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Common ragweed (*Ambrosia artemisiifolia* L.) accession in the Mid-Atlantic region resistant to ALS-, PPO-, and EPSPS-inhibiting herbicides

Frank D’Amico Jr.[1], Thierry Besançon[2], Alyssa Koehler[3], Lovreet Shergill[4], Melissa Ziegler[5], and Mark VanGessel[6]

[1]Staff, Research and Development Scientist, Global Development Biology, FMC Corporation, Newark, DE, USA

[2]Associate Professor and Extension Specialist, Rutgers, the State University of New Jersey, Chatsworth, NJ, USA

[3]Assistant Professor and Extension Specialist, Department of Plant and Soil Sciences, University of Delaware, Georgetown, DE, USA

[4]Assistant Professor, Montana State University, Southern Agricultural Research Center, Huntley, MT, USA

[5]Research and Development Statistics Team Leader, Global Development Biology, FMC Corporation, Newark, DE, USA

[6]Professor and Extension Specialist, Department of Plant and Soil Sciences, University of Delaware, Georgetown, DE, USA

Author for correspondence: Mark J. VanGessel, University of Delaware, 16483 County Seat Highway, Georgetown, DE 19947 , **Email**: mjv@udel.edu

Running title: HR common ragweed in Mid-Atlantic

Abstract:

Common ragweed is a troublesome weed in many crops. Farmers and crop advisors in the coastal Mid-Atlantic region have reported inadequate control of common ragweed in soybean with glyphosate and other herbicide modes of action. To determine if herbicide resistance was one of the causes of poor herbicide performance, twenty-nine accessions from four states (DE, MD, NJ, VA) where common ragweed plants survived herbicide applications and produced viable seeds were used for greenhouse screening. Common ragweed seedlings from those accessions were treated with multiple rates of cloransulam, fomesafen, or glyphosate, applied as a single postemergence (POST) herbicide application. All accessions except one demonstrated resistance to at least one of the herbicides applied at twice the effective rate (2X), seventeen accessions were two-way resistant (glyphosate- and cloransulam-resistant; glyphosate- and fomesafen-resistant) and three-way resistance was present in eight accessions collected from three different states. Based on the POST study, five accessions were treated preemergence (PRE) with acetolactate synthase (ALS)-inhibiting PRE herbicides, and two accessions were treated with protoporphyrinogen oxidase (PPO)-inhibiting herbicides. All accessions treated PRE with the ALS-inhibitors chlorimuron or cloransulam demonstrated resistance at the 2X rates. Both accessions treated PRE with the PPO-inhibitor sulfentrazone, had survivors at the 2X rate. When the same accessions were treated PRE with fomesafen, one had survivors at the 2X rate, and one had survivors at the 1X rate. Results from these tests confirmed common ragweed with three-way resistance to POST herbicides is widespread in the region. In addition, this is the first confirmation that common ragweed accessions in the region are also resistant to ALS- or PPO-inhibiting herbicides when applied PRE.

Nomenclature:

Chlorimuron; cloransulam; fomesafen; glyphosate; sulfentrazone; common ragweed, *Ambrosia artemisiifolia* L.; soybean, *Glycine max* L. Merr.

Keywords: Area under the curve; herbicide resistance; multiple resistance

Introduction

Common ragweed is a summer annual, native to North America, and commonly found in agricultural fields, roadsides, and other settings where soils are frequently disturbed. Common ragweed is a major agronomic weed in multiple cropping systems throughout the United States (US) and Canada (CN) (Bassett and Crompton 1975; Van Wychen 2019). It can be found in all US states except Alaska and in all major agricultural areas of CN (NRCS 2023).

In a 2019 survey of US and CN, common ragweed was ranked seventh most common weed and most troubling weed, in soybean (Van Wychen 2019). Common ragweed ranked tenth overall across all crops for both the most common and most troublesome weeds. Yield loss in soybean to competition from two common ragweed plants per meter row caused greater than 40% reduction, while six plants per meter row caused greater than 80% reduction (Barnes et al. 2018). Yield loss from weed competition averaged more than 52% for all regions of the US and CN across a multiyear survey (2007-2013) (Soltani et al. 2017). The estimated loss in revenue in the US alone would have exceeded $16 billion, using average soybean price from the trial years.

Control of common ragweed at planting can be achieved with flumioxazin (protoporphyrinogen oxidase [PPO]-inhibiting herbicide; Group 14) or with chlorimuron or cloransulam (acetolactate synthase [ALS]-inhibiting herbicides; Group 2). Prior to glyphosate-resistant (GR) soybean, farmers relied upon postemergence (POST) herbicides from the same herbicide groups, albeit different active ingredients (PPO-inhibiting herbicides acifluorfen or fomesafen, and ALS-inhibiting herbicides cloransulam or chlorimuron). No-till soybean systems were widely adopted in the Mid-Atlantic region and put a greater emphasis on controlling weeds through herbicides than the traditional system of planting into a tilled and prepared seedbed. Successful no-till soybean systems require controlling plants present at seeding, both cover crops and weeds, normally by use of a nonselective herbicide (PSU 2022). Common ragweed emergence begins in early spring and a significant percentage of seedlings may be present at soybean planting time and are subsequently exposed to nonselective herbicides (Sweet et al. 1978).

In the US, glyphosate (5-enolpyruvyl shikimate-3-phosphate synthase [EPSPS] - inhibiting herbicide Group 9) is currently the most widely utilized herbicide. Glyphosate is registered for use in-crop and noncrop sites with just over half (56%) applied to crops with GR traits, including soybean (Benbrook 2016). In 1999, glyphosate was the sole herbicide used on

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90% of the soybean hectares in the US, leading to widespread glyphosate resistance. The Weed Science Society of America defines herbicide resistance as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA 2023).

The spread of glyphosate-resistant weeds led to a significant increase in hectares incorporating ALS- and PPO-inhibiting herbicides in their weed control programs beginning in 2010. Exclusive use of glyphosate dropped to 85% of the planted soybean by 2011 and further reduction to 70% by 2014 (Benbrook 2016).

Common ragweed resistance to ALS-inhibiting herbicides in the US was first reported in 1998 and to glyphosate in 2004 (Heap 2023b). Select populations of common ragweed from DE were confirmed in 2005 with two-way resistance to ALS- and PPO-inhibiting herbicides (Heap 2023). However, these populations remained localized and did not spread (personal communication, M. VanGessel).

Common ragweed with three-way resistance to glyphosate, cloransulam, and fomesafen herbicides was reported in MD and NJ in 2016 (Heap 2023). Since then, reports of common ragweed infestations at harvest by farmers and crop advisors have been increasing in the region. The cause of poor control has not been thoroughly investigated. Therefore, this research was designed to determine if the lack of common ragweed control in soybean fields in the Mid-Atlantic region was due to herbicide resistance. ALS- and PPO-inhibiting herbicides along with glyphosate were selected for these trials since they have been the most widely used POST herbicides applied to soybean in the region (NASS 2020).

Materials and Methods

Common ragweed seeds were collected from forty field sites throughout the coastal mid-Atlantic region of the United States, from VA to NJ. Collection sites were selected based on suspected resistant populations from fields with a recent history of poor herbicidal control as described by extension personnel, industry representatives or growers. None of the collection sites had previously been tested for herbicide resistance. Twenty-nine sites had enough viable seeds for testing (Figure 1).

Collection sites were soybean fields with common ragweed plants present in the fall. Seeds were collected from the surviving plants before soybean harvest. Common ragweed seeds

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were harvested by stripping plants manually or from the combine bin during harvest. All seeds from each site were combined into a single sample. Accessions within a state were numbered from south to north (Table 1). Collection sites were identified by the nearest municipality and the year collected and are available in Supplementary Table S1. Three accessions were chosen as susceptible checks, two were field sites where herbicides of interest all provided excellent control, and the third sample was identified as sensitive and provided by FMC (FMC Corporation, Philadelphia, PA). All seeds were stored in a refrigerated facility at the University of Delaware, Georgetown, DE, or at the FMC Stine Research Center, Newark, DE, until the start of the experiment.

Experiments were conducted in a greenhouse in Newark, DE, (39.663N, -75.785W) set to provide day temperature of 25 C (± 2 C) and night temperatures of 22 C (± 2 C). An automatic watering system was used to ensure plants received adequate water. Supplemental light via high-pressure sodium lamps were set for 16 hr photoperiod, providing 250 µmol m^{-2} s^{-1}. Fertilizer (Plantex 20-20-20, Master Plant-Prod Inc., Branpton, ON) was applied twice weekly through the watering system at 150 ppm N.

Postemergence Trial

Cloransulam (FirstRate®; Corteva Agriscience, Indianapolis, IN), glyphosate (Roundup Custom®; Bayer Crop Science, St. Louis, MO) and fomesafen (Reflex®; Syngenta Crop Protection, Greensboro, NC) were selected to evaluate resistance to POST herbicides. Herbicides were applied at 1, 2, and 4X rates with X corresponding to 17.5 and 350 g ai ha^{-1} for cloransulam and fomesafen, respectively and 1,120 g ae ha^{-1} for glyphosate. A nontreated control was also included for each accession. Seeds for the POST trial were seeded into 26L x 16W x 8D cm fiber pots (The HC Companies, Twinsburg, OH) filled with potting soil (Metro-Mix 360; SunGro Horticulture, Agawam, MA) and placed in the greenhouse and watered as needed. Seedlings were transplanted at the first true leaf stage into 6 x 6 cm square by 7.5 cm deep plastic pots (The HC Companies, Twinsburg, OH). Plastic pots were filled with the same potting mix as fiber pots. Each pot was considered a replicate and contained one seedling. Treatments were applied with a single 8001E TeeJet nozzle (TeeJet Technologies, Glendale Heights, IL) in a research track sprayer (DeVries Manufacturing Inc., Hollandale, MN) set to deliver 281 L ha^{-1} at 207 kPa. POST treatments were applied to common ragweed plants with at least four true leaves and height between 5 and 8 cm. All POST herbicide treatments included nonionic surfactant (Activator 90; Loveland Products, Greeley, CO) at 0.25% v/v. Additionally, cloransulam treatments included urea ammonium nitrate (UAN) and glyphosate treatments included ammonium sulfate (AMS). The formulation of glyphosate utilized did not contain an adjuvant, so the adjuvant type and amount were consistent for all glyphosate treatments (Burgos 2015). After application, plants were allowed to dry in the spray room, and then returned to the greenhouse. Plants were not watered for 24 h following application to ensure herbicides were properly absorbed by common ragweed seedlings. Thereafter, all plants were watered and fertilized routinely with the same procedure as described previously.

Individual plants were rated 7, 14, 21, and 28 days after treatment (DAT) on a scale of 0 (no visible herbicide injury) to 100 (complete plant death/no green tissue). The overall health of individual plants was rated, and the rating was a composite of symptoms including stunting, chlorosis, bleaching, and necrosis. For each accession, plants were compared to the nontreated plants of that accession.

After the final visual rating, aboveground plant biomass was collected by harvesting all plants at the soil line, placing them into individual paper bags, and drying them at 50 C for 48 to 72 h. Once drying was completed, the samples were weighed, and dry biomass was recorded.

The procedure was repeated in time, providing two runs for each accession; each run had five replications. Due to the number of accessions collected (40) and limited greenhouse space, planting and spraying was staggered in time by treating 6 to 10 accessions at a time.

Preemergence Trial

PRE trials were conducted on common ragweed accessions that exhibited resistance in the POST trial. Those accessions were chosen based on results from the POST trial and availability of quality seed. Evaluations for ALS-herbicide resistance included a susceptible accession, and five accessions that demonstrated resistance in the POST trial. Three accessions were selected for PPO-herbicide resistance, including two of the most resistant accessions in the POST trial and a susceptible accession for comparison. Square plastic pots, (Sq Trad TW, The HC Companies, Twinsburg, OH) 10 cm x 10 cm wide x 8.5 cm deep were filled with Matapeake silt loam (fine-silty, mixed, semiactive, mesic Typic Hapludults) field soil (pH 6.2 and 1.8% organic matter content). The soil was sifted before filling pots to remove stones and foreign debris. Each pot

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was seeded by volume with 1 ml of seed from the corresponding accession. Each pot was considered a replicate with five replicates for each treatment. Filled and seeded pots were lightly watered and then placed in a -20F freezer for 4 to 6 weeks to improve germination.

Before herbicide application, seeded pots were removed from the freezer and left at room temperature to thaw. Treatments were applied 1 d after removal from the freezer. Treatments were applied following the same procedure as described above. In addition to the ALS and PPO herbicides utilized for the POST trial, a second herbicide for each mode of action was included. In each case, the second herbicide was of a different chemical family. ALS-inhibiting chlorimuron (Classic®; Corteva Agriscience, Indianapolis, IN) and PPO-inhibiting sulfentrazone (Spartan®; FMC Corp., Philadelphia, PA) were included. The 1X rate for chlorimuron, cloransulam, fomesafen, and sulfentrazone was 35, 35, 420, and 280 g ai ha^{-1}, respectively. All herbicides were applied 0.25, 0.5, 1, and 2X, and a nontreated control was included.

All pots were rated 14, 21 and 28 DAT for visual percent control based on a scale of 0 (no visible herbicide injury) to 100 (complete plant death). Additionally, emerged seedlings were counted for each pot at each rating date. At 28 DAT, injury level of individual plants in all pots was visually assessed on a 1 to 4 scale with 1 corresponding to ≤20% injury, 2 between 21% and 50%, 3 between 51% and 80%, and 4 corresponded to >80%.

Aboveground plant biomass was collected following the methodology previously described. The process was replicated in time to produce two runs, with 10 replicates in total for each treatment.

Data Analysis

Cumulative ragweed injury was evaluated by calculating the area under the curve (AUC):

$$AUC = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$ [equation 1]

where y_i= common ragweed injury at the *ith* observation, t_i= days at the *ith* observation, and N_i= total number of observations. This calculation provides a quantitative summary of herbicide injury intensity over time for comparison across treatments (Ribeiro et al. 2021; VanGessel et al. 2016; Zhang et al. 2016). Lower values result from lower initial common ragweed injury and/or faster recovery rate.

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Parameters were analyzed using a linear mixed effect model in SAS software, version 9.4 (SAS Institute, Cary, NC). Run and replicate nested within run were considered random effects while accession, herbicide, and rate were fixed effects. Comparisons between accession by rate combinations were adjusted for multiple comparisons using Tukey's method (α = 0.05). Kenward-Rogers adjusted degrees of freedom were used. Plants deemed to be dead were not included in the dry-weight model.

Results and Discussion

Two accessions (DE3 and MD 7) did not have adequate plants available for two full runs at each treatment rate but were included in the analysis and are noted as such in Table 1. Greenhouse growing conditions often allow severely injured plants to maintain some green tissue, but these plants would normally die under field conditions. As a result, restricting "dead or controlled" plants to only those with 100% control can misrepresent what would occur in the field. As a result, we characterized plants exhibiting 80 to 100% control as susceptible ("dead or controlled"). Others have used a similar approach (Harre et al. 2017; Kruger et al. 2012; Singh et al. 2020).

Postemergence Trial

Three susceptible accessions were included as checks. Each susceptible accession had one or more plants surviving (≤80% control) one of the treatments (Table 1). No susceptible accession had any plant surviving two different herbicides. All these accessions were deemed susceptible to all three herbicides based on our criteria since there were no surviving plants (≤80% visual control) in both runs of the tests for a given herbicide at either 2X or 4X application rate.

More accessions were resistant to glyphosate than either cloransulam or fomesafen (Table 1). Twenty-seven accessions were resistant to glyphosate (either alone or as multiple-resistant), followed by cloransulam with twenty-five resistant accessions. Nine accessions were resistant to fomesafen. Finding a high percentage of glyphosate resistance was not unexpected since seeds were collected from fields with common ragweed plants present late in the season, after all herbicide applications had been applied and the widespread use of glyphosate in the region.

Herbicide resistance was prevalent in the common ragweed accessions collected. For only one site (DE3) were plants susceptible to all three herbicides. Eight accessions were resistant to all three tested modes of action, with at least two accessions from DE, MD, and NJ. Sixteen accessions were resistant to both glyphosate and cloransulam; one accession was resistant to both glyphosate and fomesafen.

The sole accession from Virginia was resistant to glyphosate (Table 1). However, VA1 was susceptible to both cloransulam and fomesafen, with no plant surviving any application of those herbicides.

All accessions from MD were resistant to glyphosate, with no accession having less than five plants surviving the 2X rate of glyphosate (Table 1). For the sixteen accessions from MD with glyphosate resistance, only one accession (MD8) was susceptible to cloransulam. Four of the accessions were resistant to all three herbicide groups. The susceptible accessions from DE (DE-S1, DE-S2) were susceptible to all three herbicides (Table 1). DE3 was also deemed susceptible but did have plants survive at all rates of glyphosate and the two lower rates of cloransulam. However, there was data for only one run, and based on our criteria (survivors in both runs) DE3 was considered susceptible.

There did not appear to be any geographical patterns related to the location of resistant accessions within or between states. Single-resistant accessions were often found near two or three-way resistant accessions. However, this project was not intended to be an exhaustive survey of regional resistance. A structured sampling may provide information on patterns and distribution of resistant populations.

Dry weights were collected for individual common ragweed plants (Supplementary Table S2). Only those plants that demonstrated resistance with a visual observation for control ≤80% at 28 DAT were included in the statistical analyses for dry weights. If an accession had less than three plants survive a specific dose of that herbicide, no statistical comparison was determined for that accession at that rate.

As expected, no analysis could be performed for biomass of the susceptible accessions (DE-S1, DE-S2, or MD-S1) at any rate for any herbicide, as none had more than a single plant survive any treatment. Average biomass per nontreated plant varied widely among the various accessions. The lowest biomass was 1.6 g plant^{-1} for DE1, while the greatest biomass was 5.1 g plant^{-1} for MD4. For each accession, a comparison was made between the biomass of the

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nontreated plants and the surviving plants from each treatment. The dry weight of treated plants was reduced compared to that of the nontreated of the same accession in all cases except for DE1 when treated with cloransulam at the 1X or 2X rate (Supplementary Table S2). For those treatments, dry weight was nearly 10% greater than nontreated plants.

Area under the curve (AUC) was determined for all accessions at each treatment rate when there were three or more surviving plants. The AUC value is unitless, yet it allows all accessions with surviving plants to be compared over all evaluation intervals. Higher AUC values result from plants being severely injured or injury symptoms developing rapidly. The maximum AUC value obtainable was 2100, representing 100% control for all plants at all rating timings. Herbicides that kill weeds slower, like cloransulam or glyphosate, may not have the maximum value if susceptible plants have not died before the first rating. Accessions with AUC values less than DE-S1 exhibited a lower level of injury. DE-S1 was used as the comparison for statistical analyses for all other accessions at each corresponding rate. Lower values demonstrate a higher level of resistance. AUC is commonly used in plant pathology to describe the progression of disease epidemics (Madden et al. 2007).

Twenty-one accessions receiving glyphosate treatments were different from the susceptible check (DE-S1) for AUC at $P \leq 0.05$ with the 4X rate (Table 2). Twelve accessions were different for AUC at the 4X rate of cloransulam, while three accessions were different for fomesafen.

The high frequency of resistance to both glyphosate and cloransulam is in line with the extended use of those herbicides for many years in soybean production. Before the release of glyphosate-resistant soybean, ALS-inhibiting herbicides were the most widely used family of herbicides in soybean and resistance to this family is widely reported (Heap 2023). ALS herbicides experienced a general decline in use in soybean during the period of 2002 through 2007 but have experienced an increase since then (USGS 2023).

Glyphosate has been used on many hectares of soybean both as preplant burndown and as an in-crop POST treatment since the mid-1990s with the release of glyphosate-resistant soybean. The use of fomesafen applied POST in soybean is much more recent to help control glyphosate-resistant biotypes. The number of soybean fields treated with PPO herbicides continues to increase as glyphosate-resistant populations spread (USGS 2023).

Starting in 2005 and until 2017, there were 127 new reported cases of herbicide resistance associated with soybean in the United States (Heap 2023). Of those reported cases, 75 reports were glyphosate resistance alone, with an additional 29 cases reported with resistance to herbicides with multiple modes of action, including glyphosate. The relationship between the increase in reported cases of glyphosate-resistant weeds and the increase in both ALS and PPO herbicides cannot be overlooked. Also of note, from 2010 to 2016, there were 24 new reports of ALS resistance in soybean and 14 cases of PPO resistance.

At the 1X glyphosate field rate, all accessions exhibited >50% survival of common ragweed (Table 1). With the 1X rate of cloransulam, twenty-four accessions showed ≥50% survivorship. Six of the accessions exhibited ≥50% survivorship of the 1X fomesafen application. This proportion of resistant plants in those populations will most certainly result in unacceptable levels of control and likely yield loss. The presence of just two common ragweed plants m^{-1} of soybean rows (soybean planted in 76-cm rows), resulted in a 40% to 76% yield reduction depending on the year (Barnes et al. 2018).

Preemergence Trial

ALS-inhibiting herbicides. All accessions treated with ALS herbicides in the PRE trial demonstrated resistance (Figures 2 and 3). This was determined by all accessions having plants emerge and survive at the 2X rates of chlorimuron or cloransulam. The susceptible accession had no plants survive the 0.5X rate of either herbicide. The number of surviving plants (<80% injury 28 DAT) was similar to the nontreated control for all accessions, except the two highest rates of chlorimuron, yet over 50% of the plants survived.

The dry weight per pot for accessions DE1, DE4, and DE6 treated with chlorimuron or cloransulam was similar to the nontreated pot for the respective accession (Table 3). The dry weight for MD4 treated with chlorimuron did not differ from the nontreated check at any rate; but plants from pots treated with cloransulam at 1X and 2X had lower dry weights. The dry weight per pot for MD14 treated with chlorimuron at 0.5X, 1X, or 2X was less than the nontreated pot, but the average weight was similar among these three treatments. Like MD4, MD14 treated with cloransulam had lower dry weights than the nontreated check at the 1X and 2X rates.

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Comparing plants by percent injury level, DE1, DE4, and MD14 had similar trends when treated with chlorimuron (Figure 2). At 1X or lower rates, less than 20% of all accessions had emerged plants with a visual injury rating >80%. At the highest application rate, all three accessions had >40% of plants with observed injury ≤50%. DE6 and MD4 both had a stronger response to chlorimuron treatments than DE1, DE4, or MD14. DE6 and MD4 treated pots had fewer plants with <20% injury compared to other accessions for all rates of chlorimuron.

MD4 accession treated with cloransulam at 0.5, 1, or 2X rates had <50% of the plants with an injury rating 50% or less (Figure 3). DE1 was similar, with nearly 40% of all plants at those application rates having a visual injury level of 50% or more. DE4 and DE6 had a majority of plants rated less than 50% injured with all application rates of cloransulam. MD14 was intermediate; at the 1X rate approximately 60% of the plants exhibited less than 50% injury, while for the 2X rate nearly 40% of the plants demonstrated that response.

PPO-inhibiting herbicides. Accessions treated with PRE applications of PPO-inhibiting herbicides were classified as resistant based on our results from the POST study. The NJ5 accession treated with fomesafen had at least 20% of emerged plants survive with <80% injury at 28 DAT (Figure 4). Results were similar between the four rates. The NJ6 accession had similar results when treated with the 0.5 or 1X rate of fomesafen. More than 70% of emerged plants exhibited less than 80% control at the 0.25X rate of fomesafen, while no plants demonstrated less than 80% control at the 2X rate. Sulfentrazone applied to DE-S2 at the 1X rate resulted in 75% of plants exhibiting >80% control, all plants of that accession were controlled (>80%) at the 2X rate of sulfentrazone (Figure 5). NJ5 accession at the 1X and 2X sulfentrazone rates, had 80% and 40% of emerged plants exhibited <80%, respectively, demonstrating a high level of survivorship. NJ6 had more than 70% of emerged plants with levels of control <80%.

Accession NJ5 treated with fomesafen resulted in a dry weight reduction, with a clear rate response observed with mean dry weights of 0.30 g (0.25X), 0.21 g (0.5X), 0.12 g (1X), and 0.07 g (2X) (Table 4). There was an insufficient number of emerged plants for calculating means for NJ6 with 1X or 2X rate of fomesafen. The dry weight per pot with accession NJ6 was reduced compared to the nontreated check with all PPO herbicide treatments. NJ5 had dry weights similar to the nontreated when sulfentrazone was applied at the 0.25X or 0.5X rate, but the 1X and 2X rates reduced dry weight.

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All the accessions with suspected herbicide resistance that were treated POST were confirmed resistant to one or more of the herbicides in the tests, with one exception (DE3). In the example of DE3, although not classified as resistant due to the criteria stated previously, this accession had multiple plants survive at the 2X rate of glyphosate, which could indicate a transition to a resistant population. Similar results were observed when NJ6 was treated with cloransulam.

Twenty-five accessions had two- or three-way resistance. Only nine accessions had resistance to fomesafen. The lower incidence of resistance to POST applications of the PPO-inhibiting herbicide fomesafen versus ALS-inhibiting herbicides corresponds with the more recent introduction and use of these herbicides as compared to glyphosate or ALS-inhibiting herbicides.

This is the first confirmation of common ragweed exhibiting resistance to PRE applications of ALS- or PPO-inhibiting herbicides. Levels of resistance appeared to be similar for a given accession when treated either PRE or POST with ALS herbicides. For instance, MD14 had a high AUC value (high level of injury, lower level of resistance) (Table 2). When treated with chlorimuron or cloransulam PRE, MD14 was the accession with the greatest reduction in dry weight compared to the nontreated pots (Table 3). In contrast, DE1-treated POST with cloransulam had a lower value for AUC, demonstrating a high resistance level (Table 2). Dry weights for DE1 PRE with either chlorimuron or cloransulam were similar to that of the nontreated pots at all rates, also demonstrating a high level of resistance (Table 3). For PPO herbicides, there was not sufficient data to make comparisons between PRE and POST applications.

There were two accessions separated by collection time, Milestown (MD10 and MD10a) and Chipatico (MD11 and MD11a) (Supplementary Table S1). Each was collected in 2016 and again in 2018. Both accessions demonstrated a change from two-way resistance with cloransulam and glyphosate in the 2016 collection, to three-way resistance in the 2018 collection. Such a small sample size would make any broad conclusion unwise, however the results are concerning for area growers.

The loss of three commonly used POST herbicide modes of action to area growers due to ineffective control of common ragweed would prove costly and mandate integrated weed management focusing on non-herbicidal approaches. POST herbicides used in other areas pose unique problems due to the nature of farming in the mid-Atlantic region, including proximity of high-value crops and neighborhoods. Volatile products such as dicamba may not be an option to many growers of the mid-Atlantic. The risk of off-target movement and injury to sensitive crops has been documented in the area (Wasacz et al. 2022). Current herbicide recommendations for soybean production include applying chlorimuron or cloransulam PRE, with a POST application of a Group 2, 9, or 14 herbicide, assuming resistance is not present. When glyphosate-resistant weeds are suspected, recommendations include planting glufosinate-resistant soybean that allow for a POST application of glufosinate (PSU 2020).

Common ragweed plants present in the field at harvest are problematic. Common ragweed can impede combine operation during harvest, stems and seeds can contaminate the grain and cause dockage, and seeds can be distributed on the soil and contribute to weed populations in future seasons. However, even more troubling is the likelihood of a herbicide-resistant population of common ragweed in that field, perhaps resistant to several herbicide classes. In the mid-Atlantic region, common ragweed plants in the field at harvest are likely to be glyphosate- and ALS-herbicide resistant. There is also a chance that those common ragweed populations are also PPO-resistant. These resistant traits not only provide resistance to POST applications of herbicides, but to PRE applications of the same herbicide group as well. Additional research is required to characterize the nature of resistance for these accessions.

Practical Implications

The presence of large weeds at soybean harvest can lead to many issues, such as harvesting difficulty, contributing to foreign matter in the harvested grain, and adding weed seeds to the soil seedbank. Understanding why these plants are present is essential to developing a sustainable weed control program, as there can be a number of factors that allow for these weeds to survive. Herbicide resistance is one factor. Our research shows that all of the fields we sampled were resistant to commonly used herbicides to control common ragweed. Furthermore, we sampled an additional thirteen fields with common ragweed at harvest time, but we did not have enough viable seed to include in our trial. Eighty-six percent of the fields we sampled were resistant to two or more herbicides. This study demonstrates the need for farmers and crop advisors to immediately implement non-chemical weed control strategies to manage these biotypes, prevent further seed production, and prevent weed seed movement to uninfested fields.

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Table 1. Percent survival at 28 days after treatment of common ragweed accessions treated POST with cloransulam, fomesafen, or glyphosate (≤80% visual control). An accession is designated resistant (R) if at least one plant survived (≤80% visual control) in both runs at either 2X or 4X application, susceptible (S) had no surviving plants in all runs at either 2X or 4X rate.

	Cloransulam				Fomesafen				Glyphosate			
Accession	R/S	1X	2X	4X	R/S	1X	2X	4X	R/S	1X	2X	4X
	------- % survivors -------				------- % survivors -------				------- % survivors -------			
DE-S1	S	0	0	0	S	0	0	0	S	25	0	0
DE-S2	S	0	13	13	S	0	0	0	S	0	0	0
MD-S1	S	0	0	0	S	0	0	0	S	13	0	0
DE1	R	88	100	100	S	0	0	0	R	100	88	100
DE2	R	50	50	50	S	0	0	0	R	100	100	25
DE3[a]	S	13	13	0	S	0	0	0	S	63	50	25
DE4	R	75	63	50	R	38	38	13	R	100	100	100
DE5	R	75	88	88	R	63	38	25	R	100	100	88
DE6	R	50	75	75	S	0	0	0	R	100	100	38
MD1	R	75	75	25	S	0	0	0	R	100	100	100
MD2	R	75	75	50	S	0	0	0	R	75	63	63
MD3	R	75	75	75	S	0	0	0	R	100	100	100
MD4[b]	R	86	83	67	S	0	0	0	R	86	86	43
MD5	R	50	63	38	S	0	0	0	R	75	63	38
MD6	R	63	38	38	S	0	0	0	R	88	88	50
MD7[a]	R	100	75	50	S	0	0	0	R	100	100	50
MD8	S	0	0	0	S	0	0	0	R	100	100	100
MD9	R	75	75	75	R	50	38	25	R	100	100	100
MD10a[a,c]	R	50	100	75	S	0	0	0	R	100	100	100
MD10	R	100	100	100	R	50	25	0	R	100	88	88
MD11a[b,c]	R	86	86	63	S	29	13	0	R	100	86	71
MD11	R	100	88	88	R	0	25	0	R	88	75	88
MD12	R	75	38	63	S	0	0	0	R	100	100	75

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MD13	R	100	100	63	R	38	25	0	R	100	100	50
MD14	R	88	63	50	S	0	0	13	R	100	75	63
NJ1[b]	R	86	57	57	S	29	14	29	R	100	57	29
NJ2	R	75	25	50	S	0	0	0	S	100	13	13
NJ3	R	50	63	50	S	0	0	0	R	63	50	38
NJ4	R	13	63	63	S	0	0	0	R	63	38	50
NJ5	R	88	63	13	R	100	88	50	R	100	75	63
NJ6[b]	S	29	29	0	R	86	71	100	R	100	100	43
NJ7	R	100	88	75	S	13	13	0	R	100	63	13
NJ8	R	50	38	50	R	88	63	63	R	88	50	88
VA1	S	0	0	0	S	0	0	0	R	100	100	100

Note: two runs of 4 plants each were completed with each treatment for all accessions with the following exceptions: [a]1 run, 4 reps; [b]2 runs, < 8 reps.

[c]Samples were collected from the same fields but at different times. MD10a and MD11a were collected in 2016 and MD10 and MD11 were collected in 2018.

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Table 2. Area under the curve for common ragweed accessions treated POST with cloransulam, fomesafen, or glyphosate. Only surviving plants (survived at 80% threshold) were included in the analysis. Individual accession is compared with the susceptible check (DE-S1); lower values indicate a higher resistance level.

	Cloransulam						Fomesafen						Glyphosate					
Accession	1X		2X		4X		1X		2X		4X		1X		2X		4X	
DE-S1	1985		1992		2009		2098		2100		2100		1990		2052		2072	
DE1	811	***	643	***	1066	***		+		+		+	817	***	1198	***	1329	***
DE2		+		+		+		+		+		+	1382	***	1579	***		+
DE3		+		+		+		+		+		+	1578	***	1702	**		+
DE4	1469	**	1440	**	1456	**	1866	*	1913	NS		+	351	***	873	***	1011	***
DE5	1529	**	1286	***	1448	**	1868	*		+		+	688	***	843	***	1313	***
DE6	1293	***	1114	***	1382	***		+		+		+	915	***	1303	***	1685	***
MD1	945	***	1227	***		+		+		+		+	686	***	826	***	1155	***
MD2	1124	***	1288	***	1803	NS		+		+		+	1137	***	1474	***	1601	***
MD3	1141	***	1418	***	1250	***		+		+		+	361	***	697	***	914	***
MD4	839	***	1211	***	1220	***		+		+		+	1091	***	1412	***	1702	**
MD5	1669	NS	1427	***	1778	NS		+		+		+	1362	***	1673	***	1859	NS
MD6	1575	*		+		+		+		+		+	1148	***	1543	***	1615	***
MD7	547	***	1347	**		+		+		+		+	1375	***	1544	***		+
MD8		+		+		+		+		+		+	846	***	1183	***	1253	***
MD9	1043	***	1566	*	1526	**	1906	NS	1862	**		+	853	***	1154	***	1355	***
MD10	471	***	530	***	1057	***		+		+		+	940	***	1283	***	1477	***

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MD11	836	***	1064	***	1442	***		+		+		+	1181	***	1426	***	1444	***
MD12	1125	***	1670	NS	1503	**		+		+		+	718	***	1199	***	1437	***
MD13	1434	**	1176	***	1624	*	1922	NS		+		+	951	***	1441	***	1666	***
MD14	1526	**	1600	*	1578	**		+		+		+	967	***	1165	***	1478	***
NJ1	1066	***	1648	NS	1576	*		+		+		+	1149	***	1654	***		+
NJ2	1361	***		+	1626	*		+		+		+	1469	***		+		+
NJ3	1459	**	1690	NS	1799	*		+		+		+	1516	***	1624	***	1932	**
NJ4		+	1741	NS	1623	*		+		+		+	1515	***	1880	*	1821	*
NJ5	1044	***	1502	**		+	1476	***	1560	***	1781	***	1219	***	1375	***	1617	***
NJ6		+		+		+	1514	***	1649	***	1434	***	837	***	1159	***	1521	***
NJ7	468	***	1001	***	1422	***		+		+		+	1337	***	1653	***		+
NJ8	1736	NS	1809	NS	1846	NS	1491	***	1653	***	1785	***	1122	***	1429	***	1500	***
VA1		+		+		+		+		+		+	652	***	968	***	1231	***

Values are the sum of visual control ratings with a maximum possible value of 2100, which represents 100% control for all plants at each rating timing. Therefore, lower values for area under the curve represent higher levels of resistance for that population. Significance is designated as: *** denotes P<0.01; ** denotes P=0.05 to 0.01; * denotes P=0.1 to 0.05; NS denotes P≥0.1; + denotes <3 surviving plants, no statistical comparison made.

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Table 3. Dry weight of common ragweed accessions at 28 days after PRE application of chlorimuron or cloransulam. Mean dry weight (g) per pot is presented for 0.25X, 0.5X, 1X, and 2X of the respective herbicide. Statistical comparison of differences from the nontreated check (0X) of same accession.

		Chlorimuron								Cloransulam							
Accession	0X	0.25X		0.5X		1X		2X		0.25X		0.5X		1X		2X	
	g / pot																
DE1	0.24	0.13	NS	0.23	NS	0.21	NS	0.19	NS	0.17	NS	0.16	NS	0.16	NS	0.23	NS
DE4	0.43	0.49	NS	0.30	NS	0.29	NS	0.35	NS	0.59	NS	0.38	NS	0.39	NS	0.39	NS
DE6	0.22	0.12	NS	0.16	NS	0.10	NS	0.13	NS	0.13	NS	0.15	NS	0.17	NS	0.10	NS
MD4	0.35	0.32	NS	0.30	NS	0.27	NS	0.26	NS	0.39	NS	0.30	NS	0.16	**	0.15	**
MD14	0.46	0.39	NS	0.30	*	0.29	*	0.27	*	0.43	NS	0.45	NS	0.21	***	0.16	***

Significance is designated as: *** denotes P<0.01; ** denotes P=0.05 to 0.01; * denotes P=0.1 to 0.05; NS denotes P≥0.1

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Table 4. Dry weight of common ragweed accessions at 28 days after PRE application of fomesafen or sulfentrazone. Mean dry weight (g) per pot is presented for 0.25X, 0.5X, 1X, and 2X of the respective herbicide. Statistical comparison of differences from the nontreated check (0X) of same accession.

		Fomesafen								Sulfentrazone							
Accession	0X	0.25X		0.5X		1X		2X		0.25X		0.5X		1X		2X	
	g / pot																
NJ5	0.79	0.30	***	0.21	***	0.12	***	0.07	***	0.73	NS	0.83	NS	0.59	*	0.31	***
NJ6	0.61	0.09	***	0.19	***	+		+		0.22	***	0.27	***	0.39	*	0.20	***

Significance is designated as: *** denotes P<0.01; ** denotes P=0.05 to 0.01; * denotes P=0.1 to 0.05; NS denotes P≥0.1; + denotes <3 pots with surviving plants, no statistical comparison made.

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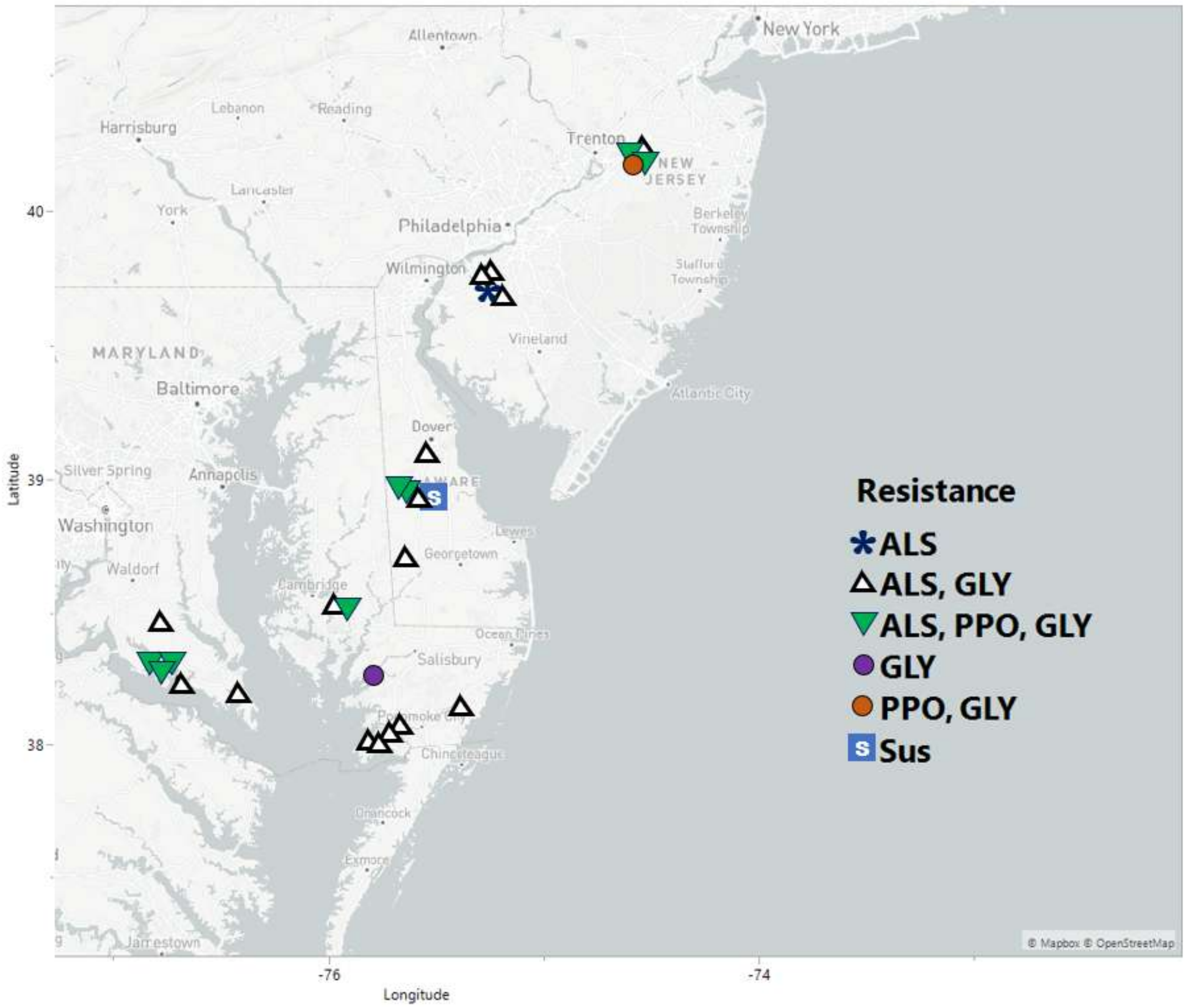

Figure 1. Map representing common ragweed collection sites in DE, MD, and NJ and confirmed resistance at each location. The VA site is located near Lawrenceville, VA and was resistant to only glyphosate. Resistance is designated as Sus= susceptible, ALS= resistance to acetolactate synthase-inhibiting herbicides; GLY= resistance to glyphosate; PPO= resistance to protoporphyrinogen oxidase-inhibiting herbicide.

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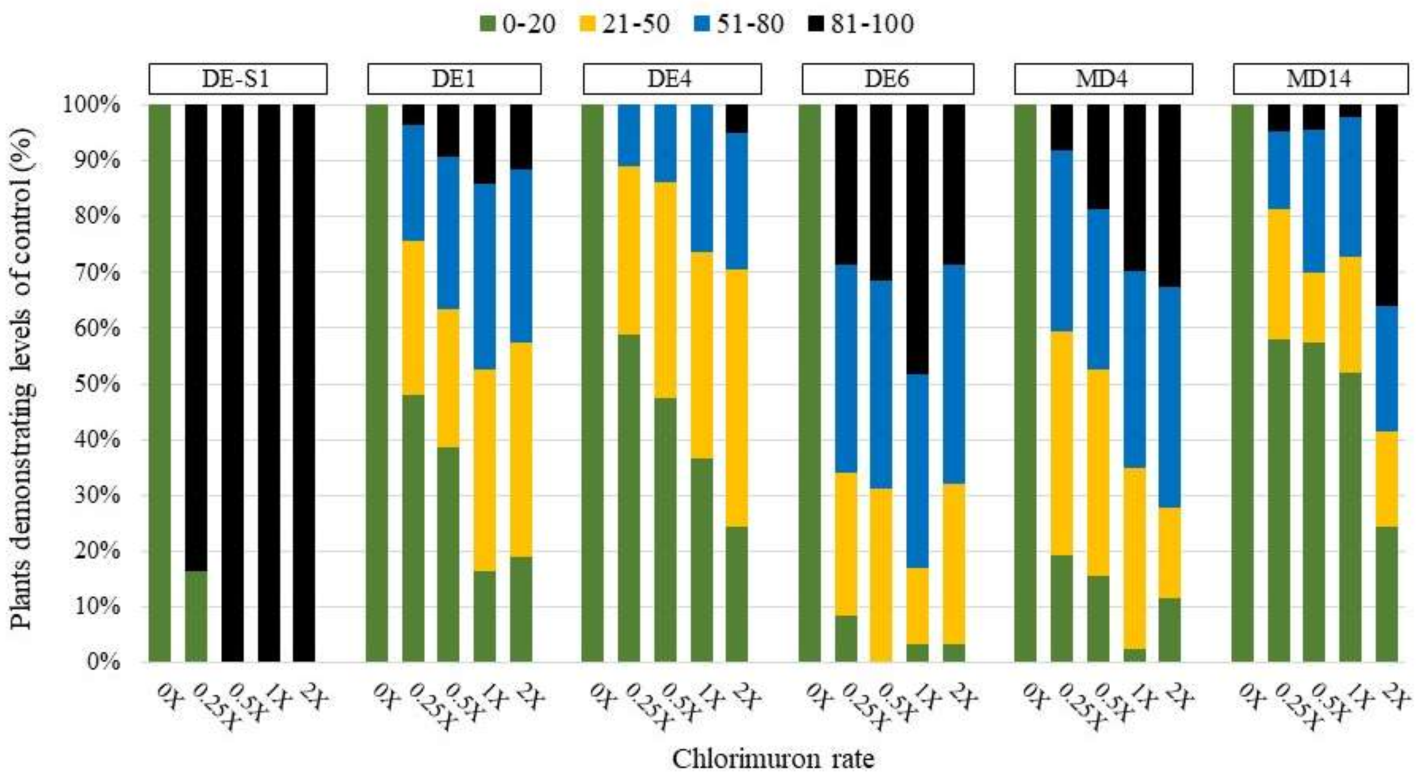

Figure 2. Common ragweed seedling emergence 28 DAT when treated PRE with chlorimuron. Chlorimuron 1X rate is 35 g ha^{-1}. Green segments = % of plants controlled 0-20% (healthy plants), yellow = % of plants controlled 21-50%, blue = % of plants controlled 51-80%, black = % of plants controlled 81-100% (considered severely damaged/dead).

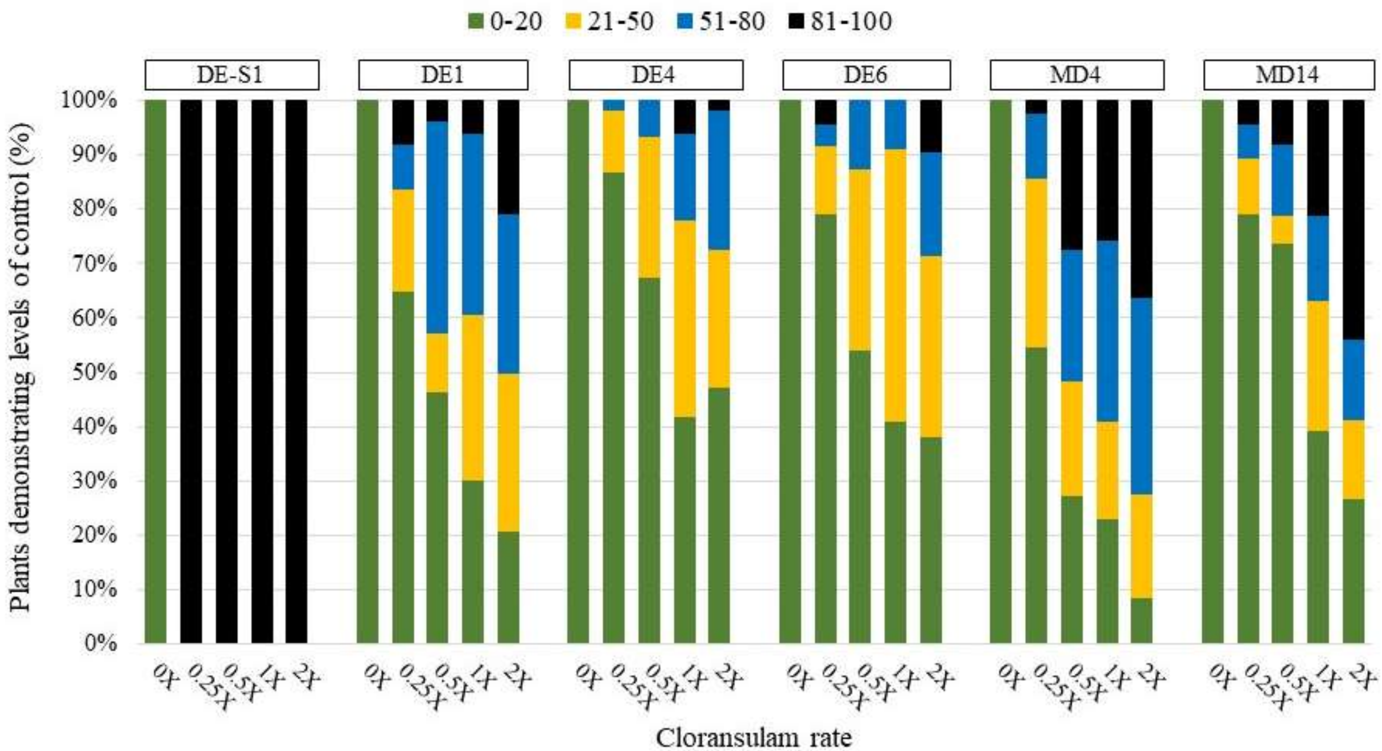

Figure 3. Common ragweed seedling emergence 28 DAT when treated PRE with cloransulam. Cloransulam 1X rate is 35 g ha^{-1}. Green segments = % of plants controlled 0-20% (healthy plants), yellow = % of plants controlled 21-50%, blue = % of plants controlled 51-80%, black = % of plants controlled 81-100% (considered severely damaged/dead).

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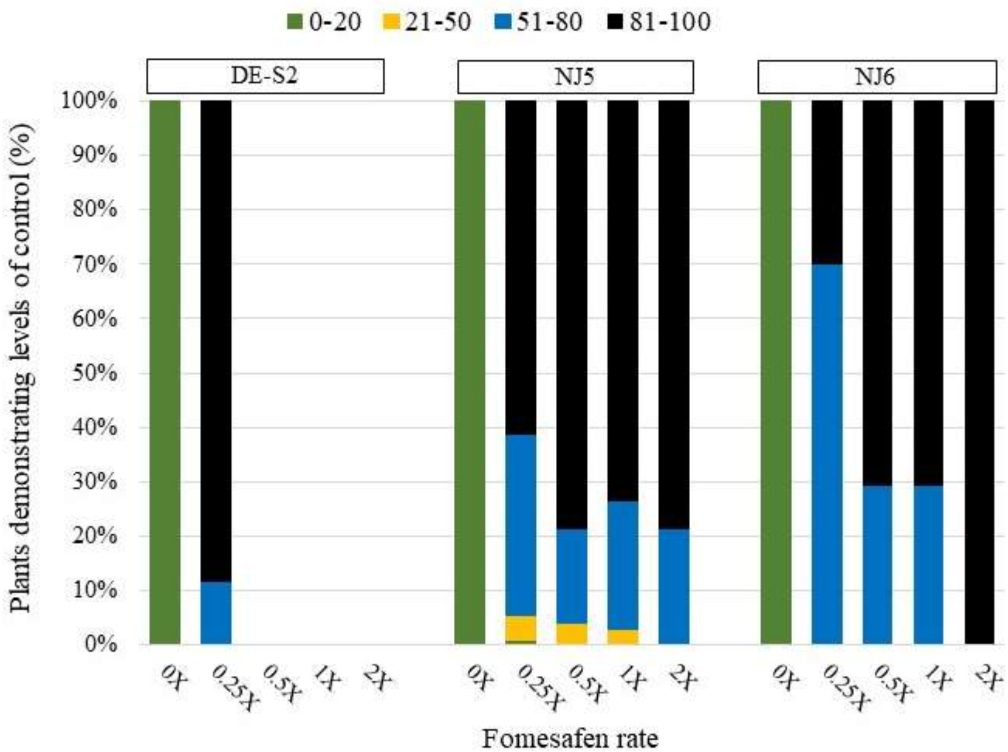

Figure 4. Common ragweed seedling emergence 28 DAT when treated PRE with fomesafen. Fomesafen 1X rate is 420 g ha^{-1}. Green segments = % of plants controlled 0-20% (healthy plants), yellow = % of plants controlled 21-50%, blue = % of plants controlled 51-80%, black = % of plants controlled 81-100% (considered severely damaged/dead). No plants emerged for DE-S2 at 0.5X, 1X or 2X rate of fomesafen.

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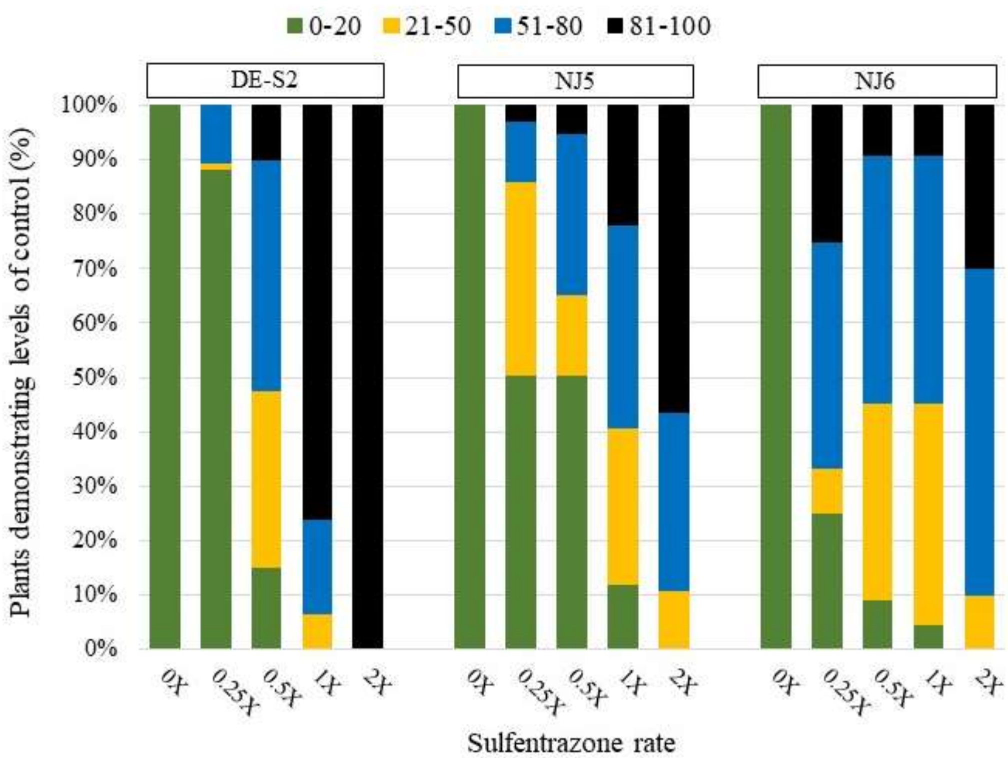

Figure 5 Common ragweed seedling emergence 28 DAT when treated PRE with sulfentrazone. Sulfentrazone 1X rate is 280 g ha^{-1}. Green segments = % of plants controlled 0-20% (healthy plants), yellow = % of plants controlled 21-50%, blue = % of plants controlled 51-80%, black = % of plants controlled 81-100% (considered severely damaged/dead).

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