed to be resistant, indicating an estimated frequency of 18.8% of the population possessing the resistance trait. Moreover, recent findings identified two new potential mutations in Palmer amaranth, namely PPX2 (R98G, R98M), which are associated with resistance to the PPO-inhibitor fomesafen. These discoveries provide further insights into the genetic mechanisms that contribute to herbicide resistance in Palmer amaranth populations, enhancing our understanding of its resistance dynamics and implications for weed management strategies (Giacomini et al. 2017). Based on these data, the control efficacy of Palmer amaranth for the two post-emergence PPO-inhibiting herbicides, lactofen, and fomesafen, was found to be 20%. This lower efficacy observed for lactofen and fomesafen, in comparison to other herbicides, could be the contributing factor for the higher biomass observed in the lactofen treatment. The reduced control efficacy of these specific herbicides might have allowed for increased plant growth and survival, leading to higher biomass in the treated plots compared to other herbicide treatments.

Male-to-female sex ratio

Population sex ratio in 2015. Paired t-tests on the proportions of males to females (testing deviations from 1:1 sex ratio) indicated that non-treated control plots, plots treated with pyroxasulfone+fluthiacet-methyl, pendimethalin, saflufenacil, and lactofen gave a significant departure from 1:1 male-to-female sex ratio (female > male) (Figure 5a). Tests of deviations from non-treated controls indicated that pyroxasulfone+fluthiacet-methyl and acetochlor led to a significant departure from the non-treated control male-to-female sex ratio (Table 7). Treatment with pyroxasulfone+fluthiacet-methyl led to a smaller proportion of females than in the non-

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

treated plots (while still deviating from a 1:1 ratio), whereas acetochlor had a higher proportion of males, albeit not significantly different from a 1:1 ratio.

Population sex ratio in 2016. Paired t-tests on the proportions of males to females (testing deviations from 1:1 sex ratio) indicated that non-treated control, pendimethalin, flumioxazin-1 and -2, and lactofen gave a significant departure from 1:1 male-to-female sex ratio (female > male) (Figure 5b). Tests of deviations from non-treated controls indicated that pendimethalin and flumioxazin -2, led to a significant departure from the non-treated control male-to-female sex ratio with an exclusively female population (Table 7).

Palmer amaranth has developed resistance to multiple herbicide modes of action (Heap 2023). Effective management practices are essential to control the invasive species of Palmer amaranth in agricultural fields. One integrated approach for control involves manipulating the weed population's sex ratios through herbicide applications or other environmental factors to reduce the density of female plants in the field. This research indicates that different herbicides can influence the sex ratio of the Palmer amaranth population in varying ways, which may also influence resulting population dynamics in herbicide resistance evolution. Some herbicide treatments led to a shift in the female-biased population sex ratios towards a 1:1 ratio by promoting a higher male population compared to non-treated control plants. The increased production of male plants through herbicide exposure may be linked to reduced levels of cytokinin in plant cells. Cytokinin is a plant growth regulator associated with sexually differentiated tissues, favoring female production under normal growth conditions and male production under stressful conditions. This phenomenon has been observed in other dioecious plants like annual mercury (*Mercurialis annua* L.) and Kentucky coffeetree (*Gymnocladus dioica* (L.) K. Koch), and it may provide insights into the sex determination of other dioecious species, including Palmer amaranth. These findings highlight the potential of manipulating sex ratios through herbicide treatments to manage Palmer amaranth populations effectively in agricultural settings (Hautala et al. 1986).

This study highlights the significance of comprehending the factors influencing the sex ratio of Palmer amaranth populations under different management scenarios and in response to various environmental conditions. The findings underscore the potential to manipulate the sex ratio of these weed populations, presenting a promising avenue for improving management

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

strategies. Additionally, female-biased sex ratio outcomes in herbicide-resistant populations treated with herbicide

Moreover, the study of survivorship curves and factors affecting plant mortality offers essential information on the species' adaptive response to environmental conditions, such as drought and competition. Understanding the drivers of mortality and population dynamics is critical for devising sustainable and effective weed management practices. Sustainability in weed management practices refers to methods that effectively control weed populations, minimize environmental impacts, and promote long-term agricultural productivity.

Moving forward, continued research efforts in Palmer amaranth biology and ecology are necessary to refine our understanding of the species' behavior and the impact of environmental factors on its population dynamics. With such knowledge, we can develop innovative and ecologically sound strategies to curtail the spread and impact of this invasive weed in agricultural landscapes. Ultimately, a comprehensive and adaptive approach that combines herbicide management, sex ratio manipulation, and ecological considerations may lead to successful and sustainable control of Palmer amaranth populations. By leveraging these insights, we can progress toward enhanced weed management scenarios that contribute to agricultural systems' long-term productivity and sustainability.

Acknowledgments

We are grateful to the University Farms of Southern Illinois University and Dr. David Barfnecht for support during this research.

Funding: No external funding was received for this research.

Competing Interests: Competing interests: The authors declare none.

References

Bazzaz FA, McConnaughay KDM (1992) Plant-plant interactions in elevated CO2 environments. Aust J Bot 40:547–563

Busi R, Powles SB, Beckie HJ, Renton M (2020) Rotations and mixtures of soil-applied herbicides delay resistance. Pest Manag Sci 76:487–496

Costea M, Weaver SE, Tardif FJ (2005) The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. Can J Plant Sci 85:507-522

Fisher RA, Bennett H, eds (2011) The Genetical Theory of Natural Selection: A Complete Variorum Edition. Oxford University Press. 318 p.

Gage KL, Gibson DJ, Young BG, Young JM, Matthews JL, Weller SC, Wilson RG (2015) Occurrence of an herbicide-resistant plant trait in agricultural field margins. Ecol Evol 5:4161–4173

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new PPX2 mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559–1563

Goncalves NettoA, NicolaiI M, Carvalho SJP, Malardo MR, Lopez-Ovejero RF, Christoffoleti PJ (2019) Control of ALS- and EPSPS-resistant *Amaranthus palmeri* by alternative herbicides applied in pre- and post-emergence. Planta Daninha 37:e019212505

Guo P, Al-Khatib K (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:869–875

Hautala E, Stafford A, Corse J, Barker PA (1986) Cytokinin variation in the sap of male and female *Gymnocladus Dioica*. J Chromatogr A 351:560–565

Hawthorn WR, Cavers PB (1976) Population dynamics of the perennial herbs *Plantago Major* L. and *P. Rugelii* Decne. J Ecol 64:511-527

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Heap I (2023) The International Herbicide-Resistant Weed Database. https://www.weedscience.org

Jenkins ME, Krausz RF, Matthews JL, Gage KL, Walters SA (2017) Control of volunteer horseradish and palmer amaranth (*Amaranthus palmeri*) with dicamba and glyphosate. Weed Technol 31:852–862

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204

Klemow KM, Raynal DJ (1983) Population biology of an annual plant in a temporally variable habitat. J Ecol 71:691-703

Lemen C (1980) Allocation of reproductive effort to the male and female strategies in wind-pollinated plants. Oecologia 45:156-159

Liebman M, Menalled FD, Buhler DD, Richard TL, Sundberg DN, Cambardella CA, Kohler KA (2004) Impacts of composted swine manure on weed and corn nutrient uptake, growth, and seed production. Weed Sci 52:365-375

Lonsdale WM (1988) Interpreting seed survivorship curves. Oikos 52:361-364

Lorestani E, Babaei S, Tahmasebi I, Sabeti P (2022) Assessment of tribenuron methyl soil residual on crops germination properties. Gesunde Pflanz 75:765–773

Massinga RA, Currie RS, Trooien TP (2003) Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Sci 51:523–531

Meyer CJ, Norsworthy JK, Young BG, Steckel LE, Bradley KW, Johnson WG, Loux MM, Davis VM, Kruger GR, Bararpour MT, Ikley JT, Spaunhorst DJ, Butts TR (2015) Herbicide program approaches for managing glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus* and *Amaranthus rudis*) in future soybean-trait technologies. Weed Technol 29:716–729

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31–62

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Reinhardt C, Vorster J, Küpper A, Peter F, Simelane A, Friis S, Magson J, Aradhya C (2022) A nonnative Palmer amaranth (*Amaranthus palmeri*) population in the Republic of South Africa is resistant to herbicides with different sites of action. Weed Sci 70: 183-197

Riggins CW, Tranel PJ (2012) Will the *Amaranthus tuberculatus* resistance mechanism to PPO-inhibiting herbicides evolve in other Amaranthus species? Int J Agron 2012(1), p.305764

Rumpa MM, Krausz RF, Gibson DJ, Gage KL (2019) Effect of PPO-inhibiting herbicides on the growth and sex ratio of a dioecious weed species *Amaranthus palmeri* (Palmer amaranth). Agron 9, 6:275

Schwartz LM, Gibson DJ (2014) The competitive response of *Panicum virgatum* cultivars to non-native invasive species. Environ Nat Resour 4, 2:80

Schwartz LM, Gibson DJ, Young BG (2016) Using integral projection models to compare population dynamics of four closely related species. Popul Ecol 58:285–292

Shaner DL (2014) Herbicide Handbook. 10th Edition. Lawrence: Weed Science Society of America. 513 p

Sosnoskie LM, Webster TM, Kichler JM, MacRae AW, Grey TL, Culpepper AS (2012) Pollen-mediated dispersal of glyphosate-resistance in palmer amaranth under field conditions. Weed Sci 60:366–373

Steckel LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567–570

Sweat JK, Horak MJ, Peterson DE, Lloyd RW, Boyer JE (1998) herbicide efficacy on four *Amaranthus* species in soybean (*Glycine max*). Weed Technol 12:315–321

Thomas AG, Dale HM (1975) The role of seed reproduction in the dynamics of established populations of *Hieracium floribundum* and a comparison with that of vegetative reproduction. Can J Botany 53:3022–3031

Tranel PJ, Riggins CW, Bell MS, Hager AG (2011) Herbicide resistances in *Amaranthus tuberculatus*: A call for new options. J Agric Food Chem 59:5808–5812

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide resistance: toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci 60:2–30

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol 27:12–27

Whitaker JR, York AC, Jordan DL, Culpepper AS, Sosnoskie LM. (2011). Residual herbicides for Palmer amaranth control. J Cotton Sci 15(1), 89-99

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

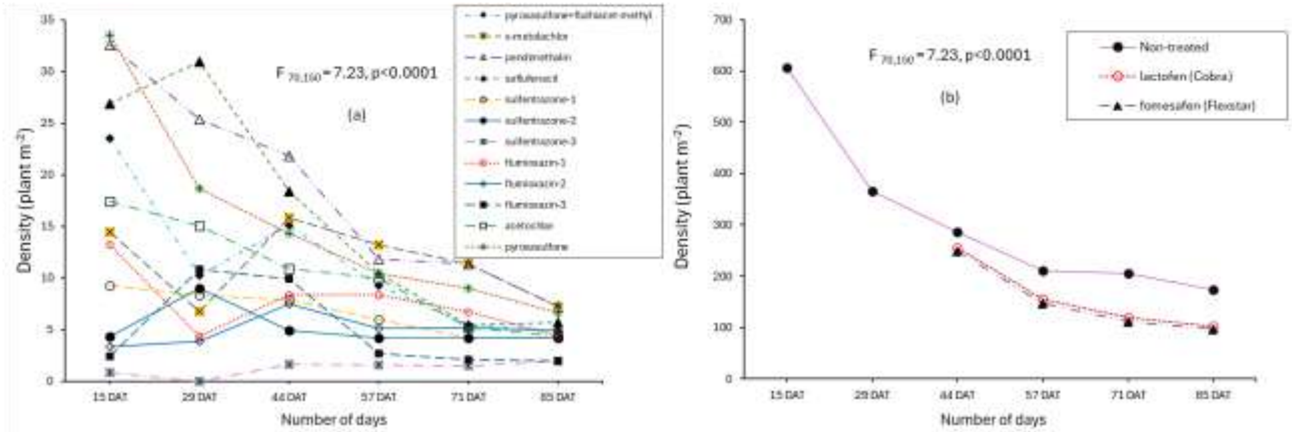

Figure 1: Effect of herbicides on Palmer amaranth density (a) preemergence (PRE) and (b) postemergence (POST) herbicide applications in 2016. Lines indicated as 3-way repeated measures mixed model.

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

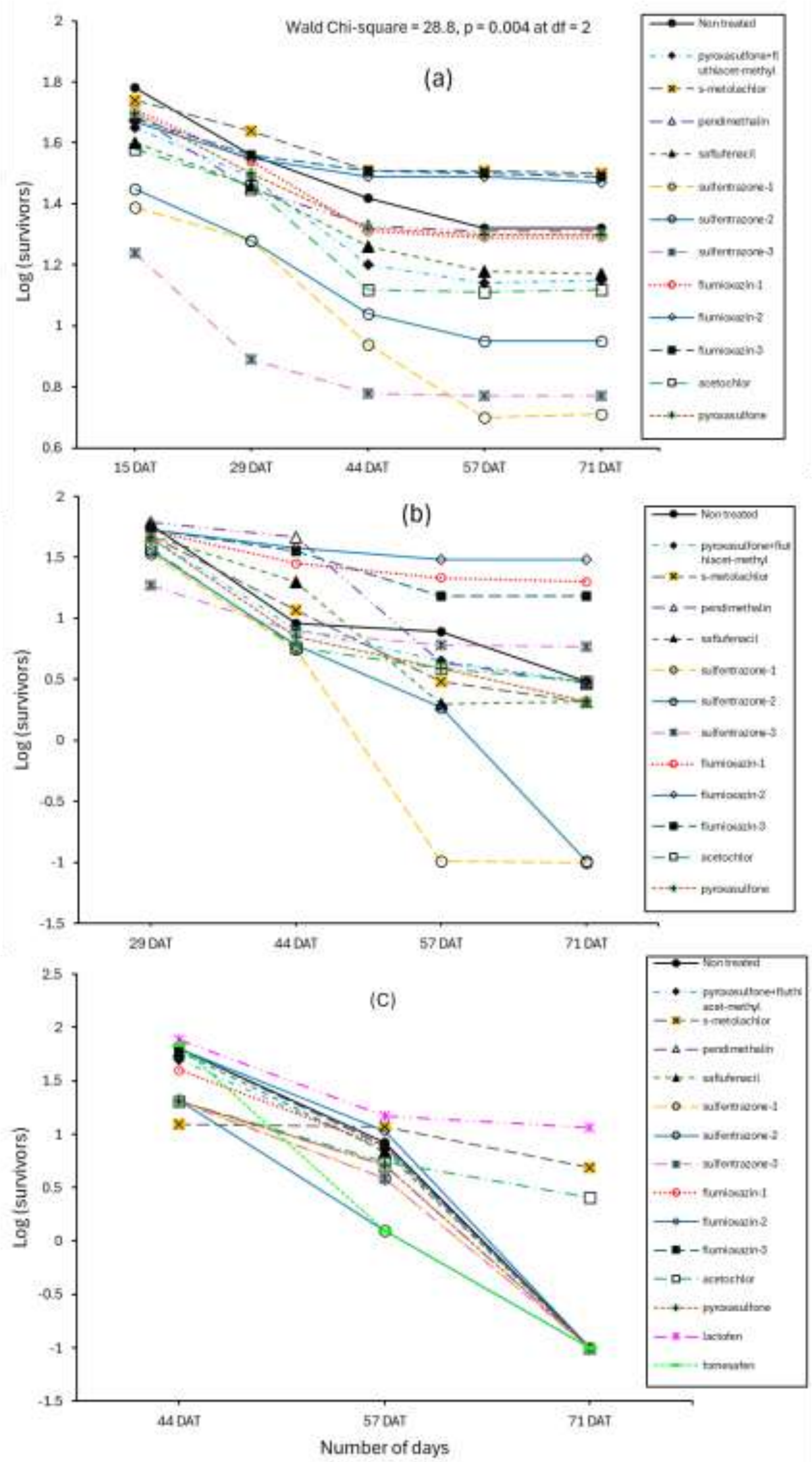

Figure 2. Survivorship curves (a) 1st cohort, (b) 2nd cohort and (c) 3rd cohort in 2016. Asterisk (*) represents significant likelihood of mortality of plants in the pendimethalin compared to the non-treated.

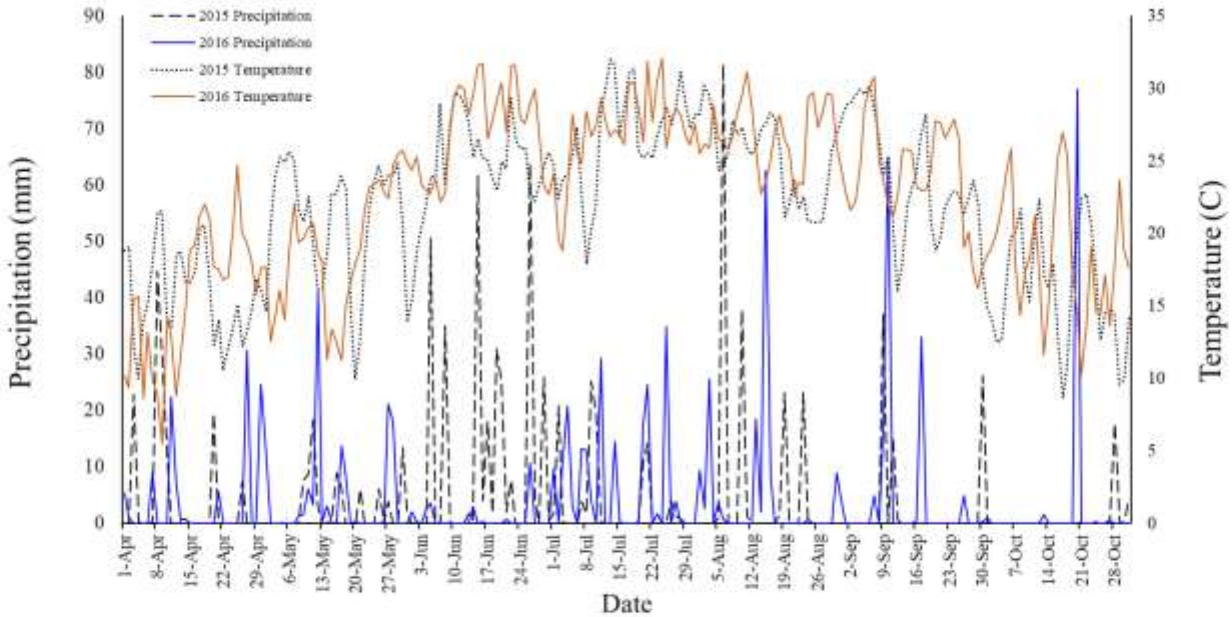

Figure 3. Total precipitation and temperature in Collinsville, Illinois during 2015 and 2016.

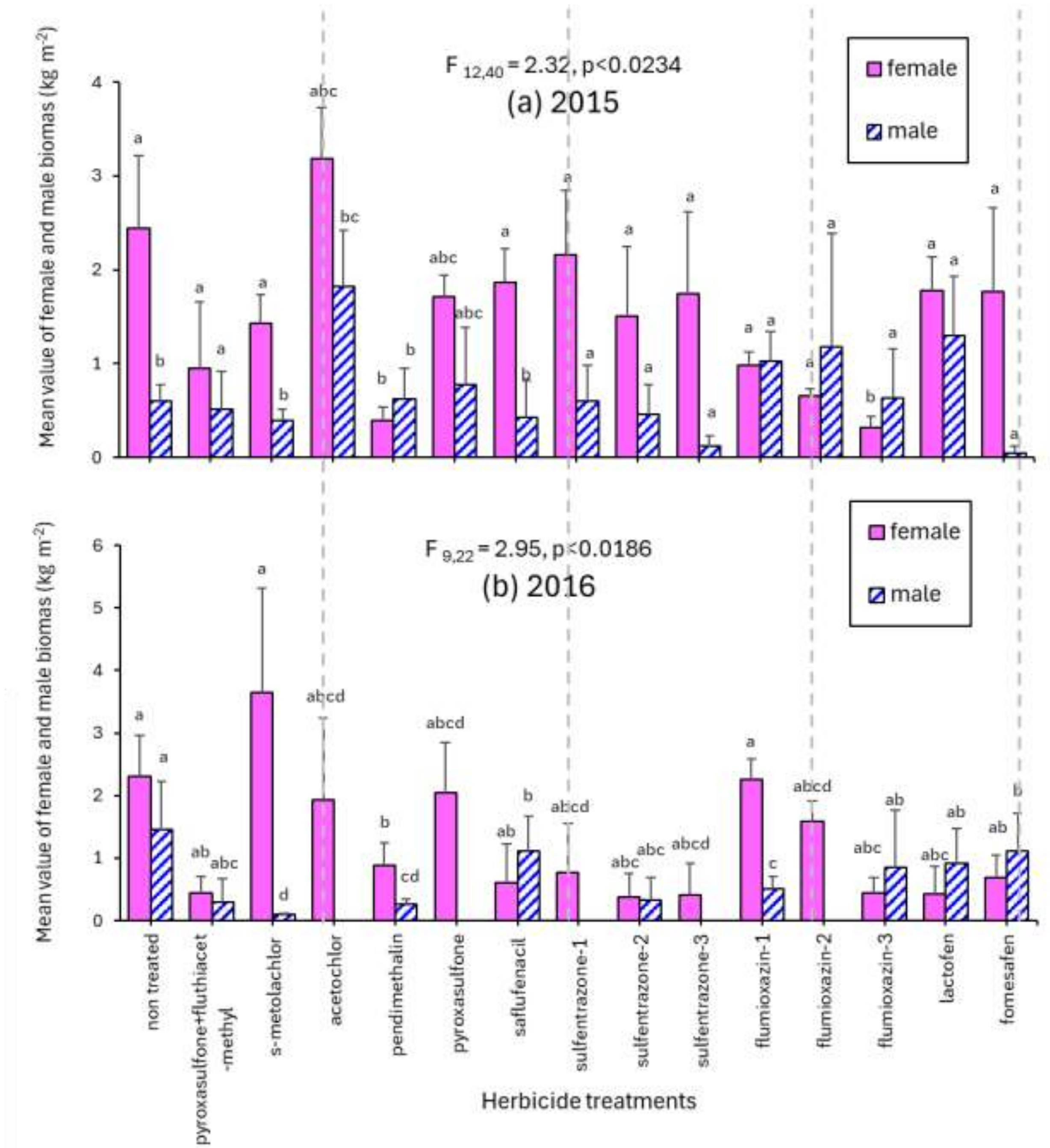

Figure 4. Effect of preemergence and postemergence herbicide on Palmer amaranth biomass (kg m^{-2}) by sex (female and male) following in (a) 2015 and (b) 2016. Bars with the same letters are statistically different (p≤0.05).

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

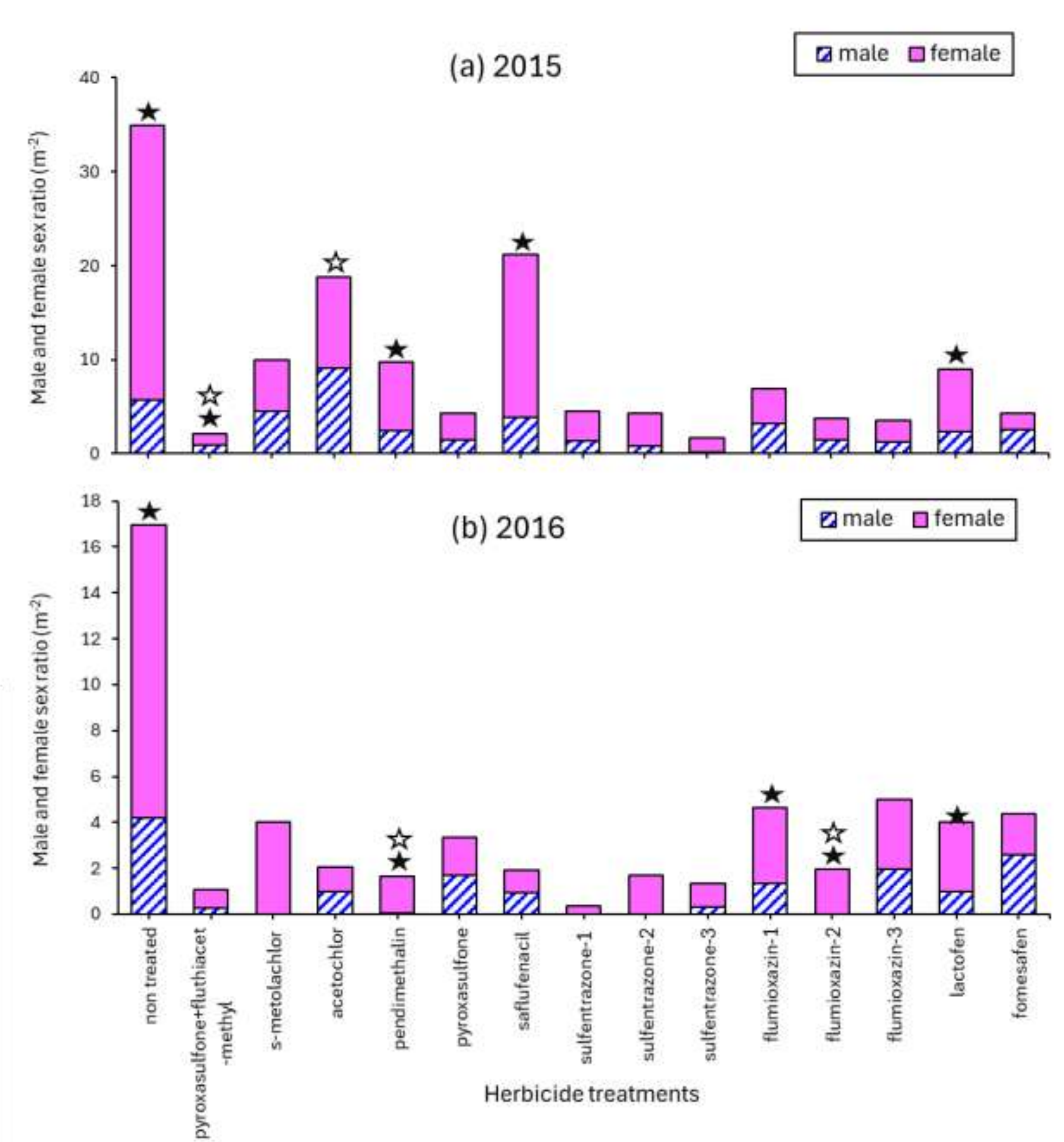

Figure 5. Effect of preemergence and postemergence herbicide on Palmer amaranth. Male-to-female sex ratios (a) 2015 and (b) 2016. Bold asterisk (★) represents significant departure from 1:1 male-to-female sex ratio (Chi-square tests, $p \leq 0.05$). Blank asterisk (☆) significant departure from non-treated control male-to-female sex ratio (t-test, H0=0.58 in 2015, H0=0.55 in 2016, $p \leq 0.05$).

Table 1. Details of herbicides and application timings.

	Herbicide treatment	Application timing	Site of action group	Rate[a]	Manufacturer
				g ai ha^{-1}	
1	pyroxasulfone+fluthiacet-methyl (Anthem)	PRE[a]	15 & 14[b]	226.80	FMC Corporation
2	*S*-metolachlor (Dual magnum)	PRE	15	756	Syngenta
3	acetochlor (Warrant)	PRE	15	1705	Syngenta
4	pendimethalin (Prowl H2O)	PRE	3	1420	BASF
5	pyroxasulfone (Zidua)	PRE	15	71	BASF
6	saflufenacil (Sharpen)	PRE	14	28	BASF
7	sulfentrazone-1 (Spartan 4F)	PRE	14	227	FMC Corporation
8	sulfentrazone-2 (Spartan 4F)	PRE	14	284	FMC Corporation
9	sulfentrazone-3 (Spartan 4F)	PRE	14	340	FMC Corporation
10	flumioxazin-1 (Valor SX)	PRE	14	57	Valent U.S.A.
11	flumioxazin-2 (Valor SX)	PRE	14	71	Valent U.S.A.
12	flumioxazin-3 (Valor SX)	PRE	14	85	Valent U.S.A.
13	lactofen (Cobra)	POST	14	340	Valent U.S.A.
14	fomesafen (Flexstar)	POST	14	680	Syngenta

[a] Abbreviations: ai = Active ingredients, PRE = preemergence application, POST = postemergence application. Note: Herbicide treatments lactofen and fomesafen were applied with COC (prime oil) and N-PAK AMS at 1% and 5% v/v, respectively.

[b] Group 3: microtubule-inhibitors, Group 14: protoporpyrinogen oxidase-inhibitors, Group 15: long-chain fatty acid elongase-inhibitors.

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Table 2. Influence of PRE and POST herbicides on Palmer amaranth control in 2016.

Herbicide treatment	Rate	15 DAT [a]	29 DAT	56 DAT
	g ai ha^{-1}	------------------%-------------------		
pyroxasulfone+fluthiacet-methyl	226.80	96 ab	88 bcd	47 defg
S-metolachlor	755.79	96 ab	87 bcde	42 fg
acetochlor	1704.78	95 ab	84 def	57 bcde
pendimethalin	1420.65	95 ab	85 def	58 bcd
pyroxasulfone	70.87	92 c	85 def	40 g
saflufenacil	28.35	95 b	80 f	58 bcd
sulfentrazone-1	226.80	96 ab	91 abc	68 abc
sulfentrazone-2	283.50	97 ab	92 ab	70 ab
sulfentrazone-3	340.19	97 a	95 a	77 a
flumioxazin-1	56.70	95 b	82 ef	43 efg
flumioxazin-2	70.87	96 ab	87 bcdef	42 fg
flumioxazin-3	85.05	96 ab	86 cdef	55 cef
		7 DAT	15 DAT	35 DAT
lactofen	340.19	90 c	20 g	33 g
fomesafen	680.39	90 c	20 g	33 g

[a] Abbreviations: PRE= preemergence application, POST = postemergence application, DAT=days after treatment for PRE and POST applications.

[b] Palmer amaranth control was evaluated based on a visible scale of 0 (no control) to 100 (complete control). Means followed by the same letters are not significantly different ($p \leq 0.05$).

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Table 3. Effect of PRE and POST herbicides Palmer amaranth density m^{-2} in 2016.

Herbicides / Time	15 DAT [a]	29 DAT	44 DAT	57 DAT	71 DAT	85 DAT
non treated	594.5±89.1[b]	320.0±10.0	230.0±19.4	148.3±29.8	139.1±29.0	107.5±18.7
pyroxasulfone+fluthiacet-methyl	23.3±20.8	10.0±5.0	15.0±9.0	9.1±4.4	5.8±3.3	5.0±3.8
s-metolachlor	14.1±4.16	6.6±3.3	15.8±8.7	13.3±5.4	11.6±5.8	7.5±3.8
acetochlor	17.5±11.4	15.0±5.2	10.8±4.4	10.0±6.2	5.0±3.8	4.1±4.1
pendimethalin	32.5±24.2	25.0±5.2	21.6±8.3	11.6±3.6	11.6±3.6	7.5±2.5
pyroxasulfone	33.3±14.8	18.3±4.6	14.1±8.2	10.8±4.6	9.1±5.4	6.6±4.1
saflufenacil	26.6±21.6	30.8±6.0	18.3±4.4	10.8±0.8	5.8±1.6	5.8±1.6
sulfentrazone-1	9.1±6.6	8.3±4.6	8.3±3.3	5.8±0.8	4.1±0.8	4.1±0.8
sulfentrazone-2	4.1±1.6	9.1±1.6	5.0±3.8	4.1±3.0	4.1±3.0	4.1±3.0
sulfentrazone-3	0.8±0.8	0.0000	1.6±1.6	1.6±1.6	1.6±1.6	1.6±1.6
flumioxazin-1	13.3±2.2	4.1±1.6	8.3±2.2	8.3±4.6	6.6±3.0	5.0±2.8
flumioxazin-2	3.3±3.3	4.1±0.8	7.5±1.4	5.0±1.4	5.0±1.4	5.0±1.4
flumioxazin-3	2.5±1.4	10.8±5.8	10.0±3.8	2.5±0	1.6±0.8	1.6±0.8
lactofen	-	-	204.1±79.0	85.8±19.5	46.6±2.2	31.6±3.6
fomesafen	-	-	196.6±67.7	82.5±2.5	43.3±9.6	27.5±6.2

[a] Abbreviations: DAT=days after treatment for preemergence (PRE) and postemergence (POST) applications.

[b] Standard error.

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Table 4. Cox proportional hazard regression model for PRE herbicides in 2016. Hazard ratios (± 95% confidence limits) provide a comparison of survivorship compared with non-treated control plants (Hazard Ratio = 1.0, p ≤ 0.05).

Herbicides / Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq	Hazard Ratio	95% Hazard Ratio
pyroxasulfone+fluthiacet-methyl	1	0.11	0.24	0.19	0.65	1.11	0.68/1.81
S-metolachlor	1	-0.41	0.26	2.39	0.12	0.66	0.39/1.11
acetochlor	1	0.09	0.26	0.13	0.71	1.10	0.65/1.83
pendimethalin	1	0.67	0.22	9.22	0.002	1.95	1.26/3.01
pyroxasulfone	1	0.07	0.24	0.09	0.75	1.07	0.66/1.75
saflufenacil	1	0.03	0.26	0.01	0.89	1.03	0.62/1.73
sulfentrazone-1	1	0.27	0.28	0.967	0.32	1.31	0.76/2.28
sulfentrazone-2	1	0.16	0.28	0.33	0.56	1.17	0.67/2.06
sulfentrazone-3	1	0.20	0.33	0.36	0.54	1.22	0.63/2.35
flumioxazin-1	1	0.08	0.24	0.10	0.74	1.08	0.66/1.76
flumioxazin-2	1	-0.35	0.27	1.73	0.18	0.69	0.41/1.19
flumioxazin-3	1	0.30	0.24	1.51	0.21	1.35	0.83/2.18

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Table 5. PRE and POST herbicide effect on survivorship of Palmer amaranth in 2016.

1st sampling period (log survivors)					
Herbicide treatment	15 DAT	29 DAT	44 DAT	57 DAT	71 DAT
non treated	1.78	1.56	1.42	1.32	1.32
pyroxasulfone+fluthiacet-methyl	1.65	1.47	1.2	1.14	1.14
S-metolachlor	1.73	1.64	1.5	1.5	1.5
acetochlor	1.58	1.45	1.12	1.12	1.11
pendimethalin	1.74	1.56	1.18	0.71	0.71
pyroxasulfone	1.70	1.50	1.32	1.30	1.30
saflufenacil	1.6	1.46	1.26	1.18	1.17
sulfentrazone-1	1.4	1.28	0.95	0.71	0.71
sulfentrazone-2	1.45	1.28	1.04	0.95	0.95
sulfentrazone-3	1.25	0.9	0.78	0.78	0.78
flumioxazin-1	1.70	1.46	1.34	1.30	1.3
flumioxazin-2	1.72	1.56	1.49	1.49	1.47
flumioxazin-3	1.67	1.56	1.18	1.18	1.17

2nd sampling period (log survivors)				
	29 DAT	44 DAT	57 DAT	71 DAT
non treated	1.78	0.95	0.9	0.47
pyroxasulfone+fluthiacet-methyl	1.74	0.84	0.6	0.49
S-metolachlor	1.70	1.00	0.3	0.30
acetochlor	1.60	0.84	0.49	0.49
pendimethalin	1.78	1.66	0.64	0.48
pyroxasulfone	1.7	1.07	0.48	0.3
saflufenacil	1.7	1.3	0.30	0.3
sulfentrazone-1	1.56	0.78	-1.0	-1.0
sulfentrazone-2	1.59	0.78	0.3	0.78
sulfentrazone-3	1.28	0.90	0.78	1.3
flumioxazin-1	1.70	1.46	1.34	1.48
flumioxazin-2	1.72	1.56	1.48	1.48

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

flumioxazin-3	1.67	1.56	1.18	1.18

3rd sampling period (log survivors)

	44 DAT	57 DAT	71 DAT
non treated	1.88	1.05	-1.0
pyroxasulfone+fluthiacet-methyl	1.71	0.8	-1.0
S-metolachlor	1.1	1.05	-1.0
acetochlor	1.3	0.7	0.41
pendimethalin	1.8	0.77	-1.0
pyroxasulfone	1.3	0.7	-1.0
saflufenacil	1.83	0.1	-1.0
sulfentrazone-1	1.33	0.1	-1.0
sulfentrazone-2	1.32	0.57	-1.0
sulfentrazone-3	1.33	0.1	-1.0
flumioxazin-1	1.6	0.88	-1.0
flumioxazin-2	1.84	1.0	-1.0
flumioxazin-3	1.80	0.58	-1.0
lactofen	1.88	1.18	1.08
fomesafen	1.88	1.05	0.7

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Table 6: Analysis of variance (ANOVA) of the effects of herbicide treatments and sex on Palmer amaranth biomass. Significant values (p ≤ 0.05) highlighted in bold.

Biomass in 2015				
Effect	DF		F value	Pr > F
Herbicide	12	40	1.71	0.1006
Sex	1	40	11.99	0.0013
Herbicide *Sex	12	40	2.32	0.0234
Biomass in 2016				
Effect	DF		F value	Pr > F
Herbicide	9	22	2.25	0.05
Sex	1	22	2.41	0.1345
Herbicide *Sex	9	22	2.95	0.0186

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press

Table 7. Comparison of the sex ratio of Palmer amaranth in 2015 and 2016, including deviations from a 1:1 male-to-female (M:F) ratio (H0=0.50) and differences between herbicide treatments and non-treated control plots (H0=0.58 in 2015, H0=0.55 in 2016). P values from paired t-tests at df=2.

Herbicides/Year	2015			2016		
	Sex ratio (M:F)	H0=0.50 Pr > |t|	H0=-0.58 Pr > |t|	Sex ratio (M:F)	H0=0.50 Pr > |t|	H0=-0.55 Pr > |t|
non treated	-0.58	0.02	0.99	-0.55	0.04	0.99
pyroxasulfone+fluthiacet-methyl	-0.11	0.03	0.05	-0.33	0.33	0.77
S-metolachlor	-0.10	0.08	0.12	-0.66	0.07	0.76
acetochlor	0.03	0.08	0.05	0.33	0.82	0.31
pendimethalin	-0.43	0.00	0.2	-1.00	<.00	<.00
pyroxasulfone	-0.30	0.23	0.61	0.22	0.55	0.19
saflufenacil	-0.61	0.02	0.86	0.33	0.82	0.31
sulfentrazone-1	-0.40	0.09	0.61	-0.33	0.13	0.57
sulfentrazone-2	-0.60	0.11	0.96	-0.33	0.13	0.57
sulfentrazone-3	-0.44	0.08	0.68	0.00	0.47	0.43
flumioxazin-1	-0.04	0.11	0.10	-0.44	0.00	0.19
flumioxazin-2	-0.52	0.16	0.91	-1.00	<.00	<.00
flumioxazin-3	-0.44	0.08	0.68	-0.33	0.33	0.77
lactofen	-0.41	0.00	0.07	-0.35	0.05	0.42
fomesafen	0.26	0.42	0.06	0.20	0.27	0.06

https://doi.org/10.1017/wet.2025.24 Published online by Cambridge University Press