DEVELOPING INTEGRATED STRATEGIES TO ADDRESS EMERGING WEED MANAGEMENT CHALLENGES IN CHRISTMAS TREE PRODUCTION

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ABSTRACT

Weeds must be well managed during the establishment phase (1-3 years) of Christmas tree production as weed competition directly relates to the rate of Christmas tree growth during this time. The objectives 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 and also to determine alternative postemergence control options for clopyralid resistant common ragweed (*Ambrosia artemisiifolia* L.), which has recently been discovered in Michigan Christmas tree farms. A field and greenhouse experiment were conducted. Four species of Christmas trees in their establishment stage were used for the field experiment: 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 applied for the field and greenhouse experiment with four replications each in complete randomized block design. The treatments were cypress bark organic mulch and the herbicides clopyralid, oxyfluorfen, and glyphosate, which were either applied alone or in combinations with each other. It was found that herbicides in combination with mulch resulted in better longer lasting weed control, but treatments involving clopyralid + glyphosate, even if mulch was also included proved to be the most phytotoxic treatments to the Christmas trees. Growth indices would likely only be affected later in the tree's life and foliar nitrogen percent levels were found not to be affected by the use of mulch or any of the treatment combinations. Overall, the best treatments for controlling clopyralid resistant common ragweed were mulch + clopyralid + glyphosate and mulch + clopyralid + oxyfluorfen.

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CHAPTER 1

LITERATURE REVIEW

Abstract

Christmas trees are sensitive to weed competition, especially during establishment. In initial stages of the tree crop, weeds can utilize available soil moisture and trees may succumb to drought stress. In later stages, weeds can even interfere with production practices. Non-chemical weed control methods alone may not provide effective weed control. Chemical weed management strategies involve the use of preemergence and postemergence herbicides at the right timing and application rates. There are many herbicides that are used in Christmas tree production in the United States, and each has specific application guidelines and weed control spectra. Moreover, crop trees vary in tolerance by species and tree age. Growers need to be careful while applying herbicides as many of these chemicals can cause injury to Christmas trees. Repeated application of herbicides with the same mechanism of action has resulted in development of herbicide resistance among several weed species. Managing herbicide resistance has now become an important issue. More research is required on identifying and managing herbicide resistance among weed species in Christmas tree production. Future research needs to focus on herbicide and mulch combinations, herbicide rotations, and tank mixing different herbicides with different mechanisms of actions and how these affect Christmas tree varieties.

1. Introduction

In the United States, Christmas trees are grown on around 15,000 farms which encompass nearly 350,000 acres [1]. This industry yields an average of $250 million in sales per year and employs more than 100,000 people [2]. There are many factors contributing to the successful production and profit margins within this industry, and these factors must be correctly implemented. In the United States, typical Christmas tree crops are produced on rotation lengths that vary from 8 to 12 years, depending on species and region of the country. Seedlings and transplants often are grown in nurseries for three to five years and then are transplanted into production fields. Throughout a given rotation, Christmas trees are sensitive to weed competition; therefore, weed control is a very pressing issue in Christmas tree production [3].

One important aspect to consider when planning for successful Christmas tree production is an adequate weed management plan, as weeds must be controlled for both aesthetic and biological reasons to create marketable Christmas trees [4]. There are some benefits to the presence of vegetative cover in Christmas tree plantations. These can include decreased erosion caused by wind and water, reduced nutrient leaching, improved microclimates, and increased biodiversity [5]. Therefore, complete elimination of non-crop vegetation (including weeds) is not necessary or recommended, but non-crop vegetation needs to be maintained at low densities [4]. The choice of chemical or non-chemical weed control often depends on weed pressure, size, and type of operations (such as wholesale vs. choose and cut), as well as growers' philosophy. Currently, there are few fully organic farms, but many growers are seeking to reduce chemical inputs. Non-chemical methods used to control weeds in Christmas tree production systems include mulching, mechanical control, livestock, cover crops, biological control, and thermal control. In our previous publication [6], we discussed the different non-chemical weed control strategies that can be applied to Christmas tree production. However, non-chemical weed control strategies alone cannot control weeds effectively and the methods are laborious, time consuming, and expensive. Therefore, chemical weed control is an essential component of effective weed management programs in Christmas tree production system. Most of the information related to chemical weed control strategies for Christmas trees is currently available in the form of extension factsheets, bulletins, newsletters, or as blogs, and hence there is strong need of a proper scientific literature review in this area. The purpose of this review is to provide an overview of chemical weed control strategies in Christmas tree production specific to the United States and to identify knowledge gaps where current practices could potentially be improved or on which further research is required. We focus our review and discussion on the United States because restrictions on the use of herbicides and the regulatory environment varies widely between the United States and Europe and between the United States and Canada.

2. Impacts of Weeds in Christmas Tree Production

During all stages of production, weeds can impede tree growth [7]. Problems caused by weeds include competition for water, nutrients, light, and space with the trees. Weeds can also harbor pathogens and pests. Weed control is especially important during the first three years after planting, when transplants are most sensitive to weed competition [7,8,9,10,11]. Weed competition, especially for water, throughout the establishment phase can reduce tree growth and can possibly result in the death of trees [12]. The level of tree growth is directly related to the extent of weed competition in the second and third years of the establishment phase [7]. Christmas trees are often grown in light-textured soils, and in these soils, weeds can take the limited available moisture which can increase tree drought stress. Seedlings and young trees can be shorter than the weeds and weeds can shade these trees this may result in reduced photosynthesis and therefore hinder leaf area development and subsequent growth [13].

For larger trees, weeds can interfere with production practices including spraying and pruning [7]. Weeds can even shade the lower branches of larger trees [14]. Weeds that are problematic in established plantations include broadleaves like 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). In addition, the seed head of grasses can grow into the trees and be difficult to remove. Common grasses include witchgrass (*Panicum capillare* L.), giant foxtail (*Setaria faberi* Herrm.), large crabgrass (*Digitaria sanguinalis* (L.) Scop) and fall panicum (*Panicum dichotoliflorum* Michx.). Vining weeds such as field bindweed, Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.), poison ivy (*Toxicodendron radicans* (L.) Kuntze), and wild grape (*Vitis spp.* L.) can grow and tangle in Christmas trees, making them difficult to remove. In situations where weeds are tangled within trees, herbicides cannot be used without risking injury to the trees [7]. The low branches of Christmas trees can be scratched by weeds, which can cause those needles to drop and brown and can cause a rough crown growth [15].

The most common means of weed control relied on by Christmas tree growers are mechanical mowing and chemical herbicide applications. However, there are several concerns with herbicides in Christmas tree plantations. Frequent applications of the same herbicides have developed herbicide-resistant weed species. For example, some Michigan Christmas tree growers have made recent reports of common ragweed resistance to clopyralid, a synthetic auxin herbicide, especially in Montcalm County [16]. Postemergence herbicides can result in severe phytotoxic injuries to Christmas trees including stunted growth, burning, and dropping of needles, chlorosis, and even complete death of the tree, particularly when new shoots have recently emerged [14]. Sensitivity to herbicides is often acute for newly planted seedlings and transplants because they often receive a larger proportion exposure from directed applications. In addition, herbicides can have an adverse environmental effect, such as herbicide leaching, drift, and run-off [4].

Weeds that are not managed can hinder Christmas trees at all stages of development. During the first three years in the plantation, adequate weed control is imperative to ensure that the trees are able to establish healthy root systems which allow them to withstand drought stress later in life [7]. This is something that needs to be considered because, after transplanting, the trees need to re-establish healthy and full root systems which have been damaged or lost during transplanting [17]. Dry summers and competition with weeds for soil moisture may cause up to an 80% mortality rate of transplants [18]. Weed competition directly relates to the rate of tree growth during their establishment phase. Thus, minimal weed competition during this establishment period will allow the trees to grow well.

When the trees get older, the main problems caused by weeds shift to issues with managing the trees by hindering activities, including spraying for pesticides and pruning the trees [2]. Large and poisonous weeds can pose a threat to the handlers who are performing these activities [7]. Weeds also can have adverse effects on the lower limbs of the trees through light competition, entanglement in the branches, and even by killing the lower branches [7]. Excessive weed growth can provide cover for mammals such as field mice, rabbits, deer, etc., which can have damaging effects on the Christmas trees. Weeds can negatively affect needle characteristics, reducing needle size and color quality (presumably due to nutrient competition)

[19]. Weeds can become a potential fire hazard during hot and dry seasons. For farms that market trees on a choose-and-cut basis, too many weeds can negatively affect the customer experience when the trees are being sold, by not allowing the customers to get close enough to the trees and/or posing a threat to the customers through the presence of dangerous weeds [20]. Similarly, weeds can interfere with harvesting operations on wholesale farms, as well.

3. Chemical Weed Control

Solely depending on non-chemical weed control methods may not result in an effective weed management in Christmas tree production. In addition, non-chemical weed control practices can be laborious, time consuming, and expensive. An effective chemical weed control includes weed identification and scouting, choosing, and applying preemergence and postemergence herbicides at their appropriate application rates and times. Growers need to choose the right herbicide product so that it does not cause any injury to the Christmas tree varieties. In this section, some of the important preemergence and postemergence herbicides that can provide effective weed control in Christmas tree production have been discussed.

3.1. Preemergence Herbicides

Preemergence herbicides are applied before the weeds have emerged. Most preemergence herbicides are applied to the soil and provide continuous control over weed emergence for longer periods of time as a residual [21]. These herbicides do not stop seeds from germinating but can prevent germinated weeds from becoming established. Preemergence herbicides work by inhibiting the growth of the roots, shoots, or both. To be effective, the herbicide must be incorporated into the soil and activated by addition of water from either rain or irrigation. These herbicides leave a residual when they are applied and, due to this, can control weeds for 8–12 weeks after application. Therefore, timing of herbicide application is critical to ensure emergence coincides with the residual window. Preemergent herbicides can be beneficial in that they often use different mechanisms of action from postemergence herbicides. However, preemergent herbicides are not very effective on their own and require combination with other (both chemical and non-chemical) weed control methods [4,22].

Young Christmas trees may be sensitive to preemergence herbicides, but once their roots are established deeper into the soil the risk of injury decreases. Preemergence herbicides should be applied soon after transplanting to reduce weed competition with the young trees. Well-established Christmas trees have a low risk of injury from preemergence herbicides because the herbicide stays near the soil surface and tree roots are much deeper [2]. There is a chance of injury if the herbicide levels build up in the soil and make their way to the root zone of the trees. This possibility can be decreased by using low solubility herbicides and altering mechanisms of action over time [7]. For best results, preemergence herbicides need to be applied to weed-free soils.

Preemergence herbicides that are labeled for use in Christmas tree production in the United States can be found in (Table 1.1). Preemergence herbicides can control both broadleaves (eudicots) and grasses (monocots). The specific herbicide indicates whether it is better utilized with broadleaves or grasses. For example, atrazine, simazine, oxyfluorfen, isoxaben, oxadiazon, and flumioxazin all control broadleaved weeds better than they control grasses, whereas napropamide, pendimethalin, s-metolachlor, oryzalin, and prodiamine all control grasses better than broadleaved weeds. For best results, growers should use a preemergence herbicide from each category [20].

Table 1.1 Preemergence herbicides labeled in the United States for use in Christmas tree production.

Active Ingredient	Trade Name	Mechanism of Action	WSSA Group [3]	Weeds Effective Against	Application Timing	Notes
Prodiamine	Barricade	Inhibits microtubule assembly	$3^{(K_1)}$ [4]	Many annual grasses [20]	After transplanting and prior to spring budbreak. Once trees are established, can be applied at any time over top or as directed spray [7].	Not recommended for trees under 1 year [7].
Prodiamine	Kerb	Inhibits microtubule assembly	$3^{(K_1)}$	Annual and perennial grasses, common chickweed (*Stellaria media* (L.) Vill.), and mustard weeds (*Sisymbrium officinale* (L.) Scop.)	In late fall when soil temperature is below 13 °C. Trees established over 1 year.	
Isoxaben	Gallery	Inhibits cell wall synthesis site B	$21^{(L)}$	Annual broadleaves [7]	Spring, before annual weeds germinate [7].	
Oxyfluorfen	Goal	PPO [1] inhibitor	$14^{(E)}$	Annual small seed broadleaves, established grasses are tolerant [4]	After seeding, or 5 weeks after seedling emergence. To established trees before budbreak or after new growth has hardened [7].	
Simazine	Princep	Photosystem II inhibitor	$5^{(C_1)}$	Many annual broadleaves and grasses as well as quackgrass (*Elymus repens* (L.) Gould) [7]	In fall or spring to dormant trees more than 2 years old [6]. To growing trees before or during rain [4].	
Pendimethalin	Pendulum AquaCap	Inhibits microtubule assembly	$3^{(K_1)}$	Annual grasses and broadleaves [7]	Over the top of trees or to soil between trees before weeds germinate. After soil has settled around new transplants [7].	
Dichlobenil	Casoron	Inhibits cell wall synthesis site A	$20^{(L)}$	Perennials like Canada thistle and horsetail [4]	In midwinter directly before a cold rain. Only use with well-established trees [4].	
Oryzalin	Surflan	Inhibits microtubule assembly	$3^{(K_1)}$	Annual grasses and some broadleaves [7]	To bare soil after transplant, requires cultivation or 13 mm rain [4].	Safe for pine and fir (not Douglas-fir) [4].

Table 1.1 (cont'd)

S-metolachlor	Pennant Magnum	VLCFA [2] synthesis inhibitor	$15^{(K_3)}$	Annual grasses, pigweeds (*Amaranthus* sp. L.), yellow nutsedge (*Cyperus esculentus* L.), nightshades (Solanaceae family) [7]	In spring prior to weed emergence [7].
Indaziflam	Marengo	Inhibits cellulose biosynthesis	$29^{(L)}$	Grasses, sedges, broadleaves [4]	Use on trees established in the field at least 1 year. As a directed spray to soil at base of trees [7].
Oxadiazon	Ronstar	PPO inhibitor	$14^{(E)}$	Many annual broadleaves and some grasses [20]	Before or at least 4 weeks after budbreak [20].
Napropamide	Devrinol	VLCFA synthesis inhibitor	$15^{(K_3)}$	Many annual grasses not good for broadleaves or perennial grasses [20]	In cool season or irrigate into soil [20].

[1] PPO: Protoporphyrinogen oxidase; [2] VLCFA: Very long-chain fatty acids; [3] WSSA stands for Weed Science Society of America. WSSA has classified herbicides based on their different mechanism of actions. [4] The number refers to the WSSA classification category and the letter plus subscript next to each number refers to the Herbicide Resistance Action Committee classification system which has both a letter and a subscript sub-category

Isoxaben provides excellent control for broadleaved weeds but does not provide good control of grasses. Isoxaben can be used to control triazine resistant weed species. It can be used on firs (*Abies spp*. Mill.), pines (*Pinus sp*. L.), and spruces (*Picea sp.* Mill.). There is little foliar activity, so isoxaben can be applied over the tops of trees [20] and should be applied before annual weeds emerge in the spring [7].

Napropamide, prodiamine, pendimethalin, and oryzalin all move slowly in the soil, meaning they need to be applied a few weeks prior to weed germination so that they can move within the soil before the weeds germinate. These preemergence herbicides can provide exemplary long-term control of annual grasses but will not control perennial grasses growing from rhizomes or stolons or broadleaved weeds. They must be applied when the temperatures are below 7 °C or irrigated in as they degrade in heat and sunlight. Prodiamine must also be applied in temperatures below 7 °C. Prodiamine provides good control of both annual and perennial grasses and some broadleaved weeds [20]. It is recommended to make one application of prodiamine in the fall. Prodiamine, pendimethalin, and oryzalin are all Weed Science Society of America (WSSA) group 3, microtubule inhibiting herbicides, which all require rainfall or other water soon after their application for activation. Oryzalin is safe for use with pine (Pinus sp.) and firs (*Abies sp*.), not including Douglas-fir {*Pseudotsuga menziesii* (Mirbel) Franco}. Prodiamine should not be used on trees in the first year after planting [4].

Oxadiazon is a WSSA group 14, protoporphyrinogen oxidase (PPO) inhibiting herbicide. It provides excellent control of annual broadleaves and good control of grasses. Oxadiazon remains on the soil surface for a long time because it has very low water solubility. It works by creating a barrier on the soil surface which kills weeds as they emerge. This works for annual broadleaves but not perennial broadleaves with established root systems. Oxadiazon can control grasses only for the short term, and hence it is recommended to combine this herbicide with one more suited for grasses [20].

Indaziflam is a WSSA group 29, cellulose biosynthesis inhibitor, which needs to be applied in fall or spring prior to weed emergence as a spray directed at the soil. It needs water/irrigation for activation. Indaziflam can cause damage to new tissue if applied over trees. It works to control grasses, sedges, and broadleaves and is persistent [4]. Indaziflam hinders root growth in the top inch of soil. It is persistent and will remain in this location for a relatively long time. It is not effective on established plants, and the presence of existing vegetation can obstruct its activity as a preemergence herbicide [23].

S-metolachlor is a group 15, very long chain fatty acid (VLCFA) inhibiting herbicide. It provides good control of annual grasses, but not as good as some others on this list, and provides outstanding control of nutsedge, which sets it apart. It should be applied in early spring, prior to budbreak of the Christmas tree. It can injure plants if sprayed over top, especially white pine (*Pinus strobus* L.) [20].

Dichlobenil is a group 20, cell wall synthesis at site A inhibitor. Dichlobenil is used to control difficult perennials such as Canada thistle (*Cirsium arvense* L.) and horsetail (*Equisetum arvense* L.). It should be applied in midwinter right before a cold rain. It should only be used on established trees and not on trees within one year of transplant [4].

Simazine, which is known under many different trade names, is a group 5, photosystem II inhibiting herbicide. It should only be applied to dormant trees unless it is raining [4]. Simazine controls broadleaved weeds and grasses [20]. Overall weed control can be improved by combining simazine with a preemergence herbicide that controls grasses. Lower rates of simazine can be used on field-grown spruce than on firs or pines [24]. The authors of [25] compared efficacy and conifer seedling mortality of simazine and atrazine. Atrazine, when mixed with simazine, was most successful at controlling weeds and had negligible impact on the seedling mortality rate [26].

3.2. Postemergence Herbicides

Postemergence herbicides are applied after the weeds have already emerged from the soil. They usually do not interact with the soil, instead killing the shoots and leaves of the weeds. Once they are absorbed by the plant, postemergence herbicides usually work by causing cellular membranes to rupture, impeding the production of essential compounds including amino acids or fatty acids, or by altering growth via hormone mimicry [4]. There are two different classifications of postemergence herbicides: systemic and contact. Translocated herbicides work better to kill perennial weeds, while contact herbicides work well against annual weeds but do not work against perennial weeds unless they are applied repeatedly. Annual weeds should be treated with contact herbicides when they are small. Perennial weeds, in contrast, should be treated with translocated herbicides when they are at least 30 cm long to provide greater area for the herbicide to be absorbed into the plant. Systemic postemergence herbicides include glyphosate, 2-4-D, and clopyralid. These herbicides can translocate through the plant and kill underground structures [4]. Examples of contact postemergence herbicides include pelargonic acid and diquat, which work through direct contact with the plant and only kill tissues with which the spray comes into contact [25]. Postemergence herbicides can also be categorized as either selective or non-selective. Selective herbicides will only kill what is on their label. On the other hand, nonselective herbicides will injure or kill nearly all plants [20].

Postemergence herbicides that are labeled for use in Christmas tree production in the United States can be found in (Table 1.2). Postemergence herbicides can be dangerous and phytotoxic to Christmas trees. The relative safety of post-emergence herbicides varies with chemical, tree species, and season, as nearly all trees are sensitive to post-emergence products during active shoot growth. If postemergence herbicides are used in the summer, they need to have good foliar activity, be safe on trees, and the sprays need to be directed to avoid tree injuries. Broadcast application of postemergence herbicides should be avoided between budbreak and the 1st of September [7].

Table 1.2 Postemergence herbicides that are labeled in the United States for use in Christmas Tree production.

Active Ingredient	Trade Name	Mechanism of Action	WSSA Group [5]	Weeds Effective Against	Application Timing	Notes
Glyphosate	Roundup	EPSPS [1] inhibitor	9(G) [6]	Most annual and perennial weeds, including woody weeds with multiple applications; does not control field horsetail [7]	After new growth has hardened in the fall. Do not contact new tree growth. Can also be applied before spring budbreak [24].	Woody weeds best controlled in September or August [24].
Sethoxydim	Segment	ACCase [2] inhibitor	1(A)	Annual and most perennial grasses [20]	To actively growing grasses [20].	
Clopyralid	Stinger	Synthetic auxin	4(O)	Controls legume, composites, plantains, nightshade, thistle, and smartweeds [7]	To susceptible weeds at 3–5 leaf stage. Canada thistle and spotted knapweed—apply a high rate before weed bud stage. Can be applied over tops of trees at any stage [7].	
2,4-D	Turret	Synthetic auxin	4(O)	Broadleaf, woody, and herbaceous weed species [7]	Before budbreak in spring. Can be applied over the top of Douglas-fir. As directed, spray for all other species. Do not spray tree foliage or apply to diseased or stressed seedlings [7].	
2,4-D	Defy Amine	Synthetic auxin	4(O)	Broadleaf, woody, and herbaceous weed species [7]	Before budbreak in spring or after new growth is hardened in late summer. Late summer applications to control woody weeds. Spray contact with tree foliage may cause injury [7].	Not safe for diseased or stressed seedlings [7].
Asulam	Asulox	DHP 3 (cell division inhibitor)	18(I)	Controls bracken ferns [7]	After hardening of new tree growth. Not by air and a maximum of one application per season [7].	
Clethodim	Envoy Plus	ACCase inhibitor	1(A)	Grasses [7]	To actively growing grasses [7].	
Fluazifop-P	Fusilade	ACCase inhibitor	1(A)	Grasses [7]	To actively growing grasses; perennial grasses may need more than one application for full control [7].	

Table 1.2 (cont'd)

Triclopyr triethyamine salt	Garlon	Synthetic auxin	4(O)	Broadleaf, woody, and herbaceous weed species [7]	In summer or early fall after conifer growth has hardened. Spray towards tree base, do not apply to trees established under 1 year [7].	Douglas-fir and white pine may be sensitive [7].
Glufosinate	Finale	Glutamine synthase inhibitor	10(H)	Many annual and perennial grasses and broadleaves [28]	Do not apply over tops of trees. Do not apply to actively growing trees [4].	
Bentazon	Basagran	PSII 4 site B inhibitor	6(C3)	Nutsedge and some broadleaved weeds [20]	Spray directly, do not apply over treetops [20].	

[1] EPSPS: 5-enolypyruvyl- shikimate- 3- phosphate synthase; [2] ACCase: Acetyl CoA Carboxylase; [3] DHP: 7,8- dihydro- pteroate synthetase; [4] PSII: Photosystem II; [5] WSSA stands for Weed Science Society of America. WSSA has classified herbicides based on their different mechanism of actions. [6] The number refers to the WSSA classification category and the letter plus subscript next to each number refers to the Herbicide Resistance Action Committee classification system which has both a letter and a subscript sub-category.

Glyphosate is a systemic, postemergence herbicide that is commonly used in Christmas tree production [20]. Glyphosate is a group 9 Shikimic acid pathway inhibitor which kills most annual and perennial weeds, and even woody weeds with multiple applications. It does not kill field horsetail. This herbicide should only be applied after growth has hardened in the fall and should not contact new growth [7]. For most Christmas tree species, glyphosate can be applied over the top of healthy completely dormant trees, though using directed sprays and avoiding direct contact with trees may be safer. By avoiding contact with trees, higher rates of glyphosate can be used to control deeply rooted weeds. Weeds need to be actively growing when glyphosate is applied, because it is absorbed through foliage and green stems and translocates throughout the plant. Therefore, it may take time for it to fully affect the weeds, especially in colder conditions. No additives should be included when applying glyphosate over top of dormant trees [15]. Glyphosate can be used with pines, spruces, and firs as well as other conifers [7]. Glyphosate becomes inactivated when it comes in contact with the soil. Therefore, tree roots are safe, and growers can plant into fields that have recently been treated with glyphosate [20]. Christmas trees can have semi directed basal sprays of glyphosate in the fall, late August, or September, or prior to spring budbreak. Douglas-fir and white pine are more vulnerable to glyphosate injury than true firs and spruces; therefore, these species should not have basal foliage sprayed until they are at least 0.6 m tall [24].

Clethodim, fluazifop-P, and sethoxydim are all group 1, Acetyl CoA Carboxylase (ACCase) Inhibitors [27]. They are selective systemic herbicides that work on annual grasses and most perennial grasses. These herbicides do not affect broadleaved weeds and are safe to use near Christmas trees in all periods of growth. They need to be applied to grasses that are actively growing [20].

Triclopyr triethylamine salt, clopyralid, and 2,4-D, are all group 4 synthetic auxin herbicides that are systemic [27]. Triclopyr triethylamine salt and 2,4-D selectively kill herbaceous and woody broadleaved weeds [20]. Clopyralid is selective to kill specific broadleaves, including legumes, composites, plantains, nightshade, thistles, and smartweeds [7]. Clopyralid does not affect grasses, sedges, or woody brush, and it is safe to spray it over established conifers [24]. Herbicides such as the volatile esters of 2,4-D are capable of causing injury to adjacent crops by movement in the vapor phase after the herbicide has dried on the soil or plant surface. The use of low-volatile esters, oil soluble amines, and dormant applications greatly reduces the hazard of injury to adjacent crops [28]. Phenoxy herbicides like 2,4-D and garlon can be dangerous to wine grapes and, considering Christmas trees are often grown with a variety of other crops or places nearby, such as grapes, organic farms, or schools, it is vital to be aware of the other crops or locations near the farm and avoid the drift of herbicides [29].

Glufosinate is a group 10, glutamine synthase inhibitor [27] that is a non-selective contact herbicide. It controls a variety of annual broadleaves and grasses. If grasses are particularly large or well tillered, then control is not as good. Glufosinate also suppresses perennial weeds. This herbicide is not active in the soil and has minimal translocation. It works best when weeds are small and actively growing [30].

Bentazon is a photosystem II site B inhibitor, group 6, selective herbicide [27]. It controls nutsedges as well as some other broadleaved weeds. Bentazon should be applied directly to the weeds as it can burn the needles of conifers, especially spruce and fir, if sprayed over top of them [20].

Asulam is a group 18 inhibitor of 7,8 dihydro-pteroate synthetase (DHP) [27]. Asulam should only be applied after new tree growth has hardened. It provides good control for many annual and perennial broadleaved weeds and grasses as well as dock species (*Rumex spp*. L.) and bracken ferns {*Pteridium aquilinum* (L.) Kuhn.} [27]. There should not be more than one application of asulam made per season [7].

3.3. Herbicides with Preemergence and Postemergence Activity

Weeds vary in their anatomy and physiology, which means that common herbicides have differing abilities to adequately control them. For example, some annual weeds can be easily controlled with preemergent herbicides; on the other hand, many perennial grasses and weeds, especially horsetails and sedges (*Cyperaceae*), are more challenging to control. One method to manage both annuals and perennials is to combine more than one herbicide in the spray tank. Often, preemergence herbicides are combined with postemergence herbicides to control both existing weeds and prevent new weeds from growing, however, it must be determined whether the herbicides are compatible with this [31]. Some herbicides can be used as both preemergence herbicides and postemergence herbicides. These herbicides can be applied over longer periods of time than herbicides that only fit into one category [4]. Herbicides with preemergence and postemergence activities that are labeled for use in Christmas tree production in the United States can be found in (Table 1.3).

Table 1.3 Herbicides with preemergence and postemergence activity that are labeled in the United States for use in Christmas Tree production.

Active Ingredient	Trade Name	Mechanism of Action	WSSA Group [4]	Weeds Effective Against	Application Timing	Notes
Hexazinone	Velpar	Photosystem II inhibitor site A	$5^{(C_1)}$ [5]	High rates effective against trailing blackberries [4]	In early April [4].	
Metribuzin + flufenacet	Axiom	Inhibits photosystem II site A and inhibits synthesis of VLCFA [1]	$5^{(C_1)}$ $15^{(K_3)}$	Chickweed (*Stellaria media* (L.) Vill.) and annual grass	To firs, including Douglas-fir, only. When trees are dormant. For trees established at least 1 year. Very early POST use only [4].	
Oxyfluorfen	Goaltender	PPO [2] Inhibitor	$14^{(E)}$	Many annual grasses and annual broadleaves [7]	Before spring budbreak and after new growth has hardened in fall. Can spray over trees unless actively growing [4]. Do not apply to stressed trees [7].	
Flumioxazin	SureGuard	PPO Inhibitor	14(E)	Selected grass and broadleaf weeds [4]	Must be applied prior to spring budbreak or after trees have hardened in fall [4].	Not safe for conifers before 2 years of emergence [4].
Flazasulfuron	Mission	ALS 3 inhibitor	2(B)	Many broadleaves and grasses less than 10 cm tall	Do not apply within 1 year of seeding trees. May be applied over the top in spring or after new growth has hardened in fall. As directed spray during growth.	

Table 1.3 (cont'd)

Hexazinone + sulfometuron	Westar	Photosystem II Inhibitor site A and ALS inhibitor	5(C1) 2(B)	Many broadleaves, annual grasses, and several perennial weed species	Broadcast to dormant trees. Out of dormancy must do directed applications to avoid contact with new growth [4].	Recommended for various firs including Douglas-fir [4].
Atrazine	Many	Inhibits Photosystem II Site A	5(C1)	Many broadleaf weeds and some grasses [7]	To soil before or after new transplants, or to dormant established trees in late fall or early spring [7].	
Lactofen	Cobra	PPO Inhibitor	14(E)	Many annual broadleaves up to 10 cm tall [7]	After seeding or transplanting and prior to budbreak. Not when conifers are stressed [7].	

[1] VLCFA: Very long-chain fatty acids [2] PPO: Protoporphyrinogen oxidase. [3] ALS: Acetolactate synthase. [4] WSSA stands for Weed Science Society of America. WSSA has classified herbicides based on their different mechanism of actions. [5] The number refers to the WSSA classification category and the letter plus subscript next to each number refers to the Herbicide Resistance Action Committee classification system which has both a letter and a subscript sub-category.

Atrazine, which is known under many different trade names, is a group 5, photosystem II inhibiting herbicide that should only be applied to dormant trees, unless it is raining [4]. Atrazine is closely related to simazine, but it is more soluble in water and better at controlling perennial weeds. Initially, applicators should treat with a mixture of the two herbicides, but once perennial grasses are under control, it is often better to just use simazine [24]. Atrazine and simazine have remarkably similar characteristics except for water solubility, as atrazine has shorter residual activity and can be absorbed by leaves, while simazine has longer residual activity but is less readily absorbed by leaves [20]. Atrazine and simazine provide excellent control of broadleaved weeds and fair control to grasses. Atrazine is most active in soils with a pH between 6.5 and 7.5. Breakdown of atrazine is slow when soil pH is below 6.5. If soil pH is raised, e.g., by liming, residual atrazine may be activated and cause injury to trees [20].

Flasaulfuron is a group 2 acetolactate synthase (ALS) inhibiting herbicide and provides for both preemergent and postemergent weed control. Flasaulfuron needs to be applied directly to avoid injury to actively growing trees, and it should not be applied within the first year of growth [4]. Flasaulfuron controls both annual grasses and annual broadleaves. This herbicide can be applied over top to dormant conifers and there must be a minimum of three months wait in between treatments [7].

Hexazinone, which is a group 5 herbicide, can be used on newly planted trees, but injuries have been observed. Hexazinone can be a groundwater hazard, therefore growers should apply it in early spring, rather than late winter, to reduce the likelihood of leaching. At high rates, this herbicide can be effective against trailing blackberries [4]. Hexazinone controls annual broadleaves and grasses well, including common ragweed, horseweed, and annual bluegrass (*Poa annua* L.) [7]. Like atrazine, hexazinone is absorbed by foliage and is only safe on most conifers during the dormant season—either before bud burst of tolerant firs, spruces, and Douglas-firs or after terminal growth has slowed in tolerant pines [32].

Hexazinone + sulfometuron is a combination of 68.6% hexazinone, a photosynthesis inhibitor, WSSA group 5% and 6.5% sulfometuron-methyl, and an acetolactate synthase inhibitor group 2. It works well with firs and Douglas-fir. Hexazinone + sulfometuron should only be applied through broadcast to dormant trees; if trees are not dormant, then applications must be directed so that the herbicide does not come into contact with new growth. This herbicide can also cause a groundwater hazard [4]. It provides good control of broadleaves and grasses as it is a combination of two herbicides [20]. It provides good control of most annual weeds, such as horseweed, common ragweed, and large crabgrass, for three to four months. Hexazinone + sulfometuron should only be used on trees that are at least four years old and have been established for at least one year, as it can stunt tree growth [7]. Metribuzin + flufenacet is a group 5 and group 15 herbicide. For Douglas-fir and true firs it should only be applied when trees are dormant and should not be applied until one year of growth has occurred [4].

Triazine herbicides (atrazine, simazine) have been largely responsible for the abundant supply of high-quality Christmas trees in the United States, as well as the success and improvement of reforestation in western coniferous forests. Due to its absorption by plant foliage as well as by roots, atrazine at agricultural use rates is not tolerated by most ornamental deciduous woody plants during active growth. Depending on dosage and plant species, atrazine can also injure actively growing conifers [33]. Since Christmas trees require several years from planting to harvest, the low cost of triazine herbicides is extremely important to the economics of Christmas tree production. The more recent herbicides registered for Christmas tree production cost 3–10 times as much as simazine or atrazine [34].

Source [35] found that hexazinone + sulfometuron can provide decent acceptable control of most weeds through July of each year. However, it did not provide adequate control of horseweed, which emerged in midsummer each year. Horseweed does well when there is less competition from other weeds, therefore horseweed increased more when weeds were controlled by hexazinone + sulfometuron plots than in the presence of other treatments, including untreated control. Hexazinone + sulfometuron treatments provided good weed control but reduced Fraser fir (*Abies fraseri* (Pursh) Poir.) height significantly when used for three years at 0.65 L Ha^{-1} at a site in Gobles, Michigan and at 0.43, 0.54, and 0.659 L Ha^{-1} at a site in Horton, Michigan [35].

Oxyfluorfen, lactofen, and flumioxazin are all WSSA group 14, protoporphyrinogen oxidase (PPO) inhibiting herbicides. Oxyfluorfen, lactofen, and flumioxazin should be applied right after transplanting, prior to budbreak, over the top of the tops of the trees or as a directed spray [4,7]. Flumioxazin and oxyfluorfen can also be applied after growth has hardened in the later season [6]. Source [36] found that many species of common Christmas tree weeds were controlled by flumioxazin, however, it showed poor control of white campion (*Silene latifolia* Poir.), dandelion (*Taraxacum sp.* Munz and I.M. Johnst.), and horseweed. There was also commercially adequate tolerance of Fraser fir and Colorado blue spruce (*Picea pungens* Engelm.) in all the trials [36].

3.4. Herbicide Resistance Management

Herbicide resistance is becoming a major problem in all crops, including Christmas trees. For example, there are reports from Michigan that common ragweed has become resistant to the clopyralid herbicide in Christmas tree production [16]. Herbicide resistance is the inherited capability of a plant to survive an herbicide application which would normally kill that plant, whereas herbicide tolerance is the ability of a species to survive and reproduce following a normal use rate of herbicide application. A species of weeds is considered resistant when an herbicide that previously controlled that weed no longer works. Herbicide resistance is more likely to occur when a singular mechanism of action of the herbicide is applied repeatedly [37]. Resistant weeds are a result of fundamental evolutionary processes. When there are certain resistant individuals in a population, upon application of that herbicide, the susceptible will die and the resistant will survive and reproduce. As the same herbicide is used increasingly, the resistant weeds will rapidly expand to become the majority population under selection pressure [38]. Herbicides (such as group 2, 9, and 5) that are only acting on a singular site of action are more likely to have weeds develop resistance than those that act on multiple sites of action. This can cause herbicide resistance to develop because it is only necessary for one gene in the plant to change in order to disrupt the binding potential of the herbicide. Not all herbicides that have the same mechanism of action will have cross resistance, because there may be different specific sites of action. As a result, it is not possible to predict if there will be cross resistance between herbicide families [39]. Weeds have developed resistance to 167 different herbicides, including 23 out of the 26 known mechanisms of action. There are herbicide resistant weeds that have been reported in 94 different crops in 71 countries [40].

Herbicide resistance can be prevented by integrating different weed control methods using chemical and non-chemical approaches. Another method to reduce the likelihood of developing herbicide resistance is to rotate among different mechanisms of action of herbicides. Using a tank mixture or combination of different mechanisms of action of herbicides together is another way to manage herbicide resistance among weed species. The speed at which resistance develops depends on the mechanism of action. For example, some group 2 herbicides are rather quick to develop resistance. Another method of resistance prevention is to monitor the weeds that are not killed by herbicides and to not let them mature and produce seed. Growers should clean equipment to prevent the spread of resistant weed seeds between areas [7]. Ground cover and mulches are another good method of weed control to manage herbicide resistance, as hard fescues {*Festuca trachyphylla* (Hack.) Krajina, nom. illeg.} have worked well for Christmas tree growers [18]. Rotating crops with different life cycles is another good method to avoid herbicide resistance, but this may not be very feasible on Christmas tree farms. Primary tillage, mechanical weed control, and field scouting for weeds are all good ways to reduce the chances of weeds becoming herbicide resistant.

Some primary examples of herbicide resistant weeds include weeds that are resistant to glyphosate, triazine herbicides, and ALS inhibiting herbicides. Glyphosate resistance is becoming a problem in annual ryegrass (*Festuca perennis* Lam.). A method to avoid glyphosate resistance is using different nonselective herbicides such as glufosinate or PPO inhibitors [19]. If glyphosate is used where there are resistant weeds, then it should be tank mixed with a different mechanism of action of herbicide and applied to the weeds while they are still small. Weeds with resistance should also not be allowed to produce seeds [19]. The triazine herbicides are another family to which resistance has developed. This includes atrazine and simazine. Weeds with resistant strains to these herbicides include pigweeds (*Amaranthus sp.* L.), lambsquarter (*Chenopodium album* L.), and horseweed. This resistance can be dealt with by using SureGuard. SureGuard can be alternated with triazine herbicides for resistance management [41]. ALS inhibiting herbicides have more plants that are resistant to them than any other mechanism of action. These herbicides are used often, and they also have a lot of soil residual. This combination lends to them being ideal targets for herbicide resistance [20]. The recurrent rate of weed populations becoming resistant to ALS inhibitors can be credited to the extensive use of these herbicides, how they are used, the strong selection pressure they employ, and the unique resistance mechanism [42]. (Table 4) lists the herbicides with different mechanisms of action that are commonly used in Christmas tree production in the United States.

Table 1.4 Common herbicides with their mechanisms of action used in Christmas trees and some common weeds that have developed resistance to respective mechanism of action. Adapted from [40].

WSSA Group [1]	Mechanism of Action	Herbicide Common Name	Resistant Weed Species
1	ACCase [2] Inhibitors	Clethodim, fluazifop-P, sethoxydim	Green Foxtail (*Setaria viridis* (L.) Beauv.) Barnyardgrass *(Echinochloa crus-galli* (L.) Beauv.)
2	ALS [3] Inhibitors	Flazasulfuron	Prickly lettuce (*Lactuca serriola*), Common Ragweed (*Ambrosia artemisiifolia* L.), Giant Ragweed (*Ambrosia trifida* L.), Common Lambsquarters (*Chenopodium album* L.), Horseweed (*Erigeron canadensis* (L.) Cronquist), Yellow Nutsedge (*Cyperus esculentus* L.), Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), Perennial Ryegrass (*Lolium perenne* L.)
3	Microtubule Inhibitors	Prodiamine, oxyfluorfen, pendimethalin, oryzalin	Green Foxtial (*Setaria viridis* (L.) Beauv.), Barnyardgrass *(Echinochloa crus-galli* (L.) Beauv.)
4	Synthetic Auxins	2,4-D, clopyralid, triclopyrtriethylamine salt	Wild Radish (*Raphanus raphanistrum* L.), Smooth Pigweed (*Amaranthus hybridus* L.), Wild Carrot (*Daucus carota* L.), Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), Prickly Lettuce (*Lactuca serriola* L.)
5	Photosystem II site A Inhibitors	Atrazine, hexazinone, simazine	Smooth Pigweed (*Amaranthus hybridus* L.), Common Ragweed (*Ambrosia artemisiifolia* L.) Common Lambsquarters (*Chenopodium album* L.), Horseweed (*Erigeron canadensis* (L.) Cronquist), Large Crabgrass (*Digitaria sanguinalis* (L.) Scop) Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), Green foxtail (*Setaria viridis* (L.) Beauv.)
6	Photosystem II Site B inhibitor 6(C3)	Bentazon	Smooth Pigweed (*Amaranthus hybridus* L.)
9	EPSPS [4] Inhibitor 9(G)	Glyphosate	Annual Ryegrass (*Festuca perennis* Lam.), Horseweed [*Erigeron canadensis* (L.) Cronquist], Bentgrasses (*Agrostis* spp. L.), Perennial Ryegrass (*Lolium perenne* L.), Common Ragweed (*Ambrosia artemisiifolia* L.), Smooth Pigweed (*Amaranthus hybridus* L.), Horseweed (*Erigeron canadensis* (L.) Cronquist), Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), Prickly Lettuce (*Lactuca serriola* L.), Perennial Ryegrass (*Lolium perenne* L.)
10	Glutamine synthase inhibitor 10(H)	Glufosinate	Perennial Ryegrass (*Lolium perenne* L.)
14	PPO [5] Inhibitors	Oxyfluorfen, flumioxazin, lactofen, oxadiazon	Smooth Pigweed (*Amaranthus hybridus* L.), Common Ragweed (*Ambrosia artemisiifolia* L.)
15	VLCFA [6] Synthesis inhibitor 15(K_3)	S-metolachlor, napropamide	Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.)
20	Inhibits cell wall synthesis site A 20(L)	Dichlobenil	Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.)
21	Inhibits cell wall synthesis site B 21(L)	Isoxaben	Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.)

Table 1.4 (cont'd)

29	Inhibits cellulose biosynthesis 29(L) [7]	Indaziflam	Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.)

[1] WSSA stands for Weed Science Society of America. WSSA has classified herbicides based on their different mechanism of action. [2] ACCase: Acetyl CoA Carboxylase. [3] ALS: Acetolactate synthase. [4] EPSPS: 5-enolypyruvyl- shikimate- 3- phosphate synthase. [5] PPO: Protoporphyrinogen oxidase. [6] VLCFA: Very long-chain fatty acids. [7] The number refers to the WSSA classification category and the letter plus subscript next to each number refers to the Herbicide Resistance Action Committee classification system which has both a letter and a subscript sub-category.

4. Conclusions

While chemical weed control can be highly effective in Christmas tree production, herbicide resistance is becoming a more pressing issue in all agricultural fields, and Christmas tree production is no exception. Currently, more research is focusing on herbicide resistance issues for agronomic crops only. However, there is a huge knowledge gap or little research is being conducted on herbicide resistance issues for Christmas tree production system. While there is research related to avoiding development of herbicide resistant weeds, there is little research on ways to manage herbicide resistance in Christmas tree production specifically. More research must be done on identification and confirmation of herbicide resistant weed species in Christmas tree farms and methods of avoiding herbicide resistance. One method that needs more research is combining multiple herbicides with different mechanisms of action to reduce the onset of herbicide resistance. New combinations or tank mixes of various herbicides with different mechanisms of action need to be tested for various weed control efficacies and their phytotoxic effects on the different Christmas tree varieties. Comparing the newer herbicide formulations with the older ones in terms of weed control efficacies is another area which has a significant knowledge gap and requires more research.

Successful weed management in Christmas tree production requires integration of chemical and non-chemical approaches. Among chemical weed control, applicators need to include and integrate preemergent and postemergent products as well as multiple mechanisms of action. In the future, research needs to focus on an integrated approach, as well. A study conducted by [43] on different organic mulch types, depths, and irrigation volume on common landscape weed control showed that pine bark mulch, when combined with a liquid formulation of preemergence herbicides at depths of 5 cm or more, can provide excellent weed control. Combining organic mulch with herbicides and understanding how these combinations interact with different Christmas tree varieties during their establishment stages require an in-depth study. Furthermore, the effects of mulch depths and particle size on herbicide leaching, efficacy, and residual effects need to be investigated for Christmas tree production.

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CHAPTER 2

DEVELOPING INTEGRATED STRATEGIES TO ADDRESS EMERGING WEED MANAGEMENT CHALLENGES IN CHRISTMAS TREE PRODUCTION

FIELD EXPERIMENT

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. We studied four species of Christmas trees in their 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) using a scale of 0% (no control/not phytotoxic) to 100% (complete control/tree death). Tree growth and foliar nitrogen concentration were also measured. Mulch combined with herbicide provided 60%-100% weed control in all cases. Combinations of mulch + clopyralid + glyphosate and clopyralid + oxyfluorfen + glyphosate resulted in the highest phytotoxicity ratings. Tree growth and foliar N did not differ among any of the treatments

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, three years post-transplant into the field [2] [3] [4] [5] [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 has 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, dropping of needles, chlorosis, and even 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 an 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, Missouri) 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 an 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 post emergence 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 control weeds. For example, in an interactive poll during an on-line 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 to be 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 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. Of methods tested, they found that sawdust was the best treatment, for long term weed control in Christmas tree production. The sawdust treatment increased 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 [15,16,17]. When organic mulches decay, it 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 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 to 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.

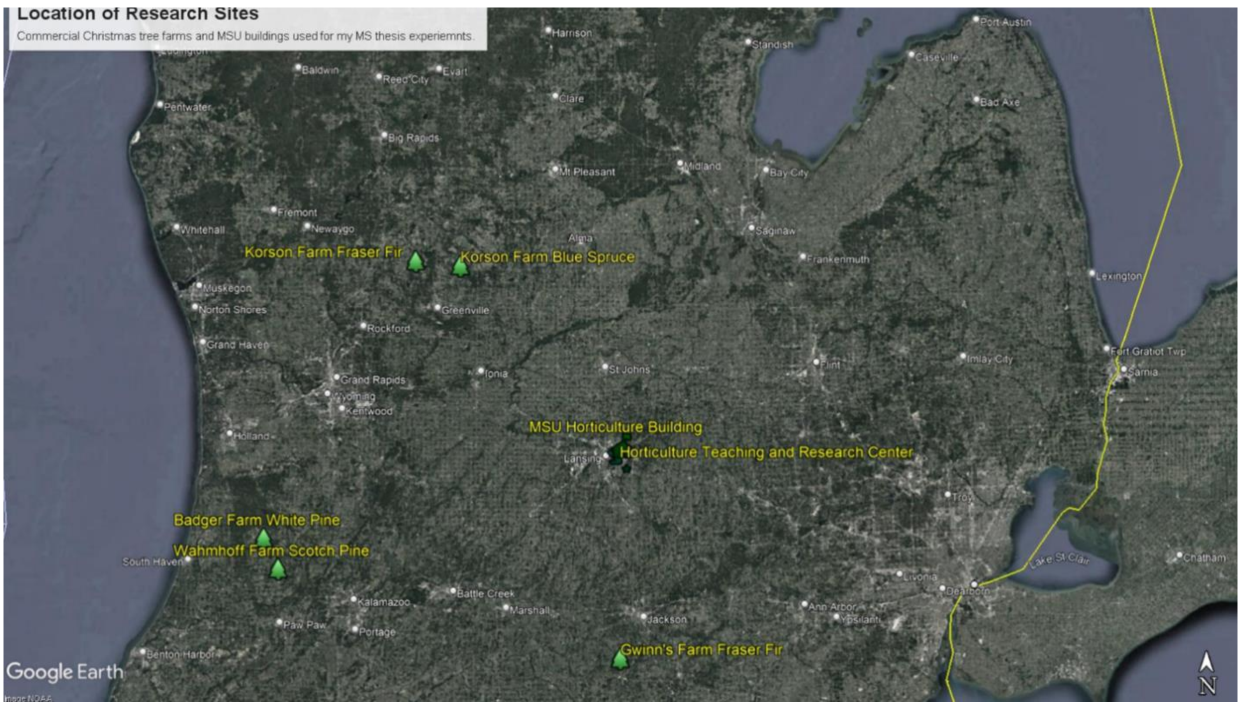
2. Materials and Methods

2.1 Plant Materials

The experiments for achieving objectives 1 and 2 were conducted at commercial Christmas tree farms located in mostly western Michigan (Fig 2.1). The trees at each farm were selected in late March to early April 2021. We installed plots in Horton, MI (Gwinn's Christmas Tree Farm) at 42° 4'32.80"N, 84°28'53.80"W, Gobles, MI (Wahmhoff Farms) at 42°20'15.70"N, 85°53'3.00"W, Allegan, MI (Badger Evergreen Nursery) at 42°25'47.60"N, 85°56'43.30"W, and Sidney, MI (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 transplants, 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).

Figure 2.1 Map of Michigan with farm locations marked with Christmas tree icons and labeled, where field experiments were conducted.



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 at Sidney farm were not irrigated but were fertilized on 2021-04-24, this was 14.5-0-6 (N-P2O5-K2O) at a rate of 113 grams per tree. Fertilizer was again applied to the blue spruce on 2022-04-20 this was 14-0-8 (N-P2O5-K2O) at a rate of 142 grams per tree. The Fraser fir at Sidney farm were irrigated at a rate of 2.12 liters per hour for 4 hours with emitters spaced every 60cm, on 2021-06-03, 2021-06-07, 2021-06-14, 2021-08-02, and 2021-08-19. The Fraser fir at Sidney farm were also fertilized on 2021-05-07, with 14.5-0-6 (N-P2O5-K2O) fertilizer at a rate of 113 grams per tree. They were again fertilized on 2022-05-19, with 14-0-8 fertilizer at a rate of 170 grams 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) (Fig 2.2). The Fraser fir at Horton farm were in three rows with four 3x4 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 one of 12 treatments *1) Clopyralid* applied at a rate of 0.58 L Ha^{-1} *2) Glyphosate* applied at a rate of 1.9 L Ha^{-1} *3*) *Oxyfluorfen* applied at a rate of 4.6 LHa^{-1} *4) Oxyfluorfen + Glyphosate* tank mixed and applied at a rate of 4.6 LHa^{-1} oxyfluorfen + 1.9 L Ha^{-1} glyphosate *5) Clopyralid + Oxyfluorfen* tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 4.6 LHa^{-1} oxyfluorfen *6) Clopyralid + Glyphosate* tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 1.9 L Ha^{-1} glyphosate *7) Mulch* (bagged cypress mulch blend (NoFloat cypress blend, Oldcastle Lawn & Garden, Atlanta, GA.) applied at a depth of 5cm and a diameter of 0.3m (Fig 2.3) *8) Mulch + Oxyfluorfen + Glyphosate* mulch applied first at a 5cm depth 0.3m diameter then shortly after oxyfluorfen + glyphosate tank mixed and applied at a rate of 4.6 LHa^{-1} oxyfluorfen +1.9 L Ha^{-1} glyphosate *9) Mulch + Clopyralid + Oxyfluorfen mulch* applied first at a 5cm depth and 0.3m diameter then clopyralid + oxyfluorfen tank mixed and applied at a rate 0.58 L Ha^{-1} clopyralid + 4.6 LHa^{-1} oxyfluorfen *10)*

Mulch + Clopyralid + Glyphosate mulch applied first at a 5cm depth and 0.3m diameter then clopyralid + glyphosate tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 1.9 L Ha^{-1} glyphosate *11) Clopyralid + Oxyfluorfen + Glyphosate* tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 4.6 LHa^{-1} oxyfluorfen + 1.9 L Ha^{-1} glyphosate *12) Control* which consisted of no herbicides or mulch applications (Table 2.1). Treatment applications for Horton farm were made on 2021-05-27. On 2021-06-11, treatments were applied to Sidney farm. Gobles and Allegan farm treatments were applied on 2021-06-17.

Table 2.1 Weed control treatments and rate of applications used in field and greenhouse experiments.

Treatments	Rate of applications (highest labeled rate)
Clopyralid	0.58 L Ha^{-1}
Glyphosate	1.9 L Ha^{-1}
Oxyfluorfen	4.6 LHa^{-1}
Oxyfluorfen + Glyphosate	4.6 LHa^{-1} + 1.9 L Ha^{-1}
Clopyralid + Oxyfluorfen	0.58 L Ha^{-1} + 4.6 LHa^{-1}
Clopyralid + Glyphosate	0.58 L Ha^{-1} + 1.9 L Ha^{-1}
Mulch only	5cm depth 0.3m diameter
Mulch + Oxyfluorfen + Glyphosate	5cm depth 0.3m diameter + 4.6 LHa^{-1} +1.9 L Ha^{-1}
Mulch + Clopyralid + Oxyfluorfen	5cm depth 0.3m diameter + 0.58 L Ha^{-1} + 4.6 LHa^{-1}
Mulch + Clopyralid + Glyphosate	5cm depth 0.3m diameter + 0.58 L Ha^{-1} + 1.9 L Ha^{-1}
Clopyralid + Oxyfluorfen + Glyphosate	0.58 L Ha^{-1} + 4.6 LHa^{-1} + 1.9 L Ha^{-1}
Control (no herbicides, no mulch)	

Figure 2.2 Example of Christmas trees in field (Horton Tree Farm).

The organic mulch used was bagged shredded cypress mulch blend (NoFloat cypress blend, Oldcastle Lawn & Garden, Atlanta, GA) at a depth of 5 cm and at a diameter of 0.3 m at the base of each tree (Fig 2.3). 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 directly over the top of the trees with a carbon dioxide ($CO_{2)}$ backpack sprayer (Bellspray R&D sprayer Inc., Opelousas, LA) calibrated to deliver 252.55 liters/hectare using an 8004 flat-fan nozzle (TeeJet Technologies, Wheaton, IL) at a pressure of 206.843 kilopascals (Fig 2.4). The herbicide band width was 81 cm, and the length was about 60 cm per each individual tree. We recorded temperature, relative humidity and windspeed and direction at the time of application using AccuWeather (Table 2.2).

Figure 2.3 Example of how organic cypress bark mulch was applied to each Christmas tree in the field experiment.

Figure 2.4 Example of how herbicides were applied at each farm to each individual tree with a Carbon dioxide backpack sprayer.

Table 2.2 Date of application, name of farm, species, soil type from soil survey, and weather conditions including temperature, humidity and wind speed for each farm used in the field experiment.

Date of Application	*Name of Farm*	*Species*	*Soil Type*	*Weather condition*
2021-05- 27	Gwinn's farm, Horton, MI	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
2021 -06-11	Korson's farm, Sidney, MI	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.
2021-06-11	Korson's farm, Sidney, MI	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.
2021-06- 17	Badger farm, Allegan, MI	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
2021-06-17	Wahmhoff farm, Gobles, MI	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. 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 percent ground cover within each plot covered with weeds and was done 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 (Fig 2.5), 60, and 90 days after the treatments (DAT) were applied.

Figure 2.5 Examples of each treatment at 30 days after treatment from all farms.

2.4.2 *Phytotoxicity*

Phytotoxicity was estimated using a visual assessment, done by the same person for all trees at all farms during each data collection, of 0% meaning no phytotoxicity (using the control treatment as a baseline) to 100% meaning complete death of the Christmas tree. Visual estimations were conducted 30 (Fig 2.5), 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, initial leader lengths, plant heights and crown widths in two perpendicular directions were recorded in centimeters for each Christmas tree. Data for leader length, plant heights, and two widths for each Christmas tree were also recorded at 30, 60, and 90 DAT. 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 groupings were: one herbicide, two or more herbicides combined, herbicide with mulch, 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 done by 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 done using PROC MIXED in SAS (Ver. 9.4, SAS Institute, Cary, NC) for checking the model, checking assumptions, checking for 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 x DAT were considered fixed effects, while blocks were random effects. Repeated measures were done for each tree for the phytotoxicity percent, weed control, and growth index variables at 30, 60, and 90 DAT. All analyses were performed at $\alpha = 0.05$ 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 done 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 2.3, 2.4, 2.5). 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 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.

Based on the contrast (Table 2.6) 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 36% 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, Horton farm, and 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 Horton and Sidney farms. The presence of oxyfluorfen provided a significant change in weed control at 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 2.3 Mean weed control (% ground cover) 90 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 5), alone or in combination, at Christmas tree farms in Michigan.

	Location/ Tree species				
Treatment	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 and $p<0.05$ level. Mean separation by Tukey's HSD test.

Table 2.4 Mean weed control (% ground cover) 60 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 5), alone or in combination, at Christmas tree farms in Michigan.

	Location/ Tree species				
Treatment	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid	9.25 bc*	6.1 cd	61.51 a	25.51 bcde	8.32 bc
Glyphosate	39.92 abc	16.15 cd	58.62 a	13.1 cde	63.68 ab
Oxyfluorfen	55.04 ab	23.03 cd	65.81 a	10.69 de	54.76 ab
Oxyfluorfen + Glyphosate	16.92 abc	76.13 ab	41.19 a	64.02 abc	49.84 ab
Clopyralid + Oxyfluorfen	68.20 ab	81.42 ab	41.31 a	60.12 abcd	52.63 ab
Clopyralid + Glyphosate	20.62 abc	33.32 bc	50 a	71.43 ab	42.57 ab
Mulch	36.56 abc	47.35 abc	83.08 a	2.41 e	87.77 a
Mulch + Oxyfluorfen + Glyphosate	79.14 a	82.57 ab	89.30 a	79.1 ab	92.69 a
Mulch + Clopyralid + Oxyfluorfen	79.14 a	87.71 a	93.89 a	87.02 a	93.89 a
Mulch + Clopyralid + Glyphosate	63.92 ab	82.68 ab	92.79 a	65.37 abc	91.26 a
Clopyralid + Oxyfluorfen + Glyphosate	79.13 a	44.37 abc	91.27 a	66.21 a	78.35 ab
Control	0 c	0 d	0 b	0 e	0 c

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

Table 2.5 Mean weed control (% ground cover) 30 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 5), alone or in combination, at Christmas tree farms in Michigan.

	Location/ Tree species				
Treatment	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid	6.25 bc	9.73 de**	41.76 b**	33.77 bc**	81.22 a**
Glyphosate	38.88 abc	23.72 cde	78.79 ab	17.56 cd	87.77 a
Oxyfluorfen	76.75 a	26.22 cde	90.74 ab	36.86 bc	76.57 a
Oxyfluorfen + Glyphosate	57.63 ab	74.91 abc	71.43 ab	60.00 abc	57.2 a
Clopyralid + Oxyfluorfen	84.54 a	89.15 a	79.73 ab	80.12 ab	78.16 a
Clopyralid + Glyphosate	49.08 ab	72.26 abc	87.54 ab	73.78 ab	82.75 a
Mulch	31.07 abc	36.08 bcd	83.92 ab	17.9 dc	90.08 a
Mulch + Oxyfluorfen + Glyphosate	80.52 a	88.99 a	88.24ab	82.57 ab	93.89 a
Mulch + Clopyralid + Oxyfluorfen	94.24 a	89.61 a	88.48 ab	92.69 a	97.95 a
Mulch + Clopyralid + Glyphosate	63.34 ab	85.99 ab	99.01 a	87.02 ab	92.37 a
Clopyralid + Oxyfluorfen + Glyphosate	79.13 a	66.21 ab	95.88 a	69.49 ab	95.35 a
Control	0 c	0 e	0 c	0 d	0 b

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

Table 2.6 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)

	Location/ Tree species				
Effect	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 x 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≤0.05; ** P≤0.01; *** p≤0.001

Table 2.7 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 60 days after treatment (DAT)

	Location/ Tree species				
Effect	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid (Clo)	1.05	17.56***	2.3	33.29**	0
Glyphosate (Gly)	0.13	23.4***	2.06	25.4***	4.28*
Oxyfluorfen (Oxy)	8.04**	76.12***	1.92	16.62***	4.43*
Clo × Gly	0.44	2.27	0.8	0.78	1.02
Clo × Oxy	0.42	1.56	2.24	1.93	0.11
Gly × Oxy	8.13**	0.06	2.03	0.03	7.07*
Clo × Gly x Oxy	1.71	1.42	7.66	2.37	0.59
Contrast: Combinations with mulch vs without	4.19*	6.88*	11.57**	1.27	5.52*

Note * p≤0.05; ** P<0.01; *** p<0.001

Table 2.8 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 30 days after treatment (DAT)

	Location/ Tree species				
Effect	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid (Clo)	1.93	35.43***	2.27	29.69***	28.76***
Glyphosate (Gly)	3.93	35.62***	8.66**	11.32**	18.45***
Oxyfluorfen (Oxy)	35.96***	57.32***	10.52**	23.67***	7.18*
Clo × Gly	0.17	0.37	1.1	0	12.91**
Clo × Oxy	0.18	0.39	5.57*	0.57	8.34**
Gly × Oxy	17.14***	3.36	22.27***	0.97	28.96***
Clo × Gly x Oxy	0.13	3.36	2.88	0.01	33.3***
Contrast: Combinations with mulch vs without	0.25	2.79	1.24	3.67	12.55**

Note * p≤0.05; ** P<0.01; *** p<0.001

3.2 Phytotoxicity

Phytotoxicity levels varied by species, with white pine and scotch pine experiencing less phytotoxic effects than the other species. At 30 DAT (Table 2.9) mulch + clopyralid + glyphosate (11% to 37%) caused a high amount of phytotoxicity at all farms except Allegan. 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 the Allegan farm. Clopyralid (8% and 9%), 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 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 phytotoxic effects decreasing to ~0% phytotoxicity. Clopyralid resulted in less than 1% phytotoxicity at 90 DAT (Table 2.11) at all farms except Horton. Based on the mulch effect contrast (Table 2.12, 2.13, 2.14) 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 or oxyfluorfen had a significant effect for Horton and Gobles farm phytotoxicity. Glyphosate significantly affected phytotoxicity at Horton, Gobles, 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 has a highly significant change in phytotoxicity from the glyphosate + oxyfluorfen treatment.

Table 2.9 Mean phytotoxicity percent 30 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 2.1), alone or in combination, at Christmas tree farms in Michigan

	Location/ Tree species				
Treatment	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	79.13 a	7.91 a	41.19 a	4.26 ab	24.03 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 and $p<0.05$ level. Mean separation by Tukey's HSD test.

Table 2.10 Mean phytotoxicity percent 60 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 2.1), alone or in combination, at Christmas tree farms in Michigan

	Location/ Tree species				
Treatment	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid	8.60 a*	9.53 ab	0 ab	4.12 bc	0a
Glyphosate	7.20 a	11.93 ab	3.20 ab	11.31 ab	2.94 a
Oxyfluorfen	9.25 a	4.26 ab	16.07 ab	8.60 ab	2.83 a
Oxyfluorfen + Glyphosate	8.60 a	34.79 a	1.86 ab	11.13 ab	0.318 a
Clopyralid + Oxyfluorfen	8.32 a	7.91 ab	2.94 ab	4.64 bc	5.14 a
Clopyralid + Glyphosate	4.99 a	16.23 ab	8.49 ab	22.24 a	5.48 a
Mulch	0 b	0 b	0 b	0 c	0a
Mulch + Oxyfluorfen + Glyphosate	11.04 a	7.23 ab	0.65 ab	18.70 ab	0.65 a
Mulch + Clopyralid + Oxyfluorfen	9.25 a	7.47 ab	1.86 ab	6.77 ab	1.27 a
Mulch + Clopyralid + Glyphosate	4.99 a	22.65 a	36.18 a	22.24 a	3.68 a
Clopyralid + Oxyfluorfen + Glyphosate	79.13 a	4.12 a	32.72 a	2.27 ab	21.07 a
Control	0 b	0 b	0 b	0 c	0a

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

Table 2.11 Mean phytotoxicity percent 90 days after treatment (DAT) of plots treated with three herbicides and organic mulch (Table 5), alone or in combination, at Christmas tree farms in Michigan

	Location/ Tree species				
Treatment	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid	0a*	7.04 abc	0 a	0.31 bc	0 c
Glyphosate	1.26 a	6.25 abc	1.27 a	17.10 a	0.3178 bc
Oxyfluorfen	1.26 a	6.10 abc	6.49 a	6.10 abc	1.2669 abc
Oxyfluorfen + Glyphosate	3.68 a	13.49 ab	2.94 a	11.31 ab	0 c
Clopyralid + Oxyfluorfen	1.85 a	3.69 bc	4.65 a	8.31 abc	0.31775 bc
Clopyralid + Glyphosate	1.26 a	13.49 ab	2.83 a	14.35 a	0 c
Mulch	0a	0 c	0 f	0 c	0 c
Mulch + Oxyfluorfen + Glyphosate	2.41 a	7.04 abc	1.27 a	11.89 ab	0.3177 bc
Mulch + Clopyralid + Oxyfluorfen	1.26 a	6.37 abc	1.51 a	4.99 abc	1.2669 abc
Mulch + Clopyralid + Glyphosate	0.31 a	19.95 ab	23.71 a	9.58 ab	11.0022 ab
Clopyralid + Oxyfluorfen + Glyphosate	79.13 a	0.31 a	25.89 a	0 a	9.98 ab
Control	0a	0 c	0 a	0 c	0 c

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

Table 2.12 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)

	Location/ Tree species				
Effect	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 x 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≤0.05; ** P<0.01; *** p<0.001

Table 2.13 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 60 days after treatment (DAT)

	Location/ Tree species				
Effect	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid (Clo)	2.09	1.98	0.13	4.13	1.85
Glyphosate (Gly)	1.04	15.31***	0.34	24.76***	1.85
Oxyfluorfen (Oxy)	9.93**	4.51*	1.7	2.68	1.58
Clo × Gly	18.38***	1.31	1.48	0.54	0.62
Clo × Oxy	13.55**	1.09	1.25	2.73	0.55
Gly × Oxy	9.29**	0.92	6.42*	2.58	6.64*
Clo × Gly x Oxy	7.43*	0.25	0.21	1.76	0.03
Contrast: Combinations with mulch vs without	0.28	0.04	0.74	5.17*	0.04

Note * p≤0.05; ** P<0.01; *** p<0.001

Table 2.14 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 90 days after treatment (DAT)

	Location/ Tree species				
Effect	Allegan White pine	Horton Fraser fir	Sidney Blue spruce	Sidney Fraser fir	Gobles Scotch pine
Clopyralid (Clo)	0.47	11.24**	0.57	0.05	4.26*
Glyphosate (Gly)	1.87	31.65***	0.01	23.13***	4.26*
Oxyfluorfen (Oxy)	2.75	9.58**	3.09	3.38	13.28**
Clo × Gly	0.95	0.22	0.13	0.72	8.14**
Clo × Oxy	0.47	3.9	1.67	0	8.14**
Gly × Oxy	1.87	0.53	7.9**	11.89**	1.62
Clo × Gly x Oxy	0.95	5.96**	0.82	0.03	13.28**
Contrast: Combinations with mulch vs without	1.38	5.1*	0.04	0.05	24.76***

Note * p≤0.05; ** P<0.01; *** p<0.001

3.3 Growth Indices

For all farms, weed control treatments did not affect (P>0.05) growth index (Table 2.16). 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 treatment x 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 2.15) trees in the control plots were larger (68.36 cm) than trees receiving the clopyralid, oxyfluorfen, oxyfluorfen + glyphosate, and mulch + clopyralid treatments (44cm - 49 cm).

Table 2.15 Gobles farm growth indices (cm) at 90 DAT for each of the 12 treatments (Table 2.1). Growth indices were measured by taking the average of two widths and one height for each tree. Out of all farms, only Gobles farm only at 90 DAT, has a significant treatment effect on growth indices, meaning only at this farm and time trees showed 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

Table 2.16 Average across DAT growth indices by farm in (cm) for all farms where there were no significant differences in growth indices between treatments (Table 2.1) in any given DAT.

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

* 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) foliar nitrogen concentration. All treatments resulted in trees that had foliar nitrogen content ranging from 1.76% to 1.96% (Table 2.17).

Table 2.17 Mean foliar nitrogen percent, for all farms combined, of the foliar samples collected from the 5 treatment groups: control (no treatment), two or more herbicides, one herbicide, mulch + herbicides, mulch only. Means within a column followed by the same letter are not different at 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	1.77a

* Foliar N percent 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 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, oxyfluorfen), as well as to test potential synergism between mulch and postemergence herbicides. The results show that weed control and phytotoxicity were variable and dependent on farm, tree species, and the weed species, but generally mulch combined with herbicides provided the best weed control.

At 90 DAT in Gobles, Allegan, and Horton, clopyralid provided the least 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 is 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 present 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 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]. 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. Mulch consistently improved the effectiveness of herbicides in controlling weeds and improved the longevity of weed control. Mulch could allow herbicides to be effective longer, prevent new weeds from emerging, and potentially have allelopathic effects to weeds. Previous studies have shown that organic mulch can bind herbicide molecules and help them to last longer by reducing leaching and runoff of herbicides. Saha et al. [23] studied the effects of various herbicide and mulch combinations and treatments at 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 in that 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, mulch was especially beneficial at Horton and Sidney blue spruce farms. At both of these farms mulch increased weed control at 90 DAT when compared to the same herbicide treatments without mulch.

The herbicides used also have varying levels of soil persistence which 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] but it will likely be washed out of sandy soils, which is where Christmas trees are commonly grown. The web soil survey from the USDA natural resources (Table 2.2) 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 herbicide 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, done 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. 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 less 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 still showed relatively low levels of phytotoxicity. Grover [31] looked at the effects of 15 herbicides on three species of Christmas trees including blue spruce and Scotch pine. Grover found that the only herbicides that reduced 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 bleaching of Scotch pine, but they recovered. After two growing seasons the shoot height of both species was drastically reduced due to many of the treatments [31]. Both studies confirm our results that pine species can withstand greater herbicide pressure than many other species. Most likely 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 in phytotoxicity.

Growth impacts of chemical weed control represent an integration of potentially off-setting effects of improved growing environment (reduce competition for light, water, nutrients) versus 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 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. Decomposition of soil organic matter by microbes utilizes soil nitrogen, however the effects of mulch on plant nutrition are variable [18,19,20]. 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 dependent on 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 to 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 to successfully control weeds in Christmas tree production as only using one weed control method is much more likely to exert excessive selection pressure on weeds.

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CHAPTER 3

DEVELOPING INTEGRATED STRATEGIES TO ADDRESS EMERGING WEED MANAGEMENT CHALLENGES IN CHRISTMAS TREE PRODUCTION

GREENHOUSE EXPERIMENT

Abstract

Common Ragweed (*Ambrosia artemisiifolia* L.) is an extremely competitive broadleaved summer annual weed found in Christmas tree production systems within Michigan. It also poses a significant allergenic risk for humans. Common ragweed has had reported resistance to glyphosate, PSII inhibitors, PPO inhibitors, and ALS herbicides. There have been recent 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 postemergence herbicide combinations and organic mulch on clopyralid-resistant common ragweed for weed control efficacy. Two stages of common ragweed were used stage 1 (6-9 leaves) and stage 2 (12-14 leaves). For common ragweed 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 greater than or equal weed control to all 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 around 20g. Based on fresh weight, the greatest plant growth occurred with glyphosate treatment in 2021 and clopyralid and mulch alone in 2022.

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; the stems are erect, branched, and up to 2m tall under favorable conditions [1, 2, 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]. Common ragweed poses a significant allergenic risk 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 reported resistance to glyphosate (Group 9), PSII inhibitors (Group 5), PPO inhibitors (Group 14), and/or ALS chemistry (Group 2) [8]. There have also been recent reports from Michigan Christmas tree growers of common ragweed resistance to clopyralid ((Stinger®, Dow AgroSciences, Indianapolis Indiana), a synthetic auxin herbicide (Group 4), in Montcalm County Michigan [10]. 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 a 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, especially those in the Asteraceae plant family, which includes Canada thistle, common ragweed, and marestail. Clopyralid is translocated through the symplast and accumulates in the growing points. Generally, it is very slowly metabolized in most plants [12]. Clopyralid was chosen for this study 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 [9].

Glyphosate (Roundup® Pro Concentrate, Monsanto Company, St. Louis, Missouri) 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 [12]. Glyphosate is rapidly metabolized by soil microbes and strongly binds soil which results in low ecotoxicity and no residual effects from year to year [12]. Glyphosate was chosen as it is the most widely used postemergence herbicide in Christmas tree production. Glyphosate is considered safe to spray in Christmas trees as long as the trees are not actively growing, and it is therefore can be applied selectively in Christmas tree production [13] [14] Glyphosate could be an alternative to clopyralid or be applied in conjunction with clopyralid to manage clopyralid resistant common ragweed however, glyphosate also has many resistant weeds including common ragweed so it might not be the best option.

The final herbicide included in this study, 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 post emergence 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 strongly absorbed by the soil and not easily desorbed. To date, there are no known cases of oxyfluorfen resistance in weeds [12]. Oxyfluorfen was chosen as it is commonly used in Christmas tree production, and it has the ability of acting as both preemergence and postemergence 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 alternative for comparison and to be used in conjunction with the chemicals outlined above. Cypress mulch has been shown to have allelopathic effects on other plants and this can help control weeds. Specifically, it has been found that cypress bark contains more phenolic compounds, which are generally thought to be allelopathic, than pinebark or pinestraw [15]. Mulch also acts as a physical barrier in the soil preventing the emergence of weed seeds though, it is unlikely work well alone when the weeds have already emerged. There is also the potential for herbicides to bind with mulch and allow continued weed control, though this is only likely with oxyfluorfen as it is the only herbicide in the trial with preemergence control [12]. We hypothesize that when herbicides and mulch are combined weed control will likely be better as there are more factors involved in preventing weeds.

In this study we looked at those three postemergence herbicides and organic shredded cypress mulch to evaluate the common ragweed control efficacy The objective of this study was to:

Objective 1: Investigate the impacts of alternative postemergence herbicide combinations and organic mulch on clopyralid-resistant common ragweed control efficacy.

Hypothesis 1: Alternative postemergence herbicide and organic mulch combinations can show early postemergence control of clopyralid-resistant common ragweed

2. Materials and Methods

2.1 Plant Materials

The experiment was conducted in a greenhouse, with a roof made of polycarbonate and the 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. 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 until 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.

Plants were grown to two stages, stage one at 6-9 leaves and the stage two 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), 10.5 cm (width) × 11.4 cm (height), were filled with commercial soilless media Suremix (composition: 70% peat moss, 21% perlite, and 9% vermiculite, manufactured by Michigan Grower Products Inc., Galesburg, MI).

Osmocote fertilizer ([17-5-11 (8 to 9 months)] (ICL Specialty Fertilizers, Dublin, Ohio) was mixed into the Surmix potting media at the manufacturer's labeled medium rate of 7.1 g/l. Twenty-five seeds were sown, in each pot and all pots were kept inside the greenhouse with 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 natural day length was approximately 13-15 hours of light per day. Weed control treatments were applied once the plants reached the desired leaf stage. Experiments were replicated twice, first in June-September 2021 and then repeated in June through September 2022.

2.2 Experimental Design and Treatments

The experiment was designed as a complete randomized block design with four replications (N=4) of each of 12 treatments during each season (2) within each stage (2). Plants were placed on greenhouse benches in randomized order within each stage. Each block contained 4 plants that were each randomly assigned one of the 12 weed control treatments (Table 3.1), treatment rates are given in rate of product: *1) Clopyralid* applied at a rate of 0.58 L Ha^{-1} *2) Glyphosate* applied at a rate of 1.9 L Ha^{-1} *3) Oxyfluorfen* applied at a rate of 4.6 LHa^{-1} *4) Oxyfluorfen + Glyphosate* tank mixed and applied at a rate of 4.6 LHa^{-1} oxyfluorfen + 1.9 L Ha^{-1} glyphosate *5) Clopyralid + Oxyfluorfen* tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 4.6 LHa^{-1} oxyfluorfen *6) Clopyralid + Glyphosate* tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 1.9 L Ha-1 glyphosate *7) Mulch* organic cypress mulch applied at a depth of 5cm and a diameter of 0.3m *8) Mulch + Oxyfluorfen + Glyphosate* mulch applied first at a 5cm depth 0.3m diameter then shortly after oxyfluorfen + glyphosate tank mixed and applied at a rate of 4.6 LHa^{-1} oxyfluorfen +1.9 L Ha^{-1} glyphosate *9) Mulch + Clopyralid + Oxyfluorfen* *mulch* applied first at a 5cm depth and 0.3m diameter then clopyralid + oxyfluorfen tank mixed and applied at a rate 0.58 L Ha^{-1} clopyralid + 4.6 LHa^{-1} oxyfluorfen *10) Mulch + Clopyralid + Glyphosate* mulch applied first at a 5cm depth and 0.3m diameter then clopyralid + glyphosate tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 1.9 L Ha^{-1} glyphosate *11) Clopyralid + Oxyfluorfen + Glyphosate* tank mixed and applied at a rate of 0.58 L Ha^{-1} clopyralid + 4.6 LHa-1 oxyfluorfen + 1.9 L Ha^{-1} glyphosate *12) Control* which consisted of no herbicides or mulch applications.

Table 3.1 Weed control treatments and rate of applications used in field and greenhouse experiments.

Treatments	Rate of applications (highest labeled rate)
Clopyralid	0.58 L Ha^{-1}
Glyphosate	1.9 L Ha^{-1}
Oxyfluorfen	4.6 LHa^{-1}
Oxyfluorfen + Glyphosate	4.6 LHa^{-1} + 1.9 L Ha^{-1}
Clopyralid + Oxyfluorfen	0.58 L Ha^{-1} + 4.6 LHa^{-1}
Clopyralid + Glyphosate	0.58 L Ha^{-1} + 1.9 L Ha^{-1}
Mulch only	5cm depth 0.3m diameter
Mulch + Oxyfluorfen + Glyphosate	5cm depth 0.3m diameter + 4.6 LHa^{-1} +1.9 L Ha^{-1}
Mulch + Clopyralid + Oxyfluorfen	5cm depth 0.3m diameter + 0.58 L Ha^{-1} + 4.6 LHa^{-1}
Mulch + Clopyralid + Glyphosate	5cm depth 0.3m diameter + 0.58 L Ha^{-1} + 1.9 L Ha^{-1}
Clopyralid + Oxyfluorfen + Glyphosate	0.58 L Ha^{-1} + 4.6 LHa^{-1} + 1.9 L Ha^{-1}
Control (no herbicides, no mulch)	

2.3 Initial Measurements and Treatment Applications

All weed control treatments were applied outside of the greenhouse. Plants were put into treatment groups and treatments were applied, then plants were returned to the greenhouse and placed in a randomized order on the bench within stage one and stage two. Organic mulch was applied before herbicides. Bagged cypress mulch blend (NoFloat cypress blend, Oldcastle Lawn

& Garden, Atlanta, GA) 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 hours of mulch application in liquid formulations at their highest labeled rate. All herbicides and their combinations were applied uniformly, directly over top of the weeds, with a carbon dioxide (CO_2) backpack sprayer (Bellspray R&D sprayer Inc., Opelousas, LA) calibrated to deliver 252.6 liters/hectare using an 8004 flat-fan nozzle (TeeJet Technologies, Wheaton, IL) at a pressure of 206.8 kilopascals. For the first round of greenhouse experiments the treatments were applied to the stage one and stage two plants on the same day, 2022-07-02, 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 2022-06-02, 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 two plants were applied on 2022-07-01, it was cloudy with a temperature of 23°C and 56% humidity with a NE wind at 11.27 km/h. 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.

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. Plants were rated, removed from benches, photographed, and returned to 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 and placed into an individual brown paper bag and weighed.

2.4.2 Statistical Analysis

Data was analyzed using PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the analysis of variance (ANOVA) and Tukey's HSD in the LSMEANS prompt of PROC GLIMMIX to separate out the means. An arcsine square root transformation was required and performed to the control percent variable to normalize 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 analysis of variance (ANOVA). Data from each evaluation were subjected to an initial two-way ANOVA. Treatments, week after treatment (WAT), and the interaction of treatment × WAT were considered fixed effects, while blocks were random effects. Mean separation was done using Tukey's HSD in the LSMEANS prompt of PROC GLIMMIX. A contrast was done in PROC GLM to compare treatments with and without mulch.

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) for checking the model, checking assumptions, and checking which transformation was needed. The arcsine square root transformation was performed to the fresh weight variable. This was done for both stages. 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 done using Tukey's HSD in the LSMEANS prompt of PROC GLIMMIX.

3. Results

3.1 Control

At 2 WAT (Table 3.2) 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 in 2021 and 2022. Mulch + clopyralid + glyphosate provided high levels (99-100%) of weed control at stage 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 least 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%, clopyralid was still low as it provided only 26% weed control.

At 8 WAT (Table 3.2) treatment effects remained largely the same as they were at 2 WAT. Notable changes included that in 2021 the control plants treated as 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 8 WAT, as it was at 2 WAT. For plants treated at stage 1 in 2022 (Fig 3.1), all of the highly effective treatments at 2 WAT increased in effectiveness by 8 WAT and provided 100% weed control. For plants treat at stage 2 in 2022 (Fig 3.2) 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%, 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 level 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 stage plants in 2022 when compared to those treated with herbicides only.

By 8 WAT (Table 3.3) many plants had completely dried up and there was nothing left so the fresh weight for these plants was N/A. Stage 1 plants treated with all mulch and herbicide combination 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 ~20g. 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

Figure 3.1 Stage 1 common ragweed plants in 2022 at 2, 4, 6, and 8 WAT and treatments listed 1-12.

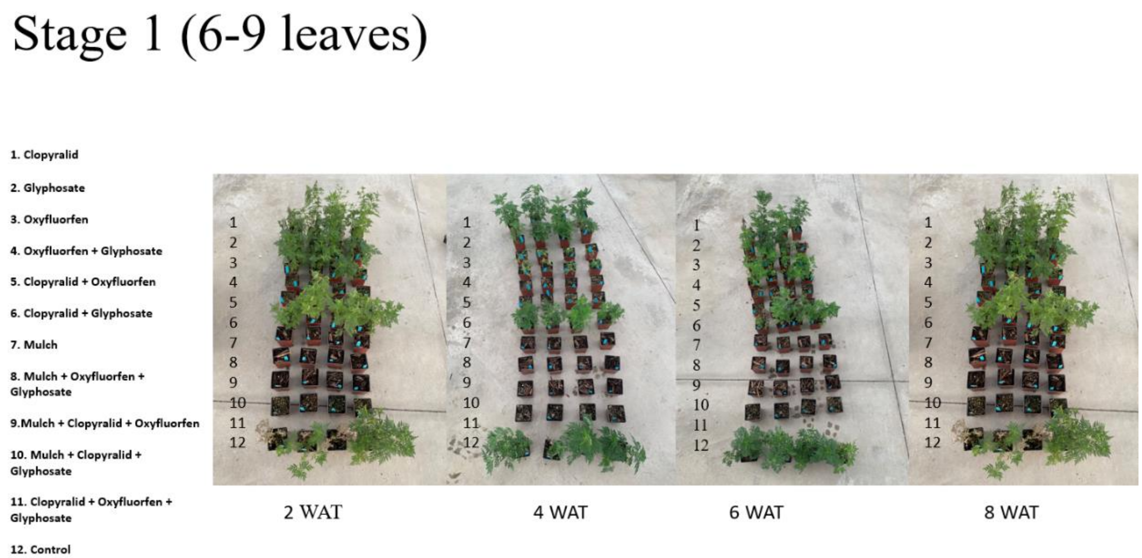

Figure 3.2 Stage 2 common ragweed plants in 2022 at 2, 4, 6, and 8 WAT and treatments listed 1-12.

Table 3.2 Control percentages (0%-100%) for common ragweed in separated by stage and year subjected to 12 weed control treatments (Table 3.1). Control percentages followed by the same letter are not significantly different within a column. Mean separation by 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

Table 3.2 (cont'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

Table 3.3 Fresh Weight (g) separated by stage and year for common ragweed, subjected to 12 weed control treatments (Table 3.1). Fresh weights followed by the same letter are not significantly different within a column. Mean separation by Tukey's HSD.

Stage 1

Treatment	2021 Fresh Weight (g)	2022 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

Table 3.3 (cont'd)

Stage 2

Treatment	2021 Fresh Weight (g)	2022 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	N/A 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 plants. There have been reports from Michigan Christmas tree growers finding clopyralid resistant common ragweed in Montcalm County Michigan [9]. Resistance is likely spreading throughout Michigan Christmas tree farms via pollen and seed 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 there is potential that they were also glyphosate resistant. Greenhouse studies were used to study potential weed management alternatives options for these common ragweed plants because it allowed for all conditions (i.e., temperature, irrigation, pest pressure, other weed competition) to be controlled and only the treatments to be held accountable. However, future studies should be done in the field to observe how outside factors affect the treatments and how these treatments scale in actual production scenarios.

Weed scientists have begun advocating for a diverse array of control options for weeds, as high selection pressures from a single method leads rapidly to resistance [6,10,11]. Indeed, the growing challenge of herbicide resistance in the United States has led to the development of integrated weed management plans [16]. These typically include using multiple herbicides or herbicides × mulch combinations amongst many other options such as mechanical control.

Herbicide options can be 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].

In our experiment we wanted to test some options for IPM weed control, especially on clopyralid resistant ragweed populations. 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 greater than or equal weed control to all 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 synergistic effects. At most evaluations oxyfluorfen provided the highest level of ragweed control as a singular herbicide treatment. This observation is consistent with other reports 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 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 postemergence 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.

5. Conclusions

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. More research is required to understand why these treatments work so well when their individual components do not work very well at controlling clopyralid resistant common ragweed postemergence. The best singular herbicide out of those tested was oxyfluorfen. Mulch alone was not effective at controlling common ragweed when it was applied post-emergence; however, it does seem to increase herbicidal effect when used in combination with the herbicides used in this study. Many of the two herbicide combination treatments as well as the two herbicides plus mulch combination treatments as well as the three herbicide combinations were effective at controlling common ragweed, which furthers the point that multi-tactic integrated weed control methods are the superior choice for controlling common ragweed.

Integrated weed management strategies are the best choice for controlling not only common ragweed but also all other weeds at the Christmas tree production system as it can help avoid 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 cycle and survival strategies. Combining nonchemical with chemical methods (such as mulch and herbicide combinations) or applying 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.

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