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Integration of Cereal Cover Crops and Synthetic Auxin Herbicides into Rowcrop Production and Weed Management

Ryan James Edwards

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Integration of cereal cover crops and synthetic auxin herbicides into rowcrop production
and weed management

By

Ryan James Edwards

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Agriculture
in the Department of Plant and Soil Science

Mississippi State, Mississippi

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2015

Integration of cereal cover crops and synthetic auxin herbicides into rowcrop production
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The occurrence of herbicide resistance weeds across the southern United States has been increasing. Research is needed to develop alternative control measures, while supporting sound agronomic practices. Greenhouse and field studies were conducted to evaluate cereal cover cropping techniques along with novel herbicides to determine their value for Mississippi growers.

Field studies were performed to determine which combination of cereal cover crops (cereal rye, wheat and oats) and residual herbicides (*S*-metolachlor + metribuzin, *S*-metolachlor + fomesafen, pendimethalin, flumioxazin, sulfentrazone + metribuzin and pyroxasulfone + flumioxazin) would maximize soybean yield in the presence of weeds. Cereal cover crop termination methods were evaluated and a partial budget was generated to examine the total costs of growing soybeans utilizing cereal cover crops and residual herbicides. Residual herbicide applications averaged across all cereal cover crops controlled *Amaranthus* spp. greater than 89% by 28 DAT. Control by the cover crops alone was 67% for of *Amaranthus* spp. In all cereal species tested, cutting the cover crops

10 cm above the soil and leaving the residue reduced weed numbers compared to other termination methods. However, high production and implementation costs may prevent widespread adoption of cereal cover crops and residual herbicides in Mississippi.

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide currently labeled for non-crop use, but has characteristics which may make it useful as a preplant burndown (PPB) herbicide. The application of AMCP prior to planting of corn and cotton were evaluated and carryover effects to soybean were also evaluated. Tank mix combinations of AMCP with residual herbicides (rimsulfuron, flumioxazin, pyroxasulfone, pyroxasulfone+ flumioxazin and atrazine) were also evaluated. A rate titration of AMCP and its impacts on crop species were evaluated in the greenhouse. Corn showed tolerance to AMCP except at 0.28 kg ai ha⁻¹ applied prior to planting. Cotton was sensitive to AMCP as rate increased closer to the planting date, but response depended upon soil texture. AMCP impacts on soybean showed greater sensitivity (90% injury) than all other species evaluated. Due to potential impacts on soybean and cotton, AMCP is not a potential PPB for use in Mississippi.

DEDICATION

This work is dedicated to my wife Elizabeth. Her love, support and motivation are the only reason that this work was completed, at all. Thank you for everything that you have sacrificed to give me the opportunity to accomplish this. You are the love of my life and this work is as much yours as it is mine. I love you, forever and for always.

*Don't ever give up on what's in your heart
Don't ever let go of what it is you believe in
Don't ever say the road is too tough and that it's better to quit*

*Don't ever think that you don't matter; Because you're important to me
Don't ever let anyone tell you that you can't follow your dreams
Don't ever think that the world doesn't need someone like you;*

*You've touched my heart and my life just by being who you are and the things you do.
There's a magic in you that no other could possess.
A quality, a warmth, a sparkle that will carry you through life's tests*

*There's a magic in you
That makes you special to me.*

Anonymous

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CHAPTER I

INTRODUCTION

Use of transgenic crops in modern agricultural production has shifted farming practices across much of the United States (Shaner 2000). Chief among transgenic crops are those with resistance to glyphosate (Shaner 2000; Kleter et al. 2007; Green 2009; Givens et al. 2009; Duke 2008). Developed in 1996, glyphosate-resistant soybean [*Glycine max* (L.) merr] was the first crop commercialized by incorporating the resistant bacterial 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) enzyme into the plant genome (Dill 2005). By 2012, glyphosate-resistant soybean, corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) accounted for 93, 90 and 90% of the total hectares planted in the United States for those respective crops (USDA 2013).

Prior to the dependence on transgenic crops, growers relied heavily on a combination of preplant burndown (PPB), preemergence (PRE) and postemergence (POST) applications of herbicides (Givens et al. 2009). Herbicides such as 2,4-D, atrazine, acetachlor, chlorimuron, simazine, diuron, *S*-metolachlor, trifloxysulfuron, pyriithiobac and MSMA were commonly used in corn, cotton or soybean production (Givens et al. 2009). Of these herbicides, and their respective mechanisms of action (MOA), many agronomic weeds are now resistant. Prior to use of transgenic crops, common weeds in high abundance on southern United States farms included sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby], bermudagrass [*Cynodon dactylon* (L.)

Pers.], morningglories (*Ipomoea* spp.), nutsedges (*Cyperus* spp.) and the *Amaranthus* spp. (Webster and Coble 1997). Use of multiple herbicidal MOA, utilization of residual herbicides and use of cultural weed control (e.g. tillage, cover crops, etc) are all methods for combatting herbicide resistance (Beckie 2006). Use of residual herbicides is one of the most successful adoptions by growers to mitigate early season weed flushes (Norsworthy 2012). Drawbacks to residual applications, such as the need for adequate rainfall for activation, possible incorporation, compatibility with conservation tillage and grower preferences for POST applications, may all limit implementation (Shaner and Beckie 2013).

Once transgenic crop technologies were released, the simplification of weed control programs led to increased applications of glyphosate while other herbicides were reduced (Givens et al. 2009). Following glyphosate prevalence, the average number of different active ingredients used in soybeans fell from 11 in 1995 to only one in 2002 (Young 2006). Similar trends were also observed in cotton and corn production over the same period of time (Young 2006, Givens et al. 2009). From 1996 and 2006, the percent of hectares where PRE applications were made fell from 67 to 28% in soybean, 90 to 78% in cotton and 73 to 61% in corn (Norsworthy et al. 2012). A grower survey in six states (Illinois, Indiana, Mississippi, North Carolina, Iowa, and Nebraska) examined the prevalence of glyphosate in cropping systems (Givens et al. 2009). Of the responses, 85, 56 and 47% of soybean, corn and cotton growers made only glyphosate applications. On average, 35% of growers who planted cotton applied glyphosate more than three times during the season while 51% of soybean growers surveyed applied two and three applications of glyphosate per yr. The survey also examined PPB applications in which

76% of all growers were identified as making a PPB application. Of these PPB applications, growers indicated that glyphosate and 2,4-D were the most common herbicides used, with glyphosate applied four to six times more frequently than 2,4-D (Givens et al. 2009). Repetitive selection by a single MOA has influenced selection pressure resulting in weed shifts and led to the evolution of glyphosate-resistant weed populations (Beckie 2006; Norsworthy et al. 2012; Powles 2008).

Currently, there are 32 weed species reported as being EPSPS resistant in the United States (Heap 2015). Chief among these are the *Amaranthus* spp. with resistance to EPSPS, acetolactate synthase (ALS), dinitroaniline (DNA), triazine and protoporphyrinogen oxidase (PPO) herbicides (Culpepper et al. 2006; Wise et al. 2009; Steckel et al. 2008; Nandula et al. 2013; Nandula et al. 2014; Legleiter and Bradley 2008; Gaines et al. 2011; Sosnoskie et al. 2012). Prior to glyphosate resistance development, 10 *Amaranthus* spp. were common in mid-western and southern United States cotton and soybean fields (Horak and Loughin 2000). Webster and Coble (1997) performed a survey of the weed species composition in the southern United States from 1974 to 1995 and found that the distribution of *Amaranthus* spp. had dramatically increased over that time period. Possible reasons for this increase in *Amaranthus* spp. populations include the adoption of reduced tillage programs, reductions in diversified herbicide programs, adoption of glyphosate-resistant cropping systems, prevalence of glyphosate-resistance in other species altering management programs, prolific seed production, ease of dispersal for both seed and pollen and their high competitiveness with crop plants (Bensch 2003; Sosnoskie et al. 2011; Horak and Loughin 2000; Mayo et al. 1995; Price et al. 2011; Shaner 2000). Many of the *Amaranthus* spp. were easily controlled (>90%) with

applications of PPO herbicides acifluorfen, lactofen and ALS herbicides chlorimuron, thifensulfuron, imazethapyr and imazaquin (Mayo et al. 1995). While easily controlled, the high competitiveness between the *Amaranthus* spp. and crops for light, water, space and nutrients, coupled with their fast growth (0.21cm per growing degree day) (Rowland et al. 1999; Horak and Loughin 2000) and prolific seed production (upwards of 600,000 seeds per plant) (Keeley et al. 1987; Morgan 2001) have made the *Amaranthus* spp. the key pest of southern United States growers (Webster 2005). Bensch et al. (2003) examined the level of soybean yield loss due to three *Amaranthus* spp.; Palmer amaranth, common waterhemp (*Amaranthus rudis* Sauer) and redroot pigweed (*Amaranthus retroflexus* L.). At the highest density (8 plants m⁻¹), soybean yield losses were 79, 56 and 38% for Palmer amaranth, common waterhemp and redroot pigweed, respectively. Hager et al. (2002) examined common waterhemp influence on soybean in detail and concluded that a negative crop response of 43% reduced yields could occur due to common waterhemp. Similarly, Palmer amaranth densities of 0.33 to 10 plants m⁻¹ can reduce soybean yields by 17 to 68% (Klingaman and Oliver 1994). Rowland et al. (1999) found that as Palmer amaranth densities increased in cotton fields, there was a negative impact on lint yield of 6 to 12%. Morgan et al. (2001) showed that one to ten Palmer amaranth plants per 9.1 m⁻¹ of row length decreased cotton yields linearly from 13 to 54%. While these studies have examined low populations in the field, annual emergence of Palmer amaranth has been shown to reach 2000 plants m² (Norsworthy et al. 2008).

By the late 1990's, Palmer amaranth resistance to ALS chemistries was widely documented in many southern states (Bond et al. 2006; Wise et al. 2009). The first documented case of glyphosate-resistant Palmer amaranth occurred in Georgia in 2004

(Culpepper 2006). Quickly, glyphosate-resistant Palmer amaranth spread throughout most of the agricultural areas of Georgia and the entire southern United States (Culpepper et al. 2011; Norsworthy et al. 2008; Steckel et al. 2008). Glyphosate-resistance is not limited to Palmer amaranth, but has been observed in common waterhemp, tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer.] and spiny amaranth (*Amaranthus spinosus* L.) (Legleiter and Bradley 2008; Nandula et al. 2013; Nandula et al. 2014). Interspecific hybridizations between glyphosate-resistant Palmer amaranth and spiny amaranth and between Palmer amaranth and tall waterhemp are suspected in transferring resistance via pollen from Palmer amaranth to other closely related members of the *Amaranthus* genus (Nandula et al. 2013; Nandula et al. 2014; Gaines et al. 2011; Sosnoskie et al. 2012).

Changes in the perception towards the glyphosate dominated paradigm employed by growers in today's agriculture will be key to the management of glyphosate-resistant weed populations. Two areas of active research that need to be pursued are the further development of cereal cover crops with residual herbicide combinations that control *Amaranthus* spp. and other weeds and incorporation of new chemistries in crops.

Cover Crops

Price et al (2011) outlined nine cultural practices that could be implemented to combat glyphosate-resistant *Amaranthus* spp. One of the proposed cultural measures was the use of cover crops that have been shown to suppress Palmer amaranth germination and growth (Price et al. 2011). Palmer amaranth seeds are fairly short lived, typically 2 to 3 yrs in the soil and only germinate within the top 5 cm of soil (Sosnoskie et al. 2011). Moldboard plowing has been shown to decrease Palmer amaranth emergence 46 to 60%

when seeds were buried 30 cm (Price et al. 2011). Working on the same principle, cover crops create unfavorable environments by decreasing the light availability for weeds to germinate (Shaner and Breckie 2013; Norsworthy et al. 2012). Cereal rye residue has been shown to decrease weed density by 90% for light sensitive weed seeds such as common lambsquarters (*Chenopodium album* L.), redroot pigweed and several grasses compared to conventional tillage (Teasdale 1998). Agronomic benefits from cover crops include reduced yearly soil erosion/water runoff, increased water infiltration into the soil strata, increased soil moisture retention and increased organic matter and nitrogen fixation (Currie and Klocke 2005). Understanding the biology of the weeds present, especially the germination requirements, reproductive development, dispersal and soil seed bank persistence are all essential in choosing the best management strategy for controlling glyphosate-resistant weeds (Norsworthy et al. 2012).

Cover crops are planted with the mindset of replacing unmanageable weed populations with a manageable, low value crop (Teasdale 1998). Another definition of a cover crop is they are living ground covers that are commonly terminated prior to planting a higher value crop, with the intention of providing a benefit to the newer crop (Hartwig and Ulrich Ammon 2002; Teasdale 1998). Both definitions work on the natural principle that terrestrial ecosystems feature natural vegetation or plant residue on the surface at all times (Currie and Klocke 2005; Vencill et al. 2012). Cover crops are typically planted in the fall, grown over the winter and terminated through broadcast applications of herbicides in the spring (Davis 2010; Holshouser et al. 2009). Two common groups associated with cover crops are the cereal grains and legumes. Several common cereal crops used as covers include cereal rye (*Secale cereal* L.), wheat

(*Triticum aestivum* L.) or oat (*Avena sativa* L.) (Teasdale 1998; Williams et al. 1998). Legumes, such as hairy vetch (*Vicia sativa* L.) or clover (*Trifolium* spp.), are useful for cover cropping situations due to their nitrogen fixation. However, research has shown that legume cover crops, in particular vetch, are hard to control and may become a weed. Holshouser et al. (2009) planted a cover crop of cereal rye and vetch in the fall of 2007. The following spring, glyphosate was applied to terminate the cover crop; the cereal rye was controlled while the vetch continued to grow. Cereal crops are preferred over legume crops because they are inexpensive, easily controlled, create more soil stabilization, decrease erosion, are a more persistent mulch and provide better suppression of weeds than legume crops (Price et al. 2002). Termination of the cereal covers by paraquat or glyphosate typically occurs 2 wks prior to the recommended planting date of the cash crop (Price et al. 2002). Once cereal covers are terminated they are either rolled with a tractor mounted roller, roller-crimper, or other farm machinery or the covers are mowed to leave stubble and the crop is planted into the residue (Davis 2010). Soybeans following cereal rye or wheat are two of the more common cover cropping systems in the southeastern US (Price et al. 2006). This is due in part to the biology of the cereal crops which allows it to withstand harsh winter conditions, reach maturity within a limited growing season, not interfere with the planting of following crop, is easily desiccated and produces an abundant biomass for sufficient cover (Koger 2002; Price et al. 2006).

Previous research using cereal cover crops to target glyphosate-resistant Palmer amaranth indicated that successful suppression of the weed was primarily due to decreased light interception by the germinating seedlings (Burgos and Talbert 1996b;

Norsworthy et al. 2012; Holshouser et al. 2009; Price et al. 2002; Currie and Klocke 2005; Price et al. 2006; Kruidhof et al. 2009). Williams et al. (1998) found that Palmer amaranth percent cover could be inhibited by 71, 58 and 67% using cover crops of cereal rye, wheat and vetch, respectively. Biomass quantity is often attributed with being the deciding factor in successful control of *Amaranthus* spp. (Vencil et al. 2012; Price et al. 2011). A residue biomass of greater than 750 kg ha⁻¹ has been shown to reduce *Amaranthus* spp. population 38 to 89% and provide 3 to 5 wks of lower *Amaranthus* spp. infestation (Williams et al. 1998). This window of time will allow crop species the opportunity to gain a competitive advantage over *Amaranthus* spp. Another benefit that has been attributed to cover crops is the added potential for dispersion of allelochemicals as the residue break down (Putnam 1983; Putnam 1988). Both wheat and cereal rye residue has been shown to exude allelopathic chemicals that may control surrounding weed plants (Kruidhof et al. 2009). Wheat residue has been shown to exude ferulic acid, which may inhibit germination and root growth of pitted morningglory (*Ipomoea lacunosa* L.), common ragweed (*Ambrosia artemisiifolia* L.) and prickly sida (*Sida spinosa* L.) (Burgos and Talbert 1996b). Cereal rye allelochemicals include DIBOA (2,4-dihydroxy-1,4-(2H)-benzoxazin-3-one), BOA (2(3H)-benzoxazolinone, β -PLA (beta-phenyllacetic acid) and β -HBA (beta-hydroxybutyric acid) (Burgos and Talbert, 1996b, Chon and Kim 2004). Both DIBOA and BOA are the more common allelochemicals, with DIBOA linked to monocot inhibition and BOA linked to dicot inhibition.

Cover cropping systems alone cannot provide season long weed control (Currie and Klocke 2005; Price et al. 2006; Williams et al. 1998; Price et al. 2011; Reeves et al. 2005). Integration of cover crops with residual herbicide application is necessary. In a 3

yr study comparing black oat (*Avena stibosa* Schreb.) to a cereal rye cover, without herbicides, the oats controlled weeds on average 36% compared to 45% with cereal rye (Reeves et al. 2005). With the addition of herbicides (pendimethalin 1.12 kg ai ha⁻¹ + fluometuron 1.7 kg ai ha⁻¹ for low input or fluometuron 1.12 kg ai ha⁻¹ + DSMA 1.7 kg ai ha⁻¹ + lactofen 0.2 kg ai ha⁻¹ + cyanazine 0.84 kg ai ha⁻¹ for high input) average weed control ranged from 71 to 82% for black oat and 81 to 86% for cereal rye (Reeves et al. 2005). Gallagher et al. (2003) applied thifensulfuron at 4.4, 2.2 and 1.1 g ai ha⁻¹ in either one application or split applications of 2.2 or 1.1 g ai ha⁻¹ to a wheat cover crop followed by soybean. Soybean yields were not impacted by the herbicide applications and the only difference noted was either the presence or absence of the cover. Similarly, Burgos and Talbert (1996a) applied imazethapyr at two rates (0.035 and 0.07 kg ai ha⁻¹) to an oat cover crop and achieved 99% Palmer amaranth control. Koger et al. (2002) applied several combinations of PRE only, PRE+POST, POST only and no herbicides to a cereal rye cover to see if herbicide application would affect weed density. Average weed control without herbicides was between 24 and 83%, but average weed control with herbicides was 81 to 100%, with Palmer amaranth controlled 100% across herbicide combinations (Koger et al. 2002). Price et al. (2006) examined the impact of three cover crops (black oat, cereal rye and wheat) in conjunction with three herbicide applications (No herbicides, PRE with pendimethalin 0.84 kg ai ha⁻¹ + metribuzin 0.43 kg ai ha⁻¹ or the PRE/POST with pendimethalin 0.84 kg ai ha⁻¹ + metribuzin 0.39 kg ai ha⁻¹ + chlorimuron 0.06 kg ai ha⁻¹ PRE followed by 8.75 g ai ha⁻¹ chlorimuron POST) to assess the impact on Palmer amaranth. They found that when cover crops received no herbicide applications, weed control was 60% averaged across three cover crops. With herbicide

addition, weed control was 93% for the PRE followed by POST applications and 90% for PRE applications alone, averaged over three cover crops. When analyzed separately, the PRE followed by POST applications had the highest control at 93, 94 and 94% for black oat, cereal rye and wheat, respectively. Soybean yields were higher when herbicides were integrated with cover crops; 5748 kg ha⁻¹ and 5823 kg ha⁻¹ for the PRE followed by POST applications and PRE only applications, respectively, compared to 4479 kg ha⁻¹ for no herbicide applications (Price 2006). Price et al. (2002) applied flumioxazin (71 or 105 g ai ha⁻¹) as a preplant to a cereal rye cover crop for cotton weed control. These treatments were compared to applications of either glyphosate isopropylamine (1.12 kg ae ha⁻¹), glyphosate trimethylsulfonium (1.12 kg ae ha⁻¹) or paraquat (1.05 kg ai ha⁻¹) for Palmer amaranth control. All applications without flumioxazin controlled Palmer amaranth less than 50%, while addition of flumioxazin provided 96 to 100% Palmer amaranth control.

Aminocyclopyrachlor.

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide currently labeled in brush management, industrial rights of way, roadsides, bare-ground, rangelands, pastures and other non-crop associated environments (Bukun et al. 2010; Anonymous 2009; Senseman 2007; Turner et al. 2009). With AMCP being used for industrial rights of way, the thought process is that introduction of AMCP into PPB programs may hold potential. In the synthetic-auxin like herbicide MOA, AMCP is the only member of the pyrimidine carboxylic acid family (Anonymous 2009; Bukun et al. 2010; Senseman 2007). Field trials have confirmed that AMCP has a response pattern similar to many other synthetic auxin herbicides (Claus et al. 2008; Bukun et al. 2010). Structurally, AMCP is similar to

the pyridine carboxylic herbicides such as picloram, aminopyralid and clopyralid (Senseman 2007). However, the AMCP molecule differs in that it possesses an additional nitrogen in its heterocyclic carbon ring structure and includes a cyclopropyl side chain (Bukun et al. 2010).

Chemically, the free acid AMCP formulation (DPX-MAT28) has a pKa disassociation constant of 4.65, making it fairly phloem mobile. Based on previous research, AMCP translocates very rapidly to meristematic regions of the plant where it acts as an auxin mimic (Anonymous 2009). Volatility of AMCP free acid is negligible due to a vapor pressure of 4.89×10^{-6} Pa (Strachan et al. 2013). The log octanol-water partitioning coefficient ($\log K_{ow}$) of -2.48 and -1.12 at pH 7 and 4, indicate AMCP is relatively water soluble (Anonymous 2009; Bukun et al. 2010). Soil activity of AMCP has been shown to be up to 2 yrs and it can be actively absorbed by plant roots (Anonymous 2009; Lindenmayer et al. 2013; Westra et al. 2008a). Absorption is primarily carried out by roots and not through emerging shoots (Oliveira et al. 2013; Bell et al. 2011). Soil half-life of AMCP has been recorded in turf studies to be from 37 to 103 days and from 72 to 128 days in bare soil (Anonymous 2009; Conklin and Lym 2013). Soil mobility for AMCP has been reported it to be fairly mobile, similar to other water soluble weak acid herbicides (Cabera et al. 2012). Soil sorption of AMCP is primarily influenced by soil organic carbon and clay content instead of by soil pH (Oliveira et al. 2011). Due to a low pKa value of 4.65 AMCP is weakly bound to soil. Once bound to soil, desorption potential of AMCP is low, indicating that once sorbed to soil it is irreversibly bound (Oliveira et al. 2011). Primary routes of AMCP

decomposition include soil microbes and photolysis (Anonymous 2009; Lewis et al. 2013; Lindenmayer et al. 2009; Oliveira et al. 2013).

Westra et al. (2008b) examined soil treated the previous yr with several rates of DPX KJM-44 (AMCP methyl ester) for crop response of several agronomic crops; corn, wheat, sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.) and soybean. Corn and sunflower exhibited tolerance to AMCP, while wheat, alfalfa and soybean showed less tolerance. Several other field studies have corroborated that wheat, cotton, alfalfa and soybean are highly sensitive to AMCP (Kniss and Lyon 2011; Strachan et al. 2011; Flessner et al. 2012). Soil concentrations of 2.0, 3.2, 5.4, and 6.2 ppb AMCP caused a 25% phytotoxicity response to soybean, cotton, alfalfa and sunflower (Strachan et al. 2011). Monocot crops, such as corn, have shown a greater tolerance to AMCP (64 g ai ha⁻¹) compared to broadleaf crops (3.3g ai ha⁻¹ AMCP for cotton and 2.2 g ai ha⁻¹ AMCP for soybean) (Strachan et al. 2011).

If AMCP could be used in crop weed management, it would serve as another tool for tank mix partners to increase the number of MOAs used and prevent glyphosate-resistance from occurring faster. Introduction of the synthetic auxins into PPB applications will serve as yet another tool for managing glyphosate-resistant weeds.

Objectives.

The encompassing objectives of this research are two fold; 1) to evaluate cereal cover crops coupled with residual herbicides for managing *Amaranthus* spp. and other aggressive weeds in Mississippi and 2) to evaluate alternative residual herbicides in PPB applications for weed efficacy and crop safety.

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CHAPTER II

THE EFFECT OF FALL SEEDED CEREAL COVER CROPS FOR CONTROL OF AMARANTHUS SPP. IN MISSISSIPPI

Abstract

Field trials were conducted to determine which combination of cereal cover crops (cereal rye, wheat and oat) and residual herbicides (*S*-metolachlor + metribuzin, *S*-metolachlor + fomesafen, pendimethalin, flumioxazin, sulfentrazone + metribuzin and pyroxasulfone + flumioxazin) would maximize soybean production through increased *Amaranthus* spp. control. Cereal cover crop termination methods were examined to determine effects on total weed populations. Cost associated with implementing a cereal cover crop with residual herbicides were compared to a glyphosate-based weed management program by calculating a partial budget based on costs (either direct or derived) from our studies. Results 28 DAT showed all herbicides averaged across all cereal cover crops controlled Palmer amaranth, spiny amaranth and tall waterhemp > 89%. Control of all *Amaranthus* spp. by cereal cover crops alone was 67% 28 DAT. No impacts on soybean yields were observed. Soybean heights were reduced 9% 21 DAT by oat averaged across all herbicide treatments. After 21 DAT, no soybean height reductions were observed. Use of different cereal cover crop termination methods showed differences in total weed populations 28 and 56 DAT. In all cereal species tested, cutting the cover crops 10 cm above the soil and leaving the residue reduced weed numbers. The

partial budget calculations showed high production and implementation costs (\$711.55 to \$886.72 ha⁻¹) for implementing cereal cover crops compared to glyphosate-based system. Subtracting the cost of implementing cereal cover crops from the soybean income showed varying profits across all combinations. Without residual herbicides, percent difference in costs by cereal cover crops alone were 57% (cereal rye), 54% (wheat) and 62% (oat). Percent difference in cost between cereal cover crops with residual herbicides ranged from 41(oat with sulfentrazone + metribuzin) to 102% (cereal rye with S-metolachlor + metribuzin).

Nomenclature: flumioxazin; fomesafen; metribuzin; pendimethalin;

pyroxasulfone; metolachlor; sulfentrazone; Palmer amaranth, *Amaranthus palmeri* S. Wats.; common waterhemp, *Amaranthus rudis* Sauer; Spiny amaranth, *Amaranthus spinosus* L.; oat, *Avena sativa* L.; soybean, *Glycine max* (L.) Meer.; cereal rye, *Secale cereale* L.; wheat, *Triticum aestivum* L.

Keywords: Cover crop, residual herbicides, partial budget, integrated weed management

Introduction

Across the southern United States, *Amaranthus* spp. populations have dramatically increased (Webster and Coble 1997). Possible reasons for this increase include agronomic paradigm adoptions of reduced tillage across the southern United States, reductions in diversified herbicide programs and wide-scale adoption of glyphosate-resistant cropping systems (Duke and Powles 2007). Physiologically, the *Amaranthus* spp. are highly competitive with crop plants through fast growth rates (0.21cm per growing degree day), prolific seed production, high numbers of annual

emergence (2000 plants m²) and ease of hybridization (Webster 2005; Bensch 2003; Sosnoskie et al. 2011; Horak and Loughin 2000; Mayo et al. 1995; Price et al. 2011; Shaner 2000; Rowland et al. 1999; Keeley et al. 1987; Morgan 2001; Norsworthy et al. 2008). All of these factors make the *Amaranthus* spp. the key agronomic weed of southern United States growers. Soybean yield losses range from 38 to 79% when populations reach 10 plants m⁻¹ depending upon the *Amaranthus* spp. present (Bensch et al. 2003; Hager et al. 2002; Klingaman and Oliver 1994).

Increased occurrence of herbicide-resistant *Amaranthus* spp. across the southern United States is of great concern (Culpepper et al. 2006; Wise et al. 2009; Steckel et al. 2008; Nandula et al. 2013; Nandula et al. 2014; Legleiter and Bradley 2008; Gaines et al. 2011; Sosnoskie et al. 2012). By the late 1990's, Palmer amaranth resistance to acetolactate synthesis (ALS) chemistries was widely documented across the southern United States (Bond et al. 2006; Wise et al. 2009). The first documented case of glyphosate-resistant Palmer amaranth occurred in Georgia in 2004 and quickly spread to surrounding states (Culpepper 2006, Culpepper et al. 2011; Norsworthy et al. 2008; Steckel et al. 2008). Interspecific pollen-mediated hybridizations transfer resistance between glyphosate-resistant Palmer amaranth to tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] and Palmer amaranth to spiny amaranth (Legleiter and Bradley 2008; Nandula et al. 2013; Nandula et al. 2014; Gaines et al. 2011).

Understanding the biology, germination, reproduction, dispersal and soil seed bank persistence are all essential in choosing the best management strategy for controlling glyphosate-resistant weeds (Norsworthy et al. 2012). To combat the increasing trend of herbicide resistance, multiple control measures have been proposed.

The adoption of cover crops has been shown to suppress Palmer amaranth germination and growth (Price et al. 2011). Cover crops are often planted with the mind set of replacing unmanageable weed populations with a manageable, low value crop (Teasdale 1998; Hartwig and Ulrich Ammon 2002). Cover crops work on the natural principle that many terrestrial ecosystems feature natural vegetation or plant residue on the soil surface at all times (Currie and Klocke 2005; Vencill et al. 2012). Agronomic benefits from cover crops include reduced soil erosion, increased water infiltration and increased organic matter incorporation into the soil (Currie and Klocke 2005). Previous research using cover crops to target glyphosate-resistant Palmer amaranth, indicate that successful weed suppression was primarily due to decreased light interception by germinating seedlings (Burgos and Talbert 1996b; Norsworthy, 2004; Holshouser et al. 2009; Price et al. 2002; Currie and Klocke 2005; Price et al. 2006; Kruidhof et al. 2009; Price et al. 2011; Shaner and Breckie 2013; Norsworthy et al. 2012; Teasdale 1998; Vencill et al. 2012; Williams et al. 1998). Two common groups associated with cover crops are legumes and cereal grains. Several common cereal crops used as covers include cereal rye, wheat and oat (Teasdale 1998; Williams et al. 1998). Legumes, such as hairy vetch (*Vicia sativa* L.) or clover (*Trifolium* spp), are beneficial as cover crops due to their nitrogen fixation. However, research has shown that legume cover crops, in particular vetch, are hard to control and may become a weed. Holshouser et al. (2009) planted a cover crop of cereal rye and vetch in the fall of 2007. The following spring, glyphosate was applied to control the cover crops; cereal rye was controlled, however the vetch continued to grow. Cereal crops are also preferred over legume crops because they are

easily controlled, provide more soil stabilization, decreased erosion, more persistent mulch and provide better weed suppression than the legume crops (Price et al. 2002).

Cereal cover crops are planted in the fall and terminated prior to seed set through broadcast applications of non-selective herbicides two wks prior to the recommended planting date of the following crop (Davis 2010; Holshouser et al. 2009; Price et al. 2002). Once cereal covers are terminated they are rolled with farm machinery and the following crop is planted into the residue (Davis 2010; Price et al. 2006; Koger 2002). Biomass quantity is often attributed with being the deciding factor in successful control of *Amaranthus* spp. (Vencil et al. 2012; Price et al. 2011; Williams et al. 1998). A soybean crop following either a cereal rye or wheat crop are two of the more common cover cropping systems in the southeastern US (Price et al. 2006). This is due to the biology of these cereal crops which withstand harsh winter conditions, reach maturity, are easily desiccated and produce an abundant biomass for sufficient cover (Koger 2002; Price et al. 2006).

However, cover cropping systems alone cannot fully provide season-long weed control (Currie and Klocke 2005; Price et al. 2006; Williams et al. 1998; Price et al. 2011; Reeves et al. 2005). Integration of cover crops with residual herbicide application is necessary. In a 3 yr study comparing black oat (*Avena stigosa* Schreb.) to a cereal rye cover without herbicides, black oat controlled weeds on average 36% compared to 45% with cereal rye (Reeves et al. 2005). With the addition of herbicides (pendimethalin 1.12 kg ai ha⁻¹ + fluometuron 1.7 kg ai ha⁻¹ for low input or fluometuron 1.12 kg ai ha⁻¹ + DSMA 1.7 kg ai ha⁻¹ + lactofen 0.2 kg ai ha⁻¹ + cyanazine 0.84 kg ai ha⁻¹ for high input) average weed control ranged from 71 to 82% for black oat and 81 to 86% for cereal rye

(Reeves et al. 2005). Gallagher et al. (2003) applied thifensulfuron at 4.4, 2.2 and 1.1 g ai ha⁻¹ in either one application or split applications of 2.2 or 1.1 g ai ha⁻¹ to a wheat cover crop for soybean. Soybean grain yields were not impacted by herbicide applications and the only difference noted was either the presence or absence of the cover. Similarly, Burgos and Talbert (1996a) applied imazethapyr at two rates (0.035 and 0.07 kg ai ha⁻¹) to an oat cover crop and achieved 99% control of Palmer amaranth. Koger et al. (2002) applied several combinations of PRE-only, PRE+POST, POST-only and no herbicides to a cereal rye cover to see if herbicide application would affect weed density. Average weed control without herbicides was from 24 to 83% while addition of herbicides increased control to 81 to 100%, with Palmer amaranth controlled 100% across herbicide combinations (Koger et al. 2002). Price et al. (2006) examined the impact of three different cover crops (black oat, cereal rye and wheat) in conjunction with three herbicide applications (none, PRE only and PRE followed by POST) to assess the impact on Palmer amaranth. Results showed that when cover crops received no herbicide applications, weed control was 60% averaged across the three cover crops. With herbicides (PRE with pendimethalin 0.84 kg ai ha⁻¹ + metribuzin 0.43 kg ai ha⁻¹ or the PRE/POST with pendimethalin 0.84 kg ai ha⁻¹ + metribuzin 0.39 kg ai ha⁻¹ followed by chlorimuron at 8.75 g ai ha⁻¹) average weed control was 93% for the PRE followed by POST and 90% for the PRE only applications, averaged over the three cover crops. When analyzed separately, the PRE followed by POST applications had the highest control at 93, 94 and 94% for black oat, cereal rye and wheat, respectively. Soybean yields were higher when herbicides were integrated with the cover crops; 5748 kg ha⁻¹ and 5823 kg ha⁻¹ for the PRE followed by POST and PRE only applications, respectively,

compared to 4479 kg ha⁻¹ for no herbicide applications (Price 2006). Price et al. (2002) applied flumioxazin (71 or 105 g ai ha⁻¹) as a PRE to a rye cover crop for cotton weed control. These treatments were compared to applications of either glyphosate isopropylamine (1.12 kg ae ha⁻¹), glyphosate trimethylsulfonium (1.12 kg ae ha⁻¹) or paraquat (1.05 kg ai ha⁻¹) for Palmer amaranth control. All applications without flumioxazin controlled Palmer amaranth less than 50%, while addition of flumioxazin provided 96 to 100% control of Palmer amaranth.

With glyphosate-resistance management, the integration of new control techniques is vital for successful sustained soybean production. Coupling weed management in an integrated fashion by using different cereal cover crop varieties and residual herbicides is an area of research that must be addressed. Previous studies have shown the utilization of residual herbicides with cereal cover crops are an effective tool for managing *Amaranthus* spp. If successful, the use of cereal cover crops in Mississippi for the control of *Amaranthus* spp. would be a benefit to growers and provide a sustainable farm practice. The objectives of this research were three fold; 1) examine implementing cereal cover crops (cereal rye, common wheat and common oat) and residual herbicides (*S*-metolachlor + metribuzin, *S*-metolachlor + fomesafen, pendimethalin, flumioxazin, sulfentrazone + metribuzin and pyroxasulfone + flumioxazin) usage in Mississippi to combat growing *Amaranthus* spp. populations, 2) compare cereal cover crop termination methods to examine the impacts of stubble presence on weed populations and 3) conduct a cost analysis to determine a partial budget to examine the total costs of utilizing cereal cover crops compared to a glyphosate-based weed management program.

Methods and Materials

Cereal cover crops and residual herbicides.

Treatments were arranged as a 4 x 6 two factor factorial in a randomized complete block design with four replications. Factor A consisted of the four cover types; no cover, wheat, oat and cereal rye. Factor B consisted of six herbicide programs labeled in soybean and included no herbicide. Treatments included pendamethalin, *S*-metolachlor + metribuzin, *S*-metolachlor + fomesafen, flumioxazin, flumioxazin + pyroxasulfone, and sulfentrazone + metribuzin (product formulations and sources listed in Table 2.2).

Field trials were initiated in 2013 and 2014 at the Mississippi Agricultural & Forestry Black Belt Experiment Station near Brooksville, MS (33.15° N by 88.33° W), the Mississippi Agricultural & Forestry R.R. Foil Plant Science Research Center near Starkville, MS (33.28° N by 88.46° W) in 2014 and two off station locations located near Eupora, MS (33.30° N by 89.16° W) and Louisville, MS (33.07° N by 89.07° W) in 2014. At the Brooksville location, trials were conducted on a non-irrigated Brooksville silty clay (Fine, smectitic, thermic Aquic Hapluderts). At the Starkville location, trials were conducted on furrow-irrigated Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquepts (all soils listed in Table 2.1). At the Eupora location, trials were conducted on a non-irrigated Oaklimer silt loam (Coarse-silty, mixed, active, thermic Fluvaquentic Dystrudepts). At the Louisville location, trials were conducted on a non-irrigated Savannah fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Fragiudults).

All cover crops were drilled in randomized alternating strips in late fall using a 1.8 m no-till seed drill at a rate of 120 kg ha⁻¹. Ammonium sulfate fertilizer (21-0-0) at

101 kg nitrogen ha⁻¹ was applied during the spring before Feekes 6 using a pulled fertilizer spreader (Large 2007). Cereals were desiccated with a broadcast spray application of 1.26 kg ae ha⁻¹ glyphosate prior to Feekes 11. Cover crops were allowed to desiccate for approximately 2 wks prior to rolling using a tractor pulled water filled steel roller used for packing rows. In 2013, cover crops were desiccated after anthesis due to waterlogged soil conditions and had to be rolled twice due to the late timing of desiccation spray. All covers were allowed a 3 day period following rolling for covers to settle on the soil surface. Soybeans were planted using a vacuum planter at a population of 339,768 seeds ha⁻¹. In 2013, Pioneer soybean 95Y70 (DuPont, Wilmington DE) was planted, while in 2014 a 95Y31 Pioneer soybean (DuPont, Wilmington DE) was planted at the Euproa and Brooksville sites and 5332 Asgrow (Monsanto, St. Louis, MO) was planted at the Louisville and Starkville sites. Plot dimensions varied between sites and were either 3.8 m x 12.12 m or 1.9 m x 9 m and set up on beds with 0.95 m centers. All residual herbicides were applied using a pressurized CO₂ powered backpack sprayer delivering 140 L ha⁻¹ at 4.68 km hr⁻¹ with four nozzles (TeeJet AIXR 11002, Spraying Systems Co., Glendale Heights, IL).

Visual weed control estimates (0 to 100%, where 100% was plant mortality) were collected 14 and 28 days after treatments (DAT). For each of our sites, key *Amaranthus* spp. targeted included Palmer amaranth, spiny amaranth and common waterhemp (Table 2.3). At the Starkville site, only common waterhemp was found, while the Brooksville site did not have any endemic populations, thus Palmer amaranth seeds were broadcast spread prior to cover crop desiccation. Both the Louisville and Eupora sites had native populations of Palmer amaranth with low levels of glyphosate-resistance (< 20%) and

only the Eupora site had spiny amaranth. Several other weeds were common in all our experiments included pitted morningglory (*Ipomoea lacunosa* L.), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), horsenettle (*Solanum carolinense* L.) and prostrate spurge (*Chamaesyce humistrata* (Engelm.ex Gray) Small). Following the last weed rating, glyphosate was applied at 1.26 kg ae ha⁻¹ as needed to control weeds within the plots. Cereal crop hts (cm) were collected after desiccation and biomass of cereals was collected using a 1 m² quadrat, cutting the plants at the soil surface. Soybean data collections were limited in 2014 at both the Starkville and Eupora sites and only weed control was collected. Soybean percent injury data (0 to 100%, where 100% is plant mortality) were collected 14 and 28 DAT. Soybean hts (cm) were collected 21, 45 and 86 DAT in Brooksville (2013 and 2014) and Louisville (2014). Soybean yields (kg ha⁻¹) were collected at both Brooksville and Louisville using a two row small plot combine.

Cereal cover crop termination.

Treatments were arranged as a 4 x 4 two factor factorial in a randomized complete block design with four replications. Factor A consisted of the four cover types of no cover, wheat, oat and cereal rye. Factor B consisted of four termination techniques and included no termination as well. Termination techniques examined included rolling, mowing, a stubble termination and a clean-bed termination. For rolling, covers were rolled using the same tractor pulled roller in the previous experiment. The mowing termination consisted of cutting the cereal stems approximately 10 cm above the soil surface and leaving the residue where it fell. The stubble termination consisted of cutting the cereal stems 25 cm above the soil and removing all residue thus leaving a tall stubble

in the field. The clean-bed termination consisted of cutting the cereal stems 10 cm above the soil and removing all residue, leaving only the short stubble.

A field trial was initiated at the Mississippi Agricultural & Forestry Black Belt Experiment Station near Brooksville, MS in 2014. Plots were 1.9 m x 9 m and set up on beds with 0.95 m centers. All cereal cover crop establishment parameters were the same as the previous study. Once all covers had dried following glyphosate desiccation spray, plots with the mowing, stubble or clean-bed terminations were performed. Following cuttings, remaining plots were rolled. No herbicides were applied for the duration of the experiment to assess the direct impacts of cereal cover terminations. Weed population stand counts (number of stems per 0.5 m²) were collected 21 and 56 DAT using a 0.5m² quadrat placed at three random points along the two center rows of the plot. All weeds present [smell melon (*Cucumis melo* L.), yellow nutsedge (*Cyperus esculentus* L.), barnyardgrass, prostrate spurge, horsenettle] were counted within the quadrat. Weed population data were pooled for each data collection to assess average weed pressure per termination method.

Data analysis.

All data were analyzed in SAS 9.3 using the PROC GLIMMIX procedure (SAS Institute Inc., Cary, NC). For the cereal cover crop and residual herbicide experiment, all weed control data were transformed using an arcsine square root function. To account for the different species at each of our sites (Table 2.3), *Amaranthus* spp. were pooled across sites. Analysis by ANOVA showed pooling was acceptable and Palmer amaranth, tall waterhemp and spiny amaranth visual control data are a compilation of *Amaranthus* spp. Transformed weed control data were subjected to ANOVA and means were separated by

LSMEANS ($\alpha=0.05$) and presented in the original scale (Table 2.4, 2.5 and 2.6). Soybean ht data were transformed using percent from the untreated, pooled across sites and yrs and subjected to ANOVA. Soybean hts collected 21 DAT showed NS interaction, but were significant for the main effect of cover type and means were separated by LSMEANS ($\alpha=0.05$) (Table 2.6). For the cereal cover crop termination experiment, weed population data 21 and 56 DAT were subjected to ANOVA and means were separated by LSMEANS ($\alpha=0.05$) (Table 2.7).

Partial budget.

A cover crop implementation partial budget was generated to assess the economic costs of installing cover crops in comparison to a glyphosate-based weed management program (Table 2.8). Values used were generated with assistance from Mississippi State agricultural economists using the 2015 Mississippi Soybean Planning Budget (Anonymous 2014), local vendors, co-ops and results from our field experiments. Herbicides used in the budget reflect the herbicides used for direct comparison with our results (Table 2.2). All label recommendations on rates and surfactant additions were followed and included in the budget. Implement costs were derived directly from the 2015 planning budget and are comprised of average labor costs, fuel cost, repair and maintenance costs, average fuel consumption and the direct cost of owning the piece of machinery to determine the total cost (\$ ha⁻¹) (Anonymous 2014). The number of POST applications of glyphosate were determined from empirical number of average sprays conducted in our trials across all sites and yrs and compared to the average number of POST sprays conducted by growers (Givens et al. 2009). For each combination of residual herbicides and cereal cover crops and each residual herbicide application in

glyphosate program, all costs associated were tabulated (Table 2.8). Total cost was tabulated for four categories associated with cereal cover crop implementation; 1) cover crop selection, 2) broadcast applications of herbicides, 3) residual herbicide selection and 4) miscellaneous costs (e.g. planting, fertilizer, spraying, etc.) (Table 2.8). A current commodity price of soybean used in the budget generation was \$10.42 bu⁻¹ (indexmundi.com). Harvest yields from our experiments for each treatment combination were used to calculate income (\$ ha⁻¹) using the current market price of soybean (Table 2.9). Using the calculated income and subtracting the costs from Table 2.8, profit (\$ ha⁻¹) was calculated for all treatment combinations and no residual herbicide combinations (cereal cover crop only). All cereal cover crops and residual combinations were compared to the glyphosate-based system and percent difference was calculated (Table 2.9).

Results and Discussion

Cereal cover crop and residual herbicides.

Our results were similar to earlier studies for *Amaranthus* spp. control with cereal cover crops and residual herbicides (Teasdale 1998; Putnam et al. 1983; Currie and Klocke 2005; Price et al. 2006; Williams et al. 1998; Price et al. 2011; Reeves et al. 2005). Control of *Amaranthus* spp. 14 DAT was 96 to 100% across all treatment combinations except for flumioxazin + pyroxasulfone (79%) with no cover (Table 2.4). There was no interaction or main effect of cover selection 28 DAT; however herbicide treatment main effect was significant and data were averaged across cereal cover crops. Control across all herbicide treatments ranged from 89% (*S*-metolachlor + metribuzin or pendimethalin alone) to 96% (sulfentrazone + metribuzin), while control by the cover

crops alone averaged 67% (Table 2.5). The need for continual monitoring of any weed escapes will be necessary based upon our results to ensure all *Amaranthus* spp. are controlled and do not set seed. *Amaranthus* spp. populations on each of our sites offered light to moderate weed pressure (<10 plants m^2). With populations reported to exceed 2000 plants m^2 (Norsworthy et al. 2008), our study shows that smaller populations of *Amaranthus* spp. can be effectively controlled with cereal cover crops and residual herbicides. As populations of *Amaranthus* spp. increase, it is likely that the benefit of cover crops would be economically more attractive as herbicide inputs would have to be higher without their use.

Control of pitted morningglory 14 DAT ranged from 93 to 100% across all treatment combinations (Table 2.4). Pitted morningglory control 28 DAT showed no significant interaction or main effects and the data are not shown. Control of pitted morningglory 28 DAT ranged from 97 to 100% across all cereal cover crops and residual combinations. Barnyardgrass control showed no significant interaction but was significant for both main effects of herbicide treatment and cereal cover. For residual herbicide main effect, control 14 DAT ranged from 90 to 100% across all herbicide applications averaged across all cereal cover crops and was significantly different than the no herbicide control (69%) (Table 2.5). By 28 DAT, barnyardgrass control ranged from 86 to 91% (Table 2.5). Flumioxazin + pyroxasulfone provided 91% control as compared to 86% for pendimethalin or *S*-metolachlor + metribuzin. All herbicide combinations provided greater barnyardgrass control than the 61% provided by the cover crops (Table 2.5). For cover selection averaged over herbicides, all covers provided greater barnyardgrass control (87 to 98%) than the no cover (72 to 73%) at both 14 and

28 DAT. Cereal rye provided the best barnyardgrass control 14 DAT and cereal rye or wheat were the best by 28 DAT (Table 2.6). Prostrate spurge (14 DAT) showed no significant interaction or main effects and the data are not shown. Prostrate spurge control 14 DAT ranged from 95 to 100% across all cereal cover crops and residual combinations. By 28 DAT, prostrate spurge control across all herbicide treatments was 89 to 97% (Table 2.5). Horsenettle (14 DAT) showed no significant interaction or main effects and the data are not shown. Horsenettle, due to its perennial nature, was harder to control and showed 44 to 53% average control by 14 DAT. By 28 DAT, horsenettle control across all herbicide treatments was 58 to 61% (Table 2.5).

Part of our studies were to evaluate the effect that the cereal cover crop itself may have on soybean growth and development. Cereal hts and cereal biomass showed NS interaction or main effects and the data are not shown. Once the wheat cover had reached Feekes 11, stem hts were 1.24 m and similar to cereal rye (1.7m) and oat (1.49 m). Average biomass production across all sites in our study were similar with cereal rye (12564.81 kg ha⁻¹) producing more biomass then wheat (11195.89 kg ha⁻¹) and oat (9875 kg ha⁻¹). Quantity of cereal biomass has been attributed as being the deciding factor in successful control of *Amaranthus* spp. (Vencil et al. 2012; Price et al. 2011). Proper establishment of the cover crop is essential in creating a barrier to prevent sunlight from reaching the soil surface (Teasdale 1998). Soybean heights showed a 9% ht reduction for an oat cover compared to 1% for cereal rye and 3% for a wheat cover 21 DAT (Table 2.6). After 21 DAT, soybean heights were unaffected by cereal cover types averaged across residual herbicides and support other findings that the presence of cereal covers does not impact soybean development (Williams et al. 1998; Price et al. 2006). Soybean

yield responses to cereal cover crops vary in the literature. Price et al. (2006) showed that cereal cover crops can increase soybean yields (5913 to 6249 kg ha⁻¹) compared to non-cover soybean yields (4031 kg ha⁻¹). However, Williams et al. (1998) showed that both a cereal rye and wheat cover crop did not impact soybean yields. Our results support the findings of Williams et al. (1998) and showed that cereal cover crops and residual herbicides have no impact on soybean yields (Table 2.4).

For each cover, the way the stems laid over greatly influenced the prolonged weed control offered by the cover, when not in conjunction with a residual herbicide. A cereal rye cover created a more complex mat of stems that were more tightly interconnected and overlapped compared to wheat and oat. Due to their thicker stems, wheat and oat plants tended to lay over in the direction the roller travelled and did not overlap horizontally like cereal rye. Gaps in the cover were observed more frequently in both wheat and oat, exposing the soil surface and opening a potential area for weed seeds to establish or germinate from the soil seed bank. For oat, since the stems were thicker, there was more potential for them to retain moisture and not lay flat when rolled. The rolling pattern of oats may also explain the early season decreases in soybean hts that were observed. Another possible explanation could be the presence of residual levels of allelochemicals exuded by the oat roots impacting the root growth and development of these soybeans. Another benefit that has been attributed to cover crops is the added potential for dispersion of allelopathic chemicals. Both wheat and cereal rye residue has been shown to exude these compounds that may control surrounding weedy plants (Kruidhof et al. 2009). Wheat residue has been shown to exude ferulic acid, which may inhibit germination and root growth of pitted morningglory, common ragweed (*Ambrosia*

artemisiifolia L.) and prickley sida (*Sida spinosa* L.) (Burgos and Talbert 1996b). Cereal rye allelochemicals include DIBOA (2,4-dihydroxy-1,4-(2H)-benzoxazin-3-one), BOA (2(3H)-benzoxazolinone, β -PLA (beta-phenyllacetic acid) and β -HBA (beta-hydroxybutyric acid) (Burgos and Talbert, 1996b). Both DIBOA and BOA are the more common allelochemicals, with DIBOA linked to monocot inhibition and BOA linked to dicot inhibition. These compounds have been shown to inhibit root growth of many crop species, but are very short lived in soils and degrade easily (Putnam 1988).

Cereal cover crop termination

Cover crops are traditionally rolled to bend the desiccated stems over the soil surface to create a mat of vegetation that prevents the transmittance of light (Teasdale 1998; Price et al. 2006). Our results show different cover crop terminations other than rolling may be a potential. By 28 DAT, wheat with the clean termination (cut low and remove biomass) average weed populations were similar (27.3 plants per 0.5 m²) to the non-cover control (25.3 plants per 0.5 m²). Weed populations under the cereal rye and rolled termination (13 plants per 0.5 m²) were different from the check but not different from wheat with the clean termination. Weed populations were lower than rye with rolling termination in the rye with mowing termination (2 plants per 0.5 m²), wheat with mowing termination (0.3 plants per 0.5 m²), and oat with all terminations except stubble termination (1 plant in clean, 2.3 plants in mowed and 2.3 plants per 0.5 m² in the stubble terminations). By 56 DAT, wheat with the clean termination (22 plants per 0.5 m²) were similar to the non-cover control (28.7 plants per 0.5 m²) (Table 2.7) and cereal rye with rolled termination (18.7 plants per 0.5 m²). Cereal rye with rolled termination was different then the non-cover control and higher significantly then all other treatment

combinations (Table 2.7). However, practical means of cutting and removing the majority of the stems to simulate a harvest situation has not been evaluated in the literature. The intention was to examine the implications of growers planting directly into cereal stubble instead of rolling, consuming time and resources to accomplish the same ends. This removal of the cereal grain may be an economic incentive to growers that could supplement the cost of cereal cover crop implementation. Further study is needed to assess the implications of cereal cover terminations and the impacts on economic returns for growers.

Partial budget.

Our results for monetary profits from growing soybeans in cereal cover crops with residuals showed large differences compared to a glyphosate-based weed management program. The price for implementing a cereal cover crop with residual herbicides ranged from \$711.55 to \$886.72 ha⁻¹ compared to a glyphosate dependent program (\$331.47 ha⁻¹ to \$399.41 ha⁻¹) (Table 2.8). A cereal rye cover crop with residual herbicides was the most expensive cover to implement (\$818.77 to \$886.72 ha⁻¹) compared to wheat (\$711.55 to \$779.49 ha⁻¹) and oat (\$735.09 to \$803.04 ha⁻¹). The most expensive treatment combination, based upon residual herbicide selection, was a cereal rye cover crop and flumioxazin (\$886.72 ha⁻¹) compared to the cheapest combination of wheat with *S*-metolachlor + fomesafen (\$711.55 ha⁻¹). Cost of implementing just the cereal cover crop ranged from \$666.85 to \$752.09 ha⁻¹. The largest costs associated with implementing cereal cover crops and residual herbicides are the fertilizer (\$296.00 ha⁻¹) and cereal seed costs (\$73.33 to \$158.57 ha⁻¹) (Table 2.8).

Yield from soybeans grown in cereal cover crops, while not significantly different, did vary across all cereals and herbicides (Table 2.4). Subtracting the cost of implementing cereal cover crops from the soybean income showed varying profits across all combinations (Table 2.9). Without residual herbicides, cereal cover crops alone provided a 57% difference (cereal rye), 54% difference (wheat) and 62% difference (oat) in reduced profits compared to a glyphosate-based system without residuals. Percent difference between cereal cover crops with residual herbicides and a glyphosate-based system ranged from 41 to 102%. The least costly treatment combination was oat with sulfentrazone + metribuzin (41% difference) compared to the most costly which was a combination of cereal rye with *S*-metolachlor + metribuzin (102% difference). Reddy (2001) found similar results, where net return showed negative economic returns using a PRE application with a wheat ($-\$19 \text{ ha}^{-1}$), oat ($-\3 ha^{-1}) or cereal rye ($-\$42 \text{ ha}^{-1}$) cover compared to PRE applications with field cultivation ($\$117 \text{ ha}^{-1}$).

Cover crop adoption rates are historically low among growers, but there is often high interest in the concept (Snapp et al. 2005; Sarrantonio and Gallandt 2003). Nowak (1992) indicated 7 reasons that limit crop residue management options; 1) information on implementation was lacking, 2) cost of implementation too high, 3) system is too complex, 4) system as a whole too expensive, 5) there is too much labor involved, 6) planning/implementation period is too short and 7) access to support system is limited. Grower perceptions toward relevance of glyphosate-resistant weeds are also important in disseminating and adoption of weed management strategies (Vencill et al. 2012). Anecdotal evidence has shown that many growers will not adopt recommendations perceived as being too expensive, time consuming and complicated beyond the standard

farming practices currently used (Shaner and Breckie 2013). Economics limitations associated with cover crops are cited as the primary limiting factor for adoption (Norsworthy et al. 2012; Snapp et al. 2005; Sarrantonio and Gallandt 2003; Nowak 1992; Reddy 2001). Reddy (2003) found that cover crop adoption was 2.5 to 3 times more expensive compared to conventional tillage.

The additional costs associated with implementing cereal cover crops with residual herbicides quickly negate any positive advantages that these treatment combinations may provide to growers. This difference between the two systems (cereal cover crops with residual herbicides and a glyphosate-based weed management program) may make it difficult for growers to fully adopt the financial implications associated with cereal cover crops (Snapp et al. 2005; Sarrantonio and Gallandt 2003). The highest cost involved in cover crops comes from fertilizer addition needed to provide biomass (Snapp et al. 2005; Reddy 2001). The added costs from these two variables dramatically increase the amount of money needed to implement the cereal cover. Further research into reduced rates of nitrogen fertilizer or nitrogen fertilizer type to maximize biomass production is needed. If a reduced rate of nitrogen fertilizer can be utilized, the cost of implementation per hectare for each cereal cover crop and residual herbicide combination will be reduced and make them more economical. Implementation of cereal cover crops is also a long term process with high input costs and incremental benefits. The increasing trend in tenant farmers, roughly 38% of US farmland, may influence the adoption and persistent usage of cereal cover crops (Norsworthy et al. 2012; Carolan et al. 2004). Commonly, tenant farmers are slow to adopt sustainable farming practices like cover crops for several reasons; 1) reluctance to inform landowners of intentions, 2) uncertainty over long-term

leases 3) lack of knowledge, and 4) emphasis on production and profitability (Carolan et al. 2004).

The use of cereal cover crops has been shown to be an effective tool for controlling *Amaranthus* spp. weeds. Our results show that adoption of either a cereal rye, wheat or oat cereal cover crop in conjunction with a residual herbicide can control populations of *Amaranthus* spp. weeds and will not impede soybean yields. While technical information is not lacking concerning the implementation of cover crops, high establishment costs may influence wide spread adoption. To make cover crops a viable control option for Mississippi, continued research into preparation techniques, fertilizer usage, economic incentives and farmer adoption must be conducted. Cover crops represent a best management option for glyphosate-resistant weed management that need support to continue development and endorsement for growers to accept and implement.

Table 2.1 Physical and chemical characteristics of soils for all cereal cover crop field trials in 2013 and 2014

Location	Series	Sand ^a	Silt % by wt	Clay	OM ^b %-----	pH	CEC ^b ---meq ^b ----
Starkville	Leeper	49	46	5	1.3	7.2	17.3
Brooksville	Broosville	11	74	15	2.5	7.5	21.8
Louisville	Savannah	18	79	3	4.7	5.9	17
Eupora	Oaklimiter	10	89	1	1.2	6.9	7

^a Soil texture and analysis by the Mississippi State University soil testing lab

^b Abbreviations: OM = organic matter; CEC = cation exchange capacity; meq = meq⁺/100g soil

Table 2.2 Product formulations, herbicide rates and source information for all herbicide treatments applied to cover crops

Common name	Herbicide rate (kg ai ha ⁻¹)	Trade name	Source
flumioxazin	0.1	Valor [®] 51% DG	Valent U.S.A, Walnut Creek, CA
flumioxazin + pyroxasulfone	0.05 + 0.07	Fierce [®] 76% WDG	Valent U.S.A
pendimethalin	0.8	Prowl H ₂ O [®] 3.8 ME	BASF, Research Triangle Park, NC
S-metolachlor + fomesafen	1.2 + 0.3	Prefix [®] 5.3 SL	Syngenta
S-metolachlor + metribuzin	1.4 + 0.4	Boundary [®] 6.5 EC	Syngenta, Greensboro, NC
sulfentrazone + metribuzin	0.07 + 0.1	Authority MTZ [®] 45% DF	FMC, Philadelphia, PA

Table 2.3 Amaranthus species present in cover crop research trials based upon sites

Location	Amaranthus spp. present	Scientific name
Starkville	Tall waterhemp	<i>Amaranthus tuberculatus</i> (Moq.) Sauer
Brooksville	Palmer amaranth	<i>Amaranthus palmeri</i> S. Wats
Louisville	Palmer amaranth ^a	<i>Amaranthus palmeri</i> S. Wats
	Spiny amaranth	<i>Amaranthus spinosus</i> L
Eupora	Palmer amaranth ^a	<i>Amaranthus palmeri</i> S. Wats

^a Palmer amaranth present were glyphosate-resistant

Table 2.4 Effect cereal cover crops and residual herbicides for control weed control 14 DAT (amaranthus spp. and pitted morningglory) and soybean yield averaged over sites

Cereal Cover	Treatment(s) ^c	Herbicide rate (kg ai ha ⁻¹)	Percent Weed Control			Soybean yield (kg ha ⁻¹)	Yield (bu ha ⁻¹)
			Amaranthus spp. 14 DAT ^b	Pitted morningglory 14 DAT			
Cereal rye	flumioxazin	0.796	100 A ^a	99 AB	2549	94	
	S-metolachlor + fomesafen	1.4 + 0.43	99 A	100 A	2213	81	
	S-metolachlor + metribuzin	1.15 + 0.32	100 A	99 AB	2231	82	
	pendimethalin	0.067	99 A	100 A	2435	89	
	sulfentrazone + metribuzin	0.052 + 0.067	100 A	100 A	2361	87	
Wheat	flumioxazin + pyroxasulfone	0.068 + 0.102	99 A	99 AB	2360	87	
	Cereal rye alone		100 A	100 A	2687	99	
	flumioxazin	0.796	100 A	94 E	2172	80	
	S-metolachlor + fomesafen	1.4 + 0.43	100 A	98 ABC	2458	90	
	S-metolachlor + metribuzin	1.15 + 0.32	100 A	98 ABC	2347	86	
Oats	pendimethalin	0.067	97 A	97 A-E	2261	83	
	sulfentrazone + metribuzin	0.052 + 0.067	100 A	99 AB	2143	79	
	flumioxazin + pyroxasulfone	0.068 + 0.102	100 A	98 ABC	2303	85	
	Wheat alone		99 A	96 A-E	2520	93	
	flumioxazin	0.796	100 A	100 A	2392	88	
No Cover	S-metolachlor + fomesafen	1.4 + 0.43	100 A	100 A	2306	85	
	S-metolachlor + metribuzin	1.15 + 0.32	100 A	96 B-E	2361	87	
	pendimethalin	0.067	99 A	99 AB	2182	80	
	sulfentrazone + metribuzin	0.052 + 0.067	97 A	100 A	2703	99	
	flumioxazin + pyroxasulfone	0.068 + 0.102	96 A	99 ABC	2249	83	
No Cover	oat alone		98 A	97 A-E	2446	90	
	flumioxazin	0.796	97 A	98 A-D	2186	80	
	S-metolachlor + fomesafen	1.4 + 0.43	96 A	93 E	2427	89	
	S-metolachlor + metribuzin	1.15 + 0.32	100 A	100 A	2426	88	
	pendimethalin	0.067	97 A	99 AB	2152	79	
No Cover	sulfentrazone + metribuzin	0.052 + 0.067	96 A	95 CDE	2039	75	
	flumioxazin + pyroxasulfone	0.068 + 0.102	79 B	98 A-D	2224	82	
	none		0 C	0 F	2470	91	
					NS		

^a Means within a column followed by similar letters NS different based on LSMeans P<0.05

^b Abbreviations: DAT; days after treatment

^c All herbicides applied are labeled in soybean production and applied PRE

Table 2.5 The effect of residual herbicide selection averaged over cereal cover crops, sites, and years for weed control

Herbicide(s) ^a	Rate (kg ai ha ⁻¹)	Visual Percent Weed Control					
		Amaranthus spp.		Barnyardgrass		Horsetail	Prostrate spurge
		28 DAT ^c	14 DAT	28 DAT	28 DAT	28 DAT	28 DAT
		----- (%) -----					
flumioxazin	0.796	93 AB ^b	91 A	88 AB	59 A	89 A	
<i>S</i> -metolachlor + fomesafen	1.4 + 0.43	94 A	90 A	90 AB	61 A	91 A	
<i>S</i> -metolachlor + metribuzin	1.15 + 0.32	89 B	90 A	86 B	59 A	97 A	
pendimethalin	0.067	89 B	100 A	86 B	61 A	97 A	
sulfentrazone + metribuzin	0.052 + 0.067	96 A	93 A	90 AB	58 A	95 A	
flumioxazin + pyroxasulfone	0.068 + 0.102	95 A	90 A	91 A	60 A	94 A	
No herbicide		67 C	69 B	61 C	15 B	61 B	

^a Herbicides used labeled for soybean applications and all applied PRE

^b Means within a column followed by similar letters are not significantly different based on LSMeans P<0.05

^c Abbreviations: DAT, days after treatment

Table 2.6 Barnyardgrass control and soybean height reductions averaged across herbicide treatments, sites, and years

Cereal cover	Percent Barnyardgrass Control		Soybean Height Reduction
	14 DAT ^b	28 DAT	21 DAT
	------(%)-----		------(%)-----
Cereal rye	98A ^a	98A	1B
Wheat	90B	94A	3B
Oat	89B	87B	9A
No Cover	73C	72C	0B

^a Weed control means within a column followed by similar letters not significantly different based on LSMeans at P<0.05

^b Abbreviations: DAT; days after treatment

Table 2.7 The interaction of cereal cover type and preparation method on the total weed population present in Brooksville, MS at 28 and 56 DAT

Cover	Preparations	Weed population stand counts	
		28 DAT ^b	56 DAT
		------(# stems per 0.5 m ²)-----	
Cereal rye	Clean	5BCD ^a	5C
	Mowed	2CD	4.7C
	Rolled	13B	18.7B
	Stubble	9.7BCD	6.7C
Wheat	Clean	27.3A	22AB
	Mowed	0.3D	2C
	Rolled	11.3BC	8.7C
	Stubble	11.3BC	0.7C
Oat	Clean	1CD	2.7C
	Mowed	2.3CD	6C
	Rolled	2.3CD	3.7C
	Stubble	2.7BCD	7.7C
No cover	Control	25.3A	28.7A

^a Weed control means within a column followed by similar letters not significantly different based on LSMeans at P<0.05

^b Abbreviations: DAT; days after treatment

Table 2.8 Partial budget comparisons of soybeans grown in cereal cover cropping systems compared to a chemical glyphosate-based weed control system

Agronomic inputs	Weed management system			
	Cover crop		Chemical	
	-----(\$ ha ⁻¹)-----			
Cover crop selection				
cereal rye	158.57			
wheat	73.33			
oat	96.87			
Broadcast applications				
glyphosate (Desiccation)	14.82			
glyphosate + 2,4-D (Burndown)				20.45
paraquat + NIS				35.96
glyphosate (POST 1)	14.82			14.82
glyphosate (POST 2)				14.82
Residual application				
flumioxazin + NIS	67.95			
flumioxazin				40.68
S-metolachlor + metribuzin	46.28			46.28
flumioxazin + pyroxasulfone	34.45			34.45
sulfentrazone + metribuzin	46.78			46.78
S-metolachlor + fomesafen	30.28			30.28
pendimethalin	24.89			24.89
Miscellaneous costs				
Planting cover (tractor + implement)	37.07			
Fertilizer (AMS)	296.00			
Fertilizer spreader	20.99			
Sprayer (desiccation)	9.08			
Sprayer (burndown)				9.08
Sprayer (PRE application)	9.08			9.08
Sprayer (POST 1)	9.08			9.08
Sprayer (POST 2)				9.08
Roller (tractor + implement)	12.89			
Soybean Seed (RR2 variety)	146.96			146.96
Planter (tractor + implement)	31.81			31.81
Total costs ^c	CEREAL RYE	WHEAT	OAT	CHEMICAL
	-----(\$ ha ⁻¹)-----			
flumioxazin	886.72	779.49	803.04	399.41
S-metolachlor + fomesafen	865.06	757.84	781.38	377.75
S-metolachlor + metribuzin	853.22	758.90	782.44	365.92
pendimethalin	865.55	771.22	794.76	378.25
sulfentrazone + metribuzin	818.77	711.55	735.09	331.47
flumioxazin + pyroxasulfone	843.67	736.45	759.99	356.36
No residual	752.09	666.85	690.39	292.06

^a Cover crop type (cereal rye, wheat or oat) are individually listed in partial budget

^b Residual herbicide applications are individually listed in partial budget

^c Total costs of production listed by weed management system (cover crop or chemical weed management) and residual herbicides, shaded columns separate weed management system

Table 2.9 Partial budget profit calculations and percent difference for soybeans grown in cereal cover cropping systems compared to a glyphosate-based weed control program; , shaded rows separate cereal cover crop types

Chemical Weed Control					
	Herbicide(s)	Income ^a	Cost ^b	Profit ^c	
		-----(\$ ha ⁻¹)-----			
	flumioxazin	833.6	399.41	434.19	
	S-metolachlor + fomesafen	927.38	331.47	595.91	
	S-metolachlor + metribuzin	916.96	377.75	539.21	
	pendimethalin	823.18	356.36	466.82	
	sulfentrazone + metribuzin	781.5	378.25	403.25	
	flumioxazin + pyroxasulfone	854.44	365.92	488.52	
	No residual (glyphosate alone)	948.22	292.06	656.16	
Winter Planted Cereal Cover Crops					
Cover	Herbicide	Income ^a	Cost ^b	Profit ^c	Cover as % of chem weed control
		-----(\$ ha ⁻¹)-----			
					---(%-Difference)---
CEREAL RYE	flumioxazin	979.48	886.72	92.76	79
	S-metolachlor + fomesafen	844.02	818.77	25.25	96
	S-metolachlor + metribuzin	854.44	865.06	-10.62	102
	pendimethalin	927.38	843.67	83.71	82
	sulfentrazone + metribuzin	906.54	865.55	40.99	90
	flumioxazin + pyroxasulfone	906.54	853.22	53.32	89
	No residual	1031.58	752.09	279.49	57
WHEAT	flumioxazin	833.60	779.49	54.11	88
	S-metolachlor + fomesafen	937.80	711.55	226.25	62
	S-metolachlor + metribuzin	896.12	757.84	138.28	74
	pendimethalin	864.86	736.45	128.41	72
	sulfentrazone + metribuzin	823.18	771.22	51.96	87
	flumioxazin + pyroxasulfone	885.70	758.9	126.8	74
	No residual	969.06	666.85	302.21	54
OAT	flumioxazin	916.96	803.04	113.92	74
	S-metolachlor + fomesafen	885.70	735.09	150.61	75
	S-metolachlor + metribuzin	906.54	781.38	125.16	77
	pendimethalin	833.60	759.99	73.61	84
	sulfentrazone + metribuzin	1031.58	794.76	236.82	41
	flumioxazin + pyroxasulfone	864.86	782.44	82.42	83
	No residual	937.80	690.39	247.41	62

^a Income = Soybean yield (bu ha⁻¹) (Table 2.4) * commodity price (\$10.42 bu soybean)

^b Cost: Table 2.8

^c Profit: Income – Cost

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CHAPTER III
USE OF AMINOCYCLOPYRACHLOR AS A ROWCROP PREPLANT
BURNDOWN HERBICIDE

Abstract

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide currently labeled for non-crop use but has characteristics which may make it useful as a PPB herbicide. The soil breakdown of AMCP applied at 0.018, 0.035, 0.070, 0.140 and 0.281 kg ai ha⁻¹ and five application timing prior to planting of corn and cotton were evaluated. Carryover effects on soybean due to AMCP were also evaluated. Single and tank mix combinations of AMCP with several burndown herbicides labeled in corn (rimsulfuron, flumioxazin, pyroxasulfone, pyroxasulfone+ flumioxazin and atrazine) were also evaluated for weed control efficacy. A rate titration of AMCP half-lives and its impacts on corn, cotton and soybean were evaluated in the greenhouse. Corn injury and yield in the timing study showed tolerance to AMCP except at 0.28 kg ai ha⁻¹ applied prior to planting. Cotton was sensitive to AMCP as rate increased closer to the planting date, but response depended upon soil texture for NACB, injury, heights and yield. AMCP carryover impacts on soybean (90% injury) indicate soybean are highly sensitive to AMCP (0.28 kg ai ha⁻¹) applied greater than 1 year earlier to soil. Henbit control (77% 28 DAT) with no tank mix partner by AMCP was comparable to all treatments except tank mixture with atrazine (84%) and industry standard of paraquat (84%). Wild garlic (93% 28 DAT)

control by no tank mix with AMCP was similar to all standards except rimsulfuron (82%) mixture. AMCP showed lower control on annual bluegrass (68% 28 DAT) compared to tank mixture with atrazine (80%) and industry standard of paraquat (82%). Rate effects of soil applied AMCP half-life depend upon soil type and followed similar trends to field experiments with soybean > cotton > corn in regards to sensitivity to AMCP rate.

Nomenclature: aminocyclopyrachlor; atrazine; flumioxazin; pyroxasulfone; rimsulfuron; wild garlic, *Allium vineale* L.; henbit, *Lamium amplexicaule* L.; annual bluegrass, *Poa annua* L.; soybean, *Glycine max* L.; cotton, *Gossypium hirsutum* L.; corn, *Zea mays* L.

Keywords: Burndown, auxin, winter annuals, half-life, carryover

Introduction

Use of transgenic crops in modern agriculture has influenced farming practices across much of the United States (Shaner 2000). Chief among the transgenic crops are those with glyphosate resistance (Shaner 2000; Kleter et al. 2007; Green 2009; Givens et al. 2009; Duke and Powles 2008). Released in 1996, glyphosate-resistant soybean were the first row crop created by incorporating the resistant bacterial 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) enzyme into the plant genome (Dill 2005). By 2012, transgenic soybean, corn and cotton accounted for 93, 90 and 90% of the total planted in the United States for those respective crops (USDA 2013).

Prior to the use of transgenic crops, preplant burndown (PPB) and PRE applications of 2,4-D, atrazine, acetachlor, chlorimuron, simazine, diuron, *S*-metolachlor, trifloxysulfuron, pyriithiobac and MSMA were common in corn, cotton or soybean (Givens et al. 2009). Prior to transgenics, common weeds in high abundance on southern

farms included sicklepod (*Senna obtusifolia* (L.) H.S. Irwin & Barneby), bermudagrass (*Cynodon dactylon* (L.) Pers), and the *Amaranthus* spp. (Webster and Coble 1997). Once transgenic crop technologies were released, the simplification of weed management lead to continual applications of glyphosate and reduced applications of other herbicides (Givens et al. 2009). The average number of different active ingredients used in soybean fell from 11 in 1995 to only one in 2002 (Young 2006). Similar trends were also observed in cotton and corn production over the same period of time (Young 2006; Givens et al. 2009). From 1996 to 2006, the percent of hectares where PRE applications were made fell from 67 to 28% in soybean, 90 to 78% in cotton and 73 to 61% in corn (Norsworthy et al. 2012). A grower survey was performed in six states (Illinois, Indiana, Mississippi, North Carolina, Iowa, and Nebraska) to examine the use of glyphosate in cropping systems (Givens et al. 2009). Of the responses, 85% of soybean growers, 56% of corn growers and 47% of cotton growers made only glyphosate applications. On average, 35% of farmers who planted cotton applied glyphosate more than three times during the season while 51% of soybean growers surveyed applied two and three applications of glyphosate per yr. Of all growers, 76% indicated they used a PPB application. Of these PPB applications, glyphosate and 2,4-D were the most common herbicides used, with glyphosate applied four to six times more frequent then 2,4-D (Givens et al. 2009). However, repetitive use of a single mechanism of action (MOA) has forced selection pressure, influenced weed shifts and lead to the evolution of glyphosate-resistant populations (Beckie 2006; Norsworthy et al. 2012; Powles 2008). Currently, 32 species of weeds are reported to be glyphosate-resistant (Heap 2015). Key to the management of glyphosate-resistant weeds are herbicide MOA rotation, utilization

of residual herbicides and utilization of cultural options (e.g. cover crops, tillage, etc) (Beckie 2006). Use of residual herbicides remains one of the most successful adoptions by growers to mitigate early season weed flushes (Norsworthy et al. 2012). However, drawbacks to residual applications, such as the need for adequate rainfall for activation, possible incorporation, compatibility with conservation tillage and grower preference for POST applications all limit implementation (Shaner and Beckie 2013). The need to re-incorporate residual herbicide applications through either PRE or PPB applications is vital for reducing the expansion of herbicide glyphosate-resistant weeds.

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide currently labeled in brush management, industrial rights of way, roadsides, bare-ground, rangelands, pastures and other non-crop associated environments (Bukun et al. 2010; Anonymous 2009; Senseman 2007; Turner et al. 2009). In the synthetic-auxin like herbicide MOA, AMCP is the only member of the pyrimidine carboxylic acid family (Anonymous 2009; Bukun et al. 2010; Senseman 2007). Field trials have confirmed that AMCP has a weed efficacy pattern similar to many other synthetic auxin herbicides like picloram, aminopyralid and clopyralid (Claus et al. 2008; Bukun et al. 2010). However, the AMCP molecule differs in that it possesses an additional nitrogen in its heterocyclic carbon ring structure and a cyclopropyl side chain (Bukun et al. 2010).

Chemically, the free acid formulation of AMCP (DPX-MAT28) has a pKa disassociation constant of 4.65 making it fairly phloem mobile. Based on previous research, AMCP translocates very rapidly to meristematic regions of the plant, where it acts as an auxin mimic (Anonymous 2009). Volatility of AMCP free acid is negligible due to a vapor pressure of 4.89×10^{-6} Pa (Strachan et al. 2013). The log octanol-water

partitioning coefficient ($\log K_{ow}$) of -2.48 and -1.12 at pH 7 and 4, indicate AMCP is relatively water soluble (Anonymous 2009; Bukun et al. 2010). Soil activity of AMCP has been shown to be up to 2 yrs and can actively be absorbed by plant roots (Anonymous 2009; Lindenmayer et al. 2013; Westra et al. 2008a). Absorption of AMCP is primarily carried out by roots and not through emerging shoots (Oliveira et al. 2013; Bell et al. 2011). Soil half-life of AMCP has been recorded in turf studies from 37 to 103 days as compared to 72 to 128 days in bare soil (Anonymous 2009; Conklin and Lym 2013). Soil mobility for AMCP has reported fair mobility similar to other water soluble weak acid herbicides (Cabera et al. 2012). Soil sorption of AMCP is primarily influenced by soil organic carbon and clay content instead of pH (Oliveira et al. 2011). Due to the low pKa value AMCP (4.65) at soil pH levels above the pKa, AMCP is weakly bound to soil. Once bound to soil, desorption potential of AMCP is low, indicating that once sorbed to soil it was irreversibly bound (Oliveria et al. 2011). Primary routes of decomposition of AMCP include soil microbes and photolysis (Anonymous 2009; Lewis et al. 2013; Lindenmayer et al. 2009; Oliveira et al. 2013).

Westra et al. (2008b) examined the response of several agronomic crops [corn, wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.) and soybean] to soil treated the previous yr with several rates of DPX KJM-44 (AMCP methyl ester). Corn and sunflower exhibited tolerance to AMCP, while wheat, alfalfa and soybean were shown to be less tolerant. Several other field studies have also corroborated that wheat, cotton, alfalfa and soybean are highly sensitive to AMCP (Kniss and Lyon 2011; Strachan et al. 2011; Flessner et al. 2012). Soil concentrations of 2.0, 3.2, 5.4, and 6.2 PPB AMCP caused 25% phytotoxicity to soybean, cotton, alfalfa and

sunflower (Strachan et al. 2011). Monocot crops such as corn have shown a greater tolerance (64 g ai ha^{-1} AMCP methyl) compared to broadleaf crops (cotton, 3.3 g ai ha^{-1} and soybean 2.2 g ai ha^{-1}) (Strachan et al. 2011). Corn is mentioned as having a unspecified metabolic pathway that promotes tolerance to AMCP (Anonymous 2009).

The need to incorporate alternative PPB chemistries other than glyphosate into current farming practices prompts us to examine the use of alternative chemistries not previously used in crop management. Thus, the objectives of this research were to 1) to evaluate the use of AMCP as a PPB herbicide for corn and cotton 2) examine tank mix partners labeled in corn for suitability with AMCP applied PPB 3) examine the effects of AMCP on subsequent crops like soybean following one year after application and 4) examine the impacts of AMCP through a rate titration on three soils for agronomic crop impacts.

Methods and Materials

Timing of AMCP application.

Treatments were arranged as a 5×4 (corn) or a 5×5 (cotton) two factor factorial in a randomized complete block design with four replications, except in 2013 when the cotton trial had only three replicates. Factor A consisted of five AMCP rates ($0.02, 0.03, 0.07, 0.14$ and $0.28 \text{ kg ai ha}^{-1}$) plus a non-treated control. Factor B consisted of application timings (2, 1, 0.5 and 0 mo prior to planting (MPP) for corn and 3, 2, 1, 0.5 and 0 MPP for cotton).

All field trials were initiated at the Mississippi Agricultural and Forestry Experiment Station Black Belt Branch Station near Brooksville, MS (33.15° N by 88.33° W) and the Mississippi Agricultural and Forestry Experiment Station R.R. Foil Plant

Science Research Center near Starkville, MS (33.28° N by 88.46° W) in 2013 and 2014. At the Brooksville location, all trials were conducted on a furrow-irrigated Brooksville silty clay (Fine, smectitic, thermic Aquic Hapluderts) (all soil properties listed in Table 3.1). At the Starkville location, 2013 and 2014 corn trials and 2013 cotton trials were conducted on furrow-irrigated Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquepts). The 2014 cotton trial in Starkville was conducted on a furrow-irrigated Marietta fine sandy loam (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts). Plot dimensions varied between sites and were either 3.8 m x 12.12 m (Starkville) or 1.9 m x 9 m (Brooksville) and set up on beds with 0.95 m centers. A DeKalb DKC 6469 corn hybrid (Monsanto, St. Louis, MO) was planted at 64,318 seeds ha⁻¹ at both sites and yrs. A Deltapine DP 1321 cotton (Monsanto, St. Louis, MO) was planted in 2013 at both sites while in 2014 a Phytogen PHY 375 cotton (Dow AgroSciences, Indianapolis, IN) was used, with both planted at approximately 109,480 seeds ha⁻¹. Nitrogen fertilizer (UAN 32%) was applied at 56 kg ha⁻¹ at planting for both crops and 168 kg ha⁻¹ as a side dress 1 mo after corn planting or 112 kg ha⁻¹ 1 mo after cotton planting. All herbicides were applied using a pressurized CO₂ powered backpack sprayer delivering 140 L ha⁻¹ at 4.68 km hr⁻¹ with four nozzles (TeeJet XR 8002, Spraying Systems Co., Glendale Heights, IL). All treatments of AMCP were tank mixed with 1.26 kg ae ha⁻¹ glyphosate to control any preexisting weeds within plots. Glyphosate was applied at 1.26 kg ae ha⁻¹ as needed to control weeds through the growing season.

Corn injury (0 to 100%, where 100% is plant mortality) was collected 21 and 56 days after planting (DAP) along with number of days to tassel. Corn hts (cm) were collected 56, 84 and 112 DAP and corn yields (kg ha⁻¹) were collected by harvesting

plants with a two row plot combine. Cotton injury (0 to 100%, where 100% is plant mortality) was collected 21 and 56 DAP and cotton plant heights (cm) were collected 21 and 75 DAP. Nodes above cracked boll (NACB) prior to defoliation of cotton were collected sampling six plants from each plot to assess developmental differences. Seed cotton yield was picked using a two row cotton picker.

In 2014 at the Starkville location, auxin like phytotoxic damage was observed 21 DAP previous AMCP treated plots with Asgrow 5332 soybeans (Monsanto, St. Louis, MO). Treatment randomizations from previous AMCP experiment were overlaid on the impacted soybean area. Soybean hts (cm) and soybean injury (0 to 100%, where 100% is plant mortality) was collected 21, 40, 64 and 86 DAP for the most recent applications of AMCP 372 days previously. Soybean yields (kg ha^{-1}) were collected using a two row plot combine to assess yield differences.

AMCP PPB tank mix partners.

Treatments were arranged as a 3 x 6 two factor factorial in a randomized complete block design with four replications. Factor A consisted of AMCP rate (0.035, 0.07 and $0.11 \text{ kg ai ha}^{-1}$). Factor B consisted of tank mix partners (product formulations, rates and sources listed in Table 3.2) plus an untreated control and a paraquat treatment ($1.12 \text{ kg ai ha}^{-1}$) for comparisons as the industry standard. Studies were conducted at the same sites as the previous experiment with the same parameters for corn. All herbicides were applied using a pressurized CO_2 powered backpack sprayer delivering 140 L ha^{-1} at 4.68 km hr^{-1} with four TeeJet XR 8002 nozzles. All treatments, except the paraquat standard, were tank mixed with $1.26 \text{ kg ae ha}^{-1}$ glyphosate to control any preexisting weeds within plots. All burndown applications were applied 45 days prior to planting, in accordance

with the flumioxazin + pyroxasulfone label for cotton. Visual weed control data (0 to 100%, where 100% is plant mortality) were collected 14 and 28 d after treatment (DAT) for annual bluegrass, henbit, Carolina geranium (*Geranium carolinianum* L.) and wild garlic. Following the last weed rating, glyphosate was applied at 1.26 kg ae ha⁻¹ as needed to control weeds. Corn visual injury (0 to 100%, where 100% is plant mortality) was collected 21 and 56 DAP along with number of days to tassel. Corn hts (cm) were collected 56, 84 and 112 DAP and corn yield (kg ha⁻¹) was collected by harvesting plants with a two row plot combine

AMCP half-life titration.

Treatments were arranged as a 3 x 8 two factor factorial in a randomized complete block design with three replications and four total runs of the experiment. Factor A consisted of AMCP rate (0.002, 0.004, 0.008, 0.015, 0.031, 0.062, 0.123 and 0.245 mg ai ha⁻¹). Factor B consisted of three field soils of a known sand (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts), silt (fine, smectitic, nonacid, thermic Vertic Epiaquepts) and a clay (Fine, smectitic, thermic Aquic Hapluderts) (Table 3.1).

The experiments were repeated at the R.R. Foil Plant Science Research Center greenhouses operated using a 14 hour day with a 33 °C day temperature and a 29 °C night temperature with light supplemented by 400 lm high pressure sodium bulbs. Soils were collected from three sites where soils had previously been identified and sieved through a 50 mm mesh screen. Soils were weighed (0.33 kg) and separately placed into 3.5 x 3.5 x 3 cm square greenhouse pots. Two seeds of DeKalb 6469 corn, Asgrow 5633 soybean and DeltaPine 1321 cotton (Monsanto, St. Louis, MO) were planted to a depth of 2 cm. A series of dilutions was performed so that 5 ml of solution would apply the correct

amount of AMCP per pot. Pots were watered with a light mist for the first 24 hr to avoid leaching AMCP too quickly. Visual percent crop injury (0 to 100%, where 100% is plant mortality) was collected at 7 and 21 DAT for each crop species. Plant hts (cm) were collected 21 DAT. At 21 DAT, biomass (g) was evaluated by cutting the plants at the soil surface and drying them in a 46 °C oven dryer for 4 days.

Data Analysis.

All analyses were performed using SAS 9.3 by ANOVA using the Proc GLIMMIX procedure and means separated by LSMEANS or Fischer's Protected LSD ($\alpha=0.05$) (SAS Institute Inc., Cary, NC). All weed control and crop injury data were transformed by arcsine square root function and all data are presented in the original scale. All crop ht and yields were transformed using percent reduction from the untreated. For corn timing of application and the corn burndown experiment, no site or yr interaction occurred and all data were pooled. A site interaction was observed for all cotton data and sites were analyzed separately. For soybean injury due to AMCP carryover, data were subjected to ANOVA and means separated by LSMEANS. Data were also subjected to linear regression using the Proc REG procedure in SAS 9.3. In the half-life study, crop injury data were transformed by arcsine square root function (7 and 21 DAT) for each crop.

Results and Discussion

Timing of AMCP application.

The effect of AMCP on development and growth depends upon crop. Visual corn injury due to AMCP (9%) occurred only at the highest rate (0.28 kg ai ha⁻¹) applied 0

MPP and only up to 21 DAP (Table 3.3). Corn quickly outgrew any symptomology observed, which included goose necking of the stalk, lodging and malformations to the brace roots. Corn yield reduction was unaffected by all treatment combinations except for the 0.28 kg ai ha⁻¹ rate applied 0 MPP, which reduced yield by 26% (Table 3.3). Number of days to tassel and corn ht reductions showed NS interaction or main effects and the data are not shown. Corn number of days to tassel ranged from 72 to 74 across all treatments. Corn heights averaged across all treatments at both sites were 74 cm (56 DAP), 142cm (84 DAP) and 206cm (112 DAP). Our results support earlier evidence that have shown that AMCP has limited activity on corn (Westra et al. 2008a; Strachan et al. 2011; Anonymous 2009).

Cotton injury 21 and 56 DAT showed NS interaction but was significant for both main effects of timing of application (MPP) and AMCP rate (Table 3.4). Injury due to timing of application from 3 to 0 MPP was averaged across all rates of AMCP applied. Injury 21 DAP ranged from 46 to 67% in Starkville and 35 to 58% in Brooksville (Table 3.4). By 56 DAP, injury had decreased to 35 to 59% in Starkville and 22 to 47% in Brooksville (Table 3.4). Injury due to rate of AMCP was also significant and averaged across all timings. Cotton injury 21 DAP ranged from 40 to 78% in Starkville and from 37 to 55% in Brooksville (Table 3.4). By 56 DAP, cotton injury was from 24 to 76% in Starkville and 19 to 44% in Brooksville (Table 3.4). Cotton ht reductions, yield reduction and NACB showed significant interaction (Table 3.5, Table 3.6). In Brooksville, AMCP applications of 0.14 and 0.28 kg ai ha⁻¹ did not prevent germination of cotton, but only stunted cotton growth by 21 DAP when applied 1 to 0 MPP (Table 3.5). Cotton hts were reduced in Starkville where AMCP was applied to soil at 0.14 and

0.28 kg ai ha⁻¹. Cotton grown in AMCP treated soil at 0.28 kg ai ha⁻¹ in Starkville, failed to emerge by 21 DAP and ht reductions were 100% (Table 3.5). Seed cotton yield reductions in Brooksville showed 33 to 80% reductions at AMCP applications of 0.14 and 0.28 kg ai ha⁻¹ applied 1 through 0 MPP (Table 3.6). In Starkville, yields were reduced 40 to 100% with higher AMCP rates applied closer to planting having more of an effect (Table 3.6). An AMCP soil rate of 0.28 kg ai ha⁻¹ prevented most cotton emergence in Starkville when applied 1 and 0 MPP, thus no yields were recorded. For NACB, a similar trend was observed with less bolls present as rate increased closer to the planting date, especially in Starkville (Table 3.6). Both cotton and soybean are reported to be highly sensitive to AMCP (Westra et al. 2008b; Strachan et al. 2011; Strachan et al. 2013; Flessner et al. 2012). Cotton has been shown to be more tolerant to AMCP than soybean (Strachan et al. 2011; Flessner et al. 2012).

At 21 DAP, there was NS interaction or main effects for soybean injury (0% injury) across all treatments due to AMCP carryover, presumably since soybean roots had not reached the AMCP layer in the soil profile. After 21 DAP, soybean injury differed from the untreated check at all collections periods at an AMCP rate of 35.1 g ai ha⁻¹ applied 372 days previously (Figure 3.1). Soybean injury at all ratings showed a linear increase in response as AMCP rate increased (Figure 3.1). Soybean height reductions differed from the untreated check for all collections periods at an AMCP rate of 70 g ai ha⁻¹ applied 372 days previously (Figure 3.2). Similar to injury, soybean height reductions at all ratings showed a linear increase in rate response as AMCP rate increased. Soybean yield reductions followed the same trend as injury and height reductions, with reductions

differing from the untreated check at an AMCP rate of 70 g ai ha⁻¹ applied 372 days previously (Figure 3.3).

AMCP PPB tank mix partners.

Number of days to tassel, corn yield reduction and corn hts showed NS interaction or main effects and the data are not shown. Number of days to tassel ranged from 70 to 73 days across all treatments. Corn heights averaged across all treatments at both sites, were 70 cm (56 DAP), 127cm (84 DAP) and 212cm (112 DAP). Corn yield reduction was -13.8% averaged across all treatments.

There was neither a significant interaction nor a main effect of AMCP rate for weed control, but there was a significant main effect of tank mix partner. Annual bluegrass control by AMCP without tank mix partners at 14 (63%) and 28 DAT (68%) was similar to all treatments except atrazine (72% by 14 DAT and 80% by 28 DAT) and paraquat (80% by 14 DAT and 82% by 28 DAT) (Table 3.7). Control of henbit by AMCP without tank mix partners at 14 (73%) and 28 DAT (77%) was similar to all treatments except atrazine (87% by 14 DAT and 84% by 28 DAT) and paraquat (87% by 14 DAT and 84% by 28 DAT) (Table 3.7). Percent control of wild garlic (14 DAT) showed NS difference among treatments and the data are not shown. Average control of wild garlic 14 DAT was 87 to 90% averaged across all treatments. Wild garlic control 28 DAT showed AMCP without tank mix (93%) was comparable to all treatments except rimsulfuron (82%) addition (Table 3.7). Percent control of Carolina geranium (14 and 28 DAT) showed NS difference among treatments and the data are not shown. Average control of Carolina geranium was 97 to 100% by 14 DAT and 89 to 99% by 28 DAT.

AMCP half-life titration.

Soil texture plays a critical role in AMCP activity (Oliveria et al. 2011). Plant heights collected for the half-life study showed no interaction or main effects for all species and the data are not shown. Average crop heights were 7.9 cm for corn, 2.1 cm for cotton and 2.5 cm for soybean. At 7 DAT, an AMCP rate of 0.123 mg ai ha⁻¹ (14% injury) or 0.245 mg ai ha⁻¹ (10% injury) injured corn in the sand, compared to 0.245 mg ai ha⁻¹ in both silt (13%) and clay (15%) compared to the untreated (Table 3.8). By 21 DAT, only the clay showed any significant injury to corn (9%) at the 0.245 mg ai ha⁻¹ rate compared to the untreated. Corn biomass was increased as AMCP rate increased across all soils; 0.002 mg ai ha⁻¹ in sand, 0.123 mg ai ha⁻¹ in silt and 0.008 mg ai ha⁻¹ in clay (Table 3.8).

At both 7 and 21 DAT, soybean injury occurred in all soils compared to the untreated check at the lowest AMCP rate (0.002 mg ai ha⁻¹). Soybean biomass was reduced compared to the untreated check by 0.031 mg ai ha⁻¹ or greater AMCP in sand, 0.004 mg ai ha⁻¹ or greater AMCP in silt and 0.062 mg ai ha⁻¹ or greater AMCP in clay (Table 3.9). Cotton injury 7 and 21 DAT was different from the untreated check at the lowest rate tested (0.002 mg ai ha⁻¹) in both the sand and silt soils but not the clay soil, where injury was different at the second lowest rate of AMCP (0.004 mg ai ha⁻¹) (Table 3.10). Cotton biomass was reduced at higher rates of AMCP except in the silt soil (0.123 mg ai ha⁻¹ in sand, 0.002 mg ai ha⁻¹ in silt and 0.062 mg ai ha⁻¹ in clay) (Table 3.10).

At the Starkville location where the soil is predominantly sand, injury due to AMCP was higher compared to the clay soils in Brooksville (Tables 3.1). Soil components related to herbicide efficacy include soil cation exchange capacity (CEC), soil pH, organic matter (OM) content and clay content (Blumhorst et al. 1990; Cabrera et

al. 2012; Oliveira et al. 2011; Oliveira et al. 2013). Oliveira et al. (2011) found that AMCP sorption to soil was closely associated with soil OM or clay content and was weakly sorbed to low OM soils and soils high in sand textures. With a K_{oc} value of 28, AMCP is very weakly bound to soil and susceptible to leaching (Anonymous 2009). After 365 days, AMCP was reported to be found 70 to 90 cm deep in soil tests (Ryman et al. 2010). With less sorption, more available AMCP could be absorbed by plant roots, leading to increased injury. Penetration deeper into the soil profile may limit microbial degradation of AMCP and could lead to carry over effects of the herbicide, as observed in our experiment. Conklin and Lym (2013) indicated that DT_{50} values for AMCP ranged from 3 to >112 days depending upon soil texture and temperature. Soil activity of AMCP has been shown to be up to 2 yrs (Anonymous 2009; Lindenmayer et al. 2013; Westra et al. 2008a). Based upon our results for soybean injury, ht reductions and yield reductions (Figure 3.1, 3.2 and 3.3) we infer that AMCP applications would require greater than 372 days for soybean to be planted to reduce economic impacts (Anonymous 2009). Further research is needed to determine impact of AMCP soil persistence on other sensitive crops.

Crop responses to AMCP are varied and differ depending upon the species (Westra et al. 2008b; Strachan et al. 2011; Strachan et al. 2013; Flessner et al. 2012). To limit AMCP injury to sensitive crops, growers will have to allow significant amounts of time for AMCP to degrade in soils before sensitive species can be planted. Therefore, while AMCP has potential as a burndown compound for use in corn production, due to the compounds long residual period and potential to injure sensitive crops, AMCP is not a viable option for a burndown herbicide in agronomic crops.

Table 3.1 Physical and chemical characteristics of soils for all aminocyclopyrachlor field trials and greenhouse bioassay for aminocyclopyrachlor carryover

Location	Series	Sand ^a	Silt	Clay	OM ^b	pH	CEC ^b
		-----% by wt-----			----%--		-----meq ^b -----
Starkville	Leeper	49.5	45.5	5	1.29	6.6	12.7
Brooksville	Broosville	11.25	73.75	15	2.54	7.5	21.8
Starkville	Marietta	44.5	50.5	5	1.25	7.2	17.3

^a Soil texture analysis by the Mississippi State University soil testing lab

^b Abbreviations: OM = organic matter; CEC = cation exchange capacity; meq = meq⁺/100g soil

Table 3.2 Product formulations, herbicide rates and source information for all treatments in the aminocyclopyrachlor burndown study

Herbicide(s)	Herbicide rate (kg ai or ae ha ⁻¹)	Trade name	Source
AMCP	0.035	DPX-MAT28 50% SG	DuPont, Wilmington, DE
	0.07		
	0.11		
atrazine	1.12	Aatrex [®] 4L	Syngenta, Greensboro, NC
	1.68		
	2.24		
flumioxazin	0.036	Valor [®] 51 WDG	Valent U.S.A, Walnut Creek, CA
	0.054		
	0.1		
flumioxazin + pyroxasulfone	0.105 + 0.009	Fierce [®] 76 WDG	Valent U.S.A
paraquat	1.12	Gramoxone [®] 2 SL	Syngenta
pyroxysulfone	0.09	Zidua [®] 85 WDG	BASF, Research Triangle Park, NC
	0.12		
	0.15		
rimsulfuron	.008	Resolve [®] 25 SG	DuPont
	0.018		
	0.03		

Table 3.3 Visual corn injury 21 DAP and grain yield as influenced by AMCP rate and application timing averaged over sites and years

MPP ^a	Rate (kg ai ha ⁻¹)	Corn injury	Yield reduction
		21 DAP ^a	
		------(%)-----	------(%)-----
2	0.018	1 B ^b	-2 BCD
	0.035	2 B	0 BCD
	0.07	1 B	-8 BCD
	0.14	1 B	-2 BCD
	0.28	2 B	3 BCD
1	0.018	1 B	-5 BCD
	0.035	1 B	-7 BCD
	0.07	2 B	4 BCD
	0.14	1 B	-3 BCD
	0.28	1 B	-11 D
0.5	0.018	1 B	-14 D
	0.035	1 B	-10 CD
	0.07	1 B	-3 BCD
	0.14	3 B	-2 BCD
	0.28	1 B	12 AB
0	0.018	0 B	-9 CD
	0.035	2 B	-1 BCD
	0.07	0 B	10 ABC
	0.14	3 B	0 BCD
	0.28	9 A	26 A
UTC	0	0 B	0 BCD

^a Abbreviations: DAP; days after planting, MPP; months prior to planting

^b Weed control means within a column followed by similar letters not significantly different based LSMeans P<0.05

Table 3.4 The main effects of application timing (MPP) and AMCP rate (kg ai ha⁻¹) on visual cotton injury 21 and 56 days after planting (DAP) averaged over AMCP rate and years

Main Effect	Percent cotton injury			
	Starkville		Brooksville	
	21 DAP ^a	56 DAP	21 DAP	56 DAP
	------(%)-----		------(%)-----	
MPP ^a				
3	46 B ^b	35 B	35 B	23 C
2	53 AB	41 B	36 B	22 C
1	66 A	53 AB	46 AB	26 BC
0.5	67 A	59 A	44 B	47 A
0	63 A	54 AB	58 A	38 AB
UTC	0 C	0 C	0 C	0 D
Rate (kg ai ha ⁻¹)				
0.018	40 B	24 D	37 D	19 C
0.035	45 B	33 CD	30 CD	27 BC
0.07	56 B	45 BC	43 BC	29 BC
0.14	78 A	64 AB	53 AB	37 AB
0.28	75 A	76 A	55 A	44 A
UTC	0 C	0 E	0 E	0 D

^a Abbreviations: DAP; days after planting, MPP; months prior to planting

^b Weed control means within a column followed by similar letters not significantly different based LSMeans P<0.05, shaded rows separate main effects

Table 3.5 The interaction of AMCP (kg ai ha⁻¹) and application (MPP) for cotton ht reductions in Starkville and Brooksville averaged across years

		Cotton height reductions							
MPP ^a	Rate	Brooksville				Starkville			
		21 DAP ^a		75 DAP		21 DAP		75 DAP	
kg ai ha ⁻¹		------(%)-----							
3	0.018	11	FGH ^b	9	DE	15	G-J	-6	D
	0.035	2	H	2	DE	12	G-J	-11	D
	0.07	6	H	7	DE	27	E-I	-8	D
	0.14	0	H	1	DEF	54	CDE	-1	D
	0.28	6	H	-2	DEF	53	CDE	10	CD
2	0.018	3	H	-5	DEF	3	IJ	-8	D
	0.035	4	H	-1	DEF	17	F-J	-4	D
	0.07	7	GH	2	DE	15	G-J	-8	D
	0.14	-3	H	-2	DE	51	CDE	-11	D
	0.28	-2	H	-6	EF	50	CDE	63	B
1	0.018	4	H	-24	F	-5	J	-3	D
	0.035	-2	FGH	-2	DEF	14	G-J	-6	D
	0.07	8	H	-1	DEF	53	CDE	-3	D
	0.14	21	D-G	13	CDE	58	BCD	53	B
	0.28	39	CDE	13	CDE	90	AB	100	A
0.5	0.018	10	FGH	0	DEF	30	D-H	-4	D
	0.035	31	C-G	21	DC	13	G-J	-6	D
	0.07	52	BC	36	BC	22	E-I	1	D
	0.14	64	AB	61	AB	67	ABC	61	B
	0.28	82	A	74	A	99	A	100	A
0	0.018	17	E-G	5	DE	-3	J	-7	D
	0.035	6	H	2	DE	-7	E-I	-5	D
	0.07	32	C-F	11	CDE	24	D-H	35	BC
	0.14	42	BCD	47	B	35	D-G	64	B
	0.28	42	BCD	50	AB	100	A	100	A
UTC		0	H	0	DEF	0	HIJ	0	D

^a Abbreviations: DAP; days after planting, MPP; months prior to planting

^b Weed control means within a column followed by similar letters not significantly different based LSMeans on at P<0.05, shaded columns separate sites

Table 3.6 The interaction of AMCP rate and application timing for node above cracked boll (NACB) and cotton yield reductions at two field locations averaged over years

MPP ^a	Rate	Brooksville		Starkville	
		NACB ^a	Yield weduction	NACB	Yield reduction
	kg ai ha ⁻¹	-----(#)----	------(%)-----	-----(#)----	------(%)-----
3	0.018	4.3BCD ^b	23B-F	3.7B-F	14D-H
	0.035	3.3DE	-1FGH	4.3BCD	13D-H
	0.07	3.5CD	-7FGH	4.5BC	24D-G
	0.14	3.4DE	8E-H	4.1BCD	48BCD
	0.28	3.4CDE	-10GH	3.8B-E	50BCD
2	0.018	3.7CD	-15H	4.2BCD	3E-H
	0.035	3.4CDE	-14H	4.6ABC	4E-H
	0.07	3.3DE	13E-H	3.7BCD	16D-H
	0.14	2.9EF	-15H	4C-F	50BCD
	0.28	3.5CD	-15H	4.8AB	70ABC
1	0.018	2.9EF	12E-H	3.8B-E	-17H
	0.035	4BCD	-7FGH	3.4DEF	17D-H
	0.07	3.5CD	-8FGH	4.4BCD	49BCD
	0.14	3.8BCD	20C-G	4.7AB	70ABC
	0.28	5.1AB	45BC	- ^c G	100A
0.5	0.018	3.1DE	-1FGH	4.1BCD	20D-H
	0.035	4.1CD	19C-G	3.7B-F	22D-G
	0.07	3.3F	33B-E	4.1BCD	46BCD
	0.14	4.9EF	80A	2.8EF	80AB
	0.28	2.4G	54AB	- ^c G	100A
0	0.018	4.5BC	-14H	4.6ABC	-11GH
	0.035	3.8CD	1E-H	4.5BC	37C-F
	0.07	5.7A	13D-H	4.8AB	47BCD
	0.14	2.9FG	44BCD	2.7F	40CDE
	0.28	2.3H	6E-H	- ^c G	100A
UTC		3.5CDE	0FGH	5.6A	0FGH

^a Abbreviations: NACB; nodes above cracked boll, MPP; months prior to planting

^b Weed control means within a column followed by similar letters not significantly different based Fischer's protected LSD on at P<0.05, shaded columns separate sites

^c Missing values denote not plants within plots as all plants failed to emerge due to AMCP application

Table 3.7 Visual percent weed control of annual bluegrass, henbit and wild garlic (14 and 28 DAT) for tank mix partners averaged over AMCP rates averaged over sites and years

Tank mix(s)	Percent weed control									
	annual bluegrass					henbit				wild garlic
	14 DAT ^a		28 DAT	14DAT		28DAT		28DAT		
	------(%)-----									
None	63	C ^b	68	B	73	B	77	B	93	AB
flumioxazin	68	BC	72	B	75	B	82	AB	88	ABC
rimsulfuron	66	BC	68	B	79	B	81	AB	82	C
pyroxasulfone	68	BC	68	B	78	B	75	B	84	BC
atrazine	72	AB	80	A	87	A	84	A	98	A
flumioxazin + pyroxasulfone	68	BC	72	B	76	B	80	AB	88	ABC
paraquat	80	A	82	A	87	A	84	A	96	A

^a Abbreviations: DAT; days after treatment

^b Weed control means within a column followed by similar letters not significantly different based LSMEANS on at P<0.05

Table 3.8 The effect of aminocyclopyrachlor rate in different soils (sand, silt and clay) for corn visual injury (7 and 21 DAT) and final dry biomass; data pooled across runs of the experiment

Rate	Sand ^a			Silt ^b			Clay ^c		
	Injury		Biomass	Injury		Biomass	Injury		Biomass
	7DAT ^d	21DAT	(g)	7DAT	21DAT	(g)	7DAT	21DAT	(g)
mg ai ha ⁻¹									
0.245	10 AB	1	0.49 A	13 A	0	0.51 B	15 A	9 A	0.37 A
0.123	14 A	9	0.4 AB	7 B	0	0.62 A	4 B	0 B	0.36 A
0.062	8 ABC	4	0.47 AB	2 B	0	0.42 C	1 B	0 B	0.31 AB
0.031	1 C	0	0.4 ABC	5 B	0	0.4 C	0 B	0 B	0.29 A-D
0.015	1 C	0	0.34 CD	4 B	0	0.41 C	0 B	0 B	0.32 AB
0.008	6 ABC	0	0.4 ABC	1 B	0	0.38 C	1 B	0 B	0.31 ABC
0.004	4 BC	4	0.4 ABC	0 B	0	0.35 C	1 B	0 B	0.25 BCD
0.002	0 C	0	0.38 BC	1 B	0	0.38 C	4 B	0 B	0.23 CD
0	0 C	0	0.28 D	0 B	0	0.35 C	0 B	0 B	0.22 D
LSD ($\alpha=0.05$) ^e	8.93	NS	0.1	6.7	NS	0.08	6.54	5.4	0.0819

^a Sand = Marietta fine sandy loam

^b Silt = Lepper silty clay loam

^c Clay = Brooksville silty clay

^d Abbreviations: DAT; days after treatment

^e Weed control means within a column followed by similar letters not significantly different based Fischer's protected LSD on at P<0.05, shaded columns separate soil types (sand, silt and clay)

Table 3.9 The effect of aminocyclopyrachlor rate in different soils (sand, silt and clay) for soybean visual injury (7 and 21 DAT) and final dry biomass averaged over runs of the experiment

Rate	Sand ^a			Silt ^b			Clay ^c		
	Injury		Biomass	Injury		Biomass	Injury		Biomass
	7DAT ^d	21DAT	------(g)-----	7DAT	21DAT	------(g)-----	7DAT	21DAT	------(g)-----
mg ai ha ⁻¹	------(%)-----	------(%)-----	------(g)-----	------(%)-----	------(%)-----	------(g)-----	------(%)-----	------(%)-----	------(g)-----
0.245	80 A	96 A	0.04 E	73 A	87 A	0.03 E	67 A	91 A	0.04 D
0.123	60 B	83 AB	0.06 DE	61 A	84 A	0.05 DE	44 B	82 A	0.06 D
0.062	32 C	76 BC	0.08 CDE	44 B	70 B	0.06 CDE	37 BC	65 B	0.09 CD
0.031	30 C	68 C	0.09 B-E	37 BC	62 BC	0.1 C	28 BC	54 BC	0.13 BC
0.015	35 C	65 C	0.09 A-D	37 BC	51 CD	0.08 CD	41 B	61 BC	0.16 AB
0.008	35 C	50 D	0.12 ABC	30 BC	40 DE	0.01 CD	31 BC	50 C	0.19 A
0.004	19 C	40 D	0.13 AB	27 C	38 DE	0.1 CD	31 BC	48 C	0.18 AB
0.002	25 C	45 D	0.14 A	23 C	34 E	0.16 B	20 C	28 D	0.2 A
0	0 D	0 E	0.14 A	0 D	0 F	0.22 A	0 D	0 E	0.17 AB
LSD ($\alpha=0.05$) ^e	16.1	12.8	0.05	14.96	13.9	0.05	17.35	13.76	0.061

^aSand =Marietta fine sandy loam

^bSilt = Lepper silty clay loam

^cClay = Brooksville silty clay

^dAbbreviations: DAT; days after treatment

^eWeed control means within a column followed by similar letters not significantly different based Fischer's protected LSD on at P<0.05, shaded columns separate soil types (sand, silt and clay)

Table 3.10 The effect of aminocyclopyrachlor rate in different soils (sand, silt and clay) for cotton visual injury (7 and 21 DAT) and final dry biomass averaged over runs of the experiment

Rate	Sand ^a			Silt ^b			Clay ^c		
	Injury		Biomass	Injury		Biomass	Injury		Biomass
	7DAT ^d	21DAT	------(g)-----	7DAT	21DAT	------(g)-----	7DAT	21DAT	------(g)-----
mg ai ha ⁻¹	------(%)-----	------(%)-----	------(g)-----	------(%)-----	------(%)-----	------(g)-----	------(%)-----	------(%)-----	------(g)-----
0.245	78 A	82 A	0.04 D	71 A	80 A	0.06 D	66 A	79 A	0.09 BC
0.123	71 A	79 A	0.06 CD	61 AB	67 AB	0.06 D	53 AB	70 AB	0.09 C
0.062	53 B	64 B	0.11 AB	50 BC	51 BC	0.1 BCD	44 BC	61 B	0.09 BC
0.031	52 BC	61 B	0.11 AB	44 BC	39 CDE	0.1 CD	35 BCD	38 C	0.15 A
0.015	50 BCD	52 B	0.13 A	39 CD	42 CD	0.12 ABC	36 BC	39 C	0.14 A
0.008	35 DE	35 C	0.09 ABC	34 CD	43 CD	0.13 ABC	39 BC	36 C	0.13 AB
0.004	36 CDE	30 C	0.09 ABC	33 CD	26 DE	0.15 A	25 CD	28 CD	0.17 A
0.002	29 E	24 C	0.08 BC	20 D	24 E	0.14 AB	16 DE	14 DE	0.17 A
0	0 F	0 D	0.1 AB	0 E	0 F	0.1 CD	0 E	0 E	0.16 A
LSD ($\alpha=0.05$) ^e	16	12.94	0.04	18.9	17.3	0.05	20.5	16.5	0.04

^aSand =Marietta fine sandy loam

^bSilt = Lepper silty clay loam

^cClay = Brooksville silty clay

^dAbbreviations: DAT; days after treatment

^eWeed control means within a column followed by similar letters not significantly different based Fischer's protected LSD on at P<0.05, shaded columns separate soil types (sand, silt and clay)

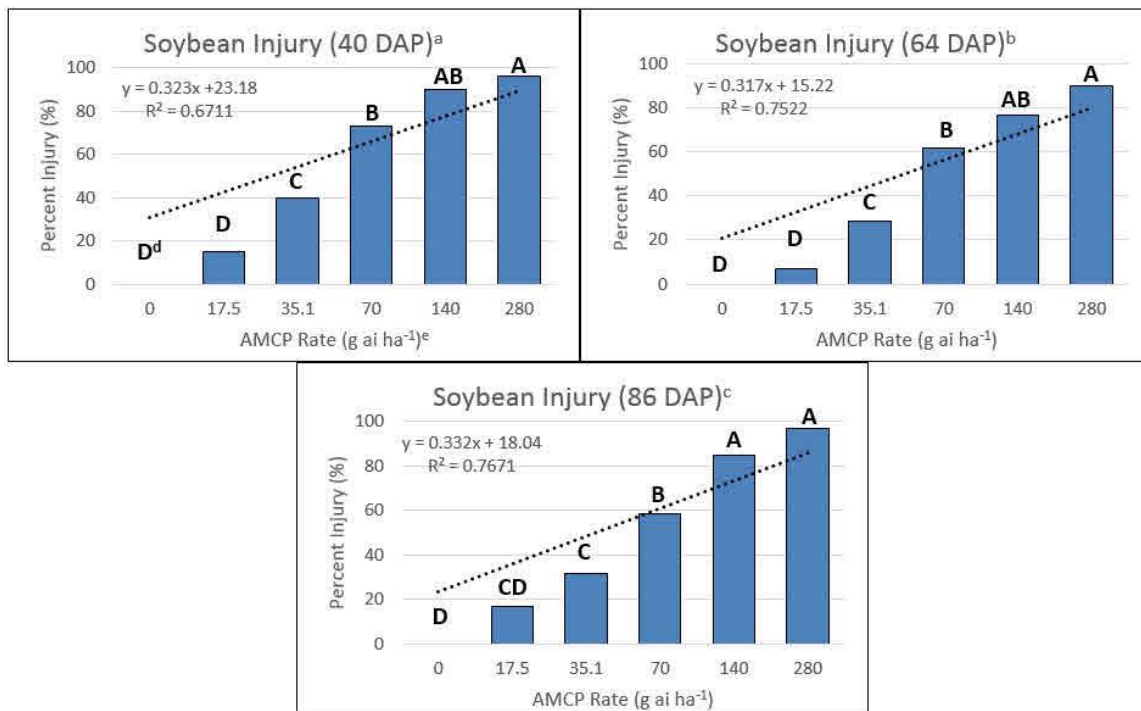


Figure 3.1 Percent soybean injury, measured at 40, 64 and 86 DAP, due to AMCP carryover with original application (g ai ha⁻¹) made 372 days prior.

a Equation for 40 DAP; $y=0.323x + 23.18$, $r^2 = 0.6711$, $SE=23.08$

b Equation for 64 DAP; $y=0.317x+15.22$, $r^2 = 0.7522$, $SE=18.53$

c Equation for 86 DAP; $y=0.332x+18.04$, $r^2 = 0.7671$, $SE=18.63$

d Injury (0 to 100%, where 100% is plant mortality) means within a plotted area followed by similar letters not significantly different based LSMEANS at $P<0.05$

e AMCP rate (g ai ha⁻¹) is indicated as original field rates applied 372 days prior to planting of soybean.

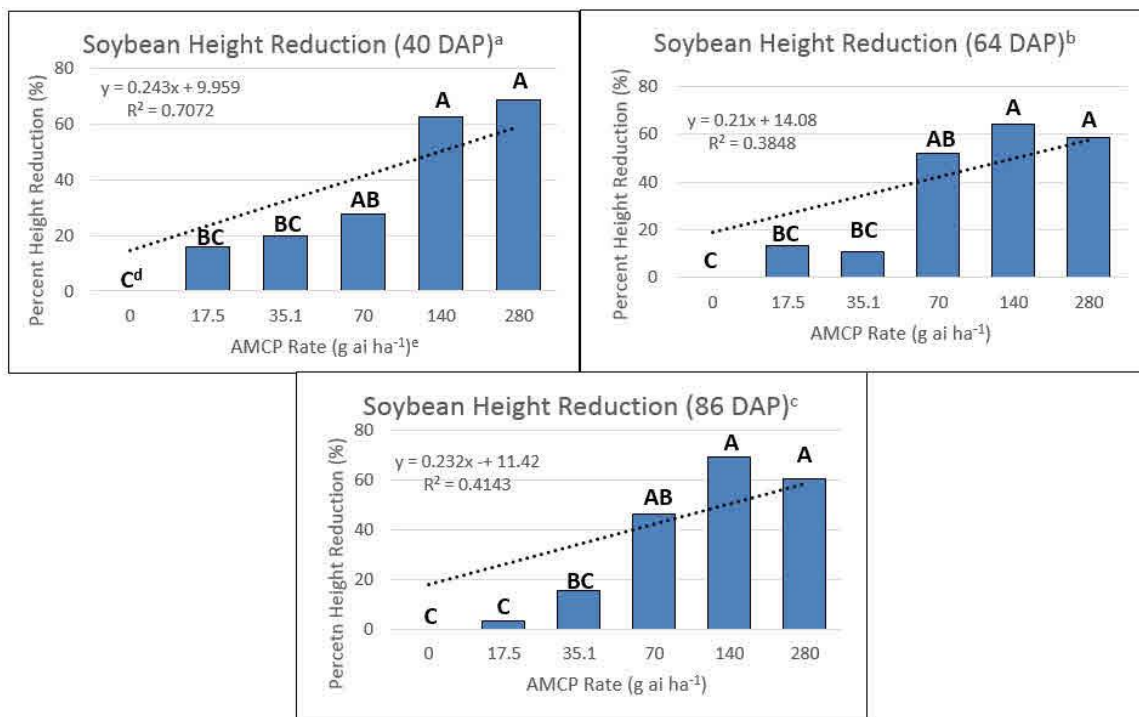


Figure 3.2 Percent soybean height reductions, measured 40, 64 and 86 DAP, due to AMCP carryover with original applications (g ai ha⁻¹) made 372 days prior.

a Equation for 40 DAP; $y = 0.243x + 9.959$, $r^2 = 0.7072$, SE = 17.14

b Equation for 64 DAP; $y = 0.21x + 14.08$, $r^2 = 0.3848$, SE = 27.14

c Equation for 86 DAP; $y = 0.232x + 11.42$, $r^2 = 0.4143$, SE = 28.11

d Injury (0 to 100%, where 100% is plant mortality) means within a plotted area followed by similar letters not significantly different based LSMEANS at $P < 0.05$

e AMCP rate (g ai ha⁻¹) is indicated as original field rates applied 372 days prior to planting of soybean.

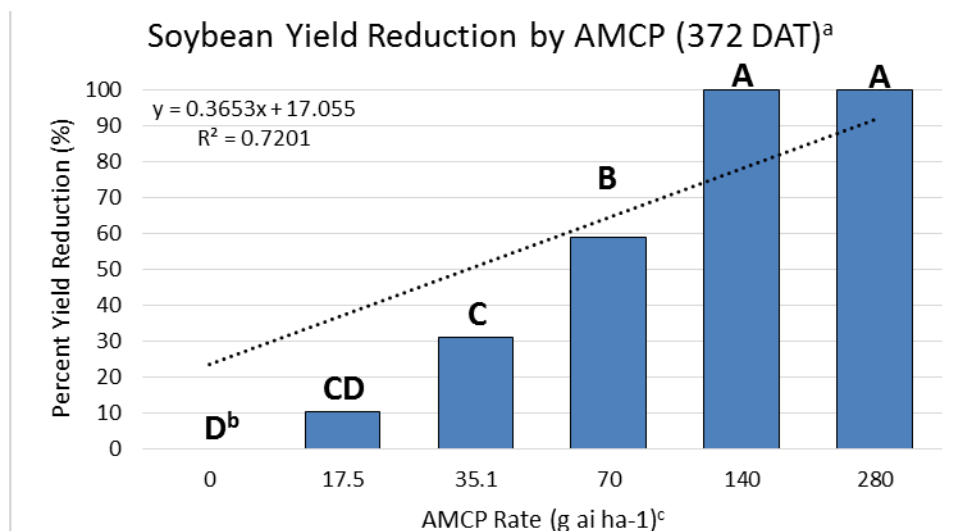


Figure 3.3 Percent soybean yield reduction due to AMCP carryover with original application (g ai ha⁻¹) made 372 days prior

a Equation for 40 DAP; $y = 0.365x + 17.05$, $r^2 = 0.7201$, $SE = 23.21$

b Injury (0 to 100%, where 100% is plant mortality) means within the plotted area followed by similar letters not significantly different based LSMeans at $P < 0.05$

c AMCP rate (g ai ha⁻¹) is indicated as original field rates applied 372 days prior to planting of soybean.

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CHAPTER IV

EXAMINATION OF AMINOCYCLOPYRACHLOR SITE OF UPTAKE INTO ROOTS
AND SHOOTS THROUGH BIOASSAY

Abstract

The effects of aminocyclopyrachlor (AMCP) uptake by crop species is not well understood, nor is the impact that uptake may have on developing roots and shoots. A greenhouse study was conducted to examine isolation of AMCP in treated layers separated by activated carbon. Each treated layer would isolate root and shoot absorption effects on germinating corn, cotton, soybean and hemp sesbania seeds. Seven experimental types of cone-tainers were employed to test different scenarios of absorption and corresponding controls to rule out carbon influence on seed development. Sand was used as the planting media to avoid soil binding of AMCP. AMCP was evaluated at a rate equivalent of 70 g ai ha⁻¹. Root and shoot lengths (cm) were measured 3, 7, 14 and 21 days after treatment. Corn was shown to be tolerant to AMCP root or shoot absorption and showed minimal responses except when both root and shoot absorption occurred simultaneously. Cotton, soybean and hemp sesbania were more sensitive to AMCP especially through root absorption. When both root and shoot absorption occurred, all sensitive seeds would germinate, but rarely grew beyond a few tenths of a cm. Results on absorption of AMCP show that root absorption is the primary pathway for absorption compared to shoot absorption.

Nomenclature: aminocyclopyrachlor; hemp sesbania, *Sesbania herbacea* (P. Mill.) McVaugh; soybean, *Glycine max* (L.) Merr.; cotton, *Gossypium hirsutum* L.; corn, *Zea mays* L.

Keywords: Activated carbon, root absorption, shoot absorption, bioassay, separation of treated layers

Introduction

Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide currently labeled in non-crop environments (Bukun et al. 2010, Anonymous 2009, Senseman 2007, Turner et al. 2009). Currently, AMCP is the only member of the pyrimidine carboxylic acid family of synthetic auxin-like herbicides (Anonymous 2009, Bukun et al. 2010, Senseman 2007). Field trials have shown that AMCP has a spectrum of efficacy similar to the pyridine carboxylic acid herbicides like aminopyralid, picloram and clopyralid (Claus et al. 2008, Bukun et al. 2010).

Chemically, the free acid of AMCP has a pKa disassociation constant of 4.65, making it a fairly phloem mobile herbicide that translocates very rapidly to meristematic regions of the plant where it acts as an auxin mimic (Anonymous 2009; Bukun et al. 2010). With a vapor pressure of 4.89×10^{-6} Pa, AMCP is considered non-volatile when formulated as a free acid (Strachan et al. 2013). Water solubility of AMCP based on log octanol-water partitioning coefficient or log K_{ow} (-2.48 to -1.12 at pH of 7 and 4, respectively) shows AMCP is fairly water soluble (Anonymous 2009, Bukun et al. 2010). With a low pKa value, AMCP is weakly bound to soil similar to other weak acid herbicides (Anonymous 2009; Cabera et al. 2012). Soil half-life of AMCP has been

shown in turf to range from 37 to 103 days as compared to 72 to 128 days in bare soil (Anonymous 2009, Conklin and Lym 2013). Soil sorption of AMCP is primarily mediated by soil organic carbon content and percent clay composition instead of pH (Oliveira et al. 2011). Once bound to soil, AMCP is irreversibly bound (Oliveria et al. 2011). Primary routes of decomposition of AMCP include soil microbes or photolysis (Anonymous 2009; Lewis et al. 2013; Lindenmayer et al. 2009; Oliveira et al. 2013).

Westra et al. (2008b) examined crop response of corn, wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.) and soybean to soil treated the previous yr with AMCP methyl ester. Both corn and sunflower showed tolerance to AMCP, while wheat, alfalfa and soybean were less tolerant. Several other studies have also shown that wheat, cotton, alfalfa and soybean are highly sensitive to AMCP (Kniss and Lyon 2011; Strachan et al. 2011; Flessner et al. 2012). Soil concentrations of 2.0, 3.2, 5.4, and 6.2 ppb caused 25% phytotoxicity to soybean, cotton, alfalfa and sunflower, respectively, while grass crops such as corn have shown a greater tolerance to AMCP (Strachan et al. 2011; Anonymous 2009).

The use of bioassays for determination of herbicidal effects is well documented (Streibig 1988; Horowitz 1976). A bioassay is an experiment for estimating the potency of a herbicide by analyzing the reaction following its application to living organisms (Streibig 1988). In a bioassay, key indicator species are utilized to test the effects of various herbicides on living systems of plants under controlled and reproducible conditions (Horowitz 1976). Use of activated carbon to absorb herbicides started with Lucas and Hammer (1947) who showed 2,4-D became inactivated by carbon application. Utilization of activated carbon was studied to safen crops from applications of soil

applied herbicides (Chandler et al. 1978; Linscott and Hagin 1967; Colquhoun et al. 2006). Linscott and Hagin (1967) applied activated carbon at several rates (0, 56, 112 and 168 kg ha⁻¹) in 3.15 cm bands over the top of drilled alfalfa seedlings to protect them from applications of triazine herbicides, with the highest rates of carbon being necessary to protect the seedlings from the highest rates of herbicides. Chandler et al. (1978) performed a similar study applying carbon (0, 111 and 222 kg ha⁻¹) over cotton seedlings for protection from diuron (3.55 kg ha⁻¹) with cotton injury ranging from 28, 3 and 8% over the rates of carbon, respectively. Multiple bioassays looking at site of herbicide uptake have utilized activated carbon to separate layers of applied herbicides to study shoot or root uptake (Wehtje et al. 1997; Narsaiah and Harvey 1977; Blair 1978; Salzman and Renner 1992). Narsaiah and Harvey (1977) outlined a pot description that could be utilized in developing herbicide placement studies, using layers of activated carbon to separate different layers of treated soil. When treated soil is above a target seed (separated by a carbon layer) as developing shoots grow into the treated soil, they will absorb herbicide and exhibit shoot absorption (Narsaiah and Harvey 1977). Likewise, if a treated layer is below a carbon layer and seeds above, root growth deeper into the treated layer will exhibit root absorption (Narsaiah and Harvey 1977). Wehtje et al. (1997) examined the uptake of sulfentrazone utilizing activated carbon to distinguish layers for yellow nutsedge (*Cyperus esculentus* L.) uptake. Four different soil treatments were described (treated soil below, treated soil above, treated soil above and below and an untreated check) using a 1 cm thick layer of activated carbon as the buffer. Using this method, yellow nutsedge exposure to sulfentrazone was identified to be affected mainly through root absorption. Using a similar procedure, Salzman and Renner (1992) used a

0.5 cm thick layer of activated carbon to examine several soil applied herbicides effects on soybean either above or below treated layers. They concluded that heavy influxes of water shortly after herbicide application may move herbicides deeper into the soil which may increase phytotoxic damage to developing seeds (Salzman and Renner 1992). Blair (1978) used an activated carbon layer of only a few millimeters (1 to 2mm) to separate layers of soil to isolate absorption of chlortoluron, isoproturon and metoxuron from wheat seeds. At harvest, wheat seed impacts by activated carbon showed no impacts by the carbon. Results showed all herbicides injured wheat seedlings when they were placed below the seed (root absorption) or where carbon was absent.

Interception of auxin herbicides using bioassays with activated carbon could be used to better understand impacts of AMCP on root and shoot absorption of crop plants. Therefore, the objective of this experiment was to isolate the absorption of AMCP to shoot, root or a combination of shoot and root absorption and observe how AMCP impacts developing seeds. This research may shed more light on the impacts of ACMP soil applications and its interactions with crop species.

Methods and Materials

Experimental design.

A greenhouse study was initiated at the Mississippi Agricultural and Forestry Experiment Station R.R. Foil Plant Science Research Center near Starkville, MS (33.28° N by 88.46° W) in 2013 and 2014. Experiments were conducted in a greenhouse operated using a 14 hour day with a 33.3 °C day temperature and a 29.4 °C night temperature with light supplemented by 400 lm high pressure sodium bulbs. All treatments were carried out in

white circular polypropylene plant propagation cone-tainers (Stuewe & Sons, Inc., Ray Leach Cone-tainers RLC-4, Tangent, OR).

Experiments were conducted where differing cone-tainer types were used to test for AMCP absorption into shoots and roots (Figure 4.1). The seven different cone-tainer types each simulated different avenues for herbicide absorption; 1) Shoot (treated) where AMCP treated soil above the carbon and seeds planted below carbon layer, 2) Shoot (control) where untreated soil above the carbon and seeds planted below carbon layer, 3) Root (treated) where seeds planted above carbon layer and AMCP treated soil below the carbon, 4) Root (control) where seeds planted above carbon layer and untreated soil below the carbon, 5) Shoot + Root (treated with no carbon) where seeds planted without a carbon layer and AMCP treated soil, 6) Shoot + Root (treated with carbon) where seeds planted and AMCP treated soil above carbon layer and 7) Control (no carbon) with the untreated control with no carbon (Figure 4.1). Each experiment was designed as a randomized complete block with six replications and the experiment was repeated three times. A paper towel plug was inserted at the bottom of each cone-tainer as a permeable wick for water capillary flow. A granular sand was used in all cone-tainers to prevent soil absorption or microbial degradation. All cone-tainers were contained in 98 well cone holders and separately suspended in 57 L water baths mixed with 0.18 kg of all purpose plant food (The Scotts Company, LLC, Miracle Grow (24-8-16), Marysville, OH). Each treatment was separately housed in water baths to eliminate potential for cross contamination between different cone-tainer types. Fertilizer was added to the water bath instead of over the top to prevent water infiltration from above the carbon layer containing the herbicide and to demonstrate wicking potential of the cones to supply

water to the developing roots. Cone-tainers were planted with either two seeds of DeKalb 6469 corn (Monsanto, St. Louis, MO), Asgrow 5633 soybean (Monsanto, St. Louis, MO), Phytogen 375 cotton (Dow AgroSciences, Indianapolis, IN) or 5 seeds of hemp sesbania.

The total weight of the sand was measured and used to determine the appropriate amount of AMCP to be added for a soil rate equivalent to 70 g ai ha⁻¹ AMCP. Three cone-tainers (types three, four and six) were filled with 100 g of sand, while three other cone-tainers (types one, two, five and seven) were filled with 150 g of sand (Figure 4.1). All cones were then shaken until level. A 1 cm thick layer of 100 mesh particle size activated carbon powder (Sigma-Aldrich, DARCO 242276, St. Louis, MO) was used to separate layers in the cones, in accordance with Narsaiah and Harvey (1977) and Blair (1978). Activated carbon was added into the cones, measured to the appropriate thickness and leveled. Cone-tainers were filled with an additional 90 g of sand (types three, four and six) or 40 g of sand (types one, two, five and seven) (Figure 4.1). Using a 5 ml syringe, AMCP solution was added to each cone such that 5 ml of solution would add the amount of material needed. Plants were evaluated 3, 7, 14 and 21 days after planting (DAP) by collecting root and shoot lengths (cm). All data were pooled across runs and all analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC) by ANOVA using the Proc GLIMMIX procedure and means separated by Fischer's Protected LSD ($\alpha=0.05$).

Results and Discussion

Corn.

Corn shoot and root development did not differ among the controls with respect to carbon presence or absence and seed placement in relation to the carbon (Table 4.1). This indicates that the presence of the carbon layer did not affect growth in the absence of herbicides. Where AMCP was placed above the carbon layer to allow only shoot absorption, shoot lengths did not differ from the untreated control 7 or 21 DAT (Table 4.1). At 7 DAT, this treatment exhibited 6.1 cm shoots which is an increase over the 4.8 cm shown by the control. By 21 DAT, shoot length ranged from 10.7 to 11.9 cm but did not differ between treatments. Where AMCP was placed below the carbon layer to allow only root absorption, roots were longer in the treated sand (11 cm) as compared to the shoot control (9.7 cm) (Table 4.2). By 21 DAT, roots in the treated sand continued to be longer (15 cm) when compared to the respective control (10.8 cm). Treatments that allowed both root and shoot absorption did not differ due to the presence or absence of the carbon layer (0.3 to 1.7 cm), but in all cases were different from the untreated check (8.7 to 12.6 cm) by 7 and 21 DAT.

Cotton.

At 7 DAT, shoot development was less than the untreated check in all cases where seeds were placed below the carbon layer regardless of the presence or absence of AMCP indicating an effect by the carbon layer. By 21 DAT, shoot development ranged from 1.8 to 5.9 cm among all control treatments but they did not differ indicating that the carbon layer was no longer affecting shoot development. At 7 DAT, shoot length was

unaffected by shoot exposure alone (4.5 cm) relative to its control (4.6 cm). When exposed to the roots only, shoots were 2.1 cm which was less than the 5.2 cm observed in its control. Exposure to both root and shoot absorption resulted in no shoot development regardless of carbon presence or absence. By 21 DAT, shoot development was less than each respective check regardless of exposure method. Exposure to roots alone or a combination of roots and shoots resulted in the greatest injury with 0 shoot growth indicating injury from root absorption was greater than from shoot absorption. Root development 7 DAT was effected by the presence of carbon regardless of seed placement. By 21 DAT, the carbon negatively affected root development only where seeds were below the carbon layer. At 7 DAT, root development was reduced only where exposed to shoot uptake alone or a combination of both root and shoot uptake. By 21 DAT, only plants exposed to root absorption alone or a combination of shoot and root resulted in less root development (0.5 to 1.7 cm) than the untreated check.

Soybean.

Similar to cotton, soybean have also been shown to be highly sensitive to AMCP (Strachan et al. 2011). Presence or absence of the carbon layer did not affect soybean shoot and root development with the exception of the shoots at 21 DAT where soybeans above the carbon layer (3.4 cm) resulted in less shoot development than the untreated check (5 cm). Shoot development was inhibited regardless of exposure method, 7 DAT with the greatest injury where exposure was to both roots and shoots. Root development 7 DAT where plants were exposed to root absorption alone or root and shoot absorption (0 to 0.9 cm) was less than the checks which ranged from 3.5 to 3.9 cm. The trend

continued at 21 DAT where only plants with root or a combination of root and shoot exposure resulted in reduced root development. This indicates that soybean injury is greatest when developing roots are exposed to AMCP.

Hemp sesbania.

Hemp sesbania root development did not differ among the controls with respect to carbon presence or absence and seed placement to carbon 7 and 21 DAT. Shoot development was impacted at both 7 (0.9 cm) and 21 (1.2 cm) DAT when seeds were planted above the carbon compared to the untreated check (1.9 cm 7 DAT and 3.1 cm 21 DAT). This indicates shoot development may have been impacted by carbon presence. For shoot absorption, there was NS difference between shoot lengths at 7 (2.4 cm) and 21 (4.6 cm) DAT compared to the respective checks (1.9 cm 7 DAT and 4.2 cm 21 DAT). When AMCP was placed below the carbon for root absorption, there was NS difference 7 DAT between the treated (1.2 cm) and untreated check (1.2 cm). By 21 DAT, root absorption in the treated cones showed shorter roots (0.4 cm) compared to the respective check (1.9 cm), indicating root inhibition by AMCP. Treatments that allowed both shoot and root absorption were all significantly different than the check for both root and shoot absorption 7 and 21 DAT.

As previous studies have also shown, herbicide proximity to the seeds often result in more phytotoxic effect (Blair 1978; Narsaiah and Harvey 1977; Burr et al. 1972; Salzman and Renner 1992). In previous studies, AMCP absorption has been shown to be primarily carried out by roots and not through emerging shoots (Oliveira et al. 2013; Bell et al. 2011; Bukun et al. 2010). A possible explanation for the reduced effects on shoots

is in part due to reduced time of uptake as shoots quickly can grow through a treated layer that may limit shoot absorption, while roots are continually exposed to leaching effects of the herbicide (Parker 1966). However, effects from other auxin herbicides have conflicting reports on impacts of both shoot and root absorption (Phillips et al. 1972, Prendeville et al. 1967). Use of this bioassay technique to detect AMCP absorption further supports those findings. Use of an activated carbon layer successfully separated AMCP absorption based on roots and shoots for all species tested.

Salzman and Renner (1992) hypothesized as herbicide vertical movement is mediated by water intrusion, a hypothetical application followed by intense rainfall may force the herbicide deeper into the soil profile, detrimentally impacting susceptible crop species rather than weed seeds located in the top centimeters of the soil. Overall soil mobility of AMCP has shown the compound to be very mobile both vertically and laterally in the soil (Oliveira et al. 2011; Oliveira et al. 2013). After 365 days, AMCP has been reported to be found 70 to 90 cm deep in soil tests (Ryman et al. 2010). Soil activity of AMCP has been shown to be up to 2 yrs (Anonymous 2009; Lindenmayer et al. 2013; Westra et al. 2008a). Long soil persistence and mobility within soil can place AMCP within the rooting zone of susceptible species like soybean and cotton. Based on our results, root absorption of AMCP can decrease root lengths of susceptible plants and limit their growth and development.

Table 4.1 The effect of AMCP absorption site on shoot development of corn, cotton, soybean and hemp sesbania averaged over runs of the experiment

Cone	Description ^b	7 DAT ^a				21 DAT			
		Corn	Cotton	Soybean	Hemp sesbania	Corn	Cotton	Soybean	Hemp sesbania
1	Shoot (treated)	6.1 A	4.5 B	2.3 BC	2.4 A	11.9 A	1.7 B	4.9 A	4.6 A
2	Shoot (control)	4.8 B	4.6 B	5.5 A	1.9 A	10.7 A	5.9 A	5.3 A	4.2 AB
3	Root (treated)	5.6 AB	2.1 C	0.9 C	0.5 B	10.5 A	0 C	4 AB	1 CD
4	Root (control)	4.6 B	5.2 AB	4.2 AB	0.9 B	11.3 A	1.8 A	3.4 B	1.2 C
5	Shoot + Root (no carbon)	0 C	0 D	0 C	0 B	0 B	0 C	0 C	0 D
6	Shoot + Root (carbon)	0 C	0 D	0 C	0 B	0 B	0 C	0 C	0 D
7	Control (no carbon)	5.1 AB	6.4 A	4.8 A	1.9 A	11.2 A	5.5 A	5 A	3.1 B
LSD ($\alpha=0.05$) ^c		1.15	1.46	2.38	1.05	2.67	1.51	1.29	1.19

^a DAT: Days after treatment

^b Shoot (treated): AMCP treated soil above the carbon and seeds planted below carbon layer

Shoot (control): untreated soil above the carbon and seeds planted below carbon layer

Root (treated): seeds planted above carbon layer and AMCP treated soil below the carbon

Root (control): seeds planted above carbon layer and untreated soil below the carbon

Shoot + Root (treated with no carbon): seeds planted without a carbon layer and AMCP treated soil

Shoot + Root (treated with carbon): seeds planted above carbon layer and AMCP treated soil above the carbon

^c Means within a column separated by Fisher's protected LSD ($\alpha=0.05$), shaded areas represent treated cones and their respective untreated controls

Table 4.2 The effect of AMCP absorption site on root development of corn, cotton, soybean and hemp sesbania averaged over runs of the experiment

Cone	Description ^b	7 DAT ^a				21 DAT			
		Corn	Cotton	Soybean	Hemp sesbania	cm			
1	Shoot (treated)	10.3 A	1.5 C	1.6 BC	4.9 A	13.7 AB	5.1 BC	4.1 B	1.8 B
2	Shoot (control)	7.4 B	4.1 B	3.2 AB	2.5 ABC	12 AB	4.1 C	3.4 B	1.9 AB
3	Root (treated)	11 A	0.9 CD	0.9 C	1.2 BC	15 A	1.7 D	0.9 C	0.4 C
4	Root (control)	9.7 A	1.5 C	3.9 A	1.2 BC	10.8 B	6.5 A	6 A	1.9 AB
5	Shoot + Root (no carbon)	1.7 C	0.3 CD	0.1 C	0.2 C	0.3 C	0.5 D	0.4 C	0.1 C
6	Shoot + Root (carbon)	1.7 C	0.2 D	0 C	0.1 C	0.3 C	0.5 D	0.4 C	0 C
7	Control (no carbon)	8.7 AB	5.4 A	3.5 AB	3.1 AB	12.6 AB	5.6 AB	4.4 AB	3 A
LSD ($\alpha=0.05$) ^c		1.97	1.26	1.85	2.77	3.65	1.44	1.62	1.1

^a Abbreviations: DAT; days after treatment

^b Shoot (treated): AMCP treated soil above the carbon and seeds planted below carbon layer

Shoot (control): untreated soil above the carbon and seeds planted below carbon layer

Root (treated): seeds planted above carbon layer and AMCP treated soil below the carbon

Root (control): seeds planted above carbon layer and untreated soil below the carbon

Shoot + Root (no carbon): seeds planted without a carbon layer and AMCP treated soil

Shoot + Root (carbon): seeds planted above carbon layer and AMCP treated soil above the carbon

^c Means within a column separated by Fisher's protected LSD ($\alpha=0.05$), shaded areas represent treated cones and their respective untreated controls

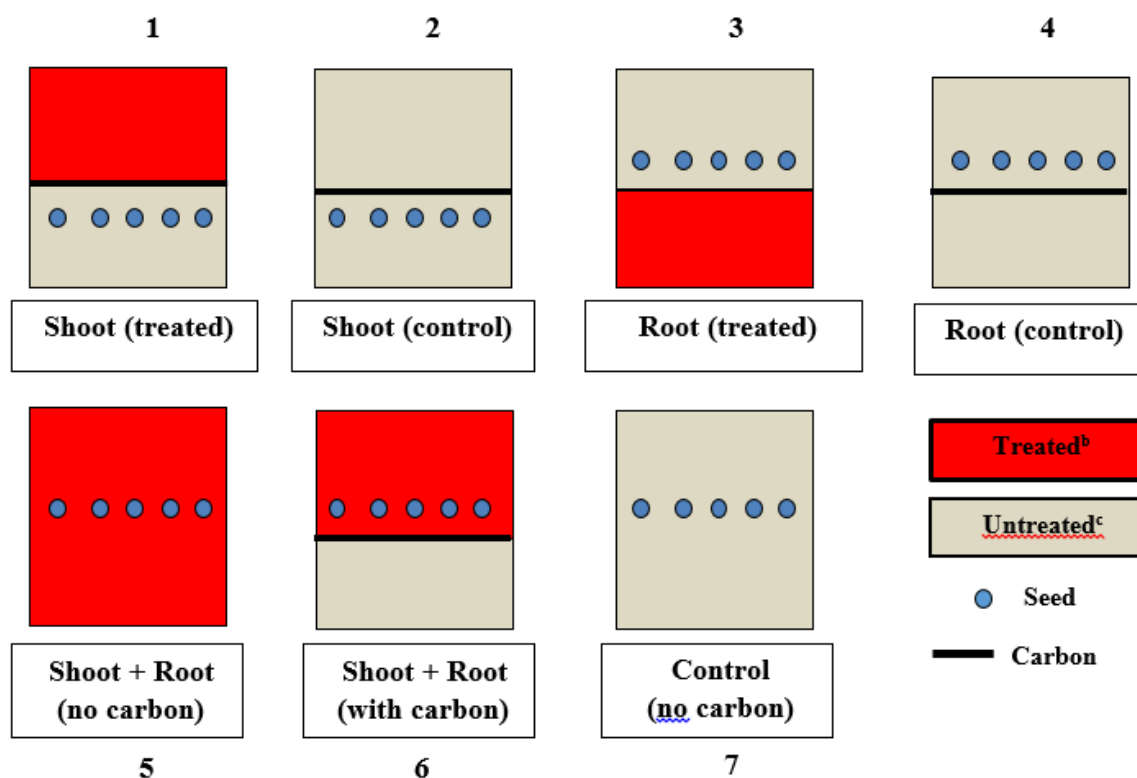


Figure 4.1 Cone types used in experiment, with shaded regions signifying treated soil regions.

^a 1) Shoot (treated): AMCP treated soil above the carbon and seeds planted below carbon layer

2) Shoot (control): untreated soil above the carbon and seeds planted below carbon layer

3) Root (treated): seeds planted above carbon layer and AMCP treated soil below the carbon

4) Root (control): seeds planted above carbon layer and untreated soil below the carbon

5) Shoot + Root (treated with no carbon): seeds planted without a carbon layer and AMCP treated soil

6) Shoot + Root (treated with carbon): seeds planted and AMCP treated soil above carbon layer

7) Control (no carbon)

^b Figure key; red shaded area represents soil partition treated with AMCP, tan shaded area represents soil partition that is untreated with AMCP, blue circles represent the placement of agronomic and weed seed tested, solid black line represents the carbon layer (1cm) placed to separate treated zones and isolate absorption of AMCP to root, shoot or root and shoot.

^c Soil medium used in experiment was granular sand to prevent soil binding of AMCP

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