horticulturae

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Alternative Integrated Weed Management Options for Clopyralid-Resistant Common Ragweed

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Abstract: Common ragweed (*Ambrosia artemisiifolia* L.) is an extremely competitive broadleaved summer annual weed found in Christmas tree production systems within Michigan. Common ragweed has been reported to have resistance to glyphosate, PSII inhibitors, PPO inhibitors, and ALS herbicides. There have been reports from Michigan Christmas tree growers of common ragweed resistance to clopyralid, a synthetic auxin herbicide, in Montcalm County, Michigan. The objective of this study was to test alternative post-emergence herbicide combinations and organic mulch on clopyralid-resistant common ragweed for weed control efficacy. The following two stages of common ragweed were used: stage 1 (6–9 leaves) and stage 2 (12–14 leaves). For common ragweed in stage 1 in 2021 and 2022, as well as stage 2 in 2022, at all evaluation dates, mulch + clopyralid + oxyfluorfen provided the highest level of weed control. For stage 1 in 2022, this treatment combination provided 100% control from 2 weeks after treatment (WAT) and always showed better or equal weed control compared to all the other treatments. The combination of mulch + clopyralid + glyphosate provided 100% control by 2 WAT when plants were treated at stage 2 in 2022. For the plants treated at stage 1 in 2022, many of the treatments reached a fresh weight of 0 g, but in 2021, those same treatments resulted in a fresh weight of around 20 g. Based on fresh weight, the greatest plant growth occurred with glyphosate treatment in 2021 and clopyralid and mulch alone in 2022. This is likely due to common ragweed's resistance to these herbicides.

Keywords: common ragweed; clopyralid; stinger; *Ambrosia artemisiifolia*; herbicide resistance; glyphosate; roundup; oxyfluorfen

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Citation: Gallina, G.; Cregg, B.; Patterson, E.; Hill, E.; Saha, D. Alternative Integrated Weed Management Options for Clopyralid-Resistant Common Ragweed. *Horticulturae* **2023**, *9*, 985. https://doi.org/10.3390/horticulturae9090985

Academic Editors: Alessandra Carrubba and Anoop Kumar Srivastava

Received: 21 July 2023
Revised: 24 August 2023
Accepted: 30 August 2023
Published: 31 August 2023

1. Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) is an extremely competitive broadleaved summer annual weed found in most agricultural settings, including Christmas tree production systems, within Michigan. The plant is usually hairy; its stems are erect, branched, and up to 2 m tall under favorable conditions [1–3]. Common ragweed is an early emerger in the Midwestern United States, emerging in mid-April to late-May [4]. Common ragweed is monoecious but can both self-pollinate and outcross [5], and is not only a problem in Christmas trees as it is listed as the ninth most common and troublesome weed in all broadleaf crops according to surveys conducted by the Weed Science Society of America (WSSA) [6,7].

Common ragweed has had been reported to have resistance to glyphosate (WSSA group 9), PSII inhibitors (WSSA group 5), PPO inhibitors (Group 14), and/or ALS chemistry (WSSA group 2) [8]. There have also been reports from Michigan Christmas tree growers of common ragweed resistance to clopyralid (Stinger®, Dow AgroSciences, Indianapolis, IN, USA), a synthetic auxin herbicide (WSSA group 4), in Montcalm County, Michigan [9]. Resistance in common ragweed can be caused by both target site (changes to the herbicide target site to confer resistance) and non-target site (changes to physiological processes to confer resistance) resistance depending on the population and mode of action. Herbicide resistance can spread quickly throughout populations because ragweed can both self-pollinate and outcross.

To overcome current herbicide resistance and delay the development of new resistance, an integrated weed management approach should be used to manage common ragweed. Using a singular herbicide will not be effective if there is already resistance to that herbicide, or it may exert high selection pressure and cause additional resistance to develop [10,11]. In this experiment, we looked at alternative weed control methods for clopyralid-resistant common ragweed, including the herbicides clopyralid, glyphosate, and oxyfluorfen, as well as shredded cypress bark organic mulch. Clopyralid is a synthetic auxin herbicide (WSSA group 4) in the picolinic acid chemical family. It controls annual and perennial broadleaved weeds. Clopyralid is particularly good at controlling weeds in the *Asteraceae*, such as Canada thistle, common ragweed, and horseweed. Clopyralid is translocated through the symplast, accumulates in the growing points, and is very slowly metabolized [12]. Clopyralid was selected for this experiment because it usually provides adequate weed control for common ragweed and is labeled for use in Christmas tree production. However, Michigan Christmas tree growers in Montcalm County, MI, have reported clopyralid-resistant common ragweed, which threatens its continued effectiveness [9]. The mechanism of resistance to clopyralid is not yet known.

Glyphosate (Roundup® Pro Concentrate, Monsanto Company, St. Louis, MO, USA), a 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase inhibitor (WSSA group 9), interrupts the shikimic acid pathway. Glyphosate is in the organophosphorus chemical family. Glyphosate is a nonselective herbicide and is translocated in the symplast; it accumulates in meristems, underground tissues, and immature leaves. There have been four reported mechanisms of resistance to glyphosate, including target site copy number variation, target site mutations, metabolism, and sequestration in the vacuole [12]. Glyphosate is promptly metabolized by soil microbes and binds to soil, causing minimal ecotoxicity and no year-to-year residual effects [12]. Glyphosate was selected as it is the most extensively used post-emergence herbicide in Christmas tree production. Glyphosate is considered safe to apply to Christmas trees when they are not actively growing [13,14]. There is potential for glyphosate to be used as a replacement to or in conjunction with clopyralid; however, there are also many weeds that have developed resistance to glyphosate.

The final herbicide included in this study, oxyfluorfen (Goaltender®, Dow AgroSciences, Indianapolis, IN, USA), is a protoporphyrinogen oxidase inhibitor (WSSA group 14). Oxyfluorfen is in the diphenylether chemical family. Oxyfluorfen can act as a pre-emergence and post-emergence herbicide, controlling numerous annual small-seeded broadleaf weeds and certain annual grasses. It is a contact herbicide with low translocation. Oxyfluorfen is tightly absorbed by the soil and not readily desorbed. Currently there are two recognized cases of oxyfluorfen resistance in weeds, including common ragweed in soybeans [8,12]. Oxyfluorfen was chosen as it is commonly used in Christmas tree production, and it has the ability to act as both pre-emergence and post-emergence weed control, making it an interesting potential alternative to clopyralid.

Organic shredded cypress mulch was included in the trial as a non-chemical weed control option. It was compared to and used with the chemicals outlined above. Cypress mulch can have allelopathic effects on other plants, which can help control weeds. Cypress bark contains a higher amount of allelopathic phenolic compounds than pine bark or pine straw [15]. Additionally, mulch can act as a physical barrier and prevent the emergence of weeds seeds. The herbicides may also bind to the mulch, allowing for persistent weed control, but out of the herbicides used in this study, oxyfluorfen is the only one with that potential because it has pre-emergence control [12]. We hypothesize that the combination of mulch and herbicides will improve weed control as there are more factors to inhibit weeds. The objective of this study was to investigate the impacts of alternative post-emergence herbicide combinations and organic mulch on clopyralid-resistant common ragweed control efficacy. Our hypothesis was that alternative post-emergence herbicide and organic mulch combinations can show early post-emergence control of clopyralid-resistant common ragweed.

2. Materials and Methods

2.1. Plant Materials

This experiment was conducted in a greenhouse, with a roof made of polycarbonate and walls made of double-sided polyethylene, at Michigan State University Horticulture Teaching and Research Center, located at 3291 College Rd, Holt, MI 48842, in 2021 and 2022. Greenhouse studies were used because they allowed for all conditions (i.e., temperature, irrigation, pest pressure, other weed competition) to be controlled and only the treatments to be held accountable. Clopyralid-resistant common ragweed seeds were collected from known clopyralid-resistant common ragweed plants by Dr. Erin Hill (Weed Diagnostician, Plant and Pest Diagnostics, MSU Extension). The seeds were stored in a mesh bag outside from mid-November to mid-January in East Lansing, MI, where they were exposed to the naturally occurring variable temperatures and precipitation to break dormancy, a method known as overwintering.

The plants were grown, in the above-mentioned greenhouse, to the following two stages: stage 1 at 6–9 leaves and stage 2 at 12–14 leaves. These stages were kept consistent between each season. Plastic square 767 mL pots (manufactured by East Jordan Plastics Inc., East Jordan, Michigan), measuring 10.5 cm (width) $\times$ 11.4 cm (height), were filled with commercial soilless media from Suremix (composition: 70% peat moss, 21% perlite, and 9% vermiculite, manufactured by Michigan Grower Products Inc., Galesburg, MI, USA). Osmocote fertilizer ((17-5-11 (8 to 9 months)) (ICL Specialty Fertilizers, Dublin, OH, USA) was mixed into the Surmix potting media at the manufacturer's labeled medium rate of 7.1 g/L. Twenty-five common ragweed seeds were sown in each pot and all pots were kept inside the greenhouse at a minimum temperature of 21 °C, maximum temperature of 26.6 °C, and average temperature of 23.8 °C. All plants received 1.27 cm of irrigation daily via two irrigation cycles through overhead sprinklers (throughout the experiment). There was no supplemental lighting and the natural day length was approximately 13–15 h of light per day. Weed control treatments were applied once the plants reached the desired leaf stage. The experiments for both stages were replicated twice, first in June through September 2021, and then, repeated in June through September 2022.

2.2. Experimental Design and Treatments

This experiment was designed with a complete randomized-block design with four replications (n= 4) of each of the 12 treatments during each season (2) within each stage (2). The plants were placed on greenhouse benches in a randomized order within each stage. Each block contained 4 plants that were each randomly assigned one of the 12 weed control treatments (Table 1).

2.3. Initial Measurements and Treatment Applications

All weed control treatments were applied outside of the greenhouse. The plants were put into treatment groups and the treatments were applied; then, the plants were returned to the greenhouse and placed in a randomized order on the bench within stage 1 and stage 2. Organic mulch was applied before herbicides. Bagged cypress mulch blend (NoFloat cypress blend, Oldcastle Lawn & Garden, Atlanta, GA, USA) at a depth of 5 cm was used. When more than one herbicide was applied, they were tank-mixed and applied simultaneously. Herbicides were applied within 2 h of mulch application in liquid formulations at their highest labeled rate based on the manufacturer's recommendations and directions. All herbicides and their combinations were applied uniformly, directly over top of the weeds, using a carbon dioxide (CO_2) backpack sprayer (Bellspray R&D sprayer Inc., Opelousas, LA, USA) calibrated to deliver 252.6 L/hectare using an 8004 flat-fan nozzle (TeeJet Technologies, Wheaton, IL, USA) at a pressure of 206.8 kilopascals. For the first round of greenhouse experiments, the treatments were applied to the stage 1 and stage 2 plants on the same day, 2 July 2022 and according to Apple Weather, the weather was partly cloudy with a temperature of 18 °C, with 60% humidity and wind at a rate of 17.7 km/h N. For the second round of greenhouse experiments, the treatments were applied on two separate days for the stage 1 and stage 2 plants. For the stage 1 plants, the treatments were applied on 2 June 2022, and according to Apple Weather, the weather was mostly cloudy with a temperature of 15 °C with 73% humidity and wind at 8.05 km/h SW. The treatments for the stage 2 plants were applied on 1 July 2022, and according to Apple Weather, it was cloudy with a temperature of 23 °C and 56% humidity with wind at a rate of 11.27 km/h NE. The treatments were applied when the ragweed plants reached the desired leaf stage, and due to differences in planting time, this happened on the same day in 2021 but on separate days in 2022.

Table 1. Weed control treatments and rates of application used in field and greenhouse experiments.

Treatments	Rate of Application (Highest Labeled Rate)
Clopyralid	0.58 L Ha^{-1}
Glyphosate	1.9 L Ha^{-1}
Oxyfluorfen	4.6 L Ha^{-1}
Oxyfluorfen + glyphosate	4.6 L Ha^{-1} + 1.9 L Ha^{-1}
Clopyralid + oxyfluorfen	0.58 L Ha^{-1} + 4.6 L Ha^{-1}
Clopyralid + glyphosate	0.58 L Ha^{-1} + 1.9 L Ha^{-1}
Mulch only	5 cm depth, 0.3 m diameter
Mulch + oxyfluorfen + glyphosate	5 cm depth, 0.3 m diameter + 4.6 L Ha^{-1} + 1.9 L Ha^{-1}
Mulch + clopyralid + oxyfluorfen	5 cm depth, 0.3 m diameter + 0.58 L Ha^{-1} + 4.6 L Ha^{-1}
Mulch + clopyralid + glyphosate	5 cm depth, 0.3 m diameter + 0.58 L Ha^{-1} + 1.9 L Ha^{-1}
Clopyralid + oxyfluorfen + glyphosate	0.58 L Ha^{-1} + 4.6 L Ha^{-1} + 1.9 L Ha^{-1}
Control (no herbicides, no mulch)	

2.4. Assessments

2.4.1. Weed Control

Weed control was estimated every two weeks until 8 weeks after treatment (WAT) by the same person within each year on a scale of 0%, meaning completely green and healthy, to 100%, meaning completely dead. The plants were rated, removed from the benches, photographed, and returned to the benches in a randomized order. Weed control was also assessed at 8 WAT, by taking the fresh weight for each individual plant. Plants from each pot were cut at the soil line, placed into an individual brown paper bag, and weighed.

2.4.2. Statistical Analysis

The data were analyzed using PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC, USA) to conduct an analysis of variance (ANOVA) (Table 2) and Tukey's HSD in the LSMEANS prompt of PROC GLIMMIX to separate out the means. An arcsine square root transformation was performed on the control percentage variable to normalize the residuals. Analysis was carried out separately within each stage and within each year, at a significance level of alpha equal to 0.05, using PROC GLIMMIX to perform an analysis of variance (ANOVA). The data from each evaluation were subjected to an initial two-way ANOVA. The treatments, weeks after treatment (WAT), and the treatment × WAT interaction were considered fixed effects, while the blocks were random effects. Mean separation was determined using Tukey's HSD in the LSMEANS prompt of PROC GLIMMIX. A contrast was carried out in PROC GLM to compare treatments with and without mulch.

Table 2. ANOVA tables from PROC GLIMMIX in SAS 9.4 showing *p* values for fresh weight analysis for each of the ragweed stages (stage 1, stage 2). *p* values for treatment (12 treatments), week after treatment year, and treatment × year interaction are shown. All effects are significant ($p < 0.05$).

	Stage 1
Effect	
Treatment	<0.0001
Year	<0.0001
Treatment × Year	<0.0001
	Stage 2
Effect	
Treatment	0.0028
Year	0.0007
Treatment × Year	0.0058

Plant fresh weight was analyzed separately by stage. Data analysis was carried out using PROC MIXED in SAS (Ver. 9.4, SAS Institute, Cary, NC, USA) for checking the model, checking assumptions, and checking which transformation was needed. An arcsine square root transformation was performed on the fresh weight variable. This was carried out for both stages. An analysis of variance was carried out at a significance level of alpha equal to 0.05, using PROC GLIMMIX with the arcsine-transformed fresh weight variable. Mean separation was determined using Tukey's HSD in the LSMEANS prompt of PROC GLIMMIX.

3. Results

At 2 WAT (Table 3), a high level (92–100%) of weed control at stage 1 (2021) and stages 1 and 2 (2022) was observed in plants treated with mulch + clopyralid + oxyfluorfen. High (97%) weed control was also seen in plants treated with mulch + oxyfluorfen + glyphosate at stage 1 (Figure 1) in 2021 and 2022. Mulch + clopyralid + glyphosate provided high levels (99–100%) of weed control at stage 2 (Figure 2) in 2021 and stage 1 in 2022. Clopyralid + oxyfluorfen + glyphosate, clopyralid + glyphosate, and clopyralid + oxyfluorfen provided 96–99% weed control at stage 1 in 2022. At 2 WAT, the control treatment, clopyralid, and mulch provided the lowest amount of weed control (0–13%) for both stages in 2021 and also stage 1 in 2022. Glyphosate and mulch provided the lowest level of weed control for stage 2 (2022) at 15–24%, and clopyralid was still low as it provided only 26% weed control.

At 8 WAT (Table 3), the treatment effects remained largely the same as they were at 2 WAT. Notable changes included that in 2021, the control plants treated at stage 1 began to die and the only highly effective (91% control) treatment at 8 WAT was mulch + oxyfluorfen + glyphosate. For plants treated at stage 2 in 2021, mulch + clopyralid + glyphosate was the most effective treatment at 8 WAT, as it was at 2 WAT. For plants treated at stage 1 in 2022, all of the highly effective treatments at 2 WAT increased in effectiveness by 8 WAT and provided 100% weed control. For plants treated at stage 2 in 2022, oxyfluorfen, clopyralid + glyphosate, mulch + oxyfluorfen + glyphosate, and clopyralid + oxyfluorfen + glyphosate increased in effectiveness to join mulch + clopyralid + oxyfluorfen as highly effective treatments, providing 89–97% weed control. Many treatments provided low levels of weed control at 8 WAT. For plants treated at stage 1 in 2021, glyphosate and oxyfluorfen were lowest at 11%, and for stage 2 in 2021, mulch + oxyfluorfen + glyphosate showed 14% weed control. In 2022, clopyralid showed the lowest level of weed control at 33% from plants treated at stage 1, while clopyralid, glyphosate, oxyfluorfen + glyphosate, and mulch provided the lowest levels of weed control (34–42%) for plants treated at stage 2. Mulch/herbicide combination treatments showed an increase in weed control of 4.2–4.7% for stage 2 plants in 2021 and both stages of plants in 2022 when compared to those treated with herbicides only.

Table 3. Control percentages (0–100%) for common ragweed separated by stage and year subjected to 12 weed control treatments (Table 1). Control percentages followed by the same letter are not significantly different within a column. Mean separation via Tukey's HSD separated by WAT.

2021 Stage 1	2 WAT *	4 WAT	6 WAT	8 WAT
Clopyralid	6.12 de **	15.16 cd	18.92 bc	29.12 bc
Glyphosate	24.69 bcde	7.12 cd	25.56 abc	11.76 c
Oxyfluorfen	31.88 bcde	24.69 bcd	67.11 ab	11.76 c
Oxyfluorfen + Glyphosate	75.24 abc	75.24 ab	54.45 ab	40.52 abc
Clopyralid + Oxyfluorfen	80.23 ab	79.43 ab	55.45 ab	44.47 abc
Clopyralid + Glyphosate	73.50 abc	54.45 abc	34.71 ab	27.32 bc
Mulch	13.76 cde	53.46 abc	43.48 ab	37.59 bc
Mulch + Oxyfluorfen + Glyphosate	90.65 a	84.05 a	71.71 ab	26.43 bc
Mulch + Clopyralid + Oxyfluorfen	92.84 a	88.19 a	76.10 a	90.65 a
Mulch + Clopyralid + Glyphosate	84.05 ab	86.87 a	47.46 ab	26.43 bc
Clopyralid + Oxyfluorfen + Glyphosate	63.30 abcd	39.54 abc	25.56 abc	20.51 c
Control	0 e	0 c	0 c	77.79 ab
2021 Stage 2	**2 WAT ***	**4 WAT**	**6 WAT**	**8 WAT**
Clopyralid	2.86 d **	3.57 cd	22.99 bc	47.46 abc
Glyphosate	36.63 bcd	20.51 bcd	32.82 bc	61.36 abc
Oxyfluorfen	25.56 cd	11.76 bcd	48.46 abc	92.32 ab
Oxyfluorfen + Glyphosate	86.87 abc	86.87 ab	56.44 ab	70.81 abc
Clopyralid + Oxyfluorfen	93.84 abc	71.71 abc	33.76 bc	30.03 bc
Clopyralid + Glyphosate	94.77 abc	82.56 ab	54.45 abc	72.61 abc
Mulch	0 d	49.46 abcd	81.02 ab	65.22 abc
Mulch + Oxyfluorfen + Glyphosate	90.65 abc	65.22 abc	26.43 bc	14.45 c
Mulch + Clopyralid + Oxyfluorfen	98.78 ab	95.20 a	90.65 ab	90.65 ab
Mulch + Clopyralid + Glyphosate	100 a	100 a	100 a	100 a
Clopyralid + Oxyfluorfen + Glyphosate	79.43 abc	81.02 ab	61.36 ab	61.36 abc
Control	0 d	0 d	0 c	44.47 abc
2022 Stage 1	**2 WAT ***	**4 WAT**	**6 WAT**	**8 WAT**
Clopyralid	5.20 c **	9.90 e	35.66 b	33.76 c
Glyphosate	40.52 b	40.52 cd	61.36 b	64.26 b
Oxyfluorfen	50.46 b	60.38 bc	57.43 b	57.43 bc
Oxyfluorfen + Glyphosate	66.16 b	68.97 b	61.36 b	61.3 bc
Clopyralid + Oxyfluorfen	96.40 a	99.96 a	100 a	100 a
Clopyralid + Glyphosate	99.96 a	100 a	100 a	100 a
Mulch	5.20 c	20.51 de	55.45 b	37.59 bc
Mulch + Oxyfluorfen + Glyphosate	97.44 a	99.96 a	99.96 a	100 a
Mulch + Clopyralid + Oxyfluorfen	100 a	100 a	100 a	100 a
Mulch + Clopyralid + Glyphosate	99.50 a	100 a	100 a	100 a
Clopyralid + Oxyfluorfen + Glyphosate	96.77 a	99.96 a	100 a	100 a
Control	0 c	0 f	0 c	0 d
2022 Stage 2	**2 WAT ***	**4 WAT**	**6 WAT**	**8 WAT**
Clopyralid	26.43 cde **	25.56 cd	42.49 acd	42.49 b
Glyphosate	24.69 def	26.43 cd	32.82 cd	38.56 b
Oxyfluorfen	75.24 abc	85.49 ab	57.43 ab	93.84 a
Oxyfluorfen + Glyphosate	37.59 bcde	36.63 bcd	37.59 cd	39.54 b
Clopyralid + Oxyfluorfen	68.97 abcd	76.10 ab	81.8 abc	83.31 ab
Clopyralid + Glyphosate	73.49 abcd	83.31 ab	93.35 a	93.84 a
Mulch	15.16 ef	18.14 de	29.12 d	34.71 b
Mulch + Oxyfluorfen + Glyphosate	81.02 ab	89.45 a	94.77 a	94.77 a
Mulch + Clopyralid + Oxyfluorfen	88.83 a	90.06 a	97.11 a	97.44 a
Mulch + Clopyralid + Glyphosate	62.33 abcde	66.16 abcd	79.43 abc	81.01 ab
Clopyralid + Oxyfluorfen + Glyphosate	72.61 abcd	75.24 abc	88.83 ab	89.45 a
Control	0 f	0 e	0 e	0 c

* WAT represents weeks after treatment application. ** Control percentages followed by the same letter are not significantly different within a column.

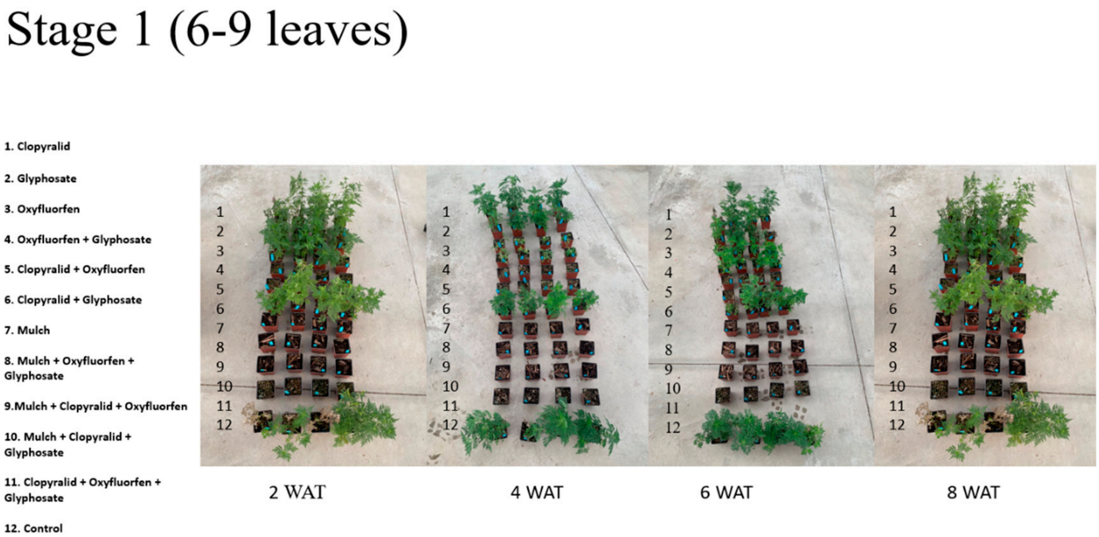

Figure 1. Stage 1 common ragweed plants in 2022 at 2, 4, 6, and 8 WAT and listed treatments 1–12.

Figure 2. Stage 2 common ragweed plants in 2022 at 2, 4, 6, and 8 WAT and listed treatments 1–12.

By 8 WAT (Table 4), many plants had completely dried up and there was nothing left, so the fresh weight for these plants was 0. Stage 1 plants treated with all mulch and herbicide combination treatments and most herbicide combination treatments in 2022 had an N/A fresh weight, but stage 1 plants in 2021 treated with the same treatments resulted in a fresh weight of ~20 g. For stage 1 plants in 2021, the plants treated with glyphosate had the heaviest fresh weight, and in 2022, the plants treated with clopyralid, and mulch plants, had the heaviest fresh weight. For the stage 2 plants in 2021, the only treatment that reached a fresh weight of N/A was mulch + clopyralid + glyphosate.

Table 4. Fresh weight (g) separated by stage and year for common ragweed, subjected to 12 weed control treatments (Table 1). Fresh weights followed by the same letter are not significantly different within a column. Mean separation via Tukey's HSD.

	Stage 1	
Treatment	2021	2022
	Fresh Weight (g)	Fresh Weight (g)
A Clopyralid	47.46 ab *	21.32 a
B Glyphosate	55.45 a	14.45 a
C Oxyfluorfen	39.54 abc	12.41 a
D Oxyfluorfen + Glyphosate	22.99 cd	13.08 a
E Clopyralid + Oxyfluorfen	22.95 cd	N/A b
F Clopyralid + Glyphosate	22.99 cd	N/A b
G Mulch	28.22 bcd	21.32 a
H Mulch + Oxyfluorfen + Glyphosate	17.38 de	N/A b
I Mulch + Clopyralid + Oxyfluorfen	6.61 e	N/A b
J Mulch + Clopyralid + Glyphosate	18.92 de	N/A b
K Clopyralid + Oxyfluorfen + Glyphosate	20.51 cde	N/A b
L Control	23.83 cd	17.38 a
	Stage 2	
Treatment	2021	2022
	Fresh Weight (g)	Fresh Weight (g)
A Clopyralid	16.63 ab *	33.76 ab
B Glyphosate	13.76 ab	23.83 abc
C Oxyfluorfen	14.45 ab	2.23 c
D Oxyfluorfen + Glyphosate	6.61 ab	30.95 abc
E Clopyralid + Oxyfluorfen	18.14 ab	15.89 abc
F Clopyralid + Glyphosate	8.18 ab	8.73 abc
G Mulch	8.18 ab	29.12 abc
H Mulch + Oxyfluorfen + Glyphosate	17.38 ab	6.61 abc
I Mulch + Clopyralid + Oxyfluorfen	0.81 ab	5.2 bc
J Mulch + Clopyralid + Glyphosate	0 b	23.83 abc
K Clopyralid + Oxyfluorfen + Glyphosate	4.76 ab	17.38 abc
L Control	24.69 a	39.54 a

* Fresh weights followed by the same letter are not significantly different within a column.

4. Discussion

The goal of this experiment was to evaluate alternative weed control options for the known clopyralid-resistant common ragweed plant. There have been reports from Michigan Christmas tree growers of clopyralid-resistant common ragweed in Montcalm County, Michigan [9]. Resistance is likely spreading throughout Michigan Christmas tree farms via pollen and seeds and there is also known glyphosate resistance in common ragweed [8]. The seeds used in this study were selected for clopyralid resistance; however, based on some of the results, it is possible that they were also glyphosate-resistant. A diverse array of control options for weeds is important as high selection pressures from a single method lead rapidly to resistance [6,10,11]. One method to combat this is the use of integrated weed management plans [16]; these typically include using multiple herbicides or herbicide $\times$ mulch combinations amongst many other options, such as mechanical control.

Herbicide options are limited in Christmas trees. Clopyralid is effective at controlling ragweed and is relatively safe for Fraser firs [17], but with the newfound resistance, clopyralid is no longer able to control some common ragweed populations. Glyphosate has been found to have poor-to-fair ragweed control and is relatively safe for Fraser firs [17]. Oxyfluorfen has been found to have poor ragweed control but is relatively safe for Fraser fir depending on the growth stage [17].

Some of the integrated weed management options that we tested worked well at controlling resistant common ragweed. In common ragweed at stage 1 in 2021 and 2022, as well as stage 2 in 2022, at all WAT, mulch + clopyralid + oxyfluorfen consistently provided the highest level of weed control. For stage 2 plants in 2021, mulch + clopyralid + glyphosate resulted in 100% control from 2 WAT and always showed better or equal weed control compared to all the other treatments. This is interesting, as ragweed is known to have both glyphosate and clopyralid resistance, but the combination of the two with mulch provided extremely good weed control, potentially indicating a synergistic effects of either the herbicide modes of action or resistance mechanisms interacting, along with the benefits from the mulch. In most evaluations, oxyfluorfen provided the highest level of ragweed control as a singular herbicide treatment. This observation is consistent with other reports, which state that it is the only herbicide of the three compared here to which common ragweed is likely not resistant [8]. The mulch + herbicide combination treatments all provided very good weed control, especially in 2022, where at stage 1, all three of those treatments, along with the three-herbicide combination, had completely killed the plants by 8 WAT. The lowest level of control was observed in the plants only treated with clopyralid, which, in many cases, was not significantly different ($p > 0.05$) from the plants that received the control treatment (no mulch or herbicides). On average, glyphosate was the second least effective treatment. It is very evident in all of the years and stages that a multiple-method approach, especially if there are three or more methods included, will work much better than a single-method approach. This agrees with Beam et al. [6], who looked at post-emergence herbicides, including glyphosate, as well as winter cover in soybean production and found that when both treatments were used, there was lower common ragweed density in soybean fields treated with both herbicides and winter cover crops. Overall, the most effective treatments out of the twelve weed control treatments for clopyralid-resistant common ragweed were mulch + clopyralid + glyphosate and mulch + clopyralid + oxyfluorfen, likely due to synergistic effects.

5. Conclusions

Integrated weed management strategies are the best choice for controlling not only common ragweed but also all other weeds in the Christmas tree production system as they can help prevent the development of herbicide resistance among the weed species. A single weed control method is not recommended as there are several types of weed species with highly diverse life cycles and survival strategies. Combining nonchemical with chemical methods (such as mulch and herbicide combinations) or applying a tank mix of herbicides with different modes of action can help in reducing resistance development among the weed species and can also mitigate herbicide-related environmental issues such as leaching and run-off. Hence, the concept of integrated weed management strategies for successful Christmas tree production is well established through this research project.

Author Contributions: Conceptualization, D.S.; methodology, D.S. and E.H.; software, G.G. and B.C.; validation, D.S. and B.C.; formal analysis, G.G. and B.C.; investigation, G.G. and D.S.; resources, D.S.; data curation, G.G.; writing—original draft preparation, G.G.; writing—review and editing, G.G., D.S., B.C., E.P. and E.H.; visualization, G.G., D.S. and B.C.; supervision, D.S., G.G. and B.C.; project administration, D.S. and G.G.; funding acquisition, D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Michigan Department of Agriculture and Rural Development and the Michigan Christmas Tree Association, Specialty Crop Block grant number 210000000477. This research was also supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture, Hatch project number MICL02670.

Data Availability Statement: Data available on request due to restrictions (ethical and privacy). The data presented in this study are available on request from the corresponding author. All data are not publicly available due to privacy and ethical issues.

Acknowledgments: We would like to thank Ashley Jeon, McKinleigh Cordahl, and Shriya Kethireddy for their contributions to this project as undergraduate research assistants. They assisted in setting up the experiment, collecting data, and caring for the plants. Without them, the project would not have been possible. In addition, we would like to extend our thanks to the statistical consulting center in the college of agriculture and natural resources at Michigan State University for their assistance in the data analysis.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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