EXPLORING INTEGRATED WEED MANAGEMENT PROGRAMS FOR IMPROVED COTTON SUSTAINABILITY AND DEVELOPING NEW HERBICIDE USES FOR RYE AND VEGETABLES

by

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(Under the Direction of A. Stanley Culpepper)

ABSTRACT

Cotton, rye, and vegetables are planted on over 567, 23, and 46 thousand ha of land in Georgia, respectively, and collectively account for a farm gate value of over $1.9 billion. These crops are not only critical for the success of local agriculture but for the nation. To maximize yields and preserve crop quality, weeds must be controlled in each of these crops.

Cotton engineered to resist dicamba provides an effective option to control Georgia's most problematic pest, glyphosate-resistant Palmer amaranth. However, overuse of dicamba postemergence in cotton is a concern and sustainable weed management programs must be implemented to delay resistance development. A study, conducted at 4 locations, demonstrated that adding 1) a rye cover crop, 2) preemergence herbicides, or 3) both the cover crop and preemergence herbicides reduced Palmer amaranth exposure to dicamba in a standard program by 65, 98, and 99%, respectively. These results are critical helping farmers implement sound diversified weed management systems.

In multi-cropped plasticulture vegetable production, plastic mulch is often used over 18 months thereby facilitating dense weed infestations. With formulation improvements and effective broadleaf weed control, 2,4-D and dicamba may serve as new tools to manage weeds between crops but only if they can be removed from the mulch. A study, conducted 9 times, used HPLC analysis and bioassay plantings of squash, watermelon, cantaloupe, broccoli, and collard to determine if 0.6 cm of overhead irrigation could remove these herbicides from the mulch. Irrigation effectively removed 2,4-D from the mulch to levels that were non-lethal for all crops. In contrast, irrigation did not successfully remove dicamba from the mulch.

Herbicide options in cereal rye are lacking, particularly for Italian ryegrass control. Cereal rye tolerance to 8 wheat herbicides was evaluated at 5 locations to potentially pursue registrations for cereal rye. Thifensulfuron-methyl + tribenuron-methyl, pyroxsulam, and halauxifen-methyl + florasulam resulted in minimal injury (≤15%) and growth reductions at the 2X rate and are candidates for potential labeling. In contrast, unacceptable injury was noted with pyroxasulfone, pyroxasulfone + flumioxazin, mesosulfuron-methyl, pinoxaden, and pinoxaden + fenoxaprop-p-ethyl.

INDEX WORDS: Weed management, cotton, Palmer amaranth, selection pressure, cover crops, residual herbicides, layby applications, dicamba, glyphosate, vegetables, crop tolerance, herbicide removal from plastic mulch, 2,4-D, HPLC analysis, squash, watermelon, cantaloupe, zucchini squash, broccoli, collard, cereal rye, Italian ryegrass, pyroxasulfone, pyroxasulfone plus flumioxazin, mesosulfuron-methyl, pinoxaden,

pinoxaden plus fenoxaprop-p-ethyl, thifensulfuron-methyl plus tribenuron-methyl, pyroxsulam, halauxifen-methyl plus florasulam

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DEDICATION

This work is dedicated to three of the most influential men in my life: my dad, John Hand, and both of my grandfathers, Jogene Hand and Joseph Milano Jr. These three men instilled in me a love for agriculture and a strong work ethic. To my dad, thank you for putting up with me for 27 years. I don't think either one of us knew it, but all those days spent with you "riding in the truck, talking on the phone, and taking people to lunch" showed me what I wanted to do when I "grew up". To my Papaw, Jogene Hand, the days spent with you in the summers working in your garden taught me to love agriculture. Of course wearing out the bream with you was fun too! You are one of the most knowledgeable people I know in the field of agriculture, and still the person I go to for advice when tending my garden. And lastly, to my late grandfather, Joseph Milano Jr. or Poppy. I'll never forget sitting with you at the bar at the Louisiana Lagniappe in Orange Beach, AL in the summer of 2015, and you were the first person to tell me that I should stay in school and get a PhD. You believed in me at a time when I didn't believe in myself, and that conversation still drives me to this day. Thank you.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Since the rise of man, weeds have been a significant problem in agriculture. Not only do weeds have the potential to reduce crop yield, but they can also delay maturity while reducing crop quality and harvesting efficiency (Brandenberger et al. 2005). For example, morningglory (*Ipomoea* spp.) can reduce harvesting efficiency in cotton and peanut (Crowley and Buchanan 1978; Chaudhari et al. 2017a). In cotton, harvesting efficiency was reduced up to 31%, and the increased presence of morningglory resulted in a higher amount of trash present in the cotton lint (Crowley and Buchanan 1978). This would lead to a lower grade for the ginned lint, resulting in the grower receiving less money for their crop. Morningglory also causes problems in peanut harvest due to its ability to become intertwined in the peanut vines, which can lead to problems when digging and inverting for picking (Chaudhari et al. 2017a). Morningglory is only one of many weeds that cause direct and indirect losses in agriculture. Knowledge and understanding of weed competition is critical to high quality crop production.

Weed management in agriculture is crucial to achieve the maximum attainable yield in crops. An integrated approach utilizing a combination of chemical, cultural, and physical methods is often necessary to adequately control problematic weeds. This includes promoting crop health through fertility and crop rotation, cover crops, deep

tillage, stale seed bed techniques, mulching, mechanical cultivation, hand-weeding, and managing the weed seed bank (Buchanan et al. 1982; Walker et al. 1989).

One of the largest problems facing agriculture today is the evolution of herbicide resistant weeds. In the United States, there have been 165 unique cases of herbicide resistant weeds reported and confirmed (Heap 2020). Over reliance on chemical control methods has led to this large number of herbicide resistant weeds. Utilization of cultural and physical control methods as well as the development of weed control strategies to mitigate further resistance are key to the future of weed control in crops. Significant research is necessary to address some of the knowledge gaps in these areas.

LITERATURE REVIEW

Weed management in plasticulture vegetable production

The loss of methyl bromide over a decade ago led to a shift in weed control practices for plasticulture vegetable production systems (Culpepper et al. 2009; Eure and Culpepper 2017; Stevens et al. 2016). Currently, one of the most common vegetable production practices in Georgia is fumigation using a 3-way system comprised of 1,3-dichloropropene, chloropicrin, and metam sodium, while covering the bed with either low-density polyethylene (LDPE) or totally impermeable film (TIF) plastic mulches (Culpepper et al. 2008; Culpepper et al. 2017). Exceptional pest control can be achieved with combinations of TIF and the 3-way fumigant systems (Culpepper et al. 2017). Due to lack of permeability, TIF mulch keeps the fumigant contained within the raised bed resulting in rotational crop intervals potentially exceeding 35 days (Culpepper et al. 2017). This, compounded with the impact of environmental conditions on fumigant

degredation, has led many growers in Georgia to fumigate their spring vegetable fields from December to January prior to planting their crop in March (Csinos et al. 2002; Desaeger et al. 2008). This allows for optimum fumigant activity as well as adequate time for fumigant degradation under the mulched bed, resulting in exceptional pest control and minimal crop injury.

Although pest control under plastic mulch is optimized in this system for the first crop, weed control between the plastic mulched beds (i.e. row middles) and for subsequently planted crops can be extremely difficult. Broadcast applied herbicides are limited by the presence of the plastic mulch. Glyphosate and paraquat, two of the most popular options among growers, provide broad-spectrum weed control and can be removed from the plastic mulch with a single rainfall or irrigation event (Boyd 2016; Culpepper et al. 2009; Grey et al. 2009). Winter annual weeds can generally be controlled by glyphosate or paraquat prior to spring vegetable planting. However, weeds that exhibit tolerance to these burndown herbicides such as wild radish (*Raphanus raphanistrum* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), and horseweed (*Conyza canadensis* (L.) Cronq.) may not be adequately controlled when they are applied alone (Culpepper et al. 2005; Eubank et al. 2008). When glyphosate or paraquat was applied alone, 80-81%, 56-60%, and 55-74% control of wild radish, cutleaf evening-primrose, and horseweed was observed, respectively (Culpepper et al. 2005; Eubank et al. 2008). Additionally, for fall planted vegetables, *Amaranthus* spp. and *Ipomoea* spp. can be problematic. With widespread glyphosate resistance in Palmer amaranth (*Amaranthus palmeri* S. Wats), paraquat is the only effective option if applied to small weeds (Culpepper et al. 2006; Chaudhari et al. 2017b). Glyphosate and paraquat provide variable and often inadequate

control of susceptible annual morningglory (Hydrick et al. 1995; Leon et al. 2016). Additionally, other vegetable producing states including California and Florida, have noted resistance to paraquat in horseweed (*Conyza canadensis* L.) (Moretti and Hanson 2016) and American black nightshade (*Solanum americanum* Mill.) (Chase et al. 1998). To control these problematic weeds and start the season with minimal weed competition, herbicide tank-mixes are necessary.

Although numerous herbicides are available to control these weeds, broadcast applications over plastic mulch increase complications. Herbicides applied over plastic mulch have been shown to wash off of the mulch with an initial irrigation or rainfall event, partially wash off of mulch over time, or bind to the mulch without release (Culpepper et al. 2009; Grey et al. 2009; Grey et al. 2018; Randell et al. 2020). For example, previous research has demonstrated that flumioxazin applied over plastic mulch persists, resulting in significant injury and yield reductions for squash and tomato (*Solanum lycopersicum* L.) (Culpepper et al. 2009; Grey et al. 2009). Halosulfuron-methyl applied over plastic mulch, even with large amounts of rainfall after application and prior to planting, damaged squash, broccoli (*Brassica oleracea* var. *botrytis* L.), and cabbage (*Brassica oleracea* var. *capitate* L.) at varying levels (Culpepper et al. 2009; Randell et al. 2020). In the case of broccoli and cabbage, halosulfuron removal from plastic mulch was more dependent on time than rainfall, with 52-58% injury for 7 DBP applications and 36-37% injury for 14 DBP applications when similar amounts of rainfall were received for both application timings (Randell et al. 2020). Therefore, some herbicides will require not just rainfall or irrigation for removal from plastic mulch, but also time. Even herbicides that can be removed may persist in the soil of old plant holes

or tears in the mulch; both scenarios pose a serious risk to high value vegetable crops. Previous research concluded that fomesafen can be removed from plastic mulch with relative ease (Culpepper and Smith 2017). However due to soil persistence, sensitive crops, such as cole crops [broccoli (*Brassica oleracea* var. *botrytis* L.), cabbage (*Brassica oleracea* var. *oleracea* L.), and cauliflower (*Brassica oleracea* var. *botrytis* L.)], cannot be planted for up to 18 months after application (Anonymous 2019a). Therefore, herbicides that can be used in multi-crop plasticulture must effectively control troublesome weeds, mix well with glyphosate or paraquat, and be easily removed from plastic mulch with rainfall or irrigation. Herbicides that meet these parameters and minimize the potential for crop injury could provide significant utility for growers using this system.

Auxin herbicides were the first group of chemicals used for selective weed control in cropping systems, with 2,4-D being the first available herbicide within this mechanism of action (Grossman 2000; Johnston et al. 2018; Norman et al. 1950). Although synthetic auxins have been available for 70 years, interest in utilizing these chemistries has been renewed due to formulation improvements along with the introduction of 2,4-D- and dicamba-tolerant crops (Anonymous 2017; Anonymous 2018a; Anonymous 2018b; Johnston et al. 2018). The use of these herbicides have demonstrated efficacy when applied for preplant burndown weed control. Culpepper et al. (2005) reported that tank-mixes of 2,4-D or dicamba plus glyphosate resulted in 94-97% control of both cutleaf evening-primrose and wild radish 28 days after treatment (DAT). Additionally, applications of glyphosate plus 2,4-D or dicamba resulted in 90-99% control of horseweed 4 weeks after treatment (Eubank et al. 2008). For *Amaranthus* and *Ipomoea*

spp., tank-mixtures of 2,4-D or dicamba plus glyphosate or paraquat have demonstrated greater than 90% control when utilized as a part of a complete herbicide program (Cahoon et al. 2015; Leon et al. 2016; Merchant et al. 2014). However, non-tolerant broadleaf crops can be extremely sensitive to low doses of auxinic herbicides. Significant injury and yield reductions due to low rates of auxin herbicides have been documented in many high-value vegetable crops such as bell pepper, cantaloupe, cucumber, potato, snap bean, squash, and watermelon (Colquhoun et al. 2014; Culpepper et al. 2018; Dittmar et al. 2016; Hand et al. 2020).

Although crop response to auxin herbicides applied at low rates to the foliage of vegetable crops has been documented, further research is needed investigating the use of 2,4-D or dicamba applications for preplant burndown applications over plastic mulch, prior to vegetable transplanting. Herbicide registrations in high value vegetable crops are rare and often entirely dependent on crop response as opposed to efficacy in weed control (Culpepper et al., 2009; Kuack, 2020). Thus, crop response and tolerance must be well understood prior to pursuing registrations of any herbicide in a vegetable crop. The interaction of 2,4-D or dicamba with plastic mulch as influenced by irrigation or application timing prior to planting vegetables has not been studied. Provided 2,4-D and dicamba can be removed from mulch with rainfall and/or irrigation, or dissipate to non-lethal concentrations rapidly on plastic mulch, the use of these herbicides would provide vegetable growers with valuable tools in their weed control program.

Reducing selection pressure using integrated weed management strategies in cotton

Problematic weeds are constantly adapting to agricultural practices (Culpepper 2006; McElroy 2014; Webster and Nichols 2012). One of the earliest examples of this

was the ability of weeds to evolve and appear similar to the cultivated crop, resulting in the inability of hand laborers to distinguish between the crop and the weed (McElroy 2014). Weed management strategies have seen a dramatic shift from primarily physical weed control methods to chemical weed control methods in the developed world (McElroy 2014; Ziska et al. 2019); yet, weeds still adapt by evolving resistance to herbicidal mechanisms of action (Busi et al. 2013; Green and Owen 2011; Gressel et al. 2016).

The selection of herbicide-resistant weeds is a function of species biology, the characteristics of the genes that confer herbicide resistance, and the number of individuals treated over time and space (Heap 2014). Glyphosate-resistant crops were commercialized in 1996, resulting in a shift to heavy reliance on glyphosate for weed control in some of the most widely produced crops in the United States such as cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) (Green 2009; Jones and Snipes 1999; Young 2006). Although many mechanisms of action were still utilized in cotton immediately following the introduction of glyphosate-resistant varieties, the number of mechanisms of action utilized in soybean production rapidly decreased from roughly five per year to just one – glyphosate (Young 2006). Although some scientists believed that weed resistance to glyphosate was extremely unlikely because of some of its properties, as little as five years after the introduction of this technology, glyphosate resistant weeds had become an issue in many production systems (Bradshaw et al. 1997; VanGessel et al. 2001; Culpepper et al. 2006). In the case of glyphosate-resistant weeds, initial mutation frequency was low, but glyphosate was used over a large area with broad-spectrum activity, selecting for many resistant weeds (Gaines et al. 2019; Heap 2014). This coupled

with the abandonment of other effective weed control practices, such as herbicide rotation and cultural or physical control methods, led to widespread herbicide resistance in many crops.

Following the selection for widespread glyphosate resistance in weeds, both industry and academic scientists recommended the reincorporation of integrated weed management strategies such as the utilization of residual herbicides or tillage (Gustafson 2008; Sosnoskie and Culpepper 2014). Although residual herbicides are important components to weed management programs in a multitude of crops, postemergence (POST) herbicide applications are still necessary for adequate weed control (Everman et al. 2009; Johnson et al. 2012). To provide greater POST herbicide flexibility to growers, crops have been engineered with resistance to glufosinate, 2,4-D, and dicamba (Meyer et al. 2015; Cahoon et al. 2015; Manuchehri et al. 2017). In the United States in 2019, nearly 55% of the cotton varieties were Xtendflex™, which are tolerant to glyphosate, glufosinate, and dicamba, while approximately 65-75% percent of soybean varieties planted in the U.S. had the Xtend™ trait package providing resistance to glyphosate and dicamba (USDA AMS 2019; E. Prostko, personal communication). These traits are widely planted because of flexibility in weed control, along with the high yield potential; as a result, dicamba use has increased in both crops. Dicamba was applied on less than 10% of acres planted to cotton in 2010 compared to 33% of planted cotton acres in 2017, while less than 1% of planted soybean acres received dicamba in 2012 compared to 27% in 2018 (USDA 2019). Dicamba-based weed management programs can improve the control of some of the most problematic weeds in the United States including Palmer amaranth (*Amaranthus palmeri* S. Watson), common waterhemp (*Amaranthus*

tuberculatus (Moq.) J. D. Sauer), horseweed (*Conyza canadensis* L.), and giant ragweed (*Ambrosia trifida* L.) (Meyer et al. 2015; Kruger et al. 2010; Barnett et al. 2013).

Although dicamba-based herbicide systems can be effective, rapid adoption and abuse of dicamba applied POST is already of great concern in regards to weed resistance development. After the introduction of glyphosate-resistant crops, overreliance on glyphosate and shifts away from integrated herbicide systems utilizing residuals led to high selection pressure for resistant individuals (Young 2006). Although the use of glyphosate outside of an integrated weed management program led to resistance issues in many problematic weeds throughout the United States, data has still shown reduced use of preemergence (PRE) herbicides. In Nebraska, over 25 and 40% of corn and soybean growers, respectively, said they did not utilize a preemergence herbicide for residual weed control (Sarangi and Jhala 2018). However, research conducted on soybean herbicide programs utilizing PRE, POST and PRE followed by POST demonstrated significantly higher control when a PRE was used and followed by a POST for problematic weeds such as common waterhemp or giant ragweed (Johnson et al. 2012). Not only can weed control be improved by including a PRE herbicide in a weed management program, but it can assist in minimizing selection pressure by reducing the number of weeds exposed to a POST application (Johnson et al. 2012). Other weed management tactics could assist in reducing selection pressure as well, such as utilizing high biomass cover crops. In cotton, even when an aggressive herbicide program was utilized applying a residual preplant burndown, two PREs with differing mechanisms of action, as well as residuals applied POST, the addition of a cereal rye cover crop reduced the total number of Palmer amaranth by nearly 2,000 plants ha^{-1} at the time of the second

POST application (Hand et al. 2019). Not only did the cover crop improve weed control, but it reduced the number of Palmer amaranth plants exposed to a POST herbicide, thus reducing selection pressure for development of further resistance. Similarly, the use of cover crops has been shown to reduce or delay weed emergence thereby reducing the number of weedy pest to be controlled with POST herbicide applications (Aulakh et al. 2012; Bunchek et al. 2020; Price et al. 2016; Wallace et al. 2019; Webster et al. 2016; Wiggins et al. 2016).

Applying multiple mechanisms of action is another way in which selection pressure can be reduced and weed control improved. In the case of Xtendflex™ cotton, dicamba and glufosinate could potentially be used as a tank-mix partner to control problematic weeds. Palmer amaranth control was highest at one to two weeks after a single postemergence application when treated with a tank-mix of dicamba and glufosinate (Vann et al. 2017). However, it is currently illegal to tank-mix these two herbicides according to the label due to off-target concerns (Anonymous 2018a; Anonymous 2018b). In many cotton growing regions, this technology is being used to control glyphosate-resistant weeds, effectively resulting in reliance on single effective mechanism of action being used to control these weeds As a result of overreliance on dicamba in field corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) production, there are populations of dicamba resistant kochia (*Bassia scoparia* (L.) A.J. Scott) in the Midwest (Cranston et al., 2001). Greenhouse studies have shown that Palmer amaranth exposed to sub-lethal rates of dicamba for three generations can lead to populations with reduced sensitivity (Tehranchian et al., 2017). Additionally, just four years after the introduction of dicamba-tolerant crops, dicamba resistant Palmer amaranth has been

identified in Tennessee (Steckel, 2020). To delay the evolution of resistance in other populations, integrated approaches such as utilizing multiple effective herbicide mechanisms of action and cultural control methods must be adopted. Therefore, integrated approaches such as utilizing multiple effective mechanisms of action and cultural control methods must be used to delay further resistance.

Improving weed control options in cereal rye produced for forage, straw, or grain

Cereal rye (*Secale cereale* L.) is commonly grown as a winter cover crop because of its extreme cold hardiness, drought tolerance, and the ability to grow well on marginal soils (Bushuk 1976). It has additional uses as a pasture forage, hay, and grain, which is fed to livestock or consumed as food and alcoholic beverages (Bushuk 1976; Hales et al. 2007; Shee et al. 2016). Rye serves multiple purposes in many states as a late-wintering forage for cattle and a summer cover crop used to mitigate erosion, moisture loss, and weed competition when planting a cash crop (Bunchek et al. 2020; Li et al. 2013; Wiggins et al. 2016). Arkansas research indicated rye fed to cattle demonstrated greater nutritional benefits compared to oat (*Avena sativa* L.) and triticale [*xTriticosecale* Wittm. Ex A. Camus (*Secale* x *Triticum*)] resulting in greater average daily gain and weight gain per area (Beck et al. 2007). Although wheat (*Triticum aestivum* L.) acreage surpassed cereal rye hectarage nationally in 2018 (47.8 and 2.0 million acres planted, respectively), Georgia growers planted a comparable amount of acres to both crops in the same year (190,000 to 200,000 acres) (USDA 2019). Furthermore, Georgia was second in the nation in acres of rye planted and harvested (190,000 and 15,000 acres, respectively) and value of rye production in 2018 ($2.8 million) (USDA 2019).

Weed management in rye is extremely challenging because of limited herbicide options. Although preplant tillage can be an effective approach to remove weeds at planting for growers not utilizing conservation production systems, weed control is needed for the entire season (Taylor and Everman 2015). In-crop tillage options are extremely limited and most often not practical, as rye seed are planted by being broadcast spread or drilled in 19 cm rows (Buntin and Cunfer 2017; Tautges et al. 2016). Thus, the success of in-season weed control in conventional production is highly dependent on herbicides. For rye, registered herbicide options are limited to 2,4-D, MCPA, bromoxynil, bromoxynil plus pyrasulfotole, and prosulfuron (Marshall 2017). Although prosulfuron is registered, a 10 month rotational restrictions to cotton (*Gossypium hirsutum* L.), peanut (*Arachis hypogaea* L.), and soybean (*Glycine max* L.) limit its use as these crops are planted immediately after rye harvest (Anonymous 2019b).

Among the most common and troublesome weeds in Georgia rye not controlled by registered herbicides include Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], henbit (*Lamium amplexicaule* L.), chickweeds (*Stellaria* spp.), and annual bluegrass (*Poa annua* L.) (Anonymous 2015, 2018c, 2018d, 2019c; Buntin and Cunfer 2017; Webster 2012). Italian ryegrass is the most problematic weed in small grain production in Georgia and challenges rye production. Fast et al. (2009) predicted that yield loss in wheat was 16% in the presence of 30 Italian ryegrass plants m^{-2}. This resulted in a reduction in grade and a significant impact on dockage, ultimately reducing the price obtained for the wheat (Fast et al. 2009). Other studies indicated that yield losses in wheat decreased 4.2% for every 10 Italian ryegrass plants m^{-2}, which can be attributed primarily to reduced tillering in wheat (Liebl and Worsham 1987).

Additionally, Italian ryegrass uptake of nitrate and potassium was twice that of wheat (Liebl and Worsham 1987). Henbit densities of 82 and 155 plants m^{-2} reduced wheat yields 13 and 38%, respectively (Conley and Bradley 2005). Differences in tiller and spike density were also detected in henbit infested treatments compared to weed-free treatments (Conley and Bradley 2005). Common chickweed has demonstrated extreme competitive abilities for both space and nutrients, reducing barley yield up to 80% (Mann and Barnes 1950).

Wheat has demonstrated high levels of tolerance to mesosulfuron-methyl, pinoxaden, and pyroxsulam (Ellis et al. 2010; Grey et al. 2012a; Howatt 2006). Additionally, these herbicides provide excellent control of susceptible populations of Italian ryegrass (Ellis et al. 2010; Grey et al. 2012a; Howatt 2006). Not only do mesosulfuron-methyl and pyroxsulam control grasses, but they also effectively control other weeds including henbit, chickweed and annual bluegrass with minimal restrictions to commonly planted rotational crops (Anonymous 2012; Anonymous 2020; Grey et al. 2012a, 2012b). Thifensulfuron-methyl plus tribenuron-methyl and halauxifen-methyl plus florasulam are also options that could provide alternative mechanisms of action and have proven safe in wheat while providing excellent broadleaf weed control including henbit and chickweed (Anonymous 2018e, 2019d; Jackson et al. 2018; Lee et al. 2015; Soltani et al. 2006). These two herbicides also provide significantly reduced rotational restrictions compared to prosulfuron with essentially no limitations to planting Georgia's major row crops once the rye is harvested (Anonymous 2018e; Anonymous 2019d).

In areas where Italian ryegrass is resistant to POST applied herbicides, pyroxasulfone and flumioxazin plus pyroxasulfone have become critical management

tools in wheat and could prove beneficial in rye production. Pyroxasulfone applied shortly after planting has become a major component of ryegrass management with minimal impacts on wheat in both the United States (Hulting et al. 2012) and Australia (Boutsalis et al. 2014). Flumioxazin plus pyroxasulfone incorporates an additional mechanism of action that broadens the weed control spectrum compared to pyroxasulfone alone and still maintains minimal injury to wheat (Crow et al. 2015; Randhawa et al. 2018).

Previous studies evaluating cereal rye tolerance to tribenuron demonstrated minimal injury but tolerance to mesosulfuron was variable (MacRae et al. 2007). Minimal information is available concerning rye tolerance to other wheat herbicides. Therefore, rye tolerance to commonly used wheat herbicides should be investigated so that weed control options can be expanded, resulting in improved weed control.

OBJECTIVES

Objective 1: Experiments were conducted to determine if 2,4-D or dicamba can be removed from the surface of plastic mulch over time or with a single irrigation event using analytical and bioassay techniques.

Objective 2: To design dicamba-based management programs that are effective and sustainable with the potential for immediate grower adoption. The influence of cover crop, PRE herbicides, and a lay-by post-directed (LPD) application using traditional herbicide chemistry were evaluated to determine their influence on selection pressure of dicamba applied POST in a cotton weed management program and overall weed control.

Objective 3: Determine tolerance of cereal rye to labeled wheat herbicides with the goal of pursuing new rye registrations, in cooperation with IR-4, for products that have the appropriate safety.

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CHAPTER 2

QUANTIFYING GLYPHOSATE PLUS 2,4-D OR DICAMBA REMOVAL FROM THE SURFACE OF TOTALLY IMPERMEABLE FILM USING ANALYTICAL AND BIOASSAY TECHNIQUES[1]

[1]Hand LC, Eason KM, Randell TM, Grey TL, Richburg JS, Culpepper AS. Accepted for publication in *Weed Technology,* 11/9/2020. Reprinted here with permission of the publisher.

Abstract

The loss of methyl bromide led to vegetable growers relying more heavily on herbicides to control weeds. Although herbicides can be effective, limited herbicide options in vegetable production challenge growers. Identifying new, effective tools to be applied over plastic mulch prior to planting for improved weed control with minimal crop injury would be beneficial. The objective of these experiments was to evaluate the persistence of preplant applications of glyphosate (1,125 or 2,250 g ae ha^{-1}) plus 2,4-D (1,065 or 2,130 g ae ha^{-1}) or dicamba (560 g ae ha^{-1}) over plastic mulch using analytical techniques and subsequent squash and watermelon response. Glyphosate and 2,4-D were not analytically detected at damaging concentrations on plastic mulch when at least 3.5 cm of rainfall was received after application and before planting. Additionally, bioassay results for squash and watermelon supported analytical results with less than 10% visual injury for either crop, with no growth or yield suppression observed. In contrast, dicamba concentrations on plastic mulch, regardless of rainfall amount or time between application and planting, remained at damaging levels. Squash yields were reduced by dicamba applied 1 to 30 days before planting while watermelon was more resilient. 2,4-D plus glyphosate applied preplant over plastic mulch can provide another herbicide option to vegetable growers. Additional research is needed to understand the impact of residual activity of 2,4-D when transplants land directly in holes present in plastic mulch at the time of application. The relationship of dicamba with plastic mulch is complex as the herbicide cannot be easily removed by rainfall. Thus, dicamba should not be included in a weed management system in plasticulture vegetable production.

Introduction

The loss of methyl bromide from the market place over a decade ago led to a shift in weed control practices for plasticulture vegetable production systems (Culpepper et al. 2009; Eure and Culpepper 2017; Stevens et al. 2016). Currently, one of the most common vegetable production practices in Georgia is to fumigate using a system comprised of 1,3-dichloropropene, chloropicrin, and metam sodium, while covering the bed with either low-density polyethylene (LDPE) mulch or totally impermeable film (TIF) (Culpepper et al. 2008; Culpepper et al. 2017). Exceptional control of many diseases, nematodes, and weeds can be achieved with this fumigant system, especially when applied under TIF (Culpepper et al. 2017). Due to lack of permeability, TIF mulch minimizes the ability of the fumigant from escaping the raised bed, improving weed control but also presenting challenges to growers due to plant back intervals potentially exceeding 35 days (Culpepper et al. 2017). This, compounded with the impact of environmental conditions on fumigant degradation, has led many growers in Georgia to fumigate their vegetable fields from December to January before planting their crop in March (Csinos et al. 2002; Desaeger et al. 2008). This allows for optimum fumigant activity as well as adequate time for fumigant degradation under the mulched bed. Although this approach provides exceptional pest control under the plastic with minimal crop injury concerns, weeds in the row middles between plastic mulched beds have become more problematic because of the extended time interval between laying the mulch and crop planting.

Weed management challenges increase for Georgia vegetable growers as they often produce three to five crops on a single installation of plastic mulch spanning over 18 to 24 months before removing the plastic. For each subsequent crop after the initial

crop, weeds not only emerge in the row middles but also through holes created in the mulch from previous plantings and natural degradation of the plastic (Boyd 2016). Broadcast-applied herbicide options are limited by the presence of plastic mulch and crop tolerance. Glyphosate and paraquat, two of the most popular options among growers, provide broad-spectrum weed control and can be removed from the plastic mulch before planting with a single rainfall or irrigation event (Boyd 2016; Culpepper et al. 2009; Grey et al. 2009). Many winter annual weeds can be controlled by glyphosate or paraquat; however, wild radish (*Raphanus raphanistrum* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), and horseweed (*Conyza canadensis* (L.) Cronq.) are often present and may not be adequately controlled by these herbicides (Culpepper et al. 2005; Eubank et al. 2008). When glyphosate or paraquat was applied alone, 80 to 81%, 56 to 60%, and 55 to 74% control of wild radish, cutleaf evening-primrose, and horseweed was observed, respectively (Culpepper et al. 2005; Eubank et al. 2008). To control these problematic weeds and start the season with minimal weed competition, additional herbicidal tools are needed.

Although numerous herbicides are available to control these weeds, broadcast applications over plastic mulch increase complications. Herbicides applied over plastic mulch have been shown to wash off of the mulch with an initial irrigation or rainfall event, partially wash off of mulch over time, or bind to the mulch without release (Culpepper et al. 2009; Grey et al. 2009; Grey et al. 2018; Randell et al. 2020). For example, previous research has demonstrated that flumioxazin applied over plastic mulch persists, resulting in significant injury and yield reductions for squash and tomato (*Solanum lycopersicum* L.) (Culpepper et al. 2009; Grey et al. 2009). Halosulfuron-

methyl applied over plastic mulch, even with large amounts of rainfall after application and prior to planting, damaged squash, broccoli (*Brassica oleracea* var. *botrytis* L.), and cabbage (*Brassica oleracea* var. *capitate* L.) at varying levels (Culpepper et al. 2009; Randell et al. 2020). Thus, identifying potential herbicide tools to improve weed control in vegetables grown on plasticulture must begin with understanding the relationship of the herbicide and the mulch.

Auxinic herbicides were the first group of chemicals used for selective weed control in cropping systems, with 2,4-D being the first available herbicide within this mechanism of action (Johnston et al. 2018). Although synthetic auxins have been available for 70 years, interest in utilizing these chemistries has been renewed due to formulation improvements along with the introduction of 2,4-D and dicamba-tolerant agronomic crops (Anonymous 2017; Anonymous 2018a; Anonymous 2018b; Johnston et al. 2018). The use of these compounds to control many troublesome weeds has been documented for decades. Culpepper et al. (2005) observed that tank mixtures of 2,4-D or dicamba plus glyphosate resulted in 94 to 97% control of both cutleaf evening-primrose and wild radish 28 days after treatment (DAT). Additionally, applications of glyphosate plus 2,4-D or dicamba resulted in 90 to 99% control of horseweed 4 weeks after treatment (Eubank et al. 2008). However, non-tolerant broadleaf crops can be extremely sensitive to low doses of these herbicides. Significant injury and yield reductions from low rates of auxinic herbicides have been documented in many high-value vegetable crops such as bell pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), snap bean (*Phaseolus vulgaris* L.), squash (*Cucumis melo* L.), and watermelon [*Citrullus*

lanatus (Thunb.) Matsum. & Nakai] (Colquhoun et al. 2014; Culpepper et al. 2018; Dittmar et al. 2016).

Although crop response to auxinic herbicides applied at low rates to the foliage of vegetable crops has been documented, further research is needed to investigate the use of preplant applications of 2,4-D or dicamba over plastic mulch prior to vegetable transplanting. Provided 2,4-D and dicamba can be removed from mulch with rainfall and/or irrigation, or dissipate to non-lethal concentrations rapidly on plastic mulch, the use of these herbicides would provide vegetable growers with valuable tools in their weed control program. Therefore, the objective of this experiment was to determine the potential for applying 2,4-D or dicamba over plastic mulch prior to transplanting squash and watermelon using analytical and bioassay techniques.

Materials and Methods

Site Selection and Trial Establishment. Studies were conducted in the summer of 2018 and 2019 near Ty Ty, GA (31.50911°N, 83.64813°W) to evaluate squash and watermelon response to 2,4-D plus glyphosate and dicamba plus glyphosate applied over plastic mulch prior to transplanting. For this site, the soil was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 90% sand, 8% silt, 2% clay, 0.64% organic matter, and a pH of 6.3 in 2018; and 84% sand, 12% silt, 4% clay, 0.86% organic matter, and a pH of 6.2 in 2019. The soil was prepared conventionally and raised beds were formed 3 months prior to planting to allow fumigant dissipation before planting. As beds were being formed, the entire trial area was treated with a fumigant system including 1,3-dichloropropene, chloropicrin, and metam sodium. The initial bedder (Hendrix & Dail,

Inc. Greenville, NC 27835) formed a prebed 20 cm tall and 81 cm wide while injecting 1,3-dichloropropene and chloropicrin (Pic-Clor 60, TriEst Ag Group, Inc., 1101 Industrial Blvd., Greenville, NC 27835) at a rate of 197 L ha^{-1} 20 cm below the bed top using 3 evenly spaced injection shanks. This was followed immediately by a combination bed shaper and plastic mulch layer (Kennco Manufacturing, Inc., Ruskin, FL 33570) that injected metam sodium (Vapam® HL™, AMVAC®, 4100 E. Washington Blvd. Los Angeles, CA 90023) 10 cm deep with injection shanks 10 cm apart at a rate of 700 L ha^{-1}. As metam sodium was injected, drip tape was placed in the center of the bed 2.5 cm below the bed surface and the entire bed was covered with black on black TIF (Guardian Agro Plastics, 10417 Greendale Drive Tampa, FL 33626).

The experimental design was a randomized complete block design with an augmented factorial arrangement of treatments, consisting of four application timings, three herbicide options, and a nontreated control for comparison. Herbicides included 2,4-D (Embed® Extra, Corteva Agriscience, 9330 Zionsville Rd., Indianapolis, IN 46268) at 1,065 and 2,130 g ae ha^{-1} and dicamba (Engenia®, BASF Corporation, 26 Dave Dr., Research Triangle Park, NC 27709) at 560 g ae ha^{-1}. Glyphosate (Roundup PowerMax II®, Bayer Cropscience, 800 N. Lindbergh Blvd., St. Louis, MO 63167) at 1,125 g ae ha^{-1} was included with the lower rate of 2,4-D and with dicamba, while 2,250 g ha^{-1} was used with the higher rate of 2,4-D. These tank mixtures, formulations, and rates were selected for potential labeling by registrants. Herbicides were applied over plastic mulch at approximately 45, 30, 15, and 1 day before planting (DBP). The 45 DBP applications were made on March 5, 2018, and February 7, 2019; 30 DBP applications were made on March 23, 2018, and February 21, 2019; 15 DBP applications were made on April 4,

2018, and March 7, 2019; and 1 DBP applications were made on April 17, 2018, and March 20, 2019. Rainfall accumulation, solar thermal radiation, and daily maximum and minimum temperature data were collected onsite at a University of Georgia Weather Monitoring Network station (https://weather.uga.edu) and are presented in Table 2.1.

Herbicide applications were broadcast directly over plastic mulch using a CO_2-pressurized backpack sprayer equipped with AIXR 11002 nozzles for 2,4-D or TTI 110015 nozzles for dicamba (Teejet Technologies, Wheaton, IL 60187). Independent spray systems were used to apply dicamba and 2,4-D treatments and sprayers were calibrated to deliver 140 L ha^{-1}. Different nozzle types were utilized for both herbicides because of labeling requirements at the time these studies were conducted. Spray booms utilized were 138 cm long with a nozzle spacing of 46 cm and booms were held 41 cm above the mulch. Air temperature at the time of application ranged from 4 to 24 C, relative humidity ranged from 39-80%, and wind speeds did not exceed 8 km h^{-1}.

Analytical Methods. To quantify the removal of 2,4-D, dicamba, and glyphosate from plastic mulch, plastic samples were collected for analysis. Sampling procedures and extraction were based on similar studies (Grey et al. 2009; Grey et al. 2018). Samples were collected approximately 2 hours after treatment [0 days after application (DAA)] as well as at the time of planting (1, 15, 30, and 45 DAA), resulting in a total of 2 samples per treated plot. Samples of mulch were collected from each plot using an open-faced wooden square frame with an inside area of 0.1 m^2. A box-cutting knife was used to harvest the mulch along the inside edge of the square. Needle-nose pliers were then used to mechanically fold the mulch inward without contacting the treated side preventing

exposure to foreign objects. Samples were stored in plastic bags, frozen upon collection, and stored at -10 °C until analysis.

Field plot replicate sample integrity was maintained throughout sample collection, preparation, and chemical analysis. For herbicide analysis, samples were removed from the freezer and allowed to equilibrate to room temperature prior to being placed in a 125-mL volumetric flask sealed with a rubber stopper. Extractions were conducted utilizing 10% methanol with high-performance liquid chromatography (HPLC) water (Fisher Scientific International, Waltham, MA 02451). The extraction volumes were 100 mL. Samples were placed on a reciprocating shaker for 2 hours at 200 rpm. Upon removal, samples passed through a 0.2 μM polytetrafloroethylene (PTFE) membrane filter (Fisher Scientific International, Waltham, MA 02451) that was fitted to a Luer-Lok™ syringe (Fisher Scientific International, Waltham, MA 02451), and then passed into a 1.5-mL microcentrifuge tube (Fisher Scientific International, Waltham, MA 02451). Microcentrifuge tubes were sealed, and centrifuged at 12,000 rpm for 5 minutes. A 1,000 μL sample was then transferred into injection vials (Fisher Scientific International, Waltham, MA 02451). Samples were analyzed with a Waters Acquity Arc Ultra-High Performance Liquid Chromatography (UHPLC) system, coupled with a Waters 2998 PDA and Waters QDa Mass Spectrometry Detector (Waters Corporation, Milford, MA 01757). Chromatographic conditions for each herbicide are provided in Table 2.2 and were adapted from Majzik et al. (2006) for 2,4-D and dicamba. Selectivity was tested by utilizing blank samples with no interfering peaks detected. Calibration curves were constructed using analytical formulations and showed a linear response for all herbicides, with correlation coefficients (R^2) >0.98 and residuals were within +/- 20% without the

use of internal standards. Recovery samples were prepared in solution based on the respective acid equivalent applied and indicated that recoveries were within acceptable range (96-105%).

Crop Establishment and Data Collection. On April 18, 2018 and March 21, 2019 transplant holes were mechanically made in the plastic mulch using a transplant hole punch wheel (Kennco Manufacturing, Inc., 1105 3rd St. NE, Ruskin, FL 33570) in preparation for planting. 'Enterprise' squash (10 cm in height) was transplanted in single rows with a spacing of 1.8 m between beds and 30 cm between plants within a row. '7197' (2018) and 'Melody' (2019) seedless watermelons (15 cm in height) were also planted on the same day as hole punching in single rows with a spacing of 3.7 m between planted beds and 30 cm between plants within a row. Pollinator diploid watermelons ('8585' in 2018 and 'Premier' in 2019) were planted at the same time as seedless watermelons and were included as every 4th plant in each plot. Plots for each crop were 5.8 m long by 0.6 m wide. Squash and watermelon management including fertility, irrigation, insect and disease management were conducted in accordance with university recommendations for the region (Kemble et al. 2019).

Visual crop injury (chlorosis, epinasty, stunting, leaf deformations) was rated on a scale of 0 to 100% (0% being no injury, 100% being crop death) every 7 days beginning one week after planting up to five weeks after planting. Reductions in squash growth were quantified by measurements across the diameter of the plant while watermelon growth was quantified by measuring the length of the longest vine. Ten plants per plot were measured weekly up to three weeks after planting. Early fresh weight biomass was obtained by removing 6 to 9 plants at the ground level and collecting weights at 16 to 22

DAP. Ten plants remained in each treated plot for harvest data. Squash harvest was initiated on May 14, 2018 and April 22, 2019. Squash were harvested a total of 30 times, 6 days per week, with fruit number and weight per plot recorded for each harvest. Harvests were then split into early harvests (1 to 10) and total harvests (1 to 30) to determine the impacts of treatments on maturity and total yield. Watermelons were harvested once when the non-treated control reached maturity. For each plot, melons were individually counted and weighed on June 27, 2018, and June 18, 2019. Watermelon harvest data was then sorted into three categories: nonmarketable watermelons (< 6.8 kg), marketable watermelons (≥ 6.8 kg), and total watermelon yield to evaluate the impact of treatments on crop maturity. Post-harvest biomass was obtained for squash only to assess herbicide damage present at the end of the season.

Statistical analysis. For analytical data, ANOVA was applied to the data combined across treatment and year to test for interactions. Year by treatment interactions were not significant; therefore, data were pooled over year. Years and replications were considered random effects. Regression analysis was performed using SAS nonlinear regression to determine if 2,4-D, dicamba, and glyphosate removal from plastic mulch could be described using the exponential decay equation: $y = B_0 e^{-B1(x)}$. In this equation, y is the herbicide concentration, B_0 is the initial concentration, B_1 is the slope, and x is sampling time in days after application (DAA). After data were regressed against time, the output from the analysis included the first-order dissipation-rate constants (k) (Ohmes et al. 2000). Data for the exponential decay equations were subjected to ANOVA using the nonlinear regression model procedure with mean separation using 95% asymptotic confidence intervals. Data were then graphed in Sigmaplot 14 (Systat Software, San Jose,

CA). Further analysis was conducted on herbicide concentration remaining at crop planting with respect to application timing. Concentrations remaining at planting were combined across application timings that received rainfall due to lack of statistical differences. Concentration means that were present following a rainfall event were analyzed in PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC 27513) and means were separated using the Shaffer-simulated method (α=0.05) (Blythe 2012).

Bioassay data were subjected to ANOVA using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC 27513) to determine if the combined treatment effects of herbicide, rate, and application timing influenced squash and watermelon growth, development, and yield. The interaction between year and treatment was evaluated and found to be not significant for all response variables; thus, data for all response variables are pooled over year. Rate effects were evaluated for 2,4-D plus glyphosate and were found to be not significant for all response variables; therefore, all 2,4-D data will be averaged over rate. Years and replications were considered random effects. All P values for tests of differences between least-squares means were compared and adjusted using the Shaffer-simulated method (α=0.05) (Blythe 2012).

Results and Discussion

Herbicide removal from plastic mulch. Dicamba at 560 g ha^{-1}, 2,4-D at 1,065 and 2,130 g ha^{-1}, and glyphosate at 1,125 and 2,250 g ha^{-1} are theoretically equivalent to 56,000; 106,500; 213,000; 112,500; and 225,000 μg ae m^{-2} applied to plastic mulch, respectively (Figure 2.1 and 2.2). The exponential decay equation accurately described the removal of all three herbicides from the surface of the mulch with first-order rate

constants (*k*) of 0.34 for dicamba, 0.24 and 0.17 for the 1 and 2X rates of 2,4-D, and 0.32 and 0.32 for the 1 and 2X rates of glyphosate (Table 2.3). First-order dissipation rate constants describing removal over time with respect to all sampling dates were similar for all herbicides (Table 2.3). For 2,4-D, dicamba, and glyphosate, rainfall was important in herbicide removal from the mulch with 3.5 to 11.3 cm of rainfall accumulated for treatments applied 15 to 45 DBP while no rainfall was observed between application and 1 DBP treatments (Figure 2.1 and 2.2; Table 2.3). These herbicides are not readily photodegraded; however, they have all demonstrated high levels of water solubility (Shaner 2014).

Although removal of 2,4-D and dicamba from the mulch appeared rapid with respect to time, it is worth noting that when averaged over the 15 to 45 DAA sample dates, there was 31 μg m^{-2} of 2,4-D (regardless of rate applied) and 494 μg m^{-2} of dicamba remaining on the plastic at planting. For both squash and watermelon, 31 μg m^{-2} is well below the rates of 2,4-D necessary to cause visual injury when applied to the foliage, assuming similar exposure mechanisms (Culpepper et al. 2018; Culpepper and Vance 2019). However, the amount of dicamba remaining on the plastic is enough to cause visual injury on both squash and watermelon when applied to the foliage. Previous research indicated that when 5 g ha^{-1} of dicamba was applied to squash foliage, it resulted in 50 to 51% visual injury at 10 and 17 days after application (Dittmar et al. 2016). This rate is equivalent to 500 μg m^{-2}. For watermelon, significant vine length reductions occurred when dicamba was applied to the foliage as low as a 1/250 field rate, which would be equivalent to 224 μg m^{-2} (Culpepper et al. 2018). Thus, damage should be acceptable from 2,4-D as long as 3.5 to 3.7 cm of rainfall occurs between application and planting

and the interval from application to planting is at least 15 days. For dicamba, neither rainfall nor time interval through 45 days will likely alleviate injury concerns.

Glyphosate removal from plastic mulch has been previously documented (Grey et al. 2009). Previous studies demonstrated that glyphosate can be removed from plastic mulch with as little as a single 1 cm rainfall or irrigation event (Culpepper et al. 2009; Grey et al. 2009). Removal of glyphosate from the surface of plastic mulch in this study followed the same pattern. Averaged over the 15 to 45 DAA application timings with at least 3.5 cm of rainfall, glyphosate at both rates was detected at 118 μg m^{-2}, which is equivalent to approximately a 1/1,000 field rate. Culpepper et al. (2009) demonstrated that tomato and squash could be safely planted after 1 cm of irrigation washed glyphosate from plastic mulch prior to transplanting. Based on the amount of rainfall received for these studies with plantings of 15 to 45 DAA, glyphosate should not negatively influence crop growth.

Squash Experiments. Pooled over years, visual injury estimates were at their maximum at 23 to 28 DAP (days after planting). Squash injury was influenced by the interaction of application timing and herbicide option ($P<0.0001$). When herbicides were applied 45, 30, 15, and 1 DBP, squash injury was 12%, 65%, 75%, and 95%, respectively, for dicamba plus glyphosate, and 2%, 4%, 7%, and 73%, respectively, for 2,4-D plus glyphosate (Table 2.4). Rainfall for each year was similar and the average amounts accumulated after application and prior to planting were 10.8, 7.8, 3.6, and 0 cm for the aforementioned application intervals, respectively (Table 2.1). Injury from 2,4-D plus glyphosate was directly related to no rainfall for the 1 DBP application (73%) versus at least 3.6 cm of rainfall for the earlier application timings (2 to 7%). In contrast, dicamba at damaging levels remained on the mulch regardless of rainfall amount or interval

between application and planting. Injury was not observed in the control. Although little work has been done utilizing these herbicides preplant in plasticulture production, 2,4-D and dicamba have both been used preplant in row crop production with sensitive crops. When applied up to 4 weeks prior to planting in non-tolerant cotton (*Gossypium hirsutum* L.), dicamba caused significant stand loss and leaf distortion while 2,4-D caused significant stand loss when applied 1 week prior to planting (York et al. 2004). In non-tolerant soybean [*Glycine max* (L.) Merr.], both 2,4-D and dicamba have the potential to cause significant injury when applied up to 2 weeks prior to planting (Thompson et al. 2007).

Squash canopy widths at 23 to 28 DAP were influenced by the interaction of application timing and herbicide option ($P<0.0001$). On average, the squash in the control were 78 cm wide (Table 2.4). Compared to the squash in the control, the 30 (47 cm), 15 (42 cm), and 1 (9 cm) DBP treatments with dicamba plus glyphosate and the 1 (25 cm) DBP treatment with 2,4-D plus glyphosate had significantly smaller width than the control. Early-season biomass at 16 to 22 DAP was influenced by the interaction of application timing and herbicide option ($P<0.0001$) and followed similar trends to squash widths. On average, early-season squash fresh weight biomass in the control weighed 66.2 g plant^{-1} (Table 2.4). Compared to the control, the 30 (36.6 g), 15 (36.4 g), and 1 (5.4 g) DBP treatments with dicamba plus glyphosate and the 1 (6.8 g) DBP treatment with 2,4-D plus glyphosate significantly reduced biomass.

Earliness of harvest is important in vegetable production as it can have a tremendous impact on fruit value (Culpepper et al. 2018). Herbicide injury has the potential to delay maturity in vegetable crops, which can lead to growers receiving less money as they miss

their predetermined market window. In an attempt to quantify the potential delay in maturity from treatments, count and weight data from the first 10 harvests were summarized and are reported. Early harvest fruit counts and weights were significantly impacted by the interaction of application timing and herbicide option ($P<0.0001$). As applications of dicamba mixtures were made closer to planting, early-season fruit counts and weights decreased, while for 2,4-D mixtures, yield loss only occurred with the application 1 DBP where rainfall was not received to remove it from the mulch. On average, squash from the control yielded 181,000 fruit ha^{-1} weighing 18,900 kg ha^{-1} during the first 10 harvests (Table 2.5). For both early-season fruit counts and weight, the 30, 15, and 1 DBP treatments with dicamba plus glyphosate and the 1 DBP treatment with 2,4-D plus glyphosate significantly reduced yield 47 to 99% compared to the control.

Cumulative fruit counts and weights after 30 harvests noted a greater yield loss with dicamba applications made closer to planting; while 2,4-D mixtures only influenced yield applied 1 DBP. On average, squash yielded 828,200 fruit ha^{-1} weighing 89,800 kg ha^{-1} in the control (Table 2.5). Season-long fruit count and weight data in response to preplant applications of dicamba or 2,4-D tank-mixtures were nearly identical. For both total fruit counts and weight, the 30, 15, and 1 DBP treatments with dicamba plus glyphosate and the 1 DBP treatment with 2,4-D plus glyphosate yielded significantly less than the control.

Following the final harvest, squash plants free of fruit were removed at the soil line and weighed to quantify the reduction in non-fruit fresh weight biomass over the entire season. Post-harvest biomass was influenced by the interaction of application timing and

herbicide option (P<0.0001). Biomass results followed similar trends to other data. On average, squash plants in the control weighed 3,987 g plant^{-1} (Table 2.4). Post-harvest biomass was significantly reduced by the 30, 15, and 1 DBP treatments with dicamba plus glyphosate and the 1 DBP treatment with 2,4-D plus glyphosate compared to the control.

Watermelon Experiments. Visual estimates of watermelon injury were at their maximum 30 DAP both years, therefore only 30 DAP visual injury is presented (Table 2.6). Watermelon injury was influenced by the interaction of application timing and herbicide option (P<0.0001). Dicamba plus glyphosate applied 45, 30, 15, and 1 DBP, injured watermelon 13%, 39%, 42%, and 94%, respectively. Previous research has noted sublethal doses of dicamba applied to foliage injure watermelon as well (Culpepper et al. 2018). For 2,4-D plus glyphosate, injury of 84% was noted when applied 1 DBP with no rainfall received after application and before planting. Once rainfall of at least 3.5 cm was received to wash the mulch, the subsequent injury was less than 5% when averaged over rate (Table 2.6).

Watermelon vine lengths taken 23 to 34 DAP were significantly influenced by the interaction of application timing and herbicide option (P<0.0001). Watermelon vines in the control averaged 68.5 cm in length (Table 2.6). Only dicamba plus glyphosate applied 30 (48 cm), 15 (48 cm), and 1 (1.5 cm) DBP, and 2,4-D plus glyphosate applied 1 (9.1 cm) DBP significantly reduced vine length. Previous research noted vine length reductions of 51% or greater when dicamba was applied to watermelon foliage at a 1/75 rate (Culpepper et al. 2018). Early-season watermelon biomass collected 16 to 22 DAP noted watermelon plants in the control averaged 19.5 g plant^{-1}. Biomass reductions were

only noted with dicamba plus glyphosate and 2,4-D plus glyphosate applied 1 DBP (1.3 and 1.6 g plant^{-1}, respectively) (Table 2.6).

Watermelon fruit counts and weights were analyzed using three categories: nonmarketable watermelons (< 6.8 kg), marketable watermelons (≥ 6.8 kg), and total watermelon yield to understand treatment effects. Yield data were analyzed and are presented in terms of weight of watermelons picked as watermelon counts followed similar patterns. Nonmarketable watermelon yield ($P<0.0001$), marketable watermelon yield ($P<0.0001$), and total watermelon yield ($P<0.0001$) are all significantly impacted by the interaction of herbicide option and application timing. For nonmarketable watermelon yield, dicamba plus glyphosate applied 1 DBP (6,026 kg ha^{-1}), as well as 2,4-D plus glyphosate applied 45 and 1 DBP (24,967 and 23,708 kg ha^{-1}, respectively) resulted in significant nonmarketable yield loss compared to the control (Table 2.7). For marketable watermelon yield, both glyphosate plus dicamba or 2,4-D applied 1 DBP (1,837 and 25,590 kg ha^{-1}, respectively) without rainfall to remove any of the herbicide from the mulch caused 40 to 96% yield loss compared to the control. Interestingly with the glyphosate plus 2,4-D treatment differences observed, less smaller nonmarketable fruit noted with the 45 DBH application was correlated with a higher numerical amount of marketable fruit while the 1 DBH application had not only less small fruit but also less marketable and total fruit when compared to the control. Previous research noted marketable watermelon yield reductions when exposed to a 1/75 rate of either 2,4-D or dicamba early in the season (Culpepper et al. 2018). Total watermelon yield followed the same trend as marketable watermelon yield with only dicamba and 2,4-D applied 1 DBP

(7,863 and 49,298 kg ha^{-1}, respectively) significantly reducing yield compared to the control.

Dicamba and 2,4-D would be extremely useful in helping vegetable producers control problematic weeds prior to planting. Although dicamba removal from plastic mulch seemed rapid with respect to time in the analytical analysis, the amount of dicamba remaining on the mulch caused significant damage to both squash and watermelon. Generally, the greater the time interval between application and planting the more crop tolerance that was observed, but excessive injury and growth reductions from dicamba on the plastic mulch prohibit potential labeling for the herbicide in plasticulture production systems. In contrast, 2,4-D demonstrated a significant potential for use in a plasticulture weed management system for vegetable growers. Minimal visual injury, growth reductions, and yield loss were observed for both squash and watermelon as long as rainfall of at least 3.5 cm occurred after application and prior to crop planting. Furthermore, this research demonstrated no differences between 2,4-D mixtures applied at a 1 or 2X rate with safety observed with applications when rainfall was received before planting. However, weed management in multi-cropped plasticulture vegetable production is extremely complex. Future research should evaluate the use of 2,4-D in a multi-cropped plasticulture system, where holes are present in the mulch, evaluating potential residual uptake of 2,4-D. Additionally, other valuable crops produced in plasticulture systems such as tomatoes, bell peppers, or cole crops should be evaluated.

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Table 2.1. Environmental data for 2,4-D plus glyphosate and dicamba plus glyphosate removal from Totally Impermeable Film.

	2018				2019			
Treatment[a]	Temperature[b]		Rainfall[c]	Radiation[d]	Temperature		Rainfall	Radiation
	Max	Min	cm	MJ m^{-2}	Max	Min	cm	MJ m^{-2}
45 DBP	21.3	8.3	11.3	726	20.9	9.9	10.3	516
30 DBP	22.8	9.8	6.3	437	21.4	10.4	9.3	405
15 DBP	22.5	9.4	3.5	235	21.6	9.7	3.7	239
1 DBP	23.5	4.4	0.0	25	17.7	3.8	0.0	22

[a]Abbreviations: DBP, days before planting.

[b]Average daily maximum and minimum after application and prior to planting reported in degrees Celcius.

[c]Total rainfall after application and prior to planting.

[d]Sum of total solar radiation after application and prior to planting.

Table 2.2. Chromatographic analysis settings in methods used to determine herbicide concentrations on plastic mulch.

Herbicide	Column[a]	Mobile phase[b]	Gradient/Isocratic				Injection volume	Flow rate	Column Temperature	Mode	SIM	Cone Voltage
			Time	%A	%B	%C	µL	mL min^{-1}	°C		g mol^{-1}	V
dicamba	Cortecs C_{18} 4.6 x 50 mm with 2.7µm packing	(A) ACN:H_20 (1:9)	0.00	50	40	10	50	1.1	30	ESI Negative	219	15
		(B) ACN:H_20 (9:1)	1.80	60	40	0						
		(C) H_20	5.40	50	40	10						
2,4-D	Symmetry C_{18} 4.6 x 75 mm with 3.5µm packing	(A) ACN:H_20 (1:9)	0.00	50	40	10	50	1.1	30	ESI Negative	219	15
		(B) ACN:H_20 (9:1)	2.69	60	40	0						
		(C) H_20	8.17	50	40	10						
glyphosate	Anionic Polar Pesticide 2.1 x 100 mm with 5µm packing	(A) H_20 + 0.9% FA	0.00	10	90	-	10	0.75	40	ESI Negative	168	20
		(B) ACN + 0.9% FA	2.00	10	90	-						
			4.00	60	40	-						

[a]All columns are from Waters Corporation©.
[b]Abbreviations: ACN, acetonitrile; ESI, electron spray ionization; SIM, single ion monitored.

Table 2.3. First-order dissipation rate constants (*k*) of 2,4-D, dicamba, and glyphosate from Totally Impermeable Film over time from field experiments conducted in the summer of 2018 and 2019.

Herbicide	Rate	First-order rate constant[a]	
	g ae ha^{-1}	$-d^{-1}-$	
dicamba	560	0.34 (0.232)[b]	a
2,4-D	1,065	0.24 (0.250)	a
	2,130	0.17 (0.105)	a
glyphosate	1,125	0.32 (0.103)	a
	2,250	0.32 (0.155)	a

[a]First-order dissipation rate constants were calculated by nonlinear regression of the herbicide with respect to time (0 to 45 d) using the equation $y = B_0e^{-B1(x)}$. Values for each herbicide and rate for first-order rate constants within a column followed by the same letter are not significantly different at the $P<0.05$ probability level. General linear model procedures were used with mean separation using 95% asymptotic confidence intervals.

[b]Values represent first-order rate constant (± 95% asymptotic confidence interval) and are combined across years.

Table 2.4. Squash injury (23-28 DAP), canopy width (23-28 DAP), early-season fresh weight biomass (16-22 DAP), and post-harvest biomass as infuenced by herbicide and application timing. Data pooled over 2018 and 2019.

Herbicide	Application	Injury[b,c]		Widths[b,c]		Early biomass[b,c]		Late biomass[b,c]	
	DBP[a]	——%——		——cm——		——————g plant^{-1}——————			
glyphosate plus dicamba	45	12 (1.2)	d	71 (2.6)	a	51.0 (4.2)	a	3,467 (312)	ab
	30	65 (3.1)	c	47 (3.2)	b	36.6 (4.4)	a	2,455 (224)	c
	15	75 (2.8)	b	42 (2.4)	b	36.4 (4.8)	a	2,401 (225)	c
	1	95 (1.6)	a	9 (2.3)	d	5.4 (1.2)	b	283 (121)	d
glyphosate plus 2,4-D[d]	45	2 (0.6)	e	76 (2.7)	a	62.9 (3.8)	a	3,834 (176)	a
	30	4 (0.8)	e	78 (3.6)	a	59.0 (4.5)	a	3,558 (172)	a
	15	7 (1.3)	e	74 (2.3)	a	52.4 (3.4)	a	3,545 (203)	a
	1	73 (2.3)	b	25 (1.9)	c	6.8 (0.9)	b	2,712 (136)	bc
NTC[a]	NTC	0 (0)	e	78 (3.3)	a	66.2 (3.1)	a	3,987 (225)	a

[a]Abbreviations: DBP, days before planting; NTC, nontreated control

[b]Means followed by the same letter in a column do not differ significantly (P≤0.05).

[c]Values represent mean (± standard error).

[d]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, ANOVA indicated there was no significant difference between these two treatments for the response variables and they are combined for presentation.

Table 2.5. Squash early yield (Harvests 1-10) and total yield (Harvests 1-30) as influenced by herbicide and application timing. Data pooled over 2018 and 2019.

		Harvests 1-10		Harvests 1-30	
Herbicide	Application	Fruit no.[b,c]	Fruit weight[b,c]	Fruit no.[b,c]	Fruit weight[b,c]
	DBP[a]	–1,000 ha^{-1}–	–1,000 kg ha^{-1}–	—1,000 ha^{-1}—	–1,000 kg ha^{-1}–
glyphosate plus dicamba	45	171.6 (7.0) a	16.9 (1.2) a	747.6 (14.4) a	80.7 (2.9) a
	30	94.1 (20.3) b	6.9 (1.9) b	558.4 (51.2) b	50.4 (7.2) b
	15	62.8 (11.4) b	3.6 (0.8) b	501.9 (26.9) b	43.2 (3.9) b
	1	0.3 (0.3) d	0.04 (0.04) b	33.1 (17.9) c	2.5 (1.4) c
glyphosate plus 2,4-D[d]	45	183.8 (4.0) a	19.1 (1.0) a	829.6 (19.8) a	90.1 (2.5) a
	30	184.4 (5.7) a	18.6 (1.1) a	804.2 (22.2) a	86.0 (3.1) a
	15	178.7 (5.8) a	20.3 (3.1) a	797.9 (20.2) a	88.8 (4.5) a
	1	41.1 (6.3) c	2.2 (0.4) b	555.3 (23.5) b	52.9 (2.7) b
NTC[a]	NTC	181.0 (4.2) a	18.9 (0.9) a	828.2 (33.5) a	89.8 (3.5) a

[a]Abbreviations: DBP, days before planting; NTC, nontreated control

[b]Means followed by the same letter in a column do not differ significantly (P≤0.05).

[c]Values represent mean (± standard error).

[d]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, ANOVA indicated there was no significant difference between these two treatments for the response variables and they are combined for presentation.

Table 2.6. Watermelon injury (30 DAP), vine length (23-34 DAP), and fresh weight biomass (16-22 DAP) as influenced by herbicide and application timing. Data pooled over 2018 and 2019.

Herbicide	Application	Injury[b,c]		Vine lengths[b,c]		Biomass[b,c]	
	DBP[a]	——%——		——cm——		—g plant^{-1}—	
glyphosate plus dicamba	45	13 (4.8)	d	60.8 (2.7)	a	21.0 (2.8)	a
	30	39 (3.6)	c	48.0 (2.2)	b	18.0 (1.4)	a
	15	42 (5.9)	c	48.0 (5.4)	b	18.7 (2.4)	a
	1	94 (1.7)	a	1.5 (1.5)	c	1.3 (0.3)	b
glyphosate plus 2,4-D[d]	45	1 (0.4)	e	68.5 (1.6)	a	23.0 (2.0)	a
	30	2 (0.8)	e	66.1 (1.5)	a	20.8 (1.4)	a
	15	4 (1.4)	e	65.2 (3.0)	a	17.7 (1.6)	a
	1	84 (3.0)	b	9.1 (2.8)	c	1.6 (0.2)	b
NTC[a]	NTC	0 (0)	e	68.5 (1.8)	a	19.5 (2.1)	a

[a]Abbreviations: DBP, days before planting; NTC, nontreated control

[b]Means followed by the same letter in a column do not differ significantly (P≤0.05).

[c]Values represent mean (± standard error).

[d]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, ANOVA indicated there was no significant difference between these two treatments for the response variables and they are combined for presentation.

Table 2.7. Watermelon fruit weight as influenced by herbicide and application timing. Data pooled over 2018 and 2019.

Herbicide	Application	Watermelon Yield					
		< 6.8 kg		≥6.8 kg		Total	
	DBP[a]	————1,000 kg ha^{-1}————					
glyphosate plus dicamba	45	29.1 (2.8)	ab	48.4 (3.6)	ab	77.5 (2.7)	a
	30	34.9 (6.7)	ab	34.3 (6.0)	bc	69.2 (2.3)	a
	15	33.8 (3.7)	ab	33.4 (4.2)	bc	67.2 (5.1)	a
	1	6.0 (3.7)	c	1.8 (1.2)	d	7.8 (4.1)	c
glyphosate plus 2,4-D[d]	45	25.0 (2.0)	b	53.1 (2.1)	a	78.1 (1.7)	a
	30	28.8 (1.7)	ab	47.7 (2.8)	ab	76.5 (2.2)	a
	15	29.9 (2.4)	ab	47.8 (2.9)	ab	77.7 (2.4)	a
	1	23.7 (3.9)	b	25.6 (5.2)	c	49.3 (6.6)	b
NTC[a]	NTC	37.9 (3.7)	a	42.5 (5.8)	ab	80.4 (5.4)	a

[a]Abbreviations: DBP, days before planting; NTC, nontreated control

[b]Means followed by the same letter in a column do not differ significantly (P≤0.05).

[c]Values represent mean (± standard error).

[d]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, ANOVA indicated there was no significant difference between these two treatments for the response variables and they are combined for presentation.

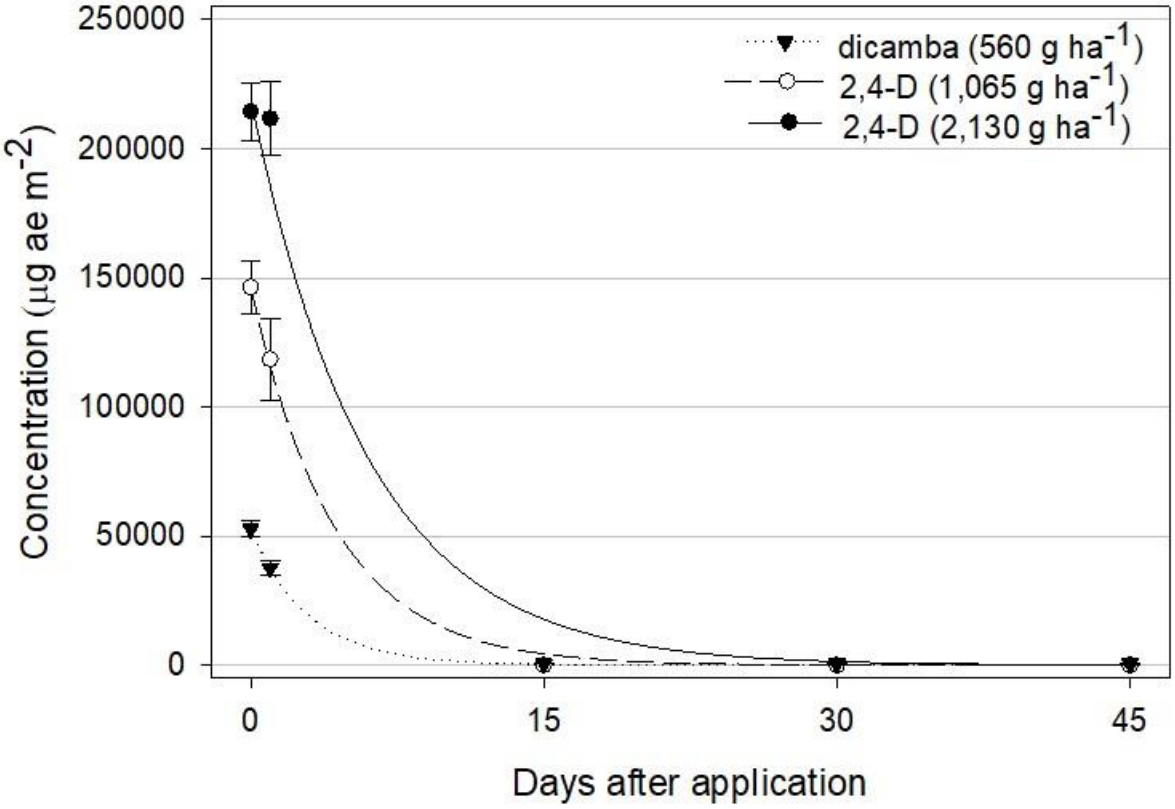

Figure 2.1. 2,4-D and dicamba removal from the surface of Totally Impermeable Film by rate in Georgia using the exponential decay equation ($y = B_0e^{-B1(x)}$). Nonlinear regression was applied for days after application. The lines represent the first-order regression equation for each treatment. Data points are the means of replications with bars indicating the standard error of the mean:

Dicamba 560 g ha^{-1}: $y = 52{,}661.7e^{(-0.339x)}$ P<0.0001 $R^2 = 0.80$

2,4-D 1,065 g ha^{-1}: $y = 146{,}866.7e^{(-0.2352x)}$ P<0.0001 $R^2 = 0.71$

2,4-D 2,130 g ha^{-1}: $y = 219{,}770.9e^{(-0.1675x)}$ P<0.0001 $R^2 = 0.83$

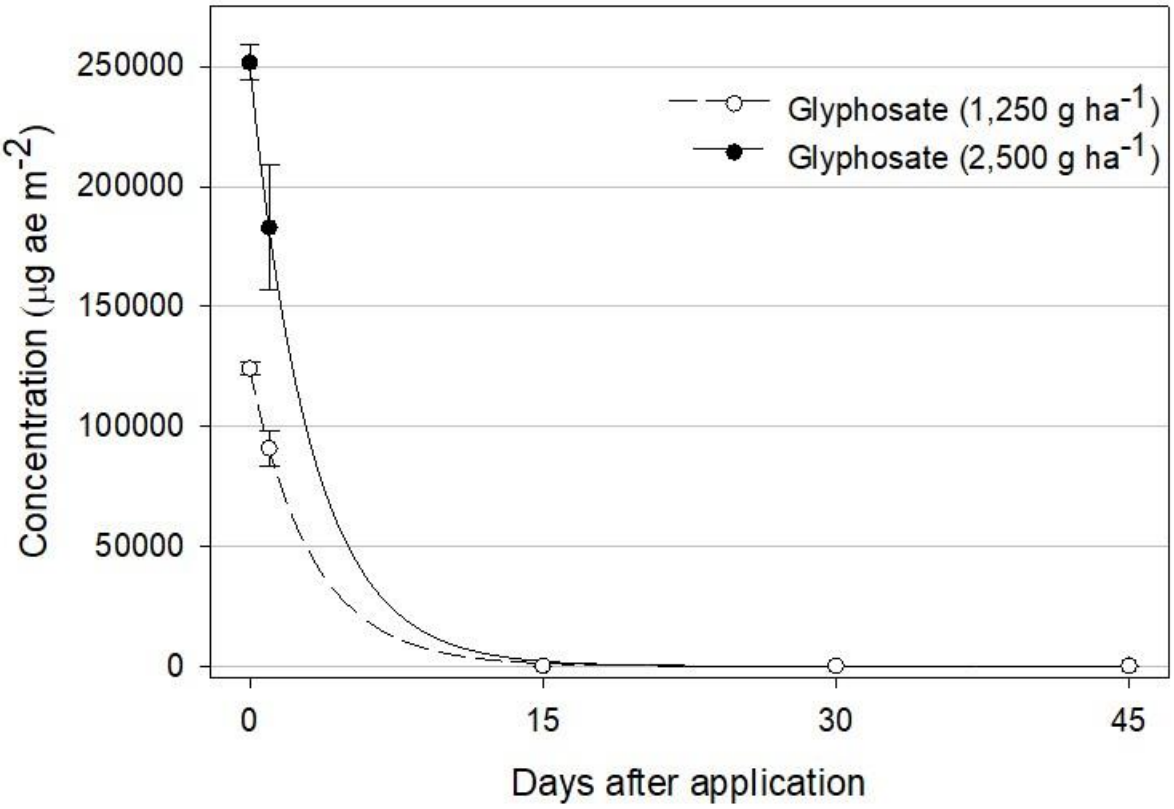

Figure 2.2. Glyphosate removal from the surface of Totally Impermeable Film by rate in Georgia using the exponential decay equation ($y = B_0e^{-B1(x)}$). Nonlinear regression was applied for days after application. The lines represent the first-order regression equation for each treatment. Data points are the means of replications with bars indicating the standard error of the mean:

Glyphosate 1,250 g ha^{-1}: $y = 124{,}220.7e^{(-0.3150x)}$ $P<0.0001$ $R^2 = 0.91$

Glyphosate 2,500 g ha^{-1}: $y = 251{,}723.7e^{(-0.3212x)}$ $P<0.0001$ $R^2 = 0.91$

CHAPTER 3

2,4-D AND DICAMBA REMOVAL FROM THE SURFACE OF PLASTIC MULCH USING OVERHEAD IRRIGATION: ANALYTICAL ANALYSIS AND CUCURBIT BIOASSAY CROP RESPONSE[1]

[1]Hand LC, Eason KM, Randell TM, Grey TL, Culpepper AS. Submitted to *Crop Protection*, 10/13/2020.

Abstract

Glyphosate and paraquat are effective preplant burndown herbicide options for multi-cropped vegetable production on plastic mulch due to their broad spectrum of activity, limited plantback concerns, and ability to be removed from plastic mulch by rainfall or irrigation. When planting vegetables, problematic weeds present such as wild radish (*Raphanus raphanistrum* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), annual morningglory (*Ipomea* spp.), or horseweed (*Conyza canadensis* (L.) Cronq.) may not be adequately controlled with these herbicides alone. With formulation improvements, 2,4-D and dicamba may be helpful in controlling these troublesome weeds prior to planting if they can be removed from plastic mulch avoiding crop damage. Experimental treatments included 2,4-D (1,065 and 2,130 g ae ha^{-1}) and dicamba (560 and 1,120 g ae ha^{-1}) applied broadcast directly over plastic mulch a day before transplanting. Just before transplanting, treatments received either overhead irrigation at 0.76 cm or no irrigation. Plastic mulch samples were collected at application and at planting to determine herbicide removal using analytical techniques, and cantaloupe and zucchini squash were subsequently transplanted on the plastic beds. Analytical analysis determined that 88-99% of the initial herbicide concentration was present at crop planting when irrigation was not implemented. At most, a 1/50 rate of dicamba and a 1/500 rate of 2,4-D was present at planting when overhead irrigation was received prior to transplanting. Maximum cantaloupe (*Cucumis melo* var. *cantalupo* Ser.) and squash (*Cucumis melo* L.) injury from 2,4-D with irrigation was 10% having no influence on plant growth, biomass, maturity and marketable yield. For dicamba with overhead irrigation, cantaloupe injury reached 35%, vine lengths were reduced 24% and maturity was delayed while squash

injury ranged from 9 to 12% without influencing growth or yield. Without irrigation to wash herbicides from the mulch prior to planting, 60-100% injury of both crops occurred with both herbicides which would have caused producers to abandon each crop. Zucchini squash was more tolerant and resilient with recovery from herbicide damage compared to cantaloupe. Experimental results demonstrated that 2,4-D can be adequately removed from the surface of plastic mulch with irrigation while dicamba removal requires more than a single irrigation event.

1. Introduction

Following the phase-out of the fumigant methyl bromide, pest management in plasticulture vegetable production has undergone significant changes (Culpepper et al., 2009; Eure and Culpepper, 2017; Stevens et al., 2016). Fumigant systems in Georgia now often consist of a combination of 1,3-dichloropropene, chloropicrin, and metam sodium injected under either low-density polyethylene (LDPE) mulch or totally impermeable film (TIF) (Culpepper et al., 2008; Culpepper et al., 2017). This system provides exceptional control of weeds, diseases, and nematodes for the first crop, however vegetable producers in the Southeast may utilize a single installation of plastic mulch for up to five crop cycles lasting 18 to 24 months. The time between the termination of one crop and the planting of another allows for the germination, emergence, and establishment of weeds in old plant holes or between the plastic mulched beds. Crop establishment free of weeds will require additional preplant burndown herbicides that can be applied broadcast over plastic mulch without crop injury.

Glyphosate and paraquat are two of the most popular herbicides for preplant burndown applications over multi-cropped plastic mulch because of their ability to be removed from plastic mulch with a single rainfall or irrigation event, minimizing plantback concerns (Culpepper et al., 2009; Grey et al., 2009; Boyd, 2016; Anonymous, 2018a). Other herbicides may not be easily removed from plastic mulch. Even herbicides that can be removed may persist in the soil of old plant holes or tears in the mulch; both scenarios pose a serious risk to high value vegetable crops. Previous research concluded that fomesafen can be removed from plastic mulch with relative ease (Culpepper and Smith, 2017). However due to soil persistence, sensitive crops, such as cole crops [broccoli (*Brassica oleracea* var. *botrytis* L.), cabbage (*Brassica oleracea* var. *oleracea* L.), and cauliflower (*Brassica oleracea* var. *botrytis* L.)], cannot be planted for up to 18 months after application (Anonymous, 2019). In contrast to fomesafen, neither flumioxazin nor halosulfuron are easily removed from plastic mulch effectively with a rainfall or irrigation event (Culpepper et al., 2009; Grey et al., 2009). In fact, broccoli, cabbage and squash were damaged by halosulfuron applications to plastic mulch after 30 days and 19 cm of rainfall occurred between applications and planting (Randell et al., 2020). Eventually halosulfuron could be removed from mulch, but often not in a realistic time frame for planting sensitive crops such as squash, broccoli, or cabbage (Grey et al., 2018; Randell et al., 2020). Therefore, herbicides that can be used in multi-crop plasticulutre must effectively control troublesome weeds, mix well with glyphosate or paraquat, and be easily removed from plastic mulch with rainfall or irrigation. Herbicides that meet these parameters and minimize the potential for crop injury could provide significant utility for growers using this system.

Interest in using 2,4-D and dicamba as preplant herbicides in multi-cropped plastic systems has increased following the introduction of auxin tolerant crops and formulation improvements (Anonymous, 2018b; Anonymous, 2018c; Anonymous, 2018d; Johnston et al., 2018). With the availability of less volatile formulations and proper stewardship of these weed control tools, these herbicides could be useful to vegetable growers. Some of the most problematic weeds when planting vegetables in the Southeastern U.S. include wild radish (*Raphanus raphanistrum* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), horseweed (*Conyza canadensis* (L.) Cronq.), and various annual morningglory species (*Ipomea* spp.). Glyphosate or paraquat alone are reported to provide 80-81%, 56-60%, 55-74% and 69-81% control of these weeds, respectively (Wilson and Worsham, 1988; Culpepper et al., 2005; Eubank et al., 2008). When 2,4-D or dicamba were tank-mixed with glyphosate or paraquat, at least 90% control was noted with all four weed species (Culpepper et al., 2005; Eubank et al., 2008; Leon et al., 2016). Although 2,4-D and dicamba would improve preplant weed control in these systems, non-transgenic broadleaf crops are extremely sensitive to these herbicides. Significant injury and yield reductions have been documented in bell pepper, potato, snap bean, squash, and watermelon when low rates of auxinic herbicides were applied to crop foliage (Colquhoun et al., 2014; Culpepper et al., 2018; Dittmar et al., 2016).

Herbicide registrations in high value vegetable crops are rare and often entirely dependent on crop response as opposed to efficacy in weed control (Culpepper et al., 2009; Kuack, 2020). Thus, crop response and tolerance must be well understood prior to pursuing registrations of any herbicide in a vegetable crop. The interaction of 2,4-D or dicamba with plastic mulch as influenced by irrigation prior to planting vegetables has

not been studied. Therefore, analytical and bioassay experiments were conducted to quantify 2,4-D and dicamba removal from plastic mulch using a single overhead irrigation event.

2. Materials and Methods

2.1 Site Selection and Trial Establishment

Four studies were conducted at the University of Georgia Ponder Research Farm (31.30'18°N, 83.39'03°W) near Ty Ty, GA from the spring of 2018 through the fall of 2019 to determine (1) the influence of overhead irrigation on either 2,4-D or dicamba removal from plastic mulch using analytical techniques, and (2) cantaloupe and zucchini squash response to 2,4-D and dicamba applied preplant broadcast over mulch, and followed by either irrigation or no irrigation prior to transplanting. Soils at the site consisted of a Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 84 to 90% sand, 8 to 10% silt, 2 to 6% clay, and 0.63 to 0.65% organic matter with a pH of 6.2 to 6.6. Soil was prepared conventionally and raised beds (20 cm tall, 81 cm wide) on 183 cm centers were formed in the winter prior to planting to allow fumigant dissipation following standard grower practices. As beds were formed, fumigants (1,3-dichloropropene, chloropicrin, and metam sodium) were applied under the mulch to keep the experimental planting area free of diseases, nematodes, and weeds. The initial bedder (Hendrix & Dail, Inc. Greenville, NC 27835) injected 1,3-dichloropropene and chloropicrin (Pic-Chlor 60, TriEst Ag Group, Inc., 1101 Industrial Blvd., Greenville, NC 27835) at a rate of 197 L ha^{-1} 20 cm below the bed top using 3 evenly spaced injection shanks. This was followed by a combination bed shaper and plastic mulch layer (Kennco Manufacturing, Inc., Ruskin, FL 33570), which injected metam sodium (Vapam® HL™,

AMVAC®, 4100 E. Washington Blvd. Los Angeles, CA 90023) 10 cm deep with injection shanks 10 cm apart, at a rate of 700 L ha^{-1}. As metam sodium was injected, drip tape (Rivulus Irrigation Ltd., Gvat, Israel 3657900) was laid in the center of the raised bed 2.5 cm below the bed surface and the entire bed was covered with black on white LDPE plastic mulch (Guardian Agricultural Plastics Corporation, 5401 West Kennedy Boulevard, Tampa, FL 33609). For the cantaloupe study in 2018 and the fall zucchini squash study in 2019, the white side of the mulch was facing up. For both spring studies in 2019, the black side of the mulch was facing up. To ensure crop response was not influenced by weed competition from row middles, glyphosate (1.12 kg ae ha^{-1}), flumioxazin (0.14 kg ai ha^{-1}), and *S*-metolachlor (1.1 kg ai ha^{-1}) were applied between plastic mulched beds approximately one week prior to planting in accordance with university recommendations for the region (Kemble et al., 2019). Row middles were subsequently hand weeded for escapes as needed.

Experimental treatments were an augmented factorial of two herbicides, two application rates, two preplant irrigation options, and a nontreated control included for comparison. Treatments were arranged in a randomized complete block design with four replications per study site. Herbicides included 2,4-D choline (Embed® Extra, Corteva Agriscience, 9330 Zionsville Rd., Indianapolis, IN 46268) at 1,065 and 2,130 g ae ha^{-1} and dicamba (Xtendimax®, Bayer CropScience, 800 N. Lindbergh Blvd., St. Louis, MO 63167) at 560 and 1,120 g ae ha^{-1}. Herbicides were applied on April 11, 2018 (cantaloupe), April 10, 2019 (cantaloupe and zucchini squash), and September 22, 2019 (zucchini squash), and allowed to dry for 24 hours. Herbicide applications were broadcast directly over plastic mulch using a CO_2-pressurized backpack sprayer calibrated to

deliver 140 L ha^{-1}. Different booms were used to apply each herbicide with all dicamba applications using TTI 110015 nozzles (Teejet Technologies, Wheaton, IL 60187); 2,4-D applications used AIXR 11002 nozzles (Teejet Technologies, Wheaton, IL 60187) in 2018 and TTI 110015 nozzles in 2019 due to changes in product labeling. Spray booms utilized were 138 cm long with a nozzle spacing of 46 cm and booms were held 41 cm above the mulch. At the time of application, air temperature ranged from 12-21 C, relative humidity ranged from 75-83%, and wind speeds did not exceed 4 km h^{-1}.

On the day after application when the plastic was dry from morning dew, custom made metal boxes were placed on top of the plastic mulched beds of plots not to receive irrigation to prevent herbicide wash off. Boxes were made of stainless aluminum weighing approximately 10 kg and measured 244 cm long, 71 cm wide, and 5 cm tall, tapered to seal against the surface of the plastic mulched beds without damaging the plastic mulch. Once the covers were in place, overhead irrigation was applied over the plastic mulch at 0.76 cm using a lateral pivot. Upon completion of overhead irrigation, the covers were removed and the mulched beds receiving irrigation were allowed time to dry prior to mulch sampling and crop planting. Metal covers remained over mulch less than 3 hr.

2.2 Analytical methods

To quantify removal of 2,4-D or dicamba with irrigation, mulch samples were taken approximately two hours after herbicide application, and again following overhead irrigation but prior to crop planting for a total of two samples per treated plot. Samples of mulch were collected from each plot using a 0.1 m^2 quadrat. A box-cutting knife was used to harvest the mulch along the inside edge of the square. Needle-nose pliers were

then used to mechanically fold the LDPE mulch inward without contacting the treated side of the mulch to prevent contamination of the treated surface with any foreign objects. Samples were subsequently stored in plastic bags, frozen upon collection, and stored at -10 °C until analysis.

Field plot replicate sample integrity was maintained throughout sample collection, preparation, and chemical analysis. For herbicide analysis, samples were removed from the freezer and allowed to equilibrate to room temperature prior to being placed in a 125-mL volumetric flask sealed with a rubber stopper. Extractions were conducted utilizing 10% methanol with high performance liquid chromatography (HPLC) water (Fisher Scientific International). The extraction volumes were 100 mL. Samples were placed on a reciprocating shaker for 2 hours at 200 rpm. Upon removal, samples were passed through a 0.2 μM polytetrafloroethylene (PTFE) membrane filter (Fisher Scientific International) that was fitted to a Luer-Lok™ syringe (Fisher Scientific International), and then passed into a 1.5-mL microcentrifuge tube (Fisher Scientific International). Microcentrifuge tubes were sealed, and centrifuged at 12,000 rpm for 5 minutes. An aliquot was then transferred into HPLC vials (Fisher Scientific International). Samples were analyzed with a Waters Acquity Arc Ultra-High Performance Liquid Chromatography (UHPLC) system, coupled with a Waters 2998 PDA and Waters QDa Mass Spectometry Detector. For analysis, the mobile phase consisted of (1) 1:9 acetonitrile:water, (2) 9:1 acetonitrile:water, and (3) water. A Cortecs C_{18} 4.6 x 50 mm column with 2.7 μm packing (Waters Corporation, 34 Maple St., Milford, MA 01757) was used with a flow rate of 1.1 mL min^{-1}, an injection volume of 47 μL, and a run time of 5 minutes per injection. Chromatographic conditions were adapted from Majzik et al. (2006). Standard curves

were generated utilizing the formulations applied in experiments and based on the acid equivalent applied.

2.3 Crop establishment and data collection

Transplant holes were hand poked in the plastic mulch using a custom made hole puncher on April 13, 2018, April 11, 2019, and September 23, 2019. The custom hole puncher, built from stainless aluminum, was used to eliminate plot-to-plot herbicide contamination. After it was used on each plot, the implement was cleaned with a mixture of ammonia and water prior to beginning on the next plot. The size of the plant hole created by the custom hole puncher was identical to a hole created by a standard transplant hole punch wheel (Kennco Manufacturing, Inc., Ruskin, FL 33570). 'Athena' cantaloupe (10 cm in height) was transplanted April 13, 2018 and April 11, 2019 in single rows with a spacing of 183 cm between beds and 30 cm between plants within a row. 'Spineless Beauty' and 'Payroll' zucchini squash (10 cm in height) were transplanted on April 11, 2019 and September 23, 2019, respectively, utilizing the same spacing as cantaloupe. One day after transplanting, all plots received 0.4 cm of irrigation to water in the transplants as well as to simulate a rainfall event in which the herbicide could move onto the foliage of the transplants or wash into the transplant hole. From that point forward, both crops were managed for irrigation, fertility and control of pests in accordance with university recommendations for the region (Kemble et al. 2019).

Estimates of visual crop injury (chlorosis, epinasty, stunting, leaf deformations) was rated on a scale of 0% to 100% (0% being no injury, 100% being crop death) every 7 days beginning one week after planting up to five weeks after planting. Reductions in cantaloupe growth were quantified by measuring the length of the longest vine, while

zucchini squash growth was quantified by measuring across the diameter of the plant. Eight plants per plot were measured weekly up to three weeks after planting. Early-season fresh weight biomass was measured by removing plants at the ground level and recording their collective weight. For cantaloupe, biomass was taken by harvesting every other plant leaving eight plants per plot spaced 60 cm apart for harvest and for squash, biomass was taken from plants on each end of the plot leaving the 8 center plants per plot spaced 30 cm apart for harvest (Coolong and Kelley 2014; Coolong and Boyhan 2017). Cantaloupe were harvested 9 to 13 times, with number of fruit and weight per plot recorded for each harvest. Harvests were then split into early harvests (1 to 4) and total harvests (1 to 13) to determine the impacts of crop damage on earliness of harvest as well as season harvest totals. Zucchini squash were harvested up to 31 times, with number of fruit and weight per plot recorded for each harvest. Zucchini squash harvests were then split into early harvests (1 to 8), and total harvests (1 to 31) to determine the impacts of crop damage on earliness of harvest as well as harvest throughout the season.

2.4 Statistical analysis

Analytical and bioassay data were subjected to an ANOVA to test for experimental run by treatment interactions. There were no significant interactions present, therefore all data are pooled over experimental run. Analytical data were compared using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC 27513) to determine if the combined treatment effects of herbicide, rate applied, and preplant irrigation impacted herbicide concentration present on the plastic mulch at planting. Concentrations were log transformed to improve normality and homogeneity of variance prior to analysis, however all data are presented in their non-transformed values.

Replications and location were included in the model as random factors. Concentration means were compared and adjusted using the Shaffer-simulated method ($\alpha = 0.05$).

Bioassay data were compared using PROC GLIMMIX in SAS to determine if the combined treatment effects of herbicide, rate, and preplant irrigation option influenced cantaloupe and zucchini squash growth, development, and yield. Replications and location were included in the model as random factors. All data concerning cantaloupe were normally distributed. Zucchini squash injury, fruit number and weight were arcsine square root, square root, and log transformed, respectively, to improve normality and homogeneity of variance prior to analysis; however, all data are presented in their back-transformed values. All P-values for tests of differences between least-squares means were compared and adjusted using the Shaffer-simulated method (α=0.05).

3. Results and Discussion

3.1 2,4-D and dicamba concentrations present on the mulch at crop planting

Dicamba application rates of 560 and 1,120 g ae ha^{-1} are equivalent to 535 and 1,070 ppm m^{-2}, respectively, while 2,4-D applied at 1,065 and 2,130 g ae ha^{-1} is equivalent to 1,059 and 2,118 ppm m^{-2}, respectively. When irrigation was not implemented, dicamba was present at 99% of the applied concentration at planting while 2,4-D was present at 88 to 91% of the initial concentration (Table 3.1). Overhead irrigation significantly reduced concentrations of both herbicides to less than 12% of the original concentration. Dicamba concentrations, even when applying half the concentration compared to 2,4-D, remained on the mulch at more than 3.5 times than observed with 2,4-D. Concentrations retained on the mulch were 1/50 to 1/100 the 1X rate of dicamba and 1/500 to 1/625 the 1X rate of 2,4-D. Previous studies have noted a high level of injury and yield loss from dicamba at

much lower rates than those observed on the mulch in this study when applied to the foliage of squash and cantaloupe (Culpepper and Vance, 2019; Dittmar et al., 2016; Hand et al., 2019). Injury from 2,4-D has not been noted from rates lower than 1/300X for either cantaloupe or squash (Culpepper and Vance, 2019).

3.2 Cantaloupe experiment

Visual injury estimates were at their maximum at 22 to 29 DAP (days after planting). Cantaloupe injury was significantly impacted by the interaction of herbicide, rate, and irrigation option ($P<0.0001$). When overhead irrigation was received prior to transplanting, 2,4-D treatments noted ≤ 5% injury, regardless of rate (Table 3.2). Dicamba applied at a 1X and 2X rate and subsequently treated with overhead irrigation resulted in 18 and 35% injury, respectively. Dicamba treatments that received overhead irrigation prior to planting recovered as the season progressed, with 10 to 15% injury noted approximately 40 DAP (data not shown).When overhead irrigation was not implemented damage was severe regardless of herbicide or rate applied; however, cantaloupe damage was 15 to 24% less with 2,4-D as compared to dicamba at comparable rates.

Cantaloupe vine lengths were also influenced by the interaction of herbicide, rate, and irrigation option when collected 27 to 28 DAP ($P<0.0001$). Similar vine lengths were observed between the control, both rates of 2,4-D, and the low rate of dicamba as long as irrigation occurred after application and before planting (Table 3.2). The 2X rate of dicamba, even with overhead irrigation, noted plant vine lengths reduced 24% compared to the control (Table 3.2). Without overhead irrigation, 2,4-D or dicamba reduced vine lengths 65 to 100%. Fresh weight biomass measured 20 to 25 DAP was influenced by the

interaction of herbicide and overhead irrigation option prior to planting ($P<0.0001$). Averaged over herbicide rate, when 2,4-D was applied and washed from the mulch, fresh weight biomass was similar to the control (Table 3.3). In contrast, even when dicamba was washed from the mulch, a reduction in fresh weight biomass of 19% was observed. When neither herbicide was removed from the mulch with overhead irrigation, fresh weight biomass reductions of 91 to 99% resulted.

Maturity or earliness of harvest is of paramount importance in vegetable production. Injury from an herbicide application causing a delay in maturity could directly impact profitability. Therefore, to quantify potential delays in maturity, the first four cantaloupe harvests were analyzed. Early-season fruit counts were only influenced by overhead irrigation option ($P<0.0001$). Averaged over herbicide and rate applied, early-season fruit count (5,746 fruit ha^{-1}) was similar to the nontreated control (6,421 fruit ha^{-1}) when irrigation was implemented (data not shown). When herbicides weren't washed from the mulch, early season fruit counts were reduced 100%. Although early-season fruit counts were only influenced by preplant irrigation option, early-season fruit weight was influenced by the interaction of herbicide and preplant overhead irrigation option ($P<0.0001$). Similar to previous trends, when 2,4-D was washed from the mulch early-season fruit weights were not influenced (Table 3.3). However, when dicamba was washed from the mulch, early-season fruit weight was reduced 32% compared to the control. Herbicides that weren't washed from the mulch eliminated early-season fruit.

Total cantaloupe yield with respect to fruit counts and weight was impacted by the interaction of herbicide, rate, and preplant irrigation option when harvested 9 to 13 times ($P<0.0001$). On average, cantaloupe yielded 24,417 fruit ha^{-1} weighing 56,677 kg

ha^{-1} in the control (Table 3.4). Fruit counts and weights were not influenced by either 2,4-D or dicamba as long as overhead irrigation was included. Even though early-season injury from dicamba with irrigation was observed, the crop was able to recover without season-long yield loss. The ability of crops to recover from low dose auxin injury early in the season has been observed many times. For example in cotton, exposure of auxin susceptible two-leaf cotton to low-doses of 2,4-D or dicamba resulted in significant injury in the first two weeks following exposure (50-57%), but impacts on yield were minimal (Everitt and Keeling 2009). Similar trends were noted in non-tolerant soybean (Solomon and Bradley 2014). When overhead irrigation was not implemented prior to planting and injury was more severe, the 1X rate of 2,4-D did not significantly reduce fruit weight but reduced fruit count by 18%. The 2X rate of 2,4-D reduced counts and weights 53 to 56%. This demonstrates a lack of a 2X safety factor in the absence of preplant overhead irrigation, which is generally required for herbicide registrations (Monaco et al., 2002). Impact was more severe with dicamba, as no fruit was harvested.

3.3 Zucchini squash experiments

Visual injury estimates for zucchini squash were at their maximum 14 to 21 DAP, and was significantly impacted by the interaction of herbicide, rate, and irrigation option ($P<0.0001$). With overhead irrigation included prior to planting, injury was similar among both rates of dicamba and 2,4-D ranging from 6 to 12% (Table 3.5). Previous research has noted zucchini squash to be more tolerant of herbicides when compared to yellow squash cultivars, which may have influenced the lower levels of injury observed with dicamba in zucchini squash compared to cantaloupe (Webster et al., 2003). When no overhead irrigation was implemented, preplant applications of 2,4-D injured zucchini

squash 60% at the 1X rate and 68% at the 2X rate which was less than the 90 to 92% noted with dicamba.

Zucchini squash plant widths, measured 21 to 27 DAP, were significantly impacted by the interaction between herbicide, rate, and irrigation option ($P<0.0001$). Zucchini squash averaged 98 cm in width in the control (Table 3.5). Regardless of herbicide, when overhead irrigation was received prior to planting, squash widths were similar to the control (97-103 cm). When irrigation was not included, 2,4-D reduced widths 28 to 39% while dicamba was more damaging and reduced widths 79 to 89%. Fresh weight biomass, collected 15 to 20 DAP, was influenced only by irrigation ($P<0.0001$; data not shown). Squash averaged a fresh weight biomass of 128 g plant^{-1} and was not influenced by either herbicide if irrigation was included. Without irrigation, 2,4-D and dicamba reduced biomass 75 and 86%, respectively.

To quantify potential delays in maturity for zucchini squash, the first eight harvests were analyzed. Earliness was not influenced by herbicide or rate when the mulch was washed prior to transplanting for fruit number or fruit weight (data not shown). However, without irrigation, a reduction in fruit count and weight harvested ranged from of 60 to 65% for both rates of 2,4-D while the impact from dicamba was generally higher ranging from 61 to 93%. To obtain total yield, squash were harvested 18 to 31 times, six days a week with both fruit number and respective weights being significantly impacted by the interaction between herbicide, rate and irrigation option ($P<0.0001$). On average, zucchini squash yielded 261,888 fruit ha^{-1} weighing 73,627 kg ha^{-1} over the entire season when auxin herbicides were not applied (Table 3.6). Similar to early-season yield, neither 2,4-D nor dicamba influenced yield as long as irrigation was implemented after herbicide

application and before transplanting. Without irrigation, fruit counts and weights were reduced 36 to 45% and 28 to 31%, respectively, by both rates of 2,4-D. Greater yield loss was noted with dicamba as fruit counts and fruit weights were reduced 83 to 96% at the 1X rate and 69 to 96% at the 2X rate.

4. Conclusions

Preplant applications of 2,4-D and dicamba could provide vegetable growers an additional mechanism of action to control problematic weeds prior to crop planting. Analytical and bioassay experiments demonstrated a single irrigation event of 0.76 cm could successfully remove 2,4-D from plastic mulch. Furthermore, doubling the rate of 2,4-D did not result in adverse effects on visual injury, crop growth, or yield in either crop, demonstrating adequate crop safety. In contrast, irrigation was not as effective in removing dicamba from the plastic mulch with significant injury, growth reduction and early-season yield loss detected with cantaloupe. Without irrigation to wash herbicides from the mulch prior to planting, damage was so severe that both crops would have been abandoned. Squash was more tolerant and resilient compared to cantaloupe.

Although 2,4-D demonstrates great potential applied preplant in a vegetable plasticulture system where irrigation can be implemented to remove it from the mulch, further research is needed evaluating its use in multi-cropped plasticulture production systems. These experiments specifically focused on the relationship of herbicides and mulch void of holes or tears in the mulch. In many cases, if labeled, 2,4-D would be applied over plastic mulch with old plant holes or tears present allowing the herbicide to contact the soil potentially providing residual activity that could damage the crop. Thus, understanding the distance to place new transplants relative to old holes or tears

containing 2,4-D and the amount of time needed between application and planting will be paramount for success. Future research should evaluate potential use patterns for 2,4-D in this system and tolerance of other crops grown in plasticulture.

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Table 3.1. Herbicide concentration remaining on plastic mulch at planting as influenced by herbicide option, rate applied, and preplant overhead irrigation option.[a]

Herbicide	Original concentration[b]	No irrigation		Irrigation	
		Concentration at planting[c,d]			
	PPM m^{-2}				
2,4-D	1,059	932	ab	2	d
	2,118	1,925	a	3	d
dicamba	535	530	b	7	c
	1,070	1,059	ab	11	c

[a]Herbicide concentrations are combined over four experimental runs.

[b]Original concentrations are concentrations that were present on plastic mulch 2 hrs after application.

[c]Concentrations present at planting were evaluated following a 1 d interval and implementation of the overhead irrigation treatment. Irrigated treatments received 0.76 cm, while treatments without irrigation were covered to prevent herbicide wash off.

[d]Means followed by the same letter with respect to herbicide concentration present on plastic at planting do not differ significantly ($P \leq 0.05$).

Table 3.2. Cantaloupe injury and vine length response as influenced by herbicide option, rate applied, and preplant overhead irrigation option.

		Injury[b]		Vine Lengths[b]	
Irrigation option[a]	Herbicide	1X rate[c]	2X rate[c]	1X rate[c]	2X rate[c]
		——————%——————		——————cm——————	
Irrigation	2,4-D	4 e	5 e	55 a	50 ab
	dicamba	18 d	35 c	48 ab	39 b
No irrigation	2,4-D	75 b	85 b	18 c	13 cd
	dicamba	99 a	100 a	1 d	0 d
	NTC[d]	0		51 a	

[a]Treatments requiring overhead irrigation received 0.76 cm, while nonirrigated treatments were covered to prevent herbicide wash off.

[b]Means followed by the same letter with respect to the response variable do not differ significantly ($P \leq 0.05$). Data are pooled over 2018 and 2019.

[c]Rates applied were 1,065 (1X) and 2,130 (2X) g ae ha^{-1} for 2,4-D and 560 (1X) and 1,120 (2X) g ae ha^{-1} for dicamba.

[d]Abbreviations: NTC, nontreated control

Table 3.3. Cantaloupe fresh weight biomass and early harvest fruit weight as influenced by overhead irrigation prior to planting and herbicide applied.[a]

Irrigation option[b]	Herbicide	Biomass[c]		Early fruit weight[c]	
		g plant^{-1}		——kg ha^{-1}——	
Irrigation	2,4-D	52	a	10,933	a
	dicamba	43	b	7,795	b
No irrigation	2,4-D	5	c	0	c
	dicamba	1	c	0	c
	NTC[d]	53	a	11,524	a

[a]Data are averaged over year and herbicide rate applied. Early harvests were the first four harvests for cantaloupe experiments.

[b]Treatments requiring overhead irrigation received 0.76 cm, while nonirrigated treatments were covered to prevent herbicide wash off.

[c]Means followed by the same letter with respect to the response variable do not differ significantly ($P \leq 0.05$).

[d]Abbreviations: NTC, nontreated control

Table 3.4. Cantaloupe yield over the entire season as influenced by herbicide option, rate applied, and preplant overhead irrigation option.

Irrigation option[a]	Herbicide	Fruit number[b]		Fruit weight[b]	
		1x rate[c]	2x rate[c]	1x rate[c]	2x rate[c]
		——————no. ha^{-1}——————		——————kg ha^{-1}——————	
Irrigation	2,4-D	23,956 ab	24,517 ab	55,545 a	57,282 a
	dicamba	25,357 a	24,517 ab	66,419 a	53,256 a
No Irrigation	2,4-D	19,994 b	10,647 c	53,521 a	26,855 b
	dicamba	0 d	0 d	0 c	0 c
	NTC[d]	24,417 ab		56,677 a	

[a]Treatments requiring overhead irrigation received 0.76 cm, while nonirrigated treatments were covered to prevent herbicide wash off.

[b]Means followed by the same letter with respect to the response variable do not differ significantly (P≤0.05). Data pooled over 2018 and 2019. Cantaloupe were harvested a total of 9 to 13 times.

[c]Rates applied were 1,065 (1X) and 2,130 (2X) g ae ha^{-1} for 2,4-D and 560 (1X) and 1,120 (2X) g ae ha^{-1} for dicamba.

[d]Abbreviations: NTC, nontreated control

Table 3.5. Zucchini squash injury and widths as influenced by herbicide, rate applied, and preplant overhead irrigation option.[a]

Irrigation option[b]	Herbicide	Injury[c]		Widths[c]	
		1X rate[d]	2X rate[d]	1X rate[d]	2X rate[d]
		——%——		——cm——	
Irrigation	2,4-D	6 d	10 d	99 a	98 a
	dicamba	9 d	12 d	103 a	97 a
No irrigation	2,4-D	60 c	68 b	71 b	60 b
	dicamba	90 a	92 a	21 c	11 c
	NTC[e]	0		98 a	

[a]All data are combined across spring and fall of 2019.

[b]Treatments requiring overhead irrigation received 0.76 cm, while nonirrigated treatments were covered to prevent herbicide wash off.

[c]Means followed by the same letter with respect to the response variable do not differ significantly ($P \leq 0.05$).

[d]Rates applied were 1,065 (1X) and 2,130 (2X) g ae ha^{-1} for 2,4-D and 560 (1X) and 1,120 (2X) g ae ha^{-1} for dicamba.

[e]Abbreviations: NTC, nontreated control

Table 3.6. Zucchini squash yield over the entire season as influenced by herbicide, rate applied, and preplant overhead irrigation option.[a]

Irrigation option[b]	Herbicide	Total fruit number[c]				Total fruit weight[c]			
		1X rate[d]		2X rate[d]		1X rate[d]		2X rate[d]	
		———no. ha^{-1}———				———kg ha^{-1}———			
Irrigation	2,4-D	248,086	a	249,593	a	70,516	ab	69,546	ab
	dicamba	262,849	a	271,894	a	70,308	ab	75,106	a
No irrigation	2,4-D	167,807	b	143,417	b	52,713	bc	50,822	c
	dicamba	43,856	c	11,100	d	22,928	d	7,523	d
	NTC[e]	261,888 a				73,627 a			

[a]Yield data are combined across spring and fall of 2019. Zucchini squash were harvested 18 to 31 times.

[b]Treatments that received overhead irrigation received 0.76 cm, while nonirrigated treatments were covered to prevent herbicide wash off.

[c]Means followed by the same letter with respect to the response variable do not differ significantly ($P \leq 0.05$).

[d]Rates applied were 1,065 (1X) and 2,130 (2X) g ae ha^{-1} for 2,4-D and 560 (1X) and 1,120 (2X) g ae ha^{-1} for dicamba.

[e]Abbreviations: NTC, nontreated control

CHAPTER 4

USING ANALYTICAL TECHNIQUES AND COLE CROP RESPONSE TO QUANTIFY 2,4-D PLUS GLYPHOSATE REMOVAL FROM THE SURFACE OF PLASTIC MULCH[1]

[1]Hand LC, Eason KM, Randell TM, Grey TL, Richburg JS, Coolong TW, and Culpepper AS. Submitted to *Hortscience*, 1/4/2021.

Abstract

Weed management in cole crop and leafy green production in the Southeast United States is extremely challenging with problematic summer and winter annual weed species. There is a wide range of planting dates for cole crops in this region from August to October and January to April, which presents a unique challenge for weed control prior to planting. Limited herbicide options confounds this issue. When these crops are grown on plastic mulch, weed diversity and intensity increase because of the lack of tillage. These crops are never grown as a first-crop on the plastic mulch, which allows weeds to germinate, grow, and establish in row middles, holes from previous crops, and areas of natural degradation of the plastic. Starting the season weed-free is further complicated by the limited number of preplant herbicide options, especially in a plasticulture system. Research was conducted to evaluate the persistence of preplant applications of 2,4-D tank-mixed with glyphosate that was applied over plastic mulch. Analytical laboratory analysis of plastic samples from field experiments, in conjunction with bioassays using broccoli (*Brassica oleracea* var. *botrytis* L.) and collard (*Brassica oleracea* var. *viridis* L.) evaluated dissipation. Analytical studies determined that 0.5 cm of irrigation after herbicide application and one d prior to planting removed 99% of 2,4-D, and 100% of glyphosate from the plastic mulch. Waiting an additional 14 d after application and overhead irrigation further reduced the amount of 2,4-D on the plastic mulch 88 to 95% from the amount remaining for the 1 d before planting treatments. For the field bioassay, preplant applications of 2,4-D tank-mixed with glyphosate resulted in 7% or less crop injury without influencing crop growth, biomass, early-season yield, or total yield for broccoli and collards as long as the plastic mulch was washed with 0.5 cm of irrigation

prior to planting. Research results suggest 2,4-D can be removed from the surface of plastic mulch with minimal impacts on transplanted broccoli and collards.

Introduction

Cole crop [broccoli, cabbage (*Brassica oleracea* var. *oleracea* L.), and cauliflower (*Brassica oleracea* var. *botrytis* L.)] and leafy green [collards and kale (*Brassica oleracea* var. *sabellica* L.)] production in the United States was comprised of 61,263 harvested ha during 2017, with nearly 22% of that occurring in the state of Georgia (Coolong, 2017; USDA, 2019). These crops have a wide planting window in the Southeast, ranging from August to October and January to April (Coolong et al., 2016; Coolong, 2017). The vast range of planting dates provide unique challenges for weed control, with potential for both problematic summer and winter annual weeds to be present prior to and at planting.

Among the most problematic weeds for these crops in Georgia include wild radish (*Raphanus raphanistrum* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), *Amaranthus* spp., and *Ipomoea* spp. (Webster, 2014). These weeds cannot be controlled with herbicides once cole crops or leafy greens are transplanted, or seedlings have emerged. Therefore, it is essential to control weeds prior to planting in conjunction with residual herbicides to minimize in-season weed emergence (Bitterlich et al., 1996; Smart et al., 2001). Controlling emerged weeds prior to seeding or transplanting can be extremely challenging, as there are few registered herbicide options for growers. Preplant burndown herbicides with broad-spectrum postemergence activity are limited to glyphosate and paraquat (Kemble et al., 2019). With widespread glyphosate resistance in

Palmer amaranth (*Amaranthus palmeri* S. Wats), paraquat is the only effective option if applied to small weeds (Culpepper et al., 2006; Chaudhari et al., 2017). Glyphosate and paraquat provide variable and often inadequate control of susceptible annual morningglory, wild radish, and primrose (Culpepper et al., 2005; Hydrick et al., 1995; Leon et al., 2016). Additionally, other vegetable producing states, including California and Florida, have noted resistance to paraquat in horseweed (*Conyza canadensis* L.) (Moretti and Hanson, 2016) and American black nightshade (*Solanum americanum* Mill.) (Chase et al., 1998). Therefore, further control options are necessary to start the season weed-free and allow for the incorporation of multiple mechanisms of action for resistance management purposes.

Tillage in bareground production systems is effective in controlling these weeds and incorporating residual herbicides (Brainard et al., 2013; Cutulle et al., 2019). However in Georgia, cole crops and leafy greens are often grown in plastic mulch systems. These systems eliminate the potential for tillage as a weed management tool, and also can increase weed densities and species present. Although an effective fumigant system is used when laying plastic mulch, it is generally only effective for the first crop produced, often tomato (*Solanum lycopersicum* L.) or pepper (*Capsicum annuum* L.), which are considered high-value crops (Csinos et al., 1997; Culpepper et al., 2017; Eure and Culpepper, 2017; Webster et al., 2001). Cole crops and leafy greens are subsequently grown in the plastic mulch system, often planted 18 to 24 mo after the initial fumigation application and plastic mulch installation (Cutulle et al., 2019). By the time cole crops or leafy greens are planted, the fumigant has degraded, allowing problematic weeds to germinate, emerge and establish not only between the plastic mulched beds (i.e. row

middles), but also in holes of the plastic mulch from previous crops or areas of natural degradation of the plastic. Thus, growers are in need of more effective herbicidal tools.

Mixtures of 2,4-D with glyphosate or paraquat could benefit growers if applied prior to planting cole crops and leafy greens due to its ability to control Palmer amaranth, annual morningglory, wild radish, and cutleaf evening-primrose (Chaudhari et al., 2017; Culpepper et al., 2005; Leon et al., 2016). While new 2,4-D formulations have been improved in regards to off-target movement (Corteva, 2018; Sosnoskie et al., 2015), its interaction with plastic mulch is unknown in terms of sorption and dissipation. Herbicide interaction with plastic mulch is variable and specific to a given herbicide. For example, herbicides have been shown to either wash off the plastic mulch with an initial irrigation or rainfall event, partially wash off of plastic mulch over time, or bind to the plastic mulch without release (Culpepper et al., 2009; Grey et al., 2009; Grey et al., 2018; Randell et al., 2020). Glyphosate and paraquat are examples of herbicides that can be easily washed off plastic mulch while other herbicides, such as carfentrazone, bind without release (Culpepper et al., 2009; Grey et al., 2009). Flumioxazin applied over plastic mulch is an example of an herbicide that partially washes off the herbicide over time (Grey et al., 2009) and has caused significant injury and yield reductions for squash (*Cucumis melo* L.) and tomato (Culpepper et al., 2009). Similarly, halosulfuron-methyl applied over plastic mulch, even after 17 to 19 cm of rainfall, damaged squash, broccoli, and cabbage (Grey et al., 2018; Randell et al., 2020).

Another factor, in addition to rainfall or irrigation, to consider when understanding the relationship of a given herbicide and plastic mulch is the time interval between application and planting. When halosulfuron was applied over plastic mulch

prior to crop planting, injury generally increased as applications were made closer to planting for squash, broccoli, and cabbage (Randell et al., 2020). In the case of broccoli and cabbage, halosulfuron removal from plastic mulch was more dependent on time than rainfall, with 52-58% injury for 7 DBP applications and 36-37% injury for 14 DBP applications when similar amounts of rainfall were received for both application timings (Randell et al., 2020). Therefore, some herbicides will require not just rainfall or irrigation for removal from plastic mulch, but also time.

Understanding the interaction of 2,4-D with plastic mulch is critical in determining the potential for using this herbicide over plastic mulch in cole crop and leafy green production in these systems. If 2,4-D dissipates from the plastic mulch with rainfall, irrigation, or degradation, then it would offer a significant improvement in preplant herbicide options. Therefore, analytical and bioassay experiments were conducted to quantify variable rates of 2,4-D removal from plastic mulch over time and with irrigation prior to planting broccoli and collards.

Materials and Methods

Site Selection and Trial Establishment. An experiment was conducted three times at the University of Georgia Ponder Research Farm (31.30’18°N, 83.39’03°W) near Ty Ty, GA in the fall of 2018 and 2019 to evaluate the tolerance of broccoli and collard to 2,4-D plus glyphosate applied preplant over plastic mulch. Soils consisted of a Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 84 to 90% sand, 8 to 10% silt, 2 to 6% clay, and 0.63 to 0.65% organic matter with a pH of 6.2 to 6.6. During the summer prior to each autumn experiment, the soil within the experimental area was tilled

to remove all plant debris and fumigants were applied and plastic mulch was installed. As bed formation occurred, the entire trial area was treated with a standard 3-way fumigant to maintain the experimental area weed-free, as well as assist in controlling nematodes and soil-borne pathogens (Culpepper et al., 2017). The initial bedder (Hendrix & Dail, Inc. Greenville, NC 27835) injected 1,3-dichloropropene and chloropicrin (Pic-Chlor 60, TriEst Ag Group, Inc., 1101 Industrial Blvd., Greenville, NC 27835) at 197 L ha^{-1} 20 cm below the bed top using 3 evenly spaced injection shanks. This was immediately followed by a combination bed shaper and plastic mulch layer (Kennco Manufacturing, Inc., Ruskin, FL 33570), which injected metam sodium (Vapam® HL™, AMVAC®, Los Angeles, CA 90023) 10 cm deep with injection shanks 10 cm apart, at 700 L ha^{-1}. As metam sodium was injected, drip tape was laid in the center of the raised bed 2.5 cm below the bed surface and the entire bed was covered with black on black totally impermeable film in 2018 and black on white low density polyethylene much in both 2019 studies (Guardian Agricultural Plastics Corporation, Tampa, FL 33609). The final formed bed consisted of an 81 cm bed top, 20 cm tall, with beds spaced 183 cm apart. A summer crop was not planted to maintain plastic mulch integrity (i.e. no holes were punched) when initiating the study as the focus of this experiment was to gain knowledge on how herbicides react with plastic mulch. All experiments were maintained weed-free with glyphosate, flumioxazin, and *S*-metolachlor between plastic mulched beds at least one week prior to planting, followed by hand-weeding as necessary (Kemble et al., 2019).

The experimental design was a randomized complete block design arranged in an augmented factorial of two application rates, four application timings, and a nontreated

control included for comparison. Four replications were included per study. Herbicides included 2,4-D choline (Embed® Extra, Corteva Agriscience, 9330 Zionsville Rd., Indianapolis, IN 46268) applied at 1,065 and 2,130 g ae ha^{-1} (1,059 and 2,118 ppm m^{-2}) tank-mixed with glyphosate (Roundup PowerMax II®, Bayer Cropscience, 800 N. Lindbergh Blvd., St. Louis, MO 63167) at 1,125 and 2,250 g ae ha^{-1} (1,119 and 2,238 ppm m^{-2}), respectively. Tank mixtures were used to represent expected grower practices of a 1 and 2 X rate. Herbicides were applied over plastic mulch at four timings prior to planting (45, 30, 15, and 1 day before planting (DBP)). The 45 DBP applications were made on September 6, 2018, September 8, 2019, and September 14, 2019; 30 DBP applications were made on September 24, 2018, September 23, 2019, and October 3, 2019; 15 DBP applications were made on October 12, 2018, October 8, 2019, and October 17, 2019; and 1 DBP applications were made on October 24, 2018, October 24, 2019, and November 2, 2019. At each timing, 2,4-D plus glyphosate was applied broadcast over the plastic mulch using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1}. Experiments in 2018 utilized AIXR 11002 nozzles (Teejet Technologies, Wheaton, IL 60187), while in 2019 experiments TTI 110015 nozzles (Teejet Technologies, Wheaton, IL 60187) were utilized due to changes in product labeling. The spray boom was 138 cm long with a nozzle spacing of 46 cm and held 41 cm above the plastic mulch. At application, air temperature was 6 to 28 C, relative humidity was 70 to 87%, and wind speeds did not exceed 5 km h^{-1}. Rainfall accumulation, solar thermal radiation, and daily maximum and minimum temperature data were collected onsite at a University of Georgia Weather Monitoring Network station (Table 4.1) (Knox, 2019).

Analytical Methods. To quantify the removal of 2,4-D and glyphosate from plastic mulch, plastic samples were collected for analysis. Samples were taken within 2 hours after treatment and at the time of planting, for 2 samples per treated plot. Sampling methods were similar to Grey et al. (2009, 2018). Samples of plastic mulch were collected from each plot using an open-faced wooden square frame with an inside area of 0.1 m^2. A box-cutting knife was used to harvest the plastic mulch along the inside edge of the square. Needle-nose pliers were then used to mechanically fold the plastic mulch inward without contacting the treated side of the plastic mulch, preventing contact of the treated surface with any foreign objects. Samples were stored in plastic bags, frozen upon collection, and stored at -10 °C until analysis.

Field plot replicate sample integrity was maintained throughout sample collection, preparation, and chemical analysis. For herbicide analysis, samples were removed from the freezer and allowed to equilibrate to room temperature prior to being placed in a 125-mL volumetric flask sealed with a rubber stopper. Extractions were conducted utilizing a 1:10 ratio of methanol and high performance liquid chromatography (HPLC) water (Fisher Scientific International, Hampton, NH 03842). The extraction volumes were 100 mL with plastic samples placed on a reciprocating shaker for 2 hours at 200 rpm. Upon removal, liquid samples were passed through a 0.2 μM polytetrafloroethylene (PTFE) membrane filter (Fisher Scientific International, Hampton, NH 03842) that was fitted to a Luer-Lok™ syringe (Fisher Scientific International), and then passed into a 1.5-mL microcentrifuge tube (Fisher Scientific International, Hampton, NH 03842). Microcentrifuge tubes were sealed and centrifuged at 12,000 rpm for 5 minutes. An aliquot of 1,000 μL was then transferred into injection vials (Fisher Scientific

International, Hampton, NH 03842). Samples were analyzed with a Waters Acquity Arc Ultra-High Performance Liquid Chromatography (UHPLC) system, coupled with a Waters 2998 PDA and Waters QDa Mass Spectometry Detector (Waters Corporation, Milford, MA 01757).

For analysis of 2,4-D, the mobile phase consisted of (1) 1:9 acetonitrile:water, (2) 9:1 acetonitrile:water, and (3) water. A Cortecs C_{18} 4.6 x 50 mm column with 2.7 µm packing (Waters Corporation, Milford, MA 01757) was used with a flow rate of 1.1 mL min^{-1}, an injection volume of 47 µL, and a run time of five minutes per injection. Chromatographic conditions were adapted from Majzik et al. (2006). For glyphosate analysis, the mobile phase consisted of (1) water with 0.9% formic acid and (2) acetonitrile with 0.9% formic acid. The Anionic Polar Pesticide 2.1 x 100 mm with 5 µm packing (Waters Corporation, Milford, MA 01757) was used with a flow rate of 0.75 mL min^{-1}, an injection volume of 10 µL, and a run time of four minutes per injection. Standard curves were generated utilizing the formulations applied in experiments and based on the acid equivalent applied.

Crop establishment and data collection. Transplant holes were hand punched in the plastic mulch using a custom-made stainless aluminum hole puncher on October 25, 2018, October 25, 2019, and November 4, 2019. The custom hole punchers were used to eliminate plot-to-plot herbicide contamination. After use on each plot, the implement was cleaned with a mixture of ammonia and water prior to beginning on the next plot. The size of the plant hole created by the custom hole punchers were identical to a hole created by a standard transplant hole punch wheel (Kennco Manufacturing, Inc., Ruskin, FL 33570). ‘Emerald crown’ broccoli and ‘Top bunch’ collard (10 cm in height) were

transplanted on the same day as hole punching for each location. Two rows of each crop were planted on each bed with a 30 cm spacing between plants both down and across the bed. One to two days after transplanting, all plots received 0.5 cm of irrigation to set the transplants as well as to simulate a rainfall event in which the herbicide could move onto the foliage of the transplants or wash into the transplant hole. Both crops were managed for irrigation, fertility and other pests according to University of Georgia Cooperative Extension Service recommendations (Coolong et al., 2016; Coolong, 2017).

Visual crop injury (chlorosis, epinasty, stunting, leaf deformations) was rated on a scale of 0 to 100% (0% being no injury, 100% being crop death) every 7 days beginning one week after planting and continuing for five weeks. Reductions in broccoli and collard growth were quantified by measuring the diameter of at least 10 plants weekly through five weeks after planting. Early-season fresh weight biomass was measured by removing 8 plants per plot at the ground level and recording their collective weight. Ten plants per plot remained for harvest data. Broccoli were harvested five times, with number of heads and weight per plot recorded for each harvest. Harvests were then split into early harvests (harvest 1 and 2) and total harvests (harvest 1 to 5) to determine the impacts of crop damage on earliness of harvest as well as season harvest totals. Broccoli head number for total harvests was the same for each treatment since one head was harvested per plant, and will not be presented. Collards were harvested three times, with number of leaves and weight per plot recorded for each harvest. Collard harvests were then split into early harvest (first harvest), and total harvests (harvest 1 to 3) to determine the impacts of crop damage on earliness of harvest as well as harvest throughout the season.

Statistical analysis. For analytical data, ANOVA was used to evaluate experimental run by treatment interactions. Regression analysis was performed using SAS nonlinear regression to determine if 2,4-D and glyphosate dissipation on plastic mulch could be described using equation 1 for exponential decay.

$$y = B_0 e^{-B_1(x)} \quad (1)$$

In this equation, *y* is the herbicide concentration, B_0 is the initial concentration, B_1 is dissipation rate, and *x* is time in days after application (DAA). After data were regressed against time, the output from the analysis included the first-order dissipation-rate constants (*k*) (Ohmes et al., 2000). Data for the exponential decay equations were subjected to ANOVA using the general linear models procedure with mean separation using 95% asymptotic confidence intervals. Data were then graphed in Sigmaplot 14 (Systat Software, San Jose, CA). Additionally, PROC GLIMMIX was used to compare means of herbicide concentrations in SAS (version 9.4, SAS Institute Inc., Cary, NC 27513). Replications and experimental runs were set as random variables. Concentration means were compared and adjusted using the Shaffer-simulated method (α=0.05).

Bioassay data were subjected to ANOVA using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC 27513) to determine if the combined treatment effects of rate and application timing influenced broccoli and collard growth, development, and yield. The interaction between experimental run and treatment was evaluated and determined not to be significant, therefore all bioassay data is combined across experimental runs. Injury, crop width, biomass, and yield were set as the fixed effects, while experimental run and replocation were considered random effects. All P values for tests of differences between least-squares means were compared and adjusted

using the Shaffer-simulated method (α=0.05). Linear regression was attempted for bioassay response variables; however, due to lack of fit, means will be presented.

Results and Discussion

Analytical analysis. Interactions between experimental run and treatment were evaluated and determined to be not significant. Therefore, analytical data are combined over experimental runs. The initial concentrations of the 1,065 and 2,130 g ae ha^{-1} rate of 2,4-D, and the 1,125 and 2,250 g ae ha^{-1} rate of glyphosate present on plastic mulch, with their respective standard error, were 1,282 (±25), 1,936 (±35), 1,303 (±54), and 3,498 (±128) ppm m^{-2}, respectively. Glyphosate was not detected in any samples taken at crop planting following irrigation, regardless of rate applied (data not shown). Previous studies have demonstrated that glyphosate can be removed from plastic mulch with a single irrigation event of 1 cm, and glyphosate is currently labeled for use over plastic mulch prior to vegetable transplanting (Bayer CropScience, 2018; Culpepper et al., 2009; Grey et al., 2009). The exponential decay equation accurately described removal of 2,4-D from plastic mulch with first order rate constants (*k*) and 95% asymptotic confidence intervals of 4.59 (±5.3) for the 1X rate of 2,4-D and 4.83 (±6.4) for the 2X rate of 2,4-D (Figure 4.1). First order rate constants were similar for both rates of 2,4-D (data not shown). Irrigation washed, on average, 99% of the 2,4-D from the plastic mulch on the afternoon of application. When concentrations present at application are removed from the graph, a better picture of herbicide removal with time and additional rainfall can be observed for the 1 to 45 DBP applications. When 2,4-D was applied and washed from the plastic mulch the afternoon of application, concentrations at planting were 12.9 and 15.4 ppm m^{-}

^{2} for the 1X and 2X rates, respectively (Figure 4.2). Including an additional 14 days prior to planting with 0 to 6.1 cm of additional rainfall, concentrations were further reduced 88 to 95%. Concentrations at planting for applications made 45, 30, and 15 DBP were similar (Figure 4.2). Although a single irrigation event did not completely remove 2,4-D from the surface of plastic mulch, additional time between application and planting resulted in further concentration reductions. For example, a 1/100 concentration of the 2,4-D applied was present after the irrigation on day one but by day 15 only a 1/1,000 of the original concentration remained. Irrigation and rainfall had the greatest influence on removal of 2,4-D and glyphosate from plastic mulch; however, application interval prior to planting could also play a role in reducing the risk of injury to sensitive crops in response to preplant applications of 2,4-D. Previous research has indicated that 2,4-D is not readily photodegraded but it has demonstrated high levels of water solubility (Shaner, 2014).

Cole crops and leafy greens have high levels of tolerance to 2,4-D when applied to the foliage. The rate required to visually detect symptomology often must be higher than a 1/75 field use rate even when applied directly to the foliage (Culpepper and Vance, 2019). Thus, crop damage should be minimal from 2,4-D regardless of application timing or rate applied so long as 0.5 cm of overhead irrigation or rainfall is received prior to crop planting.

Broccoli experiment. All response variables for broccoli were not significantly impacted by experimental run and are combined for analysis and presentation. Visual injury estimates are reported from 21 to 33 DAP (days after planting), when injury was at its maximum. Broccoli injury was not impacted by 2,4-D plus glyphosate rate or application

timing (P = 0.6465). Injury observed in broccoli was 7% or less from all treatments (Table 4.2). Herbicide labels in vegetable crops are highly dependent on the level of injury to the crop, with anything above 10% often being unacceptable (Culpepper et al., 2009). This data demonstrates sufficient crop safety when 2,4-D plus glyphosate are applied preplant over plastic mulch and washed off with rainfall or irrigation prior to broccoli planting. Mohseni-Moghadam and Doohan (2015) noted injury of 16% when a 1/100 field rate or higher of 2,4-D was applied to broccoli foliage in one year. In another year, they reported injury was lower than 10% even when a 1/50 rate was applied to broccoli foliage. When canola foliage was treated with a 1/25 field rate of 2,4-D or less, observed injury was ≤ 5% (Wall, 1996). Although the rate applied to canola is higher than the highest concentration remaining on the plastic mulch at planting in this study, similar responses were noted between canola and broccoli.

Broccoli widths at 27 to 31 DAP were not influenced by the rate applied or application timing (P=0.5036). On average, broccoli in the control was 33.6 cm wide with no differences observed among treatments (Table 4.2). Fresh weight biomass, collected 26-35 DAP, followed similar trends as 2,4-D treatments did not influence weights when compared to the control (P = 0.3675).

Earliness of harvest is important in vegetable production as it can have a tremendous impact on fruit value (Culpepper et al., 2018). Herbicide injury has the potential to delay maturity in vegetable crops, which can lead to economic losses. In an attempt to quantify the potential delay in maturity from treatments, head count and weight data from the first two harvests were summarized and are presented. Early-season broccoli head counts and weight were not impacted by rate applied or application timing

(P = 0.9043 and P = 0.9858, respectively). On average, broccoli in the control plots yielded 30,751 heads ha^{-1} weighing 13,507 kg ha^{-1} with no differences among treatments (Table 4.2). Similarly, treatments did not influence season long harvested broccoli head number (P=0.9998) or weight (P=0.9935). Over the entire season, broccoli in the nontreated plots averaged 71,729 heads ha^{-1} weighing 21,623 kg ha^{-1}.

Collard experiments. All response variables for collards were not significantly impacted by experimental run and will be combined for analysis and presentation. Visual injury estimates were at their maximum at 21 to 30 DAP and are reported (Table 4.3). Collard injury was not influenced by rate applied or application timing (P = 0.4783). Collard injury was less ≤ 7% among of treatments with results being similar to those noted with broccoli.

Collards in the control plots averaged 28.9 cm wide at 27 to 31 DAP (Table 4.3). Widths from treated plots at this time ranged from 27.7 to 28.6 cm and were similar to the control. Similarly, 2,4-D plus glyphosate treatments did not influence collard biomass. Fresh weight biomass was collected 26 to 35 DAP, weighing 59.5 g $plant^{-1}$ in the control (Table 4.3).

With respect to early season collard yield, there were no differences in leaf number (P=0.9976) or weight (P=0.9989). Collards grown in the control yielded 768,100 leaves ha^{-1} weighing 22,927 kg ha^{-1} early in the season (Table 4.3). A similar trend was noted for yield over the entire season for both leaf number (P=0.9873) and weight (P=0.7512). Collards grown in the control yielded 2,300,000 leaves ha^{-1} weighing 66,091 kg ha^{-1} over the entire season. Differences in collard leaf number by any treatment was less than 3% when compared to the control.

2,4-D could be a useful tool in helping vegetable producers control problematic weeds prior to planting. Preplant applications of 2,4-D tank-mixed with glyphosate resulted in $\leq$ 7% crop injury without influencing crop growth, biomass, early-season yield, or total yield for broccoli and collards as long the plastic mulch was washed with 0.5 cm of irrigation prior to planting. Additionally, these studies demonstrated no differences between the 1X and 2X use rates with respect to all response variables for each crop. Of note is that these studies were conducted with no holes in the plastic mulch at the time of application. The presence of holes in the plastic mulch can complicate weed management in multi-cropped plastic due to the potential for residual herbicide uptake by crops planted following application. Therefore, future research should evaluate the optimal plantback interval of vegetable crops planted to multi-cropped plastic mulch with holes present that has been treated with 2,4-D.

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Table 4.1. Environmental data for 2,4-D choline plus glyphosate removal from plastic mulch.

	2018				2019 - PD 1[z]				2019 - PD 2[z]			
Treatment[z]	Temperature[y]		Rainfall[x]	Radiation[v]	Temperature		Rainfall	Radiation	Temperature		Rainfall	Radiation
	Max	Min	cm	MJ m^{-2}	Max	Min	cm	MJ m^{-2}	Max	Min	cm	MJ m^{-2}
45 DBP	31.0	19.4	9.6	770	30.8	18.0	7.3	753	29.1	16.3	10.2	693
30 DBP	29.6	18.1	9.6	438	29.8	17.1	7.3	467	26.9	15.0	10.2	403
15 DBP	27.1	15.0	0.5	186	26.3	14.7	6.6	217	24.6	12.5	5.2	201
1 DBP	19.8	12.4	0.5	7	24.2	9.7	0.5	10	19.4	5.00	0.5	16

[z]Abbreviations: DBP, days before planting; PD, planting date.

[y]Average daily maximum and minimum after application and prior to planting.

[x]Total rainfall after application and prior to planting.

[w]Days from application to the first rainfall event.

[v]Sum of total solar radiation after application and prior to planting.

Table 4.2. Broccoli response to 2,4-D choline plus glyphosate applied over plastic mulch prior to transplanting.[z]

Rate[y]	Application timing	Injury[w]	Widths[w]	Biomass[w]	Harvests 1-2		Harvests 1-5
					Broccoli Heads[w]	Head weight[w]	Head weight[w]
	DBP[x]	%	cm	g plant^{-1}	no. ha^{-1}	kg ha^{-1}	kg ha^{-1}
1,065 + 1,125 g ae ha^{-1}	45	5 ns[x]	31.9 ns[x]	60.8 ns[x]	26,360 ns[x]	11,890 ns[x]	22,204 ns[x]
	30	5	31.9	64.2	26,444	12,000	22,967
	15	4	32.9	65.6	30,035	13,745	22,878
	1	4	32.7	65.6	27,557	12,927	23,644
2,130 + 2,250 g ae ha^{-1}	45	5	32.3	63.4	25,159	11,266	22,270
	30	4	32.0	64.6	23,407	10,940	22,634
	15	7	31.4	63.2	26,791	12,315	23,620
	1	4	32.6	64.9	26,777	12,001	23,881
NTC[x]		0	33.6	65.6	30,751	13,507	22,249

[z]All data is averaged over three experimental runs.

[y]Rates correspond to 2,4-D choline + glyphosate.

[x]Abbreviations: DBP, days before planting; ns, not significant; NTC, nontreated control.

[w]Means were compared and did not differ from the nontreated control according to the Shaffer-simulated test ($\alpha = 0.05$).

Table 4.3. Collard response to 2,4-D choline plus glyphosate applied over plastic mulch prior to transplanting.[z]

					First harvest		Harvests 1-3	
Rate[y]	Application timing	Injury[w]	Widths[w]	Biomass[w]	Leaf number[w]	Leaf weight[w]	Leaf number[w]	Leaf weight[w]
	DBP[x]	%	cm	g plant^{-1}	1,000 ha^{-1}	kg ha^{-1}	1,000 ha^{-1}	kg ha^{-1}
1,065 + 1,125 g ae ha^{-1}	45	4 ns[x]	27.9 ns[x]	55.1 ns[x]	748.4 ns[x]	22,320 ns[x]	2,281 ns[x]	66,904 ns[x]
	30	4	27.9	55.4	752.0	22,241	2,259	65,158
	15	4	28.6	57.3	766.9	22,953	2,366	72,117
	1	3	28.2	56.9	773.5	22,434	2,350	70,680
2,130 + 2,250 g ae ha^{-1}	45	7	27.8	55.7	716.7	21,158	2,232	64,018
	30	4	27.7	53.6	755.5	21,921	2,244	62,049
	15	7	27.4	54.4	735.2	21,618	2,288	70,792
	1	4	28.6	58.9	754.9	23,170	2,350	73,336
NTC[x]		0	28.9	59.5	768.1	22,927	2,300	66,091

[z]All data is averaged over three experimental runs.

[y]Rates correspond to 2,4-D choline + glyphosate.

[x]Abbreviations: DBP, days before planting; ns, not significant; NTC, nontreated control.

[w]Means were compared and did not differ from the nontreated control according to the Shaffer-simulated test ($\alpha = 0.05$).

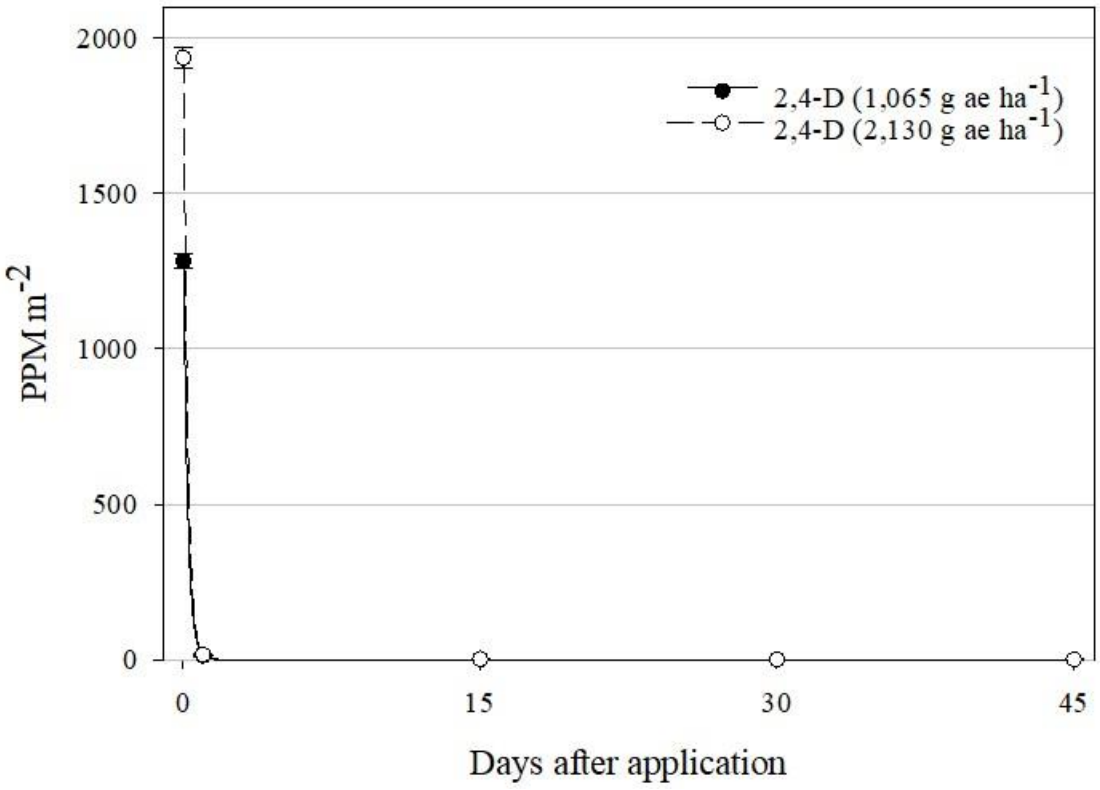

Figure 4.1. 2,4-D removal from the surface of plastic mulch in Georgia using the exponential decay equation ($y = B_0e^{-B1(x)}$). Nonlinear regression was applied for days after application. The lines represent the first-order regression equation for each rate applied. Data are averaged over three experimental runs. Data points are the means of replications with bars indicating the standard error of the mean:

2,4-D (1,065 g ae ha^{-1}): $y = 1{,}281.9e^{(-4.5886x)}$ $P<0.0001$ $R^2 = 0.97$

2,4-D (2,130 g ae ha^{-1}): $y = 1{,}936.2e^{(-4.8285x)}$ $P<0.0001$ $R^2 = 0.97$

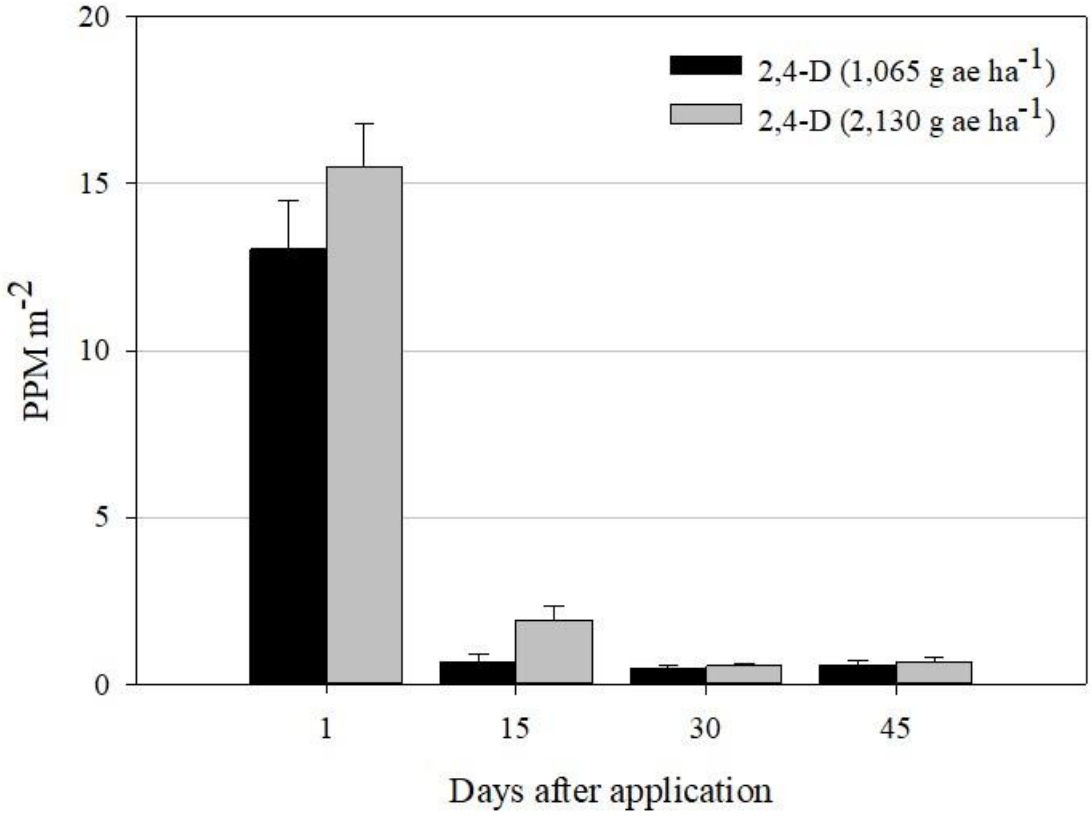

Figure 4.2. 2,4-D concentrations remaining on the surface of plastic mulch at crop transplanting as influenced by rate applied. Data averaged over three experimental runs.

CHAPTER 5

COVER CROPS, RESIDUAL HERBICIDES, AND APPLICATION METHOD REDUCE SELECTION PRESSURE TO DICAMBA POST POTENTIALLY DELAYING PALMER AMARANTH RESISTANCE[1]

[1]Hand LC, Randell TM, Nichols RL, Steckel LE, Basinger NT, Culpepper AS. To be submitted to *Agronomy Journal*.

Abstract

Dicamba-tolerant soybean (*Glycine max* L.) and cotton (*Gossypium hirsutum* L.) are the most common herbicide-tolerant technologies planted for these crops across the U.S.; thus, measures to reduce selection for dicamba-resistance in weeds is paramount. Four studies in GA and TN evaluated the potential for integrated strategies to reduce selection pressure on dicamba applied POST in cotton. A split-plot arrangement consisted of conventional tillage or cereal rye (*Secale cereale* L.) cover crop as the whole plot. The subplot included four herbicide systems: no herbicide, 3 sequential POSTs (postemergence), PRE (preemergence) fb 3 POSTs, and PRE fb 2 POSTs fb LPD (lay-by post directed). Each POST application was glyphosate plus dicamba. The cover crop reduced Palmer amaranth (*Amaranthus palmeri* S. Watson) density 75, 70, and 54% at POST 1, POST 2, and POST 3 applications, respectively. PRE herbicides reduced densities 99, 99, and 96% at the aforementioned application timings, respectively, while a combination of cover crop plus PRE herbicides resulted in similar reductions. Cumulative for the season, Palmer amaranth exposure to dicamba was reduced 65% by cover crops, 98% by PRE herbicides, and 98% by cover crop plus PRE herbicides. Replacing the third POST with an LPD application further reduced plants exposed with dicamba by 38,319 plants ha^{-1}. Crowfootgrass (*Dactyloctenium aegyptium* (L.) Willd.) and yellow nutsedge (*Cyperus esculentus* L.) followed trends observed with Palmer amaranth. Neither cover crop nor PRE herbicides effectively reduced the number of pitted morningglory (*Ipomoea lacunosa* L.) controlled by glyphosate plus dicamba POST. Also of significance, no program completely eliminated weeds at harvest, early-season cotton heights in total

POST programs were reduced at least 27% by early-season weed competition, and cotton yields were greater when using conservation tillage systems.

Introduction

Problematic weeds are constantly adapting to agricultural practices (Culpepper, 2006; McElroy, 2014; Webster and Nichols, 2012). One of the earliest recorded examples of this phenomenon was crop mimicry, or the ability of a weed species to evolve and appear similar to the cultivated crop, resulting in the inability of hand laborers to distinguish the weed from the crop visually (McElroy, 2014). Weed management strategies have seen a dramatic shift from primarily physical or mechanical weed control to chemical weed control in the developed world (McElroy, 2014; Ziska et al., 2019); yet, weeds continue to adapt by evolving resistance to herbicidal mechanisms of action (Busi et al., 2013; Green and Owen, 2011; Gressel et al., 2016).

The selection for herbicide resistant weeds is a function of species biology, characteristics of genes conferring herbicide resistance, and the number of individuals treated over time and space (Gaines et al., 2019; Heap, 2014). Glyphosate-resistant crops were commercialized in 1996, resulting in a rapid shift to heavy reliance on glyphosate for weed control in some of the most widely produced crops in the U.S., such as cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.) (Green, 2009; Jones and Snipes, 1999; Young, 2006). Immediately following the introduction of glyphosate resistant varieties, the number of different mechanisms of action utilized in soybean during the growing season decreased from five to one – glyphosate (Young, 2006). Although some scientists believed that weed resistance to glyphosate was extremely unlikely, as soon as

five years after the introduction of this technology, glyphosate-resistant weeds had become problematic in many crop production systems (Bradshaw et al., 1997; VanGessel, 2001; Culpepper et al., 2006). In the case of glyphosate-resistant weeds, initial mutation frequency was low; however, glyphosate was used over a large area, with broad-spectrum activity selecting for many resistant biotypes (Gaines et al., 2019; Heap, 2014). This, coupled with the abandonment of other effective weed control practices, such as herbicide rotation and cultural or physical control methods, led to widespread herbicide resistant weeds in many crops.

Following the selection for widespread glyphosate resistance in weeds, both industry and academia recommended the reincorporation of integrated weed management strategies, such as utilization of residual herbicides or tillage (Gustafson, 2008; Sosnoskie and Culpepper, 2014). Although residual herbicides are important components to weed management programs in a multitude of crops, postemergence (POST) herbicide applications are still necessary for season-long weed control (Everman et al., 2009; Johnson et al., 2012). To provide greater flexibility to growers as well as broaden POST herbicide options, cotton has been engineered with resistance to POST applications of glufosinate, 2,4-D, and dicamba (Meyer et al., 2015; Cahoon et al., 2015; Manuchehri et al., 2017). XtendFlex™ varieties, which are tolerant to glyphosate, glufosinate, and dicamba, accounted for nearly 55% of the cotton varieties planted in the U.S. during 2019, while approximately 65 to 75% percent of soybean varieties planted during that time had the Xtend™ trait package providing resistance to glyphosate and dicamba (USDA AMS, 2019; E. Prostko, personal communication). These traits are widely planted because they provide flexibility in weed control and have high yield potential; as

a result, dicamba use has increased. Dicamba was applied on less than 10% of hectares planted to cotton in 2010 compared with 43% of planted cotton hectares in 2019, while less than 1% of planted soybean hectares received dicamba in 2012 compared with 27% in 2018 (USDA NASS, 2020). Dicamba-based weed management programs can effectively control many of the most problematic weeds in the U.S. including Palmer amaranth (*Amaranthus palmeri* S. Watson), common waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), horseweed (*Conyza canadensis* L.), giant ragweed (*Ambrosia trifida* L.), and morningglory species (*Ipomea* spp.) (Barnett et al., 2013; Kruger et al., 2010; Leon et al., 2016; Meyer et al., 2015).

Although dicamba-based herbicide systems can be effective, rapid adoption and abuse of dicamba applied POST is already of great concern in regards to weed resistance development. As a result of overreliance on dicamba in field corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) production, there are populations of dicamba resistant kochia (*Bassia scoparia* (L.) A.J. Scott) in the Midwest (Cranston et al., 2001). Greenhouse studies have shown that Palmer amaranth exposed to sub-lethal rates of dicamba for three generations can lead to populations with reduced sensitivity (Tehranchian et al., 2017). Additionally, just four years after the introduction of dicamba-tolerant crops, dicamba resistant Palmer amaranth has been identified in Tennessee (Steckel, 2020). To delay the evolution of resistance in other populations, integrated approaches such as utilizing multiple effective herbicide mechanisms of action and cultural control methods must be adopted.

Applying multiple mechanisms of action in tank-mixtures is a method to reduce selection pressure and potentially improve weed control (Gressel et al., 2016; Vann et al.,

2017). Crops tolerant to both glufosinate and dicamba would allow for an effective dicamba POST tank mixture that can improve weed control beyond either herbicide applied alone (Vann et al., 2017) and likely delaying resistance to either herbicide mechanism of action (Gressel et al., 2016). However, current registrations prohibit this herbicide mixture because of volatility concerns (Anonymous, 2018a; Anonymous, 2018b). Thus, this restriction often forces growers to make glyphosate plus dicamba applications, ultimately applying dicamba as the only effective mechanism of action being used to control glyphosate resistant weeds.

The use of residual herbicides and cover crops diversifying management programs have also been shown to improve weed control while reducing selection pressure on POST applied herbicides. For example, Johnson et al. (2012) observed both improved common waterhemp and giant ragweed control with the addition of PRE herbicides in a total POST system. They also documented the PRE herbicide can assist in minimizing selection pressure by reducing the number of weeds exposed to the POST herbicide application (Johnson et al., 2012). Similarly, the use of cover crops has been shown to reduce or delay weed emergence thereby reducing the number of weedy pest to be controlled with POST herbicide applications (Aulakh et al., 2012; Bunchek et al., 2020; Price et al., 2016; Wallace et al., 2019; Webster et al., 2016; Wiggins et al., 2016). Thus, the objective of this experiment was to design dicamba-based management programs that are effective and sustainable with the potential for immediate grower adoption. The influence of cover crop, PRE herbicides, and a lay-by post-directed (LPD) application using traditional herbicide chemistry were evaluated to determine their

influence on selection pressure of dicamba applied POST in a cotton weed management program.

Materials and Methods

During 2018 and 2019, an experiment was conducted four times in Ty Ty, GA and Jackson, TN; site characterization including soil texture, organic matter, soil pH, cereal rye (*Secale cereale* L.) biomass, and cotton planting date are provided in Table 5.1. Treatments were arranged in a split-plot design, with the whole plot being conventional tillage or cereal rye cover crop. For the cover crop systems, cereal rye (cv. 'Wrens Abruzzi') was planted at a seeding rate of 100 kg ha^{-1} with a grain drill (Great Plains Manufacturing, Salina, KS) in the November to December prior to cotton planting. Once the cereal rye reached a minimum height of 2 m in the spring, it was rolled with a roller crimper (I & J Manufacturing, Gordonville, PA) in the direction cotton would be planted and killed with glyphosate in preparation for planting cotton. Cotton was planted using a strip-till planter system, with a two-row planter attached to the strip-till implement to reduce planting error in GA, and with a no-till planter in TN. For conventional tillage systems, bareground beds were rotary-tilled making them free of weeds and debris and planted on the same day using the same planters as noted for the conservation tillage systems. Deltapine 1646 B2XF and Deltapine 1518 B2XF at 2 seeds per 20 cm were planted 1.2 cm deep in GA and TN, respectively. Cultural practices, including fertilization, insect management, plant growth management and defoliation, were conducted as recommended by the extension service in each state (Raper, 2014; Whitaker et al., 2018).

The sub-plot factor was herbicide system, with four systems evaluated: (1) no herbicide; (2) 3 sequential applications of dicamba plus glyphosate POST (0.56 + 1.12 kg ai ha^{-1}); (3) diuron plus fomesafen (0.57 + 0.17 kg ai ha^{-1}) PRE fb 3 sequential applications of dicamba plus glyphosate POST; and (4) diuron plus fomesafen PRE fb 2 applications of dicamba plus glyphosate POST fb diuron plus MSMA (0.84 + 1.38 kg ai ha^{-1}) plus crop oil concentrate (1% v/v) directed at lay-by. PRE applications occurred 0 to 1 d after planting, POST 1 occurring 12 to 23 d after PRE applications, POST 2 occurring 18 to 30 d after POST 1, and POST 3/LPD occurring 16-21 d after POST 2. Cotton height, weed height, and maximum weed density for each location are listed in Table 5.2.

Two dicamba-based programs included three dicamba POST applications following label requirements at the time the experiment was initiated. In 2019, new requirements limited dicamba to two in-season applications but continued to allow an additional burndown application equaling a potential for three applications during the season (Anonymous, 2018a; 2018b). All herbicides were applied using a CO_2 – pressurized backpack sprayer calibrated to deliver 140 L ha^{-1}. PRE and POST applications were made using 110015 TTI nozzles, while lay-by applications were made using Floodjet TK-VS2 nozzles (Teejet Technologies, Wheaton, IL) with spray directed toward the bottom 10 cm of the cotton plant. All residual herbicides were activated with at least 0.6 cm of rainfall or irrigation within 48 hr of application. Plots were two rows wide spaced 96 cm apart and 9 m long in TN, and four or six rows wide spaced 92 cm apart and 7.5 m long in GA.

In GA, differences in cotton population relative to tillage system were visually evident thus stand was recorded; differences were not observed in TN and were not

recorded. Emerged cotton plants were counted for the entire plot 15 d after planting in GA. Cotton heights were also collected in GA on 20 plants per plot beginning 6 d after POST 2 and were measured up to 5 d after POST 3/LPD to determine the impacts of weed competition on cotton growth. Visual injury to cotton was evaluated following each herbicide application. However, injury was less than 10% for all treatments, evaluation dates, and locations and will not be reported.

To quantify the influence that cover crop, PRE herbicides, and a directed lay-by application had on the selection pressure of glyphosate plus dicamba applied POST, weeds were counted one day prior to each in-season herbicide application. In GA, the entire plot was counted, while a representative 0.25 m^2 quadrat of each plot was counted in TN. Following the first POST application, broadleaf weeds present were separated into two categories: 1) previously treated and damaged by dicamba or 2) newly emerged, which quantifies the total number of weeds being treated with dicamba plus glyphosate but also determines the number of weeds surviving at least one dicamba plus glyphosate application with potential for additional exposure. Separating weed counts into previously damaged or newly emerged occurred at the three GA locations. From these counts, exposure to glyphosate plus dicamba over the entire season can be calculated, which can assist in determining the optimum weed management system to reduce exposure to POST herbicide options in cotton. Weeds counted throughout the season included Palmer amaranth, pitted morningglory (*Ipomea lacunosa* L.), yellow nutsedge (*Cyperus esculentus* L.), and crowfootgrass [*Dactyloctenium aegyptium* (L.) Willd.]. At the end of the season, Palmer amaranth and crowfootgrass were counted for the entire plot and subsequently harvested and weighed for biomass where present in GA. Pitted

morningglory and yellow nutsedge were not evaluated at the end of the season because pitted morninglory vines were desiccated by defoliation mixtures and yellow nutsedge was eliminated by a late-season fungus. Cotton was harvested using a cotton picker for yield comparisons.

Data were subjected to ANOVA using PROC GLIMMIX in SAS, version 9.4 (SAS Institute, Cary, NC) evaluating the impact of tillage option and herbicide program on the response variables. Cotton population and height, weed counts and biomass, and cotton yield were all set as the response variables in the model, while block and location were included as random factors. Location by treatment interactions were evaluated for all response variables, and when appropriate, data was separated by location for analysis. All weed counts were square root transformed, and weed biomass was log transformed to improve normality and homogeneity of variance prior to analysis, however all data are presented in their back-transformed values. All P-values for tests of differences between least-squares means were compared and adjusted using the Shaffer-simulated method ($\alpha = 0.05$).

Results and Discussion

Cotton Population

Cotton population was influenced by soil temperature during emergence in GA. Two distinct environmental conditions were observed, with maximum soil temperatures ranging from 30 to 37 C for two locations (2018 and 2019 early planted) and 40 to 43 C for one location (2019 late planted). Soil temperature data was obtained from the UGA weather monitoring center. When combined across 2018 and 2019 early planted locations, cotton population was higher with conventionally prepared soil (112,345 plants

ha^{-1}) compared with cover crop treatments (94,144 plants ha $^{-1}$) (data not shown). Conditions at these locations were optimal for cotton germination and root development in the conventional system with maximum soil temperatures ranging from 30 to 37 C and minimum soil temperatures ranging from 18 to 23 C (McMichael and Burke, 1994; Pearson et al., 1970; Raphael et al., 2017; Snider et al., 2014). The reduction in population with the cover crop may have been influenced by cooler soil temperatures and higher moisture levels which has been previously documented (Teasdale and Mohler, 1993). In fact, Teasdale and Mohler (1993) observed predicted soil temperature decreases of 2 to 5 C in the first five weeks following cover crop termination when 3,000 kg ha^{-1} of residue was present. Although a greater plant population was observed in conventional systems, both production practices had sufficient plant populations to maximize yield potential (Whitaker et al., 2018)

At the third GA location, maximum soil temperatures ranged from 40 to 43 C for each of the first seven days after planting in the conventional system. McMichael and Burke (1994) noted root development at 40 C was nearly half that observed at 34 C. Irrigation was implemented to preserve the crop but limitations of irrigation volume and the inability to lower soil temperatures for an extended period of time limited irrigation benefits. Due to these adverse conditions, cotton population was significantly reduced when planted into conventional tillage (51,457 plants ha^{-1}), while cotton planted into a rye cover crop noted a higher population (79,072 plants ha^{-1}). As mentioned earlier, cover crops can reduce soil temperatures and help preserve soil moisture improving emergence in excessively hot or dry environments (Teasdale and Mohler, 1993). Final populations in

the conservation tillage systems were adequate to maximize yield potential while those in the conventional systems were low (Whitaker et al., 2018).

Cotton Heights

Results were not influenced by year or location, thus heights were combined for analysis. Since herbicide injury was transient, differences in cotton heights assist in quantifying the impact of early-season weed control on competition with the crop. Although less weeds were observed in the conservation tillage system when compared with the conventional system (Table 5.3), early-season weed competition was similar among tillage systems. The emerging weed population in the strip-tilled area of the conservation tillage system (10 cm on each side of the cotton plant) was similar to that observed in the respective area of the conservation tillage system leading to similar early-season weed competition on the young cotton (data not shown). In contrast, differences in herbicide systems were significant. Approximately two wks after POST 1, the tallest cotton was observed when a PRE herbicide was followed by a POST application (36 cm), where cotton treated with a POST only and cotton not treated with a herbicide were significantly shorter (25 and 26 cm, respectively) (data not shown). The weeds present in these locations (Palmer amaranth, pitted morningglory, crowfootgrass, and yellow nutsedge) are all extremely competitive with crops, competing for light, nutrients, space, and water (Zimdahl, 2004). A PRE application is vital to reduce weed competition and maximize yield in cotton (Byrd and Coble, 1991; Crowley and Buchanan, 1978; Keeley and Thullen, 1975; Rowland et al., 1999).

Palmer amaranth

Density counts were averaged over the TN and three GA locations and was influenced by the interaction of tillage option and herbicide system when evaluated 1 d before the first POST application. Nearly 2 million plants ha^{-1} were recorded in the tilled system when no herbicide was utilized (Table 5.3). The cover crop alone reduced emergence 75% which is an effective approach in reducing selection pressure of POST applied herbicides (Aulakh et al., 2012; Bunchek et al., 2020; Price et al., 2016; Wallace et al., 2019; Webster et al., 2016; Wiggins et al., 2016). Previous research has demonstrated that 5,200 kg ha^{-1} of rye biomass decreased Palmer amaranth emergence 50% (Webster et al. 2016). The application of two effective mechanisms of action PRE, with timely rainfall or irrigation, was even more effective than the cover crop reducing the number of Palmer amaranth plants present by at least 99.7%, regardless of production system. The value of residual herbicides with a cover crop are often questioned due to the cover crop preventing some of the herbicide from reaching the soil (Teasdale et al., 2003). However, this study and others have documented residual herbicides in combination with a cover crop may be one of the most effective approaches for reducing selection pressure to POST applied herbicides (Bunchek et al., 2020; Wallace et al., 2019). Additionally, the cover crop had a similar influence reducing the number of plants to be controlled by PRE herbicides thereby reducing selection pressure on those herbicide tools as well.

Just prior to the second POST application, over 1.2 million plants ha^{-1} were observed in the no-herbicide conventional system (Table 5.4). Intra- and interspecific competition has been reported with Palmer amaranth in soybean and sweet potato

[*Ipomoea batatas* (L.) Lam.], and could be why density was reduced at each application timing (Basinger et al., 2019a; 2019b). The cover crop alone or glyphosate plus dicamba POST 1 reduced the number of plants present 70 to 89% when compared with the conventional control. The cover crop was as effective as a single POST application in controlling the weed at this time. Previous research has noted sub-optimal control from one application of dicamba to Palmer amaranth (Merchant et al., 2013). Although cover crops are extremely effective early in the season, herbicides are still necessary for season-long weed control (Teasdale, 1996). The PRE fb POST program, regardless of production system, reduced the number of plants treated by the POST 2 application by at least 98.5%. Trends one d prior to POST 3 were similar, with the cover crop alone providing 54% control, and all herbicide systems providing 90% control or greater (data not shown).

With density counts taken one d prior to each POST application, the total number of Palmer amaranth exposed to dicamba plus glyphosate over the entire season within each system can be calculated. Results were influenced by the interaction of tillage option and herbicide system. The highest level of exposure was present when 3 sequential POSTs were applied in conventional tillage (2,155,409 plants ha^{-1}) (Table 5.5). The addition of the cover crop with this program reduced season-long exposure to dicamba plus glyphosate by 65%. Although the cover crop was beneficial, the PRE application was more impactful reducing exposure over 97% and 91% in conventional and conservation tillage systems, respectively. Replacing the POST 3 application with the LPD application further reduced the number of Palmer amaranth plants treated with glyphosate plus dicamba by 38,319 plant ha^{-1} when comparing relative systems. This was

a 66% reduction in exposure (18,105 vs 56,424 plants ha^{-1}) at this time (data not shown). Lay-by applications in cotton can provide a unique opportunity to further reduce selection pressure to POST herbicide options by utilizing additional mechanisms of action that can provide both POST and residual control of problematic weeds (Clewis et al., 2008; Price et al., 2008).

Reducing the number of plants treated with dicamba is paramount for farm sustainability; however, reducing the number of plants receiving multiple exposures over time may be even more important (Bagavathiannan and Davis, 2018; Tehranchian et al., 2017). In GA, it was determined that the number of plants ha^{-1} surviving glyphosate plus dicamba POST 1 to be treated with a second application was 204,472; about 10% of the emerged population at time of POST 1 (Table 5.4). The addition of a cover crop or a PRE herbicide in conjunction with the POST 1 application reduced the number of plants previously surviving a dicamba plus glyphosate application by over 43 or 97%, respectively. At time of the final herbicide application, 35 times more Palmer amaranth had survived in the total POST program (43,506 plants ha^{-1}) receiving either one or two previous exposures to dicamba as compared with the PRE fb POST program (1,208 plant ha^{-1}). Treating the same weed population with the same mechanism of action continuously places enormous selection pressure for the development of herbicide resistance (Bagavathiannan and Davis, 2018). Therefore, reducing the number of Palmer amaranth treated with a single mechanism of action multiple times in a season reduces the likelihood of developing resistance.

Palmer amaranth plants were counted and weighed at harvest to evaluate the impact of tillage option and herbicide system on population and size over the entire

season. Both end of season counts and above-ground biomass were impacted by the main effect of herbicide system. Price et al. (2016) also observed that cover crop and tillage systems had generally similar weed densities at harvest after herbicide programs were implemented. Palmer amaranth density and biomass were highest when no herbicide was used (167,641 plants ha^{-1} weighing 1,793 kg ha^{-1}) (Table 5.6). All herbicide systems reduced both population and biomass relative to the nontreated control (102 – 847 plants ha^{-1} weighing 7 – 24 kg ha^{-1}). However, when only comparing treatments receiving herbicides, both systems receiving a PRE resulted in reduced Palmer amaranth density at the end of the season compared to the 3 sequential POST system (data not shown). Of special note is that even with an ideal herbicide system, including a PRE application with two effective mechanisms of action, sequential POST applications, and a lay-by application, Palmer amaranth was not eliminated at the end of the season.

Crowfootgrass

Crowfootgrass density is combined over two GA locations and an interaction of tillage option and herbicide system was observed for the values taken one d before POST 1. The highest density of 788,759 plants $ha^{-1}$was present in conventional tillage with no herbicide (Table 5.3). The cover crop reduced the density 74% with the PRE herbicide being more effective at 92%. Both fomesafen and diuron have demonstrated substantial grass control when applied PRE (Gardner et al., 2006; Walker et al., 1998). The integrated system of cover crops and the PRE herbicide reduced the population nearly 95% when compared with the conventional control (Table 5.3). In peanuts (*Arachis hypogaea* L.), similar results were observed (Aulakh et al., 2015). Large crabgrass (*Digitaria sanguinalis* L.) control was 41% with a cover crop alone and 61% with two

effective PRE herbicides alone, but when herbicides and cover crop were used simultaneously control was 91% (Aulakh et al., 2015).

The highest crowfootgrass density at POST 2 was also observed in conventional tillage with no herbicide treatment (662,439 plants ha^{-1}) (Table 5.4). The cover crop reduced weed density 72%, however, 98% of a reduction was observed with the PRE fb POST herbicide system in both tillage systems. Over 500,000 crowfootgrass plants ha^{-1} emerged after the POST 2 application and before the final herbicide application in conventional tillage with no herbicide, documenting the need for late-season weed control in cotton (data now shown). Previous research has demonstrated the need for late-season grass control in cotton. Even when *S*-metolachlor was applied early POST with glyphosate, late-season control of grass weeds was less than 80%, resulting in yield losses of at least 23% (Clewis et al., 2006). Even after continual grass emergence, density counts at time of the final herbicide application were 49% less with the cover crop when averaged over herbicide systems and were 98% less with herbicide systems, regardless of tillage system, when compared with no herbicides (data not shown).

At harvest, crowfootgrass was counted, harvested, and weighed to determine the impact of tillage option and herbicide system on density over the entire season. Both density and biomass were only impacted by the main effect of herbicide system. Density at harvest was highest when no herbicide was used (2,636,244 plants ha^{-1}), while adding any herbicide system resulted in a reduction over 98% (Table 5.6). Similar to density, biomass was highest when no herbicide was used (1,817.2 kg ha^{-1}) but differences among herbicide systems was observed. A total POST program and the PRE fb three POST applications accumulated 18.8 and 5.2 times more biomass than the standard system of a

PRE fb sequential POST and a directed lay-by (Table 5.6). Residual activity from diuron reducing late-emerging plants was likely part of the difference. Clewis et al. (2008) also observed improved grass control when directed lay-by applications were used compared with POST only systems or when no lay-by was applied.

Yellow Nutsedge

Averaged over the two GA locations where nutsedge was present, an interaction of tillage option and herbicide system was noted 1 day prior to the first POST application (Table 3). The highest yellow nutsedge density was present with conventional tillage without herbicides (1,320,437 plants ha^{-1}). The addition of the rye cover crop reduced yellow nutsedge populations 68% (Table 5.3). When yellow nutsedge control was evaluated in peanut, no differences were noted between conventional tillage treatments compared with when a cover crop was used which the authors attributed to low biomass levels accrued and soil moisture (Aulakh et al., 2015). The PRE herbicide treatment reduced the nutsedge population 87% just prior to the first glyphosate plus dicamba application and was likely a result from fomesafen activity (Table 5.3). Previous research in multiple cropping systems has demonstrated that the residual activity of fomesafen can effectively control of yellow nutsedge (Boyd, 2015; Grichar,1992; Reed et al., 2016).

Similar to early-season observations, density counts at time of POST 2 noted the highest yellow nutsedge density was present when no herbicide was used in conjunction with conventional tillage (3,209,930 plants ha^{-1}) (Table 5.4). The addition of a cover crop alone, herbicides alone, or cover crop plus herbicide reduced the population 78 to 90%. At POST 3, tillage practices had no impact but herbicide systems continued to reduce the population at least 76% (data not shown). All treatments that received herbicide

applications included POST applications of glyphosate in tank-mix with dicamba. Previous research by Burke et al. (2008) noted significant reductions in shoot and root/tuber dry weights resulting from applications of glyphosate. The utilization of glyphosate to reduce tuber production in a weed management system plays a large part in long-term management of nutsedge species (Burke et al., 2008; Reddy and Bryson, 2009; Webster et al., 2008).

Pitted Morningglory

PRE herbicides did not reduce the morningglory population at any of the three GA locations where the weed was present (data not shown). Previous research has demonstrated that neither fomesafen nor diuron applied PRE effectively control morningglory (Gardner et al., 2006; Osborne et al., 2003). Glyphosate plus dicamba POST and the LPD application provided complete control of emerged morningglory (data not shown). However, the weed continued to emerge after each application which has been documented in morningglory species (Norsworthy and Oliveira, 2007; Oliveira and Norsworthy, 2006; Singh et al., 2012). Interestingly, at the location with the highest morningglory density (426,069 plants ha^{-1}), treatments with a cover crop noted a 71% reduction in morningglory density at the time of POST 1 compared with conventionally tilled treatments (data not shown). However, the cover crop was not beneficial at later evaluations nor was it beneficial during any evaluation at the other locations. Previous research has noted little benefit from a rye cover crop with respect to morningglory control (Koger et al., 2002).

Cotton Yield

Cotton yield, pooled over locations, was influenced by the main effect of herbicide system and the main effect of tillage option. With respect to herbicide system, seed cotton yield was highest when a PRE application was utilized (4,081 – 4,198 kg ha^{-1}) (Table 5.7). Solely relying on POST herbicides for weed control resulted in a reduction in seed cotton yield of 14%, while not using any herbicide resulted in a 95 to 96% yield loss. Previous research on the weed-free period of cotton has demonstrated that an 8-week weed free period should result in maximum cotton yields (Buchanan and Burns, 1970; Tursun et al., 2016). The use of a PRE application in this study followed by sequential POST applications allowed for minimal weed competition from planting through harvest. With respect to tillage option, cotton grown with a cover crop yielded higher than cotton grown in conventional tillage (3,226 and 2,761 kg ha^{-1}, respectively). Although stand was reduced in cover crop treatments at two locations, cotton has the ability to compensate for gaps in stand (Hasnam, 1985). Previous research has observed increased yields from cotton grown in a cover crop, demonstrating benefits outside of just weed control. Price et al. (2012) demonstrated yield benefits in early planted cover crop treatments compared with conventional tillage in two out of three years, which resulted in greater economic returns. Price et al. (2016) also noted higher yields with cover crop treatments compared with conventional tillage. Previous research has demonstrated many benefits to utilizing cover crops including soil moisture preservation, improved water infiltration, reduced erosion and runoff and reduced thrips pressure during early-season (Mirsky et al., 2012; Knight et al., 2017; Teasdale, 1996). Many of these benefits could have factored into the increased yield observed in this experiment.

Conclusions

Reducing the number of weeds, especially Palmer amaranth, that farmers need to control with POST herbicides will likely improve farm sustainability as a result of having less herbicide resistance. The data presented herein demonstrates cover crops reduced the number of Palmer amaranth and crowfootgrass plants present from cotton emergence through the final herbicide application by 49 to 75% thereby reducing selection pressure for both PRE and POST herbicide applications. PRE herbicides were more effective than the cover crop reducing Palmer amaranth, crowfootgrass, and nutsedge plants that needed to be controlled by POST herbicides by 77 to 99%. However, the combination of the cover crop and PRE herbicides was the more consistently effective option. Replacing the third POST application with an LPD application further reduced selection pressure to the POST herbicides, although much less than cover crops or PRE herbicides. Additionally, from an agronomic standpoint, a complete herbicide program improved cotton growth, development, and ultimately yield at the end of the season. Conservation tillage systems also resulted in higher cotton yields due to the many benefits associated with cover crop use.

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Table 5.1. Soil characteristics, cover crop biomass level, and planting dates for each experiment.

Year	Site	Soil texture	Organic matter	Soil pH	Cereal rye biomass	Planting date
		% sand, silt, clay	%		kg ha^{-1}	
2018	Jackson, TN	22, 49, 29	1.4	6.6	3,651	May 10
2018	Ty Ty, GA	85, 10, 5	0.65	6.2	6,019	May 16
2019	Ty Ty, GA	84, 14, 2	0.64	5.8	2,306	May 14
2019	Ty Ty, GA	88, 10, 2	0.52	6.7	1,575	May 23

Table 5.2. Cotton and weed size at each application, and maximum weed population by location.

Location	Year	Application	Cotton Heights	Palmer amaranth		Pitted Morningglory		Crowfootgrass		Yellow Nutsedge	
				Height	Max. pop.[a,c]	Height[c]	Max. pop.[a,c]	Height[c]	Max. pop.[a,c]	Height[c]	Max. pop.[a,c]
			cm	cm	plants ha^{-1}	cm	plants ha^{-1}	cm	plants ha^{-1}	cm	plants ha^{-1}
Jackson, TN	2018	POST 1[a]	25	10	125,000	-	-	-	-	-	-
		POST 2[a]	51	8		-		-		-	
		POST 3/LPD[a]	60	5		-		-		-	
Ty Ty, GA	2018	POST 1	13	18	3,553,444	13	426,069	-	-	-	-
		POST 2	36	13		0		-		-	
		POST 3/LPD	51	13		10		-		-	
Ty Ty, GA	2019-1[b]	POST 1	20	20	7,316,338	5	47,819	20	1,075,932	40	552,378
		POST 2	51	20		5		20		26	
		POST 3/LPD	71	15		10		10		15	
Ty Ty, GA	2019-2[b]	POST 1	25	46	1,154,834	13	12,792	25	914,542	36	2,312,449
		POST 2	51	28		8		18		23	
		POST 3/LPD	79	20		8		10		15	

[a]Abbreviations: POST 1, Postemergence Application 1; POST 2, Postemergence Application 2; POST 3/LPD, Postemergence Application 3 or Lay-by Post-directed; Max. pop., Maximum population.

[b]Ty Ty, GA locations in 2019 were planted at different times. 2019-1 is the first planting date, while 2019-2 is the second planting date.

[c]Maximum populations were present in conventional tillage with no herbicide for each weed, and are reported for each location. If a "-" is present under a weed for a specific location, then that weed was not evaluated at that location.

Table 5.3. Weed density one d before POST 1[a] as influenced by the interaction of tillage system and the use of a preemergence herbicide.

Tillage option	Herbicide system[a]	Palmer amaranth[b]		Crowfootgrass[b]		Yellow nutsedge[b]	
		——————————no. ha^{-1}——————————					
Conventional	None	1,961,175	a	788,759	a	1,320,437	a
	PRE	3,717	c	62,349	c	176,679	b
Cover crop	None	480,869	b	206,276	b	417,827	b
	PRE	1,524	c	42,974	c	300,793	b

[a]Abbreviations: PRE, preemergence; POST 1, first topical application.

[b]Data averaged over four locations for Palmer amaranth and two locations for crowfootgrass and yellow nutsedge. Density was evaluated for the entire plot and converted to density ha^{-1}. Means within a column followed by a different letter are significantly different according to the Shaffer-simulated method (α=0.05).

Table 5.4. Weed density evaluated one d before POST 2 as influenced by tillage and herbicide system.[a]

		Palmer amaranth				Crowfootgrass[c,e,f]		Yellow nutsedge[c,e,f]	
		Total[c,e,f]		Survived[d,f]		Total		Total	
Tillage option	Herbicide system[a,b]	——————————no. ha^{-1}——————————							
Conventional	None	1,208,924	a	-		662,439	a	3,209,930	a
	POST only	227,191	bc	204,472	a	67,644	bc	293,074	b
	PRE fb POST	9,972	d	5,272	c	5,620	c	311,837	b
Cover crop	None	368,396	b	-		184,796	b	711,409	b
	POST only	127,882	cd	116,035	b	48,389	bc	514,711	b
	PRE fb POST	17,859	d	4,701	c	10,050	c	348,527	b

[a]Abbreviations: POST 2, second topical application; POST, postemergence; PRE, preemergence.

[b]The POST only herbicide system had received one application of dicamba plus glyphosate; PRE fb POST systems had received diuron plus fomesafen at planting, followed by dicamba plus glyphosate at POST 1.

[c]Total density present for each treatment.

[d]Palmer amaranth surviving the previous application of glyphosate + dicamba at the 3 GA locations; "-" designates systems where dicamba was not applied.

[e]Data averaged over four locations for Palmer amaranth and two locations for crowfootgrass and yellow nutsedge.

[f]Means within a column followed by a different letter are significantly different according to the Shaffer-simulated method (α=0.05).

Table 5.5. Palmer amaranth exposure to dicamba plus glyphosate over the entire season as influenced by tillage and herbicide system.

Tillage option	Herbicide system	Palmer amaranth exposed[a]	
		——————no. ha^{-1}——————	
Conventional	3 POSTs[b]	2,155,409	a
	PRE fb 3 POSTs[b]	47,355	c
	PRE fb 2 POSTs fb LPD[b]	17,181	c
Cover crop	3 POSTs	744,670	b
	PRE fb 3 POSTs	66,287	c
	PRE fb 2 POSTs fb LPD	19,053	c

[a]Data are averaged over four locations. Means within a column followed by a different letter are significantly different according to the Shaffer-simulated method (α=0.05).
[b]Abbreviations: POST, postemergence; PRE, preemergence; LPD, lay-by post-directed.

Table 5.6. Palmer amaranth and crowfootgrass density and biomass at harvest as influenced by herbicide system.

	Palmer amaranth[a,b]		Crowfootgrass[a,b]	
	Density	Biomass	Density	Biomass
Herbicide system	no. ha^{-1}	kg ha^{-1}	no. ha^{-1}	kg ha^{-1}
None	167,641 a	1,793 a	2,636,244 a	1,817.2 a
3 POSTs[c]	847 b	24 b	27,275 b	17.0 b
PRE fb 3 POSTs[c]	184 b	7 b	19,929 b	4.7 c
PRE fb 2 POSTs fb LPD[c]	102 b	8 b	2,053 b	0.9 d

[a]Palmer amaranth data averaged over four locations and crowfootgrass data averaged over two locations. Means within a column followed by a different letter are significantly different according to the Shaffer-simulated method (α=0.05).

[b]Data are averaged over tillage option.

[c]Abbreviations: POST, postemergence; PRE, preemergence; LPD, lay-by post-directed.

Table 5.7. Seed cotton yield as influenced by the main effects of herbicide system and tillage.

	Seed cotton yield[a]	
Herbicide system[b,d]	kg ha^{-1}	
None	173	c
3 POSTs	3,521	b
PRE fb 3 POSTs	4,081	a
PRE fb 2 POSTs fb LPD	4,198	a
Tillage option[c]		
Conventional	2,761	b
Cover crop	3,226	a

[a]Data are averaged over four locations. Means within a column followed by a different letter are significantly different according to the Shaffer-simulated method (α=0.05).

[b]Data averaged over tillage option.
[c]Data averaged over herbicide system.
[d]Abbreviations: POST, postemergence; PRE, preemergence; LPD, lay-by post-directed.

CHAPTER 6

CEREAL RYE RESPONSE TO EIGHT COMMONLY USED WHEAT HERBICIDES[1]

[1]Hand LC, Randell TM, Culpepper AS. To be submitted to *Weed Technology*.

Abstract

Eight herbicides registered for use in wheat, at two rates, were evaluated for potential labeling in rye to expand currently limited chemical weed control options. Across five site years, halauxifen-methyl plus florasulam, pyroxsulam, and thifensulfuron-methyl plus tribenuron-methyl applied POST to rye in Zadoks scale (Z) 1.3 caused less than 15% visual injury at the 2X rate having no influence on density or yield. These herbicides at the 2X rate reduced plant heights 11% at 10 DAT with rye recovering rapidly; plant heights were not influenced with these herbicides at their 1X rate. In contrast, significant injury was observed with the 1X rate of mesosulfuron-methyl (45%), pinoxaden (27%), and pinoxaden plus fenoxaprop-p-ethyl (30%) applied POST; early-season height reduction of 19 to 26% was also noted. Residual herbicides pyroxasulfone applied as a delayed PRE, Z 1.0, or flumioxazin plus pyroxasulfone applied at spike, Z 1.1, caused 27 to 28% and 16 to 47% injury, respectively, when the 1X rate was activated by rainfall within 2 d of application. These residual herbicides also reduced plant heights and density up to 35 and 40%, respectively, at the same locations. Grain yield was not influenced by herbicide or rate applied.

Introduction

Cereal rye (*Secale cereale* L.) is commonly grown as a winter cover crop because of its extreme cold hardiness, drought tolerance, and the ability to grow well on marginal soils (Bushuk 1976). It has additional uses as a pasture forage, hay, and grain, which is fed to livestock or consumed as food and alcoholic beverages (Bushuk 1976; Hales et al. 2007; Shee et al. 2016). Rye serves multiple purposes in many states as a late-wintering forage for cattle and a summer cover crop used to mitigate erosion, moisture loss, and weed competition when planting a cash crop (Bunchek et al. 2020; Li et al. 2013; Wiggins et al. 2016). Arkansas research indicated rye fed to cattle demonstrated greater nutritional benefits compared to oat (*Avena sativa* L.) and triticale [*xTriticosecale* Wittm. Ex A. Camus (*Secale* x *Triticum*)] resulting in greater average daily gain and weight gain per area (Beck et al. 2007). Although wheat (*Triticum aestivum* L.) hectarage surpassed cereal rye hectarage nationally in 2018 (18.8 and 0.8 million ha planted, respectively), Georgia growers planted a comparable amount of acres to both crops in the same year (74,800 to 78,740 ha) (USDA 2019). Furthermore, Georgia was second in the nation in acres of rye planted and harvested (74,800 and 5,906 ha, respectively) and value of rye production in 2018 ($2.8 million) (USDA 2019).

Weed management in rye is extremely challenging because of limited herbicide options. Although preplant tillage can be an effective approach to remove weeds at planting for growers not utilizing conservation production systems, weed control is needed for the entire season (Taylor and Everman 2015). In-crop tillage options are extremely limited and most often not practical, as rye seed are planted by being broadcast spread or drilled in 19 cm rows (Buntin and Cunfer 2017; Tautges et al. 2016). Thus, the

success of in-season weed control in conventional production is highly dependent on herbicides. For rye, registered herbicide options are limited to 2,4-D, MCPA, bromoxynil, bromoxynil plus pyrasulfotole, and prosulfuron (Marshall 2017). Although prosulfuron is registered, a 10 month rotational restrictions to cotton (*Gossypium hirsutum* L.), peanut (*Arachis hypogaea* L.), and soybean (*Glycine max* L.) limit its use as these crops are planted immediately after rye harvest (Anonymous 2019c).

Among the most common and troublesome weeds in Georgia rye not controlled by registered herbicides include Italian ryegrass [*Lolium perenne* L. ssp. *Multiflorum* (Lam.) Husnot], henbit (*Lamium amplexicaule* L.), chickweeds (*Stellaria* spp.), and annual bluegrass (*Poa annua* L.) (Anonymous 2015, 2018a, 2018b, 2019b; Buntin and Cunfer 2017; Webster 2012). Italian ryegrass is the most problematic weed in small grain production in Georgia and challenges rye production. Fast et al. (2009) predicted that yield loss in wheat was 16% in the presence of 30 Italian ryegrass plants m^{-2}. This resulted in a reduction in grade and a significant impact on dockage, ultimately reducing the price obtained for the wheat (Fast et al. 2009). Other studies indicated that yield losses in wheat decreased 4.2% for every 10 Italian ryegrass plants m^{-2}, which can be attributed primarily to reduced tillering in wheat (Liebl and Worsham 1987). Additionally, Italian ryegrass uptake of nitrate and potassium was twice that of wheat (Liebl and Worsham 1987). Henbit densities of 82 and 155 plants m^{-2} reduced wheat yields 13 and 38%, respectively (Conley and Bradley 2005). Differences in tiller and spike density were also detected in henbit infested treatments compared to weed-free treatments (Conley and Bradley 2005). Common chickweed has demonstrated extreme

competitive abilities for both space and nutrients, reducing barley yield up to 80% (Mann and Barnes 1950).

Wheat has demonstrated high levels of tolerance to mesosulfuron-methyl, pinoxaden, and pyroxsulam (Ellis et al. 2010; Grey et al. 2012a; Howatt 2006). Additionally, these herbicides provide excellent control of susceptible populations of Italian ryegrass (Ellis et al. 2010; Grey et al. 2012; Howatt 2006). Not only do mesosulfuron-methyl and pyroxsulam control grasses, but they also effectively control other weeds including henbit, chickweed and annual bluegrass with minimal restrictions to commonly planted rotational crops (Anonymous 2012; Anonymous 2020; Grey et al. 2012a, 2012b). Thifensulfuron-methyl plus tribenuron-methyl and halauxifen-methyl plus florasulam are also options that could provide alternative mechanisms of action and have proven safe in wheat while providing excellent broadleaf weed control including henbit and chickweed (Anonymous 2018c, 2019a;Jackson et al. 2018; Lee et al. 2015; Soltani et al. 2006). These two herbicides also provide significantly reduced rotational restrictions compared to prosulfuron with essentially no limitations to planting Georgia's major row crops once the rye is harvested (Anonymous 2018c; Anonymous 2019a).

In areas where Italian ryegrass is resistant to POST applied herbicides, pyroxasulfone and flumioxazin plus pyroxasulfone have become critical management tools in wheat and could prove beneficial in rye production. Pyroxasulfone applied shortly after planting has become a major component of ryegrass management with minimal impacts on wheat in both the United States (Hulting et al. 2012) and Australia (Boutsalis et al. 2014). Flumioxazin plus pyroxasulfone incorporates an additional mechanism of action that broadens the weed control spectrum compared to pyroxasulfone

alone and still maintains minimal injury to wheat (Crow et al. 2015; Randhawa et al. 2018).

Previous studies evaluating cereal rye tolerance to tribenuron demonstrated minimal injury but tolerance to mesosulfuron was variable (MacRae et al. 2007). Minimal information is available concerning rye tolerance to other wheat herbicides. Therefore, rye tolerance to commonly used wheat herbicides should be investigated. The objective of this study was to determine rye tolerance to commonly used wheat herbicides generating data needed for potential registrations through IR-4.

Materials and Methods

An experiment was conducted at five locations during 2018-2019 and 2019-2020 at an on-farm location near Chula, GA (31.53’73°N, 83.64’75°W), the Ponder Research Farm near Ty Ty, GA (31.30’18°N, 83.39’03°W) and the Sunbelt Ag Expo near Moultrie, GA (31.14’15°N, 83.71’64°W) to evaluate the tolerance of cereal rye to eight commonly used wheat herbicides. Location characteristics including soil texture, organic matter, soil pH, planting dates, and herbicide application dates are shown in Table 6.1. Rye (cv. ‘Wrens Abruzzi’) was planted with a grain drill (Great Plains Manufacturing, Salina, KS) on conventionally prepared soil in rows spaced 19 cm apart, and seeds were drilled 1.25 cm deep at a rate of 100 kg ha^{-1}. Production practices were conducted as recommended by the University of Georgia Cooperative Extension Service (Buntin and Cunfer 2017). Studies were maintained weed-free using tillage prior to planting and labeled applications of MCPA or 2,4-D applied to the entire trial area at least two weeks after POST treatment applications. Locations void of ryegrass were specifically selected since management options do not exist.

Treatments were arranged in a randomized complete block design with a factorial of eight herbicides and two rates of each herbicide. Two non-treated controls were included for comparison, resulting in a total of eighteen treatments replicated eight times in 2018 – 2019, and four times in 2019 - 2020. Following label recommendations for use in wheat, three different application timings were used depending on herbicide applied including 1) delayed PRE applications when at least 80% of the rye in the Zadoks (Z) 1.0 stage of growth with a shoot of at least 1 cm long for pyroxasulfone, 2) spike applications with rye 2 to 5 cm in height in the Z1.1 stage of growth for pyroxasulfone plus flumioxazin, and 3) POST applications when rye was 10 to 13 cm in height with 3 true leaves in Z1.3 growth stages for all other herbicides. Herbicides evaluated, rates applied, and application timings for each herbicide are found in Table 6.2. All herbicide applications were made utilizing a CO_2-pressurized backpack sprayer equipped with 110012 AIXR nozzles (TeeJet Technologies, Wheaton, IL 60187). The spray boom was 138 cm long with a nozzle spacing of 46 cm, and was calibrated to deliver 140 L ha^{-1}. For delayed PRE applications, air temperatures were 15 to 24 C, soil temperatures were 11 to 22 C, relative humidity was 37 to 96%, and wind speeds ranged from 0 to 8 km h^{-1}. For spiking applications, air temperatures were 11 to 32 C, soil temperatures were 14 to 30 C, relative humidity was 40 to 88%, and wind speeds ranged from 3 to 11 km h^{-1}. For POST applications, air temperatures were 17 to 23 C, soil temperatures were 14 to 22 C, relative humidity was 57 to 71%, and wind speeds ranged from 3 to 5 km h^{-1}.

To quantify the impact of herbicide applications to cereal rye, visual injury estimates (0 to 100%), heights, density counts, and yield were evaluated. Injury, height and stand data collection and analysis were split into two groups: early-season residual

herbicide applications (pyroxasulfone and flumioxazin plus pyroxasulfone) and POST herbicide applications. Visual injury, heights and stand were evaluated for residual herbicide treatments beginning approximately 17 d after delayed PRE applications, 11 d after spiking applications and 10 d after application for POST treatments. Visual injury and heights were recorded for all treatments weekly for five additional weeks and again at harvest. Heights were quantified by measuring 10 random plants from rows 2 and 6 on the planted bed, resulting in 20 plants measured per plot. Averages per plot were calculated prior to statistical analysis. Stand was measured at the first injury evaluation for each application timing and was quantified by counting emerged plants from a 1.5 m section of rows 2 and 6 on the planted bed. Prior to statistical analysis, stand was converted to a percentage of the nontreated control. At the end of the season, a plot combine (ALMACO, Nevada, IA 50201) was used to collect grain yield from the entire plot in the 2019 – 2020 locations. Yield was not collected in 2018 - 2019 due to adverse weather conditions prior to harvest. Rye yields were adjusted to 14% moisture for statistical analysis.

For statistical analysis, data were subjected to ANOVA using PROC GLIMMIX in SAS (version 9.4, SAS Institute, Cary, NC) to determine the impact of herbicide and application rate on cereal rye injury, heights, density counts, and grain yield. Data analysis with respect to injury, heights, and density were separated into early-season residual applications and POST applications. For early-season residual applications, all response variables were separated by location due to a significant year by treatment interaction resulting from the time of activating rainfall (Table 3). For POST herbicide applications, a significant year by treatment interaction was not present; therefore, all

injury, height, and density count data is combined across locations. Grain yield was not influenced by the interaction of year by treatment and are combined across harvested locations. Injury, heights, density counts, and grain yield were set as the response variables for all herbicide applications. For all variables, replication was included in the model as a random factor. For POST applications and grain yield, location was also included in the model as a random factor. All P values for tests of differences between least-squares means were compared and adjusted using the Tukey-Kramer method (α = 0.05). The Tukey-Kramer method was chosen because it reduces Type I error compared to Fisher's protected LSD when more than three means are compared to each other, and it can also be used for balanced designs (Blythe 2012; Westfall et al. 2011).

Results and Discussion

Residual herbicides. At each location, injury was significantly influenced by the interaction of herbicide and rate applied (Table 6.4). Pyroxasulfone applied as a delayed PRE injured rye 27 to 52% at 17 DAT at two of five locations. Specific to these locations was 1.3 to 1.4 cm of rainfall that occurred within 2 d of application (Table 6.3). For the three other locations where less injury (0-16%) was observed, rainfall during this same period was 46 to 93% less. By 41 DAT, injury from all locations receiving over 2 cm of rainfall during the first 20 DAT were injured 13 to 44% with the greatest level of injury (25-44%) still observed at the two locations receiving the most rainfall during the first two days after application. Increased injury from soil-applied herbicides due to increased soil moisture along with decreased soil temperature has also been noted with chloroacetamide herbicides in corn (*Zea mays* L.) (Boldt and Barrett 1989). Rye is often slower to emerge when compared to wheat; thus, it may have been more vulnerable to a

delayed PRE application of pyroxasulfone (Clark 2007). Interestingly the pyroxasulfone label suggests to not irrigate with a delayed PRE application prior to wheat emergence suggesting there may be a potential concern if activation occurs too soon after a delayed PRE application to non-emerged wheat (Anonymous 2017). Although injury was significant at four of five locations during the first 5 weeks after application, rye recovered to less than 10% injury across locations by 130 DAT (data not reported).

Rye heights and stand were influenced by the interaction of residual herbicide and rate applied. At the two locations where visual injury was greatest (Table 6.4), rye height was reduced by pyroxasulfone 19 to 37% when compared to the control (Table 6.5). Additionally, stand was reduced 27 to 37% by the high rate at both locations with the low rate reducing stand 26% at one location (Table 6.6). Previous research has demonstrated significant stand reductions in wheat and rye in response to both partial and full rates of pyroxasulfone applied preemergence (Boutsalis et al. 2014; Palhano et al. 2018) and preplant (Price et al. 2020). At the other three locations, pyroxasulfone did not influence density and only reduced height 11% with the high rate at one location (Tables 6.5 and 6.6). Observations taken 41 DAT noted heights followed similar trends to early-season evaluations; however, by 70 DAT differences in heights were no longer detectable (data not shown).

Mixtures of flumioxazin plus pyroxasulfone applied to spiking rye caused more visual injury than pyroxasulfone applied as a delayed PRE at 4 of 5 locations (Table 6.4). Similar to pyroxasulfone, greater injury levels were observed at the locations where rainfall occurred more closely to application (Table 6.3). Flumioxazin applied prior to wheat planting has resulted in significant injury (up to 30%) and reduced wheat biomass

at tillering (Clay et al. 2010; Crow et al. 2015). Thus, injury from mixtures of flumioxazin plus pyroxasulfone are likely to be greater than pyroxasulfone alone even with different application timings. Injury ratings in this experiment at 35 DAT noted at least 18% injury at 4 of 5 locations even with the 1X application rate (Table 6.4). Similar to pyroxasulfone, rye recovery during the season was remarkable with less than 15% damage noted across locations by 120 DAT (data not reported).

Flumioxazin plus pyroxasulfone reduced plant heights with the 1X rate (14-35%) at 3 locations and with the 2X rate (9-53%) at all locations at 11 DAT (Table 6.5). Additionally, stand loss was observed at 2 and 4 locations with the 1 and 2X rate, respectively (Table 6). Height measurements documented 5 weeks after application followed identical trends to those noted during early season; however, by 65 DAT height differences were no longer detectable (data not shown). Flumioxazin applications prior to wheat planting have demonstrated significant reductions in fresh-weight biomass evaluated at tillering in previous research (Crow et al. 2015). Although biomass was reduced in the aforementioned study, wheat yield was not impacted at the end of the season. Once rye has emerged, it generally grows faster than wheat (Clark 2007). This gives rye the ability to recover from early season herbicide injury, which could lead to a similar response as wheat with minimal impact on yield.

POST herbicides. Rye injury and heights in response to POST applications were significantly influenced by the main effect of herbicide (Table 6.7); treatments did not impact rye density (data not reported). Halauxifen-methyl plus florasulam, pyroxsulam, and thifensulfuron-methyl plus tribenuron-methyl when averaged over rate injured rye less than 14% throughout the season (Table 6.7). At 10 DAT, these herbicides averaged

over rate reduced rye heights 10% compared to the control (Table 6.7). However, height differences between these herbicides and the control were no longer detectable by 30 DAT. Mesosulfuron-methyl, pinoxaden, and pinoxaden plus fenoxaprop-p-ethyl were more damaging to rye with injury ranging from 28 to 47% and height reductions ranging from 18 to 25% at 10 DAT. By 30 DAT, injury of 18 to 40% was detected with plant heights significantly less than the control with only mesosulfuron-methyl. Similar studies noted up to 25% injury in wheat treated with mesosulfuron-methyl at 15 g ai ha^{-1}, and applications to rye at a similar growth stage resulted in injury of 36% at two weeks after application (Grey et al. 2012a; MacRae et al. 2007). Similar to the delayed PRE and spike applications, rye recovered from injury by POST herbicides with 10% or less observed by 120 DAT (data not reported). Grey et al. (2012a) and MacRae et al. (2007) also noted how quickly wheat and rye recover from herbicide injury.

Rye grain yield. Upon maturity, rye grain was harvested using a small-plot combine. Rye yield ranged from 443 – 630 kg ha^{-1} when averaged over location. No treatment significantly reduced yield compared to the nontreated control (data not reported). Previous studies reported similar trends, with early-season injury not translating to yield losses in rye or wheat (Crow et al. 2015; Grey et al. 2012a; MacRae et al. 2007; Robinson et al. 2015). Interestingly, yield was not impacted even with stand losses of nearly 50% with some early-season herbicide applications. Studies on wheat response to stand loss noted no yield impacts with up to 60% stand loss in two out of three locations (Conley and Bradley 2005). As mentioned earlier, rye grows more aggressively than wheat (Clark 2007), which could contribute to the similar response noted in this experiment.

Injury from pyroxasulfone as a delayed PRE, flumioxazin plus pyroxasulfone at spike, mesosulfuron POST, pinoxaden POST, and pinoxaden plus fenoxaprop-p-ethyl POST exceeded the level of safety desired to obtain a registration for rates and application methods studied in this experiment, which would result in hesitancy from registrants due to liability issues (Fennimore and Doohan 2008). In contrast, halauxifen-methyl plus florasulam, pyroxsulam, and thifensulfuron-methyl plus tribenuron-methyl applied caused less than 15% visual injury at the 2X rate with no effect on density or yield. These herbicides at the 2X rate reduced plant heights 11% at 10 DAT with rye recovering rapidly; additionally plant heights were not influenced with 1X rates of these herbicides. With crop tolerance in rye, these herbicides offer the potential for controlling henbit, chickweed, wild radish, cutleaf evening-primrose, and susceptible populations of Italian ryegrass (Anonymous 2018c; Culpepper and Vance 2016; Ellis et al. 2010; Geier et al. 2011; Lee et al. 2015; Rauch and Campbell 2019). Further research should evaluate the use of tank-mixtures to broaden the weed control spectrum as well as different application timings.

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Table 6.1. Experimental location characteristics, planting dates, and application dates.

Location	Year	Soil texture	Organic matter	Soil pH	Planting date	Application dates[a]		
		% sand, silt, clay	%			Delayed PRE	Spiking	POST
Chula, GA	2018	88, 8, 4	0.65	6.6	10/30	11/4	11/9	11/19
Ponder[b]	2019	88, 10, 2	0.52	6.7	10/24	10/24	10/28	11/7
Ponder[b]	2019	88, 10, 2	0.52	6.7	11/11	11/11	11/19	12/5
Expo[b]	2019	86, 10, 4	1.2	6.2	11/21	11/21	11/27	12/15
Expo[b]	2019	86, 10, 4	1.2	6.2	12/5	12/5	12/11	12/23

[a]Delayed PRE, spiking, and POST applications were made at the 1.0, 1.1, and 1.3 growth stages, respectively, according to the Zadoks scale.

[b]Ponder locations were conducted at the Ponder research farm near Ty Ty, GA, and Expo locations were conducted at the Sunbelt Ag Expo near Moultrie, GA.

Table 6.2. Herbicides, rates used, and application timing of rye.

Herbicide	Trade name	Rate	Application Timing[a]	Manufacturer
		g ai ha^{-1}		
pyroxasulfone	Zidua®	59	Delayed PRE	BASF Corp., Research Triangle Park, NC 27709
		119	Delayed PRE	
flumioxazin + pyroxasulfone	Fierce®	14.3 + 18.1	Spiking	Valent U.S.A. Corp., Walnut Creek, CA 94596
		28.6 + 36.2	Spiking	
halauxifen-methyl + florasulam[b]	Quelex®	2.2 + 2.1	POST	Corteva Agrisciences, Indianapolis, IN 46268
		4.4 + 4.2	POST	
mesosulfuron-methyl[c]	Osprey®	15	POST	Bayer CropScience, St. Louis, MO 63167
		30	POST	
pinoxaden	Axial® XL	24	POST	Syngenta Crop Protection, Greensboro, NC 27419
		49	POST	
pinoxaden + fenoxaprop-p-ethyl	Axial® Bold	24.3 + 12.1	POST	Syngenta Crop Protection, Greensboro, NC 27419
		48.6 + 24.2	POST	
pyroxsulam[d]	Powerflex®	18	POST	Corteva Agrisciences, Indianapolis, IN 46268
		37	POST	
thifensulfuron-methyl + tribenuron-methyl[e]	Harmony® Extra	8.5 + 4.3	POST	FMC Corp., Philadelphia, PA 19104
		17 + 8.6	POST	

[a]Delayed PRE, spiking, and POST applications were made at the 1.0, 1.1, and 1.3 growth stages, respectively, according to the Zadoks scale.

[b]Applications of Quelex included 1% v/v of COC for the lower rate, with 2% v/v utilized with the higher rate.

[c]Applications of Osprey included 2.34 L ha^{-1} of both NIS and UAN (28-0-0) with the lower rate, with 4.68 L ha^{-1} NIS and UAN utilized with the high rate.

[d]Applications of Powerflex included 2.34 L ha^{-1} of COC with the lower rate, with 4.68 L ha^{-1} utilized with the higher rate.

[e]Applications of Harmony Extra included 2.34 L ha^{-1} of NIS with the lower rate, with 4.68 L ha^{-1} utilized with the higher rate.

Table 6.3. Rainfall data accumulated for the first 20 days after applying early-season residual herbicides by location.[a]

Herbicide	DAA[b]	2018[c]	Ponder Early[c]	Ponder Late[c]	Expo Early[c]	Expo Late[c]
			cm			
pyroxasulfone	0-2	0.7	1.3	1.4	0.4	0.1
	0-5	3.7	3.1	2.6	0.4	0.4
	0-10	9.9	3.8	2.6	1.2	2.2
	0-20	11.6	6.8	4.2	1.7	9.8
flumioxazin + pyroxasulfone	0-2	0.1	1.7	0	0	0.1
	0-5	6.3	2.3	0.5	0.8	1.9
	0-10	7.9	2.3	0.6	0.9	3.7
	0-20	8.5	6.5	1.7	4.6	9.7

[a]Cumulative rainfall for indicated days after application.

[b]Abbreviations: DAA, days after application.

[c]The 2018 study was conducted on-farm near Chula, GA. Studies at the Ponder Research Farm near Ty Ty, GA, and the Sunbelt Ag Expo near Moultrie, GA, were conducted during 2019 with two different planting dates at each location.

Table 6.4. Rye injury response to residual herbicides and rate applied early in the season.

Herbicide	Rate	17 d after delayed PRE and 11 d after spiking					41d after delayed PRE and 35 d after spiking				
		2018[b,c]	Ponder Early[b,c]	Ponder Late[b,c]	Expo Early[b,c]	Expo Late[b,c]	2018[b,c]	Ponder Early[b,c]	Ponder Late[b,c]	Expo Early[b,c]	Expo Late[b,c]
	g ai ha^{-1}	——————————————————————————————————%——————————————————————————————————									
pyroxasulfone	59	0 c	28 c	27 b	1 c	8 b	13 c	25 c	30 ab	1 bc	15 cd
	119	7 c	42 b	52 a	14 b	16 b	29 b	39 bc	44 a	2 bc	23 bc
flumioxazin + pyroxasulfone	14.3 + 18.1	30 b	47 b	20 b	16 b	39 a	33 b	45 b	18 b	7 b	43 b
	28.6 + 36.2	48 a	75 a	24 b	45 a	58 a	55 a	65 a	25 b	31 a	86 a
NTC[a]		0 c	0 d	0 c	0 c	0 b	0 d	0 d	0 c	0 c	0 d

[a]Abbreviations: DAS, days after spiking applications; NTC, nontreated control.

[b]Means followed by the same letter within a column do not differ significantly (P≥0.05).

[c]The 2018 study was conducted on-farm near Chula, GA. Studies at the Ponder Research Farm near Ty Ty, GA, and the Sunbelt Ag Expo near Moultrie, GA, were conducted during 2019 with two different planting dates at each location.

Table 6.5. Influence of early season residual herbicide and rate on cereal rye heights evaluated 17 d after delayed PRE and 11 d after spiking applications.[a]

Herbicide	Rate	2018[b]	Ponder Early[b,c]	Ponder Late[b,c]	Expo Early[b,c]	Expo Late[b,c]
	g ai ha^{-1}	cm				
pyroxasulfone	59	12.5 ab	9.5 b	9.4 c	9.8 ab	8.8 ab
	119	11.4 bc	7.8 c	8.6 c	9.7 ab	8.5 ab
flumioxazin + pyroxasulfone	14.3 + 18.1	11.0 c	8.1 c	10.8 ab	9.5 ab	7.6 b
	28.6 + 36.2	10.5 c	5.8 d	10.6 b	9.0 b	5.8 c
NTC[d]		12.8 a	12.4 a	11.6 a	10.1 a	9.4 a

[a]Heights were quantified by measuring 10 plants from rows 2 and 6 on the planted bed, resulting in 20 total plants measured per plot. Averages per plot were calculated prior to statistical analysis.

[b]Means followed by the same letter within a column do not differ significantly (P≥0.05).

[c]The 2018 study was conducted on-farm near Chula, GA. Studies at the Ponder Research Farm near Ty Ty, GA, and the Sunbelt Ag Expo near Moultrie, GA, were conducted during 2019 with two different planting dates at each location.

[d]Abbreviations: NTC, nontreated control.

Table 6.6. Rye stand as influenced by residual herbicide applied delayed PRE or at spiking and rate.[a]

Herbicide	Rate	2018[c]	Ponder Early[c,d]	Ponder Late[c,d]	Expo Early[c,d]	Expo Late[c,d]
	g ai ha^{-1}	———% of the nontreated———				
pyroxasulfone	59	97 a	74 b	78 ab	92 a	84 ab
	119	97 a	63 bc	73 b	88 a	83 ab
flumioxazin + pyroxasulfone	14.3 + 18.1	93 a	60 c	94 ab	93 a	75 b
	28.6 + 36.2	78 b	54 c	96 ab	68 b	73 b
NTC[b]		100 a	100 a	100 a	100 a	100 a

[a]Stand was quantified 11 d after flumioxazin + pyroxasulfone applications by counting emerged plants from a 1.5 m section of rows 2 and 6 on the planted bed. Stand was then converted to a percentage of the nontreated prior to statistical analysis.

[b]Abbreviations: NTC, nontreated control.

[c]Means followed by the same letter within a column do not differ significantly (P≥0.05).

[d]The 2018 study was conducted on-farm near Chula, GA. Studies at the Ponder Research Farm near Ty Ty, GA, and the Sunbelt Ag Expo near Moultrie, GA, were conducted during 2019 with two different planting dates at each location.

Table 6.7. Influence of postemergence herbicides on cereal rye injury and heights. Data are combined across herbicide rate and location.

	10 DAA[a]		31 DAA[a]	
Herbicide	Injury[b]	Heights[b,c]	Injury[b]	Heights[b,c]
	%	cm	%	cm
halauxifen-methyl + florasulam	8 c	13.7 b	6 c	20.6 ab
mesosulfuron-methyl	48 a	11.2 d	40 a	18.7 c
pinoxaden	29 b	12.3 c	18 b	19.9 bc
pinoxaden + fenoxaprop-p-ethyl	29 b	11.8 cd	18 b	19.8 bc
pyroxsulam	10 c	13.7 b	6 c	21.6 a
thifensulfuron-methyl + tribenuron-methyl	13 c	13.7 b	8 c	20.7 ab
NTC[a]	0 d	15.2 a	0 d	20.8 ab

[a]Abbreviations: DAA, days after application; NTC, nontreated control.

[b]Means followed by the same letter within a column do not differ significantly (P≥0.05).

[c]Heights were quantified by measuring 10 plants from rows 2 and 6 on the planted bed, resulting in 20 total plants measured per plot.

CHAPTER 7

CONCLUSIONS

Utilizing 2,4-D or dicamba broadcast over plastic mulch. Understanding the interaction between herbicides and plastic mulch could improve preplant weed control options in plasticulture vegetable production. With respect to the relationship between 2,4-D or dicamba and plastic mulch, analytical studies determined that with as little as 0.5 cm of overhead irrigation or rainfall after application and before planting, 2,4-D can be effectively removed from plastic mulch. Increasing the interval between application and planting further reduced the concentration of 2,4-D present on the plastic mulch at crop planting. However, the relationship between dicamba and plastic mulch is more complex. Even after a 45 day interval between application and planting, with almost 12 cm of rainfall, dicamba was not effectively removed from plastic mulch. Bioassay studies confirmed these results. Crops evaluated (squash, watermelon, cantaloupe, zucchini squash, broccoli, and collard) demonstrated minimal negative impacts as a result of preplant 2,4-D applications over plastic mulch as long as irrigation was implemented. However, all crops responded negatively to preplant applications of dicamba, with significant injury and yield loss noted. Therefore, the use of dicamba over mulch should be avoided. Although these experiments helped explain the relationship of plastic mulch with 2,4-D and dicamba, the studies did not take into account the influence of residual activity of these herbicides if holes or tears in the mulch were present during an application. Thus, further research should evaluate crop tolerance to 2,4-D applied over

multicropped mulch with holes present during application to better determine appropriate plantback intervals.

Reducing selection pressure using integrated weed management strategies. Weeds are constantly adapting to management practices. With herbicide resistance quickly evolving and few new mechanisms of action on the horizon, care must be taken to delay resistance to the mechanisms of action available, particularly in regards to POST herbicide options. In cotton, utilizing cover crops, residual applied PRE herbicides, and layby applications can assist in delaying resistance to POST herbicides. Averaged over four locations, when cotton was grown in conventional tillage and received three sequential applications of dicamba plus glyphosate POST, over 2.1 million Palmer amaranth ha^{-1} were exposed to dicamba plus glyphosate over the entire season. Adding a cover crop to that system reduced exposure 65%, while utilizing two effective herbicidal mechanisms of action PRE at planting had the greatest impact, with a 98% reduction in Palmer amaranth exposure noted over the entire season. Averaged over tillage option, switching the third POST out for a layby application further reduced exposure by 38,319 plants ha^{-1} by utilizing additional mechanisms of action. Not only did the use of residual herbicides at planting have the biggest impact on reducing exposure, but it also provided the highest level of control of Palmer amaranth, crowfootgrass, and yellow nutsedge. Neither PRE herbicides nor cover crops controlled morningglory but the weed was effectively controlled with POST herbicides. Regardless of tillage or herbicide program selected, weeds were not completely controlled further stressing the importance of implementing a diversified management system. Similar to weed control, cotton responded postiviely when including PRE herbicides by improving yield 16% across

tillage options and yield was also increased 14% with the addition of a cover crop across herbicide programs. Cover crops, PRE herbicides, and layby applications can be utilized in an integrated weed management strategy to improve weed control, delay resistance to POST herbicides, and increase cotton yield.

Improving weed control options in cereal rye. Herbicide options in cereal rye are lacking, particularly when trying to control problematic grass weeds. Additional broadleaf weed control options would also benefit producers. Numerous effective herbicides are available for wheat production but response of rye to these herbicides is unknown. Thus, eight herbicides, currently registered for use in wheat, at two rates were applied in rye to determine crop safety and the potential for future registration. Delayed PRE applications of pyroxasulfone resulted in high levels of injury, height reductions, and stand loss when irrigation or rainfall exceeding 1.3 cm occurred within 2 days of application. Flumioxazin plus pyroxasulfone applied to rye at spiking was even more injurious, reducing heights and/or stand at all locations. Halauxifen-methyl plus florasulam, pyroxsulam, or thifensulfuron-methyl plus tribenuron-methyl applied POST injured rye less than 15% at the 2X rate, with minmal height reductions while not influencing stand. In contrast, mesosulfuron-methyl, pinoxaden, and pinoxaden plus fenoxaprop-p-ethyl applied POST injured rye 29 to 48% with height reductions of 19 to 26%; but these herbicides did not influence stand. Although rye was damaged significantly by five of the eight herbicide treatments, no differences in yield were noted as rye's ability to recover from early-season herbicide damage was again documented. Halauxifen-methyl plus florasulam, pyroxsulam, and thifensulfuron-methyl plus tribenuron-methyl applied POST were the only herbicides that demonstrated adequate

crop safety for use in rye and would improve weed control options for producers. Further research should evaluate different application timings as well as tank mixtures.

APPENDIX

ANALYTICAL AND COLE CROP BIOASSAY RESULTS FOR PREPLANT DICAMBA PLUS GLYPHOSATE APPLICATIONS OVER PLASTIC MULCH

In Chapter 4 of this dissertation, the use of 2,4-D plus glyphosate over plastic mulch prior to broccoli and collard planting was thoroughly discussed. In the same experiment, dicamba plus glyphosate tank-mixtures were also evaluated, and the results are provided herein. Dicamba (Xtendimax®, Bayer CropScience, 800 N. Lindbergh Blvd., St. Louis, MO 63167) plus glyphosate (Roundup PowerMax II®, Bayer Cropscience, 800 N. Lindbergh Blvd., St. Louis, MO 63167) was applied at 560 g ae ha^{-1} plus 1,125 g ae ha^{-1}, respectively. All methods for trial establishment, applications, analytical studies, bioassay studies, and statistical analysis were identical to those described in Chapter 4 and can be found on pages 95 to 102.

Analytical results. Combined over experimental run, the initial concentration of dicamba approximately two hours after application was 1,141.3 (±35.43) ppm m^{-2}. The exponential decay equation accurately described removal of dicamba from the surface of plastic mulch with a first-order rate constant (*k*) and 95% asymptotic confidence interval of 0.26 (±0.518) (Figure A.1). Rainfall appeared to have the biggest impact on dicamba removal from plastic mulch, with at least 0.5 cm of rainfall or overhead irrigation received at each application timing. However, a single overhead irrigation event did not completely remove dicamba from the surface of the mulch, with 29.0 ppm m^{-2} remaining

at planting (data not reported). This concentration is equivalent to a 1/40 rate of dicamba. However, when applications were made 15 to 45 days ahead of plantings concentrations were significantly reduced to 1.1 ppm m^{-2} at most (data not reported). Cole crops and leafy greens have demonstrated high levels of tolerance to applications of dicamba to the foliage, with a 1/75 field rate or higher required to detect visual injury (Culpepper and Vance 2019). Therefore, theoretically, crop damage should be minimal, particularly at earlier application timings.

Broccoli results. All response variables for broccoli were not significantly impacted by experimental run and are combined for analysis and presentation. Visual injury estimates are reported from 21-33 DAP (days after planting), when injury was at its maximum. Generally, visual injury resulting from preplant applications of dicamba plus glyphosate over plastic much was higher when applications were made at earlier timings (Table A.1). When applications were made 45, 30, 15, and 1 DBP (days before planting), injury levels were 18, 17, 19, and 7%, respectively. This contradicts results from the analytical study, which demonstrated that the dicamba concentration present on plastic mulch at planting was higher when applied 1 DBP, and lower when applied at earlier timings (45, 30, and 15 DBP). Originally, it was hypothesized that the amount of time that dicamba remained on the mulch prior to a rainfall event could be the reason that it bound so tightly to the mulch and resulted in higher injury levels. A 1 d interval between application and irrigation when the application was made 1 DBP compared to intervals of 3 to 14, 1 to 12, and 19 to 28 d, across years, for applications 15, 30, and 45 DBP, respectively. However, there could be a multitude of other factors influencing these results.

Broccoli widths were evaluated 27 to 31 DAP, and broccoli grown in the nontreated plots were 33.6 cm wide (Table A.1). When mulch was treated 45, 30, and 15 DBP, broccoli widths were reduced 8 to 11% compared to the nontreated. Broccoli grown in plots where mulch was treated 1 DBP were similar to the nontreated (Table A.1). Fresh-weight biomass collected 26 to 35 DAP followed similar trends to widths. Broccoli grown in the nontreated weighed 65.6 g plant^{-1} (Table A.1) and was similar to biomass when mulch was washed 1 DBP; biomass was reduced 15 to 27% at earlier application timings (Table A.1).

Earliness of harvest is important in vegetable production as it can have a tremendous impact on fruit value (Culpepper et al. 2018). Herbicide injury has the potential to delay maturity in vegetable crops, which can lead to economic losses. In an attempt to quantify the potential delay in maturity from treatments, count and weight data from the first two harvests were summarized and are presented. For the first two harvests, broccoli in the nontreated yielded 30,751 heads ha^{-1} weighing 13,507 kg ha^{-1} (Table A.1). Applying dicamba 45 DBP resulted in a 70% reduction in head number and a 58% reduction in head weight compared to the nontreated. All other treatments yielded similar to the nontreated early in the season. Although yield was reduced in the early season, broccoli recovered and there were no differences in head number or weight for the entire season. Over the entire season, broccoli in the nontreated plots averaged 71,729 heads ha^{-1} weighing 21,623 kg ha^{-1}.

Collard results. All response variables for collard were not significantly impacted by experimental run and are combined for analysis and presentation. Visual injury estimates are reported from 21 to 33 DAP (days after planting), when injury was at its maximum.

Generally, visual injury trends were similar to that of broccoli, with greater injury when dicamba plus glyphosate was applied at earlier timings (Table A.2). When applications were made 45, 30, 15, and 1 DBP (days before planting), injury levels were 19, 18, 17, and 6%, respectively.

Collard widths were evaluated 27 to 31 DAP, and collard grown in the nontreated plots were 28.3 cm wide (Table A.2). Only dicamba plus glyphosate applied 30 DBP significantly reduced collard widths, with a 7% reduction noted. Fresh-weight biomass collected 26 to 35 DAP followed similar trends to collard widths. Collard grown in the nontreated weighed 59.5 g plant^{-1} (Table A.2). Biomass was reduced when dicamba was applied 45, 30, and 15 DBP, with 16 to 21% reductions in biomass noted. Dicamba applied 1 DBP and washed from the mulch did not reduced biomass compared to the nontreated (Table A.1).

With respect to early season collard yield, there were no differences in leaf number or weight at the first harvest (Table A.2). Collard grown in the control yielded 768,100 leaves ha^{-1} weighing 22,927 kg ha^{-1}. Similarly, no differences were noted with total collard yield with 2,300,000 leaves ha^{-1} weighing 66,091 kg ha^{-1} (Table A.2).

Conclusions. Herbicidal relationships with plastic mulch are extremely complex. Different herbicides behave differently on the surface of plastic mulch. In the case of glyphosate, a single irrigation or rainfall event of 1 cm can remove glyphosate from the surface of plastic mulch with minimal risk of injury to subsequently planted crops (Culpepper et al. 2009; Grey et al. 2009). Carfentrazone-ethyl binds so tightly to the mulch that it can’t be removed with rainfall, but it also doesn’t damage crops that are subsequently planted (Culpepper et al. 2009; Grey et al. 2009). When halosulfuron-

methyl is applied over mulch, it slowly releases over time, with time playing a bigger impact than rainfall (Culpepper et al. 2009; Grey et al. 2009; Grey et al. 2018; Randell et al. 2020). For this experiment, analytical results indicated that dicamba concentrations were higher at planting when applied 1 DBP and removed from mulch with overhead irrigation compared to earlier application timings, and bioassay studies indicated higher injury levels as a result of earlier application timing, contradictory to the analytical experiment. Currently, weed management programs for plasticulture produced vegetables should not include dicamba due to the risk of crop injury. Additional research is necessary to characterize the relationship between dicamba and plastic mulch prior to integration into current weed management practices.

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Table A.1. Broccoli response to dicamba plus glyphosate applied over plastic mulch prior to planting.[a]

				Harvests 1-2		Harvests 1-5
Application timing	Injury[c]	Widths[c]	Biomass[c]	Broccoli Heads[c]	Head weight[c]	Head weight[c]
DBP[b]	%	cm	g plant^{-1}	no. ha^{-1}	kg ha^{-1}	kg ha^{-1}
45	18 a	29.8 c	47.9 b	9,178 b	5,679 b	20,398 ns[b]
30	17 a	31.0 bc	55.8 b	19,539 a	9,997 ab	21,168
15	19 a	30.1 c	54.4 b	21,056 a	10,139 ab	21,788
1	7 b	32.2 ab	64.9 a	26,250 a	14,111 a	22,358
NTC[b]	0 c	33.6 a	65.6 a	30,751 a	13,507 a	21,623

[a]All data is averaged over three experimental runs.

[b]Abbreviations: DBP, days before planting; ns, not significant; NTC, nontreated control.

[c]Means were compared and adjusted using the Shaffer-simulated method ($\alpha = 0.05$).

Table A.2. Collard response to dicamba plus glyphosate applied over plastic mulch prior to planting.[a]

Application timing	Injury[c]	Widths[c]	Biomass[c]	First harvest		Harvests 1-3	
				Leaf number[c]	Leaf weight[c]	Leaf number[c]	Leaf weight[c]
DBP[b]	%	cm	g plant^{-1}	1,000 ha^{-1}	kg ha^{-1}	1,000 ha^{-1}	kg ha^{-1}
45	19 a	26.5 ab	47.0 b	712.5 ns[b]	20,132 ns[b]	2,201 ns[b]	61,674 ns[b]
30	18 a	26.2 b	49.4 b	716.7	20,488	2,188	62,227
15	17 a	26.4 ab	50.1 b	738.8	20,946	2,278	65,706
1	6 b	28.0 ab	55.9 a	739.4	22,242	2,303	70,937
NTC[b]	0 c	28.3 a	59.5 a	768.1	22,927	2,300	66,091

[a]All data is averaged over three experimental runs.

[b]Abbreviations: DBP, days before planting; ns, not significant; NTC, nontreated control.

[c]Means were compared and adjusted using the Shaffer-simulated method ($\alpha = 0.05$).

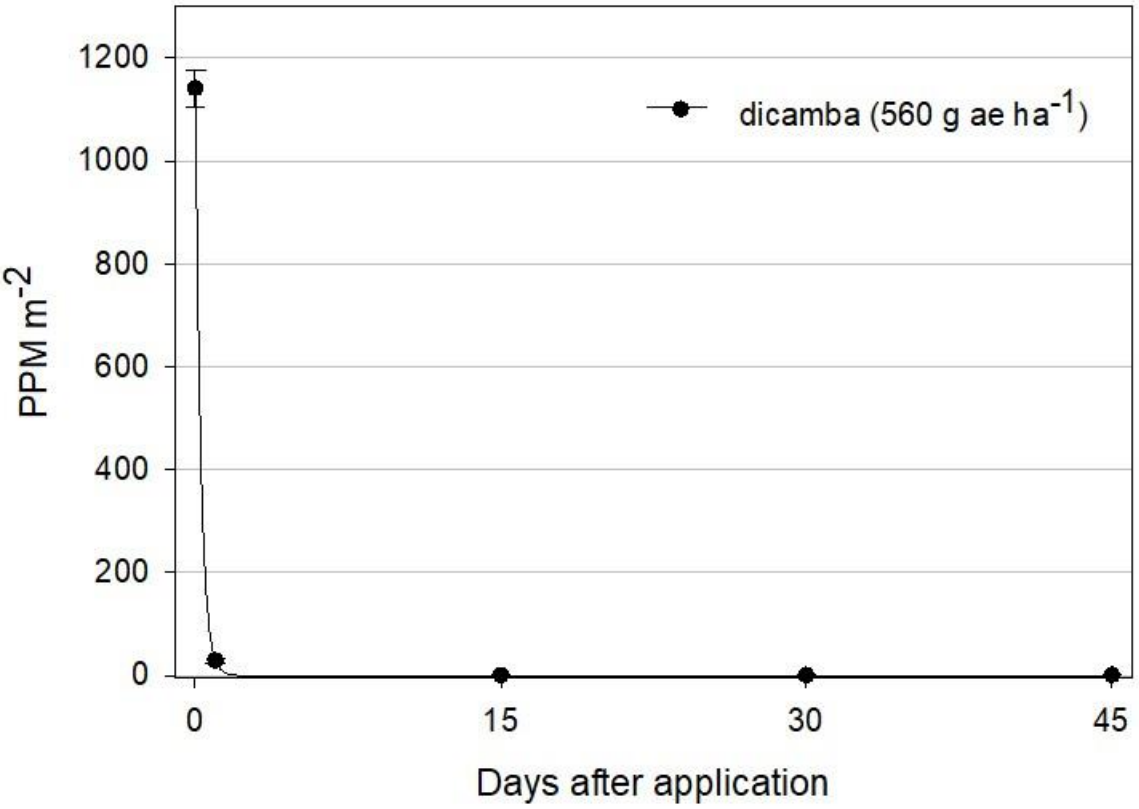

Figure A.1. Dicamba removal from the surface of plastic mulch in Georgia using the exponential decay equation ($y = B_0e^{-B1(x)}$). Nonlinear regression was applied for days after application. The regression line represents the first-order regression equation for each treatment. Data points are the means of replications with bars indicating the standard error of the mean:

Dicamba 560 g ha^{-1}: $y = 1{,}141.3e^{(-3.67x)}$ $P<0.0001$ $R^2 = 0.91$