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Developing Integrated Strategies to Address Emerging Weed Management Challenges in Christmas Tree Production

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Abstract: Weed control is an important aspect during the first few years of Christmas tree establishment, as weed competition directly relates to the rate of Christmas tree growth during this time. The objectives of this study were to evaluate the weed control efficacy of organic mulch and herbicide combinations and to determine their phytotoxic effects on four different species of Christmas trees during the establishment stage: Fraser fir (*Abies fraseri* (Pursh) Poir), blue spruce (*Picea pungens* Engelm.), white pine (*Pinus strobus* L.), and Scotch pine (*Pinus sylvestris* L.). Twelve weed control treatments were established in a complete randomized block design with four replications in each of five fields. Weed control treatments included cypress bark organic mulch and herbicides applied alone and in combinations as well as an untreated control. Herbicides included clopyralid, oxyfluorfen, and glyphosate. All herbicides were applied at their highest labeled rate. Data collection included visual estimations of weed control and phytotoxicity to trees at 30, 60, and 90 days after treatment (DAT). Tree growth and foliar nitrogen concentration were also measured. Mulch combined with herbicide provided 60%–100% weed control in all cases; at two farms, mulch provided a significant increase in weed control when compared to the same treatments without mulch. Combinations of mulch + clopyralid + glyphosate and clopyralid + oxyfluorfen + glyphosate resulted in the highest phytotoxicity ratings. Tree growth was decreased due to some treatments at Gobles farm, and foliar N did not differ among any of the treatments.

Keywords: Christmas trees; weed control; postemergence herbicides; organic mulch; glyphosate

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Citation: Gallina, G.; Cregg, B.; Patterson, E.; Saha, D. Developing Integrated Strategies to Address Emerging Weed Management Challenges in Christmas Tree Production. *Forests* **2023**, *14*, 881. https://doi.org/10.3390/f14050881

Academic Editor: Craig Nitschke

Received: 23 March 2023
Revised: 14 April 2023
Accepted: 24 April 2023
Published: 25 April 2023

1. Introduction

Effective weed control is critical in Christmas tree production systems [1], especially from the time of initial seedling survival, through the establishment phase, and three years post-transplant into the field [2–6]. Tree growth is directly related to the extent of weed competition in the second and third years of the establishment phase [2]. Christmas trees are often grown in well-drained soils, and in these soils, weeds compete for limited available moisture, which can result in the trees facing drought stress. Additionally, weeds can shade young Christmas trees and seedlings, which may result in a reduction in photosynthesis and therefore hinder leaf area development and subsequent growth [7]. A variety of common problematic weeds compete in Michigan Christmas tree production including horseweed (*Erigeron canadensis* (L.) Cronquist), field bindweed (*Convolvulus arvensis* L.), wild carrot (*Daucus carota* L.), hoary alyssum (*Berteroa incana* (L.) DC.), common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L) and hairy vetch (*Vicia villosa* Roth), witchgrass (*Panicum capillare* L.), giant foxtail (*Setaria faberi* Herrm.), large crabgrass (*Digitaria sanguinalis* (L.) Scop), and fall panicum (*Panicum dichotoliflorum* Michx.).

The most common weed control strategies used by Christmas tree growers to manage these problematic weeds are mechanical mowing and chemical herbicide applications. Chemical weed control programs, though often highly effective, are not without drawbacks. First, frequent applications of unrotated herbicides have resulted in the development of herbicide-resistant weeds in almost all plant production systems [8]. Resistant weeds decrease production and have a limited number of alternative weed control strategies. Second, post-emergence herbicide application can result in severe phytotoxic injuries to Christmas trees including stunted growth, burning, the dropping of needles, chlorosis, and even the complete death of the tree [9]. Trees are most sensitive to herbicides during the establishment stage, when weed control is critical [3]. Third, herbicides can have adverse environmental and off-target effects, such as herbicide leaching, drift, and run-off [1]. However, despite these drawbacks, both pre-emergent and post-emergent herbicides are widely used among Christmas tree producers. In the current study, we focus on post-emergent herbicides because they often represent a default approach by many growers yet pose a relatively high risk for non-target injury. In particular, the present experiment focused on three frequently used postemergence herbicides: clopyralid, glyphosate, and oxyfluorfen. In addition, we investigated the use of organic mulch as a means to control weeds by itself and in combination with post emergence herbicides.

Clopyralid (Stinger®, Dow AgroSciences, Indianapolis Indiana) is a synthetic auxin herbicide (WSSA group 4) in the picolinic acid chemical family. It controls annual and perennial broadleaved weeds, especially those in the Asteraceae plant family, which includes Canada thistle, ragweed, and marestail, but also some others such as wild buckwheat. Clopyralid is translocated through the symplast and accumulates in the growing points. Generally, it is very slowly metabolized in most plants [10]. Clopyralid was chosen as it commonly provides good weed control for common ragweed and is labeled for use in Christmas tree production. However, newly reported clopyralid resistance by Michigan Christmas tree growers in Montcalm County in central lower Michigan threatens its continued usefulness [11].

Glyphosate (Roundup® Pro Concentrate, Monsanto Company, St. Louis, MO, USA) is a 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase inhibitor (WSSA group 9), which disrupts the shikimic acid pathway, in the organophosphorus chemical family. Glyphosate is nonselective and is translocated in the symplast, it accumulates in underground tissues, meristems, and immature leaves. Four mechanisms of resistance have been reported in weeds including target site mutations, target site copy number variation, metabolism, and sequestration in the vacuole [10]. Glyphosate is rapidly metabolized by soil microbes and strongly binds soil, which results in low ecotoxicity and no residual effects from year to year [10]. Glyphosate was chosen for this study, as it is the most widely used postemergence herbicide in Christmas tree production, and many weeds have developed resistance to it. It is generally considered safe to spray in Christmas trees as long as the trees are not actively growing, and it therefore can be applied selectively in Christmas tree production [3].

The final herbicide, oxyfluorfen (Goaltender®, Dow AgroSciences, Indianapolis, Indiana), is a protoporphyrinogen oxidase inhibitor (WSSA group 14) in the diphenylether chemical family. Oxyfluorfen can be used both preemergence and postemergence, and it controls many annual small-seeded broadleaf weeds and some annual grasses. It is a contact herbicide with low translocation, primarily killing leaf tissue it comes in contact with. Oxyfluorfen is firmly absorbed by the soil and not easily desorbed. To date, there are no known cases of oxyfluorfen resistance in weeds [10].

In addition to chemical weed control, many Christmas tree producers are interested in non-chemical approaches to controlling weeds. For example, in an interactive poll during an online Christmas tree production webinar, nearly one-third of participants indicated they use mulch to help control weeds. Organic shredded cypress mulch was used as a non-chemical weed control alternative for comparison and used in conjunction with the chemicals outlined above. Cypress has been shown to have allelopathic effects on other plants, and this can help control weeds. Specifically, it has been found that cypress contains more phenolic compounds, which are generally thought to be allelopathic, than pinebark or pinestraw [12]. Mulch also acts as a physical barrier in the soil, preventing the emergence of weed seeds. There is also the potential for the herbicide to bind with the mulch and allow for continued weed control; this is only likely with oxyfluorfen, as it is the only herbicide in this trial with preemergence control [10]. When herbicides and mulch are combined, weed control will likely be improved, as there are more factors involved in preventing weeds. Arthur and Wang [13] tested various weed control options in Christmas trees, including herbicides, organic mulch, and inorganic mulch. Among the methods tested, they found that sawdust was the best treatment for long-term weed control in Christmas tree production. The sawdust treatment increased the soil microbial biomass and soil water and N content. These positive soil effects are useful when considering the long-term impacts on the soil for the duration of the life of the Christmas tree [13]. Cregg, Nzokou, and Goldy [14] looked at various weed control methods, including mulches, hand weeding, chemicals, and irrigation, and found that wood chips provided nearly 100% weed control. Wood chips also had an added benefit in non-irrigated systems when compared to other treatments, such as having comparable survival and growth rates to the irrigated plots and increasing the height and diameter of Fraser fir compared to other treatments [14]. For the above reasons, cypress bark mulch is a good mulch choice for Christmas tree weed control, as it will likely provide decent weed control, be healthy for the trees, encourage growth, and improve soil health.

As beneficial as mulch can be, it can also have adverse effects by decreasing available nitrogen levels in the soil. Organic mulch can provide carbon to soil microbes, which stimulates the growth of those microbes, causing them to have a higher demand for N [15–17]. When organic mulches decay, they can use nitrogen, which can restrict nitrogen from being taken up by the tree [18,19]. Chalker-Scott [20] reviewed that low-nutrient mulches can decrease N in soil water but do not impact plant N levels, and even low-N mulches such as straw, sawdust, and bark can increase the foliar or soil nutrient levels [20]. Experimental research has shown that using organic mulch does not immobilize N or impede growth, and in fact, it can increase the N levels of plants [20].

In this study, we looked at the three postemergence herbicides listed above and organic shredded cypress mulch to evaluate the weed control efficacy and phytotoxic effects on Christmas trees. The objectives of this study were to:

Objective 1. *Determine the weed control efficacy of different postemergence herbicide combinations and compare with organic mulch weed control efficacy.*

Hypothesis 1. *Combining organic mulch with postemergence herbicide combinations can improve weed control.*

Objective 2. *Evaluate the phytotoxic effects of postemergence herbicide combinations and organic mulching on four different types of Christmas trees during the establishment stage.*

Hypothesis 2. *Postemergence herbicide combinations alone can cause injury to some varieties of Christmas trees, but the addition of organic mulch will reduce the phytotoxic effects.*

2. Materials and Methods

2.1. Plant Materials

The experiments were conducted at commercial Christmas tree farms located in mostly western Michigan. The trees at each farm were selected in late March to early April 2021. We installed plots in Horton, MI, USA (Gwinn's Christmas Tree Farm) at 42°4′32.80″ N, 84°28′53.80″ W; Gobles, MI, USA (Wahmhoff Farms) at 42°20′15.70″ N, 85°53′3.00″ W; Allegan, MI, USA (Badger Evergreen Nursery) at 42°25′47.60″ N, 85°56′43.30″ W; and Sidney, MI, USA (Korson's Tree Farms) at 43°15′41.90″ N, 85°8′40.40″ W and 43°16′30.20″ N, 85°19′49.30″ W. Each plantation was established with bare-root transplantsl trees had been growing in the field for 1–2 years. Different species were selected on each farm based on grower availability: 2-year Fraser fir (*Abies fraseri* (Pursh) Poir) (Horton farm), 2-year blue spruce (*Picea pungens* Engelm.) and 2-year Fraser fir (Sidney farm), 1-year White pine (*Pinus strobus* L.) (Allegan farm), and 1-year Scotch pine (*Pinus sylvestris* L.) (Gobles farms).

Aside from weed control, all cultural practices were maintained based on the growers' standard practices. The Scotch pines at Gobles farm and the white pines at Allegan farm received no fertilizer or irrigation during the entire period of experiment. The blue spruce trees at Sidney farm were not irrigated but were fertilized on 24 April 2021; this was 14.5-0-6 (N-P_2O_5-K_2O) at a rate of 113 g per tree. Fertilizer was again applied to the blue spruce on 20 April 2022; this was 14-0-8 (N-P_2O_5-K_2O) at a rate of 142 g per tree. The Fraser fir trees at Sidney farm were irrigated at a rate of 2.12 L per hour for 4 h, with emitters spaced every 60 cm, on 3 June 2021, 7 June 2021, 14 June 2021, 2 August 2021, and 19 August 2021. The Fraser fir trees at Sidney farm were also fertilized on 7 May 2021 with 14.5-0-6 (N-P_2O_5-K_2O) fertilizer at a rate of 113 g per tree. They were again fertilized on 19 May 2022 with 14-0-8 fertilizer at a rate of 170 g per tree.

2.2. Experimental Design and Treatments

At each farm, we installed each experiment in a complete randomized block design with four replications (N = 4) of each of the treatments (12) at each field (5) (Figure 1). The Fraser fir trees at Horton farm were in three rows with four 3×4 blocks for the 4 replications of the 12 treatments. At the rest of the farms, the rows were not as straight, so the 4 blocks were each a row of trees; hence, there were 4 rows of 12 trees. Treatments are listed in rates of product. Each block at each field contained 12 trees that were each randomly assigned 1 of 12 treatments (Table 1). Treatment applications for Horton farm were made on 27 May 2021. On 11 June 2021, treatments were applied to Sidney farm. Gobles farm and Allegan farm treatments were applied on 17 June 2021.

The organic mulch used was a bagged shredded cypress mulch blend (NoFloat cypress blend, Oldcastle Lawn & Garden, Atlanta, GA, USA) (Figure 2). Organic mulch was applied immediately before herbicide applications. When more than one herbicide was applied, they were tank mixed and applied simultaneously. Herbicides were applied at their highest labeled rates and were in liquid formulations. All herbicides and their combinations were applied uniformly and directly over the top of the trees with a carbon dioxide (CO_2) backpack sprayer (Bellspray R&D sprayer Inc., Opelousas, LA, USA) calibrated to deliver 252.55 L/hectare using an 8004 flat-fan nozzle (TeeJet Technologies, Wheaton, IL, USA) at a pressure of 206.843 kilopascals (Figure 3). The herbicide band width was 81 cm, and the length was about 60 cm per each individual tree. We recorded the temperature, relative humidity, and windspeed and direction at the time of application using AccuWeather (Table 2).

Figure 1. Example of Christmas trees in field (Horton Tree Farm).

Table 1. Weed control treatments and rate of applications used in field experiments.

Treatments	Rate of Applications (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)	

Figure 2. Example of how organic cypress bark mulch was applied to each Christmas tree in the field experiment.

Figure 3. Example of how herbicides were applied at each farm to each individual tree with a Carbon dioxide backpack sprayer.

Table 2. The date of treatment application, name of farm, species, soil type from the soil survey, and weather conditions including temperature, humidity, and wind speed for each farm used in the field experiment.

Date of Treatment Application	Name of Farm	Species	Soil Type	Weather Condition
27 May 2021	Gwinn's farm, Horton, MI, USA	Fraser fir	Boyer-Oshtemo sandy loams, 1 to 6 percent slopes 11.5% 11B Boyer-Oshtemo sandy loams, 6 to 12 percent slopes 37.9% 11C Hillsdale-Riddles sandy loams, 6 to 12 percent slopes 50.6% 49C	Sunny, 15.5 °C, 52% humidity, wind 15.9 km/h northeast
11 June 2021	Korson's farm, Sidney, MI, USA	Fraser fir	Tekenink fine sandy loam, 6 to 12 percent slopes 100% 62C	Partly cloudy, 26.6 °C, 71% humidity, wind 2.9 km/h east.
11 June 2021	Korson's farm, Sidney, MI, USA	Blue spruce	McBride and Isabella sandy loams, 2 to 6 percent slopes 100% Mk	Partly cloudy, 26.6 °C, 71% humidity, wind 2.9 km/h east.
17 June 2021	Badger farm, Allegan, MI, USA	White pine	Metea loamy fine sand, 1 to 6 percent slopes 78.2% 27B Metea loamy fine sand, 6 to 12 percent slopes 21.8% 27C	Sunny, 26.6 °C, 48% humidity, wind 5.95 km/h southwest
17 June 2021	Wahmhoff farm, Gobles, MI, USA	Scotch pine	Spinks-Oshtemo complex, 0 to 6 percent slopes 100% 12B	Sunny, 31 °C, 48% humidity, wind 11.27 km/h southwest

2.3. Dominant Weeds for Each Farm

Dominant weed species at each farm location were also identified and recorded. The dominant weed species for the Fraser fir at Horton farm was hoary alyssum (*Berteroa incana* (L.) DC). The dominant weed species at the Sidney Fraser fir plots were mainly hoary alyssum, horseweed (*Conyza canadensis* (L.) Cronquist var. canadensis), common ragweed (*Ambrosia artemisiifolia* L.), dandelions (*Taraxacum officinale* F.H. Wigg.), and black medic (*Medicago lupulina* L.). The dominant weeds noted for Sidney farm blue spruce were white clover *(Trifolium repens* L.) and black medic. For Gobles farm, the dominant weed species recorded was horseweed. The Allegan farm primary weeds recorded were hoary alyssum, common ragweed, and horseweed.

2.4. Assessments

2.4.1. Weed Control

Weed control was estimated visually as the percent ground cover within each plot covered with weeds and was carried out by the same person for all trees at all farms during each collection. We estimated weed cover as 0%, meaning no weed control (using the control treatment as a baseline), to 100%, meaning complete weed control. Weed control percent was judged for each individual tree. Visual estimations were conducted 30, 60, and 90 days after the treatments (DAT) were applied.

2.4.2. Phytotoxicity

Phytotoxicity was estimated using a visual assessment carried out by the same person for all trees at all farms during each data collection, with 0% meaning no phytotoxicity (using the control treatment as a baseline) and 100% meaning complete death of the Christmas tree. Visual estimations were conducted 30, 60, and 90 DAT, which was from June to October 2021.

2.4.3. Growth Indices

In late May to mid-June 2021, before applying the weed control treatments, the initial leader lengths, plant heights, and crown widths in two perpendicular directions were recorded in centimeters for each Christmas tree. Data for plant heights and two widths for each Christmas tree were also recorded at 30, 60, and 90 DAT (Table S1). Growth indices were calculated for each tree as: growth index = (plant height + width 1 + width 2)/3.

2.4.4. Foliar Nitrogen Content

For foliar nitrogen analysis, treatment groups were created in order to collect enough total foliar material for grinding without severely damaging any one tree. The groups were: one herbicide, two or more herbicides combined, herbicide with mulch, and mulch only. Samples were taken from each farm. Three to five pieces of current-year growth were collected per tree. Samples were collected in May 2022 and then sent to A &L Great Lakes Laboratories, Fort Wayne, IN for foliar nitrogen analysis. Nitrogen analysis was performed using the Dumas method (1831) [21]. The Dumas method (1831) [21] is carried out by the complete combustion of the matrix in oxygen, the gases are then reduced by copper and dried while trapping CO_2, and then N is determined using a universal detector [21].

2.4.5. Statistical Analysis

Data were analyzed by farm due to there being different species at each farm. Data analysis was carried out using PROC MIXED in SAS (Ver. 9.4, SAS Institute, Cary, NC, USA) for checking the model, checking assumptions, checking for the variance–covariance structure, and checking for which, if any, transformation was needed. The ar (1) variance structure was the best fit for the weed control data, and the arh (1) variance structure was the best fit for the phytotoxicity data. An arcsine square root transformation was required and performed to the weed control percent and phytotoxicity percent variables to normalize residuals. These were consistent throughout all farms. Analysis was carried out separately within each farm/species combination, at a significance level of alpha equal to 0.05, using PROC GLIMMIX to perform analysis of variance (ANOVA). Data from each evaluation were subjected to an initial two-way ANOVA. Treatments, DAT, and the interaction of treatment $\times$ DAT were considered fixed effects, while blocks were random effects. Repeated measures were conducted for each tree for the phytotoxicity percent, weed control, and growth index variables at 30, 60, and 90 DAT. All analyses were performed at an $\alpha = 0.05$ significance level. Mean separation was completed using Tukey's HSD by DAT in the LSMEANS prompt of PROC GLIMMIX. In addition to comparing means across all treatment combinations, we conducted separate analyses to determine the extent and nature of interactions between and among the three herbicides tested. For this analysis, we used the complete factorial combination of treatments of the various herbicides, not including mulch plots. To determine the effect of adding mulch to the herbicide combinations, we constructed a priori contrasts of plots with mulch versus those without. For the foliar N data analysis, analysis was conducted for all farms together due to the limited amount of replication available.

3. Results

3.1. Weed Control

At 90 DAT across farms, the highest level of weed control was observed in plots treated with mulch and herbicide combination treatments (Table 3, Supplemental Tables S2 and S3). Mulch + clopyralid + oxyfluorfen and mulch + clopyralid + glyphosate provided 63% to 92% weed control at the farms in Horton, Sidney (blue spruce), Gobles, and Allegan. Mulch + oxyfluorfen + glyphosate provided 66% to 96% weed control across farms. Mulch alone provided a high level (88%) of weed control at the farm in Gobles. At Allegan farm, there was no significant difference in weed control between any of the treatments except for the control treatment. Notably low levels of weed control were found in plots treated with clopyralid (1.85% to 6.9%) at the Horton and Sidney (blue spruce) farms. Glyphosate also provided low levels of weed control (5% to 13%) at the farms in Sidney (both) and Horton. At the farm in Sidney (blue spruce), low weed control was also observed in plots treated with oxyfluorfen + glyphosate, clopyralid + oxyfluorfen, and clopyralid + glyphosate.

Overall, as a general rule, the treatments that included mulch combined with herbicides provided the highest amount of weed control, especially at the 90 DAT.

Table 3. Mean weed control (% ground cover) 90 days after treatment (DAT) of plots treated with three herbicides and organic mulch, alone or in combination, at Christmas tree farms in Michigan.

Treatment	Location/Tree Species				
	Allegan White Pine	**Horton Fraser Fir**	**Sidney Blue Spruce**	**Sidney Fraser Fir**	**Gobles Scotch Pine**
Clopyralid	75.23 a *	1.86 d	6.93 d	15.06 abcd	22.48 bc
Glyphosate	60.49 a	11.51 cd	13.32 d	5.82 cd	63.03 ab
Oxyfluorfen	63.59 a	18.19 bcd	24.72 bcd	12.38 bcd	67.04 ab
Oxyfluorfen + Glyphosate	51.89 a	59.02 abc	9.69 d	31.87 abc	67.13 ab
Clopyralid + Oxyfluorfen	76.91 a	59.01 abc	9.25 d	23.93 abcd	72.35 ab
Clopyralid + Glyphosate	58.16 a	43.57 bcd	10.53 d	43.57 abc	52.89 ab
Mulch	69.13 a	59.73 abc	72.34 abc	2.83 dc	88.10 a
Mulch + Oxyfluorfen + Glyphosate	82.62 a	85.18 a	83.08 ab	66.78 a	96.27 a
Mulch + Clopyralid + Oxyfluorfen	91.13 a	76.75 a	92.47 a	56.31 ab	95.31 a
Mulch + Clopyralid + Glyphosate	85.18 a	75.00 a	90.87 a	36.67 abc	63.81 ab
Clopyralid + Oxyfluorfen + Glyphosate	79.13 a	67.37 ab	23.16 cd	37.91 abc	16.63 bc
Control	0.00 b	0.00 d	0.00 d	0.00 d	0.00 c

* Means within a column followed by the same letter are not significantly different at the $p < 0.05$ level. Mean separation by Tukey's HSD test.

Based on the contrast (Table 4) comparing the treatments with mulch to their mulch-free counterparts, at 90 DAT, at Horton farm, there was 10% greater weed control seen in treatments with mulch, and in Sidney Blue Spruce, there was 46% greater weed control observed in treatments with mulch.

At 90 DAT, the presence of clopyralid provided a significant change in weed control at Allegan farm and Horton farm and for the Fraser fir plots at Sidney farm. The presence of glyphosate showed a significant change in weed control in the Fraser fir plots at both the Horton and Sidney farms. The presence of oxyfluorfen provided a significant change in weed control at the Allegan, Horton, and Gobles farms. The interaction effects of clopyralid + glyphosate and glyphosate + oxyfluorfen had a significant change in weed control at Allegan and Gobles farms. The interaction of clopyralid + oxyfluorfen had a significant effect on weed control in the Fraser fir plots at Sidney farm. The three-way treatment interaction had a highly significant effect on weed control at Allegan farm.

Table 4. Weed Control Summary Analysis of Variance (F values) for weed control of factorial combinations of three herbicides and contrast of herbicides treatments with and without mulch. Analyses based on assessments conducted 90 days after treatment (DAT).

Effect	Location/Tree Species				
	Allegan White Pine	**Horton Fraser Fir**	**Sidney Blue Spruce**	**Sidney Fraser Fir**	**Gobles Scotch Pine**
Clopyralid (Clo)	15.26 ***	4.46 *	0.25	8.18 **	0.03
Glyphosate (Gly)	2.7	10.71 **	1.35	6.32 *	1.17
Oxyfluorfen (Oxy)	8.37 **	38 ***	3.34	4.08	5.31 *
Clo × Gly	6.12 *	1.13	0.07	0	5.93 *
Clo × Oxy	2.45	0.61	0.41	3 **	3.17
Gly × Oxy	4.56 *	0.14	1.48	0.21	13.97 ***
Clo × Gly × Oxy	10.57 **	0.76	3.45	0.19	0
Contrast: Combinations with mulch vs. without	3.18	10.96 **	45.55 ***	2.05	0.03

Note * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

3.2. Phytotoxicity

Phytotoxicity levels varied by species, with white pine and scotch pine experiencing fewer phytotoxic effects than other species. At 30 DAT (Table 5), mulch + clopyralid + glyphosate (11% to 37%) caused a high amount of phytotoxicity at all farms except for Allegan farm. Clopyralid + glyphosate (7% to 38%) demonstrated a high amount of phytotoxicity at Gobles and Sidney (Fraser Fir). Oxyfluorfen + glyphosate (13% and 35%) and clopyralid + oxyfluorfen + glyphosate (7% and 41%) had high levels of phytotoxicity at Allegan and Horton. Mulch + oxyfluorfen + glyphosate (8%), had high phytotoxicity at Allegan farm. Clopyralid (8% and 9%) and clopyralid + oxyfluorfen (10% and 10%) indicated phytotoxicity at the Gobles and Allegan farms. Glyphosate (15%) also showed phytotoxic effects at the Gobles farm. At all farms, mulch and control provided 0% phytotoxicity; at 30 DAT, many of the other treatments did not show a real difference from the highest phytotoxicity levels or these 0% treatments. The only differentiated treatment was clopyralid (5%) at the Sidney (Fraser fir) farm. As time went on, many treatments significantly decreased in terms of phytotoxic effects, decreasing to ~0% phytotoxicity. Clopyralid resulted in less than 1% phytotoxicity at 90 DAT (Supplemental Table S7) at all farms except for Horton farm. Based on the mulch effect contrast (Table 6, Supplemental Tables S8 and S9), there was no significant mulch effect at 30 DAT at any farm. At 60 DAT, Fraser fir plots at Sidney farm showed a significant increase (1.29%) in phytotoxicity due to the presence of mulch. At 90 DAT, Horton and Gobles farms showed an increase in phytotoxicity of 1% and 6% in plots treated with mulch. At 30 DAT, the presence of clopyralid had a significant effect for the Horton farm phytotoxicity. Glyphosate significantly affected phytotoxicity at Horton and the Fraser fir plots at Sidney. Gobles farm had a significant effect on phytotoxicity from clopyralid + glyphosate and clopyralid + oxyfluorfen. Both species at Sidney farm had a highly significant change in phytotoxicity from the glyphosate + oxyfluorfen treatment. Gobles had a significant change in phytotoxicity due to the three-herbicide combination and the combination of glyphosate and oxyfluorfen. Allegan farm had a significant change in phytotoxicity from oxyfluorfen and the combinations of clopyralid and glyphosate and clopyralid and oxyfluorfen.

Table 5. Mean phytotoxicity percent 30 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 1), alone or in combination, at Christmas tree farms in Michigan.

Treatment	Location/Tree Species				
	Allegan White Pine	**Horton Fraser Fir**	**Sidney Blue Spruce**	**Sidney Fraser Fir**	**Gobles Scotch Pine**
Clopyralid	9.25 a *	17.19 ab	4.06 ab	5.78 bc	8.32 a
Glyphosate	3.68 ab	10.64 ab	7.20 ab	23.25 ab	15.15 a
Oxyfluorfen	6.25 ab	2.94 ab	4.99 ab	12.38 abc	4.99 ab
Oxyfluorfen + Glyphosate	13.15 a	35.62 a	7.73 ab	25.00 ab	4.12 ab
Clopyralid + Oxyfluorfen	10.21 a	6.10 ab	15.79 ab	12.12 abc	10.02 a
Clopyralid + Glyphosate	6.10 ab	11.61 ab	7.65 ab	38.51 a	7.30 a
Mulch	0 b	3.69 ab	0 b	0 c	0 b
Mulch + Oxyfluorfen + Glyphosate	8.32 a	5.50 ab	17.66 ab	31.46 ab	5.82 ab
Mulch + Clopyralid + Oxyfluorfen	5.82 ab	6.25 ab	7.30 ab	18.36 ab	2.18 ab
Mulch + Clopyralid + Glyphosate	6.65 ab	15.69 ab	32.77 a	37.36 a	11.64 a
Clopyralid + Oxyfluorfen + Glyphosate	7.91 a	41.19 a	4.26 a	24.03 ab	5.14 ab
Control	0 b	0 b	0 b	0 c	0 b

* Means within a column followed by the same letter are not significantly different at the $p < 0.05$ level. Mean separation by Tukey's HSD level.

Table 6. Phytotoxicity Summary Analysis of Variance (F values) for phytotoxicity of factorial combinations of three herbicides and contrast of herbicides treatments with and without mulch. Analyses based on assessments conducted 30 days after treatment (DAT).

Effect	Location/Tree Species				
	Allegan White Pine	**Horton Fraser Fir**	**Sidney Blue Spruce**	**Sidney Fraser Fir**	**Gobles Scotch Pine**
Clopyralid (Clo)	5.04 *	2.84	0.03	2.46	3.46
Glyphosate (Gly)	1.83	11.6 **	0.04	24.8 ***	2.98
Oxyfluorfen (Oxy)	9.47 **	2.61	3.74	1.79	0.01
Clo × Gly	6.89 *	1.59	0.34	0.11	10.14 **
Clo × Oxy	5.85 *	0.81	1.32	2.85	0.09
Gly × Oxy	0.13	3.74	5.38	5.88 *	10.3 **
Clo × Gly × Oxy	0.36	1.31	0.41	0.08	5.03 *
Contrast: Combinations with mulch vs. without	0.59	0.14	0.8	0.81	0.02

Note * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3. Growth Indices

For all farms, weed control treatments did not affect ($p > 0.05$) the growth index (Table 7). Time had a significant effect ($p < 0.05$) on growth indices for Gobles farm, Gwinn farm, and Allegan farm. At Gobles (Scotch pine) and Horton, the interaction of the treatment × DAT was significant ($p < 0.05$). The only farm that had any significant differences between treatments at any DAT was Gobles farm. At Gobles farm, at 90 DAT (Table 8), trees in the control plots were larger (68.37 cm) than trees receiving the clopyralid, oxyfluorfen, oxyfluorfen + glyphosate, and mulch + clopyralid treatments (44–49 cm).

Table 7. Average across DAT growth indices by farm (in cm) for all farms where there were no significant differences in growth indices between treatments (Table 1) in any given DAT.

Treatment	Allegan	Horton	Sidney Blue Spruce	Sidney Fraser Fir
1. Clopyralid	26.46 a *	46.20 a	43.59 a	64.72 a
2. Glyphosate	34.39 a	47.73 a	47.26 a	62.86 a
3. Oxyfluorfen	29.30 a	45.27 a	45.16 a	60.93 a
4. Oxyfluorfen + Glyphosate	30.48 a	41.45 a	48.84 a	66.49 a
5. Clopyralid + Oxyfluorfen	30.35 a	53.52 a	45.38 a	67.12 a
6. Clopyralid + Glyphosate	33.05 a	41.49 a	40.18 a	70.09 a
7. Mulch	28.89 a	39.68 a	40.29 a	67.29 a
8. Mulch + Oxyfluorfen + Glyphosate	28.89 a	41.75 a	37.25 a	58.66 a
9. Mulch + Clopyralid + Oxyfluorfen	31.42 a	40.53 a	47.49 a	65.03 a
10. Mulch + Clopyralid + Glyphosate	34.63 a	41.63 a	38.41 a	65.88 a
11. Clopyralid + Oxyfluorfen + Glyphosate	29.53 a	40.61 a	43.89 a	63.37 a
12. Control	30.40 a	44.52 a	43.86 a	64.32 a

* Growth indices followed by the same letter are not significantly different within a column.

Table 8. Gobles farm growth indices (cm) at 90 DAT for each of the 12 treatments. Growth indices were measured by taking the average of two widths and one height for each tree. Out of all farms, only Gobles farm at 90 DAT had a significant treatment effect on growth indices, meaning that only at this farm and time did trees show a size difference based on what treatments were applied.

Treatment 90 DAT **	Gobles
1. Clopyralid	44.87 b *
2. Glyphosate	58.74 ab
3. Oxyfluorfen	43.36 b
4. Oxyfluorfen + Glyphosate	44.98 b
5. Clopyralid + Oxyfluorfen	55.67 ab
6. Clopyralid + Glyphosate	51.96 ab
7. Mulch	53.76 ab
8. Mulch + Oxyfluorfen + Glyphosate	56.20 ab
9. Mulch + Clopyralid + Oxyfluorfen	48.37 b
10. Mulch + Clopyralid + Glyphosate	60.33 ab
11. Clopyralid + Oxyfluorfen + Glyphosate	64.56 ab
12. Control	68.37 a

** DAT represents days after treatment application; * Growth indices followed by the same letter are not significantly different within a column.

3.4. Foliar Nitrogen

Weed control treatments groups did not affect ($p > 0.05$) the foliar nitrogen concentration. All treatments resulted in trees that had a foliar nitrogen content ranging from 1.77% to 1.96% (Table 9).

Table 9. Mean foliar nitrogen percent, for all farms combined, of the foliar samples collected from the five treatment groups: control (no treatment), two or more herbicides, one herbicide, mulch + herbicides, and mulch only. Means within a column followed by the same letter are not different at the $p < 0.05$ level. Mean separation by Tukey's HSD.

Treatment Group	Foliar Nitrogen Percent
Control	1.96 a *
Two or More Herbicides	1.90 a
One Herbicide	1.90 a
Mulch + Herbicides	1.80 a
Mulch	1.77 a

* Foliar N percents followed by the same letter are not significantly different within a column.

4. Discussion

There are many weeds common in Christmas tree production that have developed resistance to clopyralid and glyphosate, meaning weed control options are decreasing for growers [8]. The use of organic mulch and mixing herbicides with different modes of action could allow for the control of these resistant weeds and provide novel integrated weed control measures. The goal of this research was to evaluate some of the most common postemergence herbicides used in Christmas tree weed management (glyphosate, clopyralid, and oxyfluorfen), as well as to test the potential synergism between mulch and postemergence herbicides. The results show that weed control and phytotoxicity were variable and dependent on the farm, tree species, and weed species, but generally, mulch combined with herbicides provided the best weed control.

Based on the ANOVA comparing all treatments at 90 DAT in Gobles, Allegan, and Horton, clopyralid provided the lowest level of weed control, aside from the control treatment, possibly due to the presence of clopyralid-resistant common ragweed, which was recently discovered in Michigan [11]. Glyphosate also had relatively low levels of weed control across farms. There are clopyralid-resistant horseweed and glyphosate-resistant horseweed [8], due to these being the dominant weeds at Allegan and Gobles farms; this could explain the lack of weed control. It is possible that there were new and different weeds that developed after treatments were applied at 90 DAT. It also could have had poor control because treatments were applied when the weeds were too large. It was found that singular herbicide treatments (with a single mechanism of action) were the least effective, likely due to the presence of herbicide-resistant weeds. It is easier for weeds to overcome one mechanism of action, but when more than one is combined, or combined with organic mulch, it is harder for weeds to develop resistance [22]. Based on the factorial analysis, no single herbicide had any stronger effect on weed control than any other herbicide; however, the combination of two herbicides had a very strong effect. In multiple cases, mulch improved the effectiveness of herbicides in controlling weeds and improved the longevity of weed control. Mulch could allow herbicides to be effective for longer, prevent new weeds from emerging, and potentially have allelopathic effects on weeds. Previous studies have shown that organic mulch can bind herbicide molecules and help them to last longer by reducing the leaching and runoff of herbicides. Saha et al. [23] studied the effects of various herbicide and mulch combinations and treatments on weed control in nursery production and found that, especially for large crabgrass and garden spurge, all treatments involving mulch and herbicide combinations showed very high levels of weed control, ranging from 88% to 100% in all cases. This agrees with what we found: mulch and herbicide combination treatments provide better weed control than herbicides alone. According to Derr [24], the herbicide dichlobenil, when combined with mulch, provided weed control for a year after application, but when dichlobenil was applied alone, it did not control weeds for a year. For example, combining pine nuggets with oxyfluorfen or pendimethalin provided excellent weed control [24]. In our study, based on the direct comparison of the same treatments with and without mulch (i.e., clopyralid + glyphosate and mulch + clopyralid + glyphosate), we found that the presence of mulch, when directly compared to the same treatments without mulch, significantly improved the weed control at 90 DAT for Horton and Sidney blue spruce farms. Treatments with mulch still showed higher levels of weed control than other treatments based on the standard ANOVA; however, there was only a direct increase when comparing the same treatments with and without mulch at these two farms. Future studies are needed to further characterize the benefits of mulch and the reasons for increased weed control. Further studies are also needed to determine if improved weed control with the herbicide combination treatments is due to the combination of herbicides or due to the increased overall amount of herbicide.

The herbicides used also have varying levels of soil persistence, with glyphosate having none [25] and oxyfluorfen having some persistence, as it can also be used as a preemergence herbicide [3]. Glyphosate is a water-soluble herbicide, so it will only bind with soils under certain conditions, and those conditions are usually only achieved in clay soils [26]. The web soil survey from the USDA natural resources (Table 2) indicated that most of the farms used had loamy sand or sandy loam soils. Mantzos et al. [27] looked at the persistence of oxyfluorfen in soil, water, and sunflowers. They found that oxyfluorfen moves very little in the soil and is not a threat for runoff or leaching but is therefore persistent in soil [27] and likely to provide longer weed control for Christmas trees. Notably, at Gobles and in the blue spruce plots at Sidney, oxyfluorfen showed the highest level of weed control out of the singular herbicides at 90 days, likely due to the persistence in the soil. Herbicide persistence may also lead to longer-term differences in herbicidal effects, and therefore, this may warrant future experiments that account for multiple-year effects.

Integrated weed management options with glyphosate combined with one or more herbicides showed the highest amount of phytotoxicity at most farms at 30 DAT; however, glyphosate alone had low phytotoxicity at most farms. Among the herbicides tested, clopyralid is considered safe to apply over the top of conifers [7], while glyphosate and oxyfluorfen are not [24]. For example, in a trial in Oregon conducted by Coate [28], clopyralid did not cause phytotoxicity in 10 different conifers, including western white pine [28]. The present study was conducted to reassess the level of phytotoxicity to these particular species of Christmas trees; overall, relatively low levels of phytotoxicity were observed. Naturally, the least phytotoxic treatments were mulch and control. Out of all singular herbicide treatments, oxyfluorfen was often the least phytotoxic. Richardson and Zandstra [29] conducted four studies to determine the Christmas tree tolerance and weed control of flumioxazin as well as other herbicide treatments including oxyfluorfen. The visual injury rating of Fraser fir did not exceed 6% when treated with oxyfluorfen [29]. Contrary to our original hypothesis, the addition of mulch led to a slight increase in phytotoxicity at Horton and Gobles farms, and at Gobles farm and the Fraser fir plots at Sidney farm, the most phytotoxic treatments at 30 DAT were clopyralid + glyphosate and mulch + clopyralid + glyphosate. Generally, as time went on, the phytotoxic effects decreased at all farms. Overall, there were fewer phytotoxic effects in scotch pine and white pine than in the other species. Willoughby studied broad-spectrum herbicides in forestry during the dormant season. Willoughby found that when glyphosate was applied to Scotch pine, even at three times the normal rate, there was a survival rate of 96% to 100%. [30]. In the present study, herbicides were applied to actively growing trees, which is not recommended, but they still showed relatively low levels of phytotoxicity. Grover [31] looked at the effects of 15 herbicides on 3 species of Christmas trees, including blue spruce and Scotch pine. Grover found that the only herbicides that reduced the survival of either species were norea (a preemergent photosynthesis inhibiting herbicide), which reduced Scotch pine survival to 59%, and pyrazon (a preemergence or early postemergence photosystem II inhibiting herbicide), which reduced blue spruce survival to 72%. PCP also caused the bleaching of Scotch pine, but they recovered. After two growing seasons, the shoot height of both species was drastically reduced as a result of many of the treatments [31]. Both studies confirm our results indicating that pine species can withstand greater herbicide pressure than many other species. It is most likely that all species were actively growing when treatments were applied, as they were applied during the month of June. Continued research with better species replication is required to gain a fuller understanding of the species' effect on phytotoxicity.

The growth impacts of chemical weed control represent an integration of potentially off-setting effects of an improved growing environment (reduced competition for light, water, and nutrients) versus the potential negative impacts of phytotoxic damage. No farm aside from Gobles showed any difference in growth indices, probably because subsequent-year growth is more likely to be affected due to phytotoxic effects; as conifers demonstrate, determinate growth and new-year growth may be negatively influenced by herbicides present in the plant, while meristematic tissue was being produced in the previous year [31]. Grover observed that 60% weed control was required for the optimal growth of spruce species, whereas for Scotch pine, only 40% was needed [31]. This warrants subsequent-year studies to investigate the long-term risks of using herbicides on Christmas trees, how growth is impacted over time, and how much weed control is necessary in order to have maximum tree growth.

Nutrient tie-up from organic mulch is a frequently mentioned concern among Christmas tree growers. The decomposition of soil organic matter by microbes utilizes soil nitrogen; however, the effects of mulch on plant nutrition are variable [18–20]. In the current study, mulch did not affect foliar N, which ranged from 1.75 to 1.95%. The Oregon Christmas tree nutrient management guide recommends 1.4 to 1.9% foliar N content, depending on the species, for Douglas-fir Grand fir, Nordmann fir, and Noble fir [32]. Thus, the foliar N value we reported is adequate N for all the above species, aside from Grand fir. It is important to note that the foliar N levels were slightly lower with organic mulch, and with long-term use, mulch could decrease the levels to below adequate amounts.

5. Conclusions

This experiment evaluated different weed control options for use in Christmas tree production. Herbicides in combination with mulch resulted in better, longer-lasting weed control, but integrated treatments including glyphosate, even if mulch was also included, proved to be the most phytotoxic treatments for the Christmas trees at 30 DAT. Growth indices would likely only be affected later in the life of the tree, and foliar nitrogen percent levels were found not to be affected by the use of mulch or any of the treatment combinations. The use of integrated weed management options is extremely important for successfully controlling weeds in Christmas tree production, as only using one weed control method is much more likely to exert excessive selection pressure on weeds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14050881/s1.

Author Contributions: Conceptualization, D.S.; methodology, D.S.; 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. and E.P.; 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. Limited data are available in the Supplementary Material.

Acknowledgments: We would like to thank Dan Wahmhoff, Kevin Mohrland, Rex Korson, and Mike Gwinn for providing space and Christmas trees in their farms for conducting this study. Additionally, we would thank the statistical consulting center in the College of Agriculture and Natural Resources at Michigan State University for the assistance with the data analysis. We would also like to thank Ashley Jeon (Undergraduate Research Assistant, Department of Horticulture, Michigan State University) for helping in setting up the field experiments and data collection.

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

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