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The Effect of Hose Type and Cleanout Procedure on Crop Injury due to Herbicide Residues

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The effect of hose type and cleanout procedure on crop injury due to herbicide residues

By

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Field and greenhouse experiments were conducted to determine the effect of auxin injury on soybean and cotton due to spray hose material, formulation and cleanout procedures on auxin equipment cleanout. Visual estimations of injury (VEOI) in wheat, height reduction, and yield reduction due to rimsulfuron and glyphosate titration was higher when compared to rimsulfuron only treatments with respect to 1/2X through the 1/256X treatments. Sequestration of 2,4-D within agricultural hose types did differ due to hose type and is confirmed by analytical testing, but field observation of wheat did not show differences among treatments due to VEOI, height reduction or yield reduction. Using soybean as a bio-indicator, differences did occur with respect to dicamba sequestration in agricultural hose types with respect to VEOI, height reduction, node reduction, yield reduction and ppm analyte retained. Results indicate chemical makeup of hose type in determination of ppm analyte dicamba retained. Cleaning procedures of water or ammonia do not prove to be different with respect to VEOI, height reduction, yield reduction or ppm analyte retained. Sequestration of 2,4-D within valved manifold

systems and using water or ammonia as cleanout procedures in conjunction with rinse procedures did not show differences with respect to VEOI, height reduction, nodes above cracked boll (NACB), yield reduction or ppm analyte retained. It was not until standard 2,4-D applications were applied in field experiments when differences were observed. Deactivation of dicamba and 2,4-D using the Fenton procedure within various rates, showed an interaction with respect to VEOI, height reduction, node reduction, yield reduction and ppm analyte. Using soybean as a bio-indicator showed differences with the Fenton procedure deactivating the dicamba analyte in the 1/16X, 1/64X and 1/256X rate with respect to VEOI, height reduction, node reduction, yield reduction and ppm analyte retained. Using cotton as a bio-indicator showed differences with the Fenton procedure deactivating the 2,4-D analyte in every rate with respect to VEOI, height reduction, yield reduction and ppm analyte.

DEDICATION

I would like to dedicate this work to my family and friends who stuck by me through thick and thin. Life can bring so many ups and downs, but when it comes to accomplishing something that you truly care about, only family and friends are the ones who completely understand. I dedicate this dissertation, as I did with my master's thesis, to my grandfather, Milton Cundiff, who passed away at the age of 93 on April 4th 2012. My grandfather was the first in our family to graduate from college and was an inspiration to me my entire life. Before I began this six year journey through graduate school, he wrote to me: "Hello Gary T., Hope things are going better for you. Sometimes it takes a long time for the worm to turn. I don't know if your dad ever told you that I was in an orphanage for seventeen years. As soon as some people hear this, they think that it was a hard life, but it was the best thing that ever happened to me. The woman who was the superintendent took an interest in me in every way she could. She got the board of directors to help me my first semester at Georgetown until I could get an athletic scholarship. I just wanted to point out some things that could happen even when things are bad. I wish you the best in your job and in your life and when life gets to the lowest point, that's usually when the worm turns. Good Luck, Grandpa". Just as my grandfather, without the people along the way taking an interest in me and truly hearing what I have to say, this would've been impossible to accomplish.

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CHAPTER I

INTRODUCTION

Since introduced by Monsanto in 1996, genetically engineered Roundup Ready ® (RR) crops revolutionized weed control and no-till practices (Johnson et al. 2012a). Roundup Ready® crops are resistant to the herbicide glyphosate (N-(phosphonomethyl)glycine) (Senseman 2007), enabling producers to spray the herbicide post-emergence throughout the growing season and achieve excellent broad spectrum control. Roundup Ready® soybean was introduced in the United States in 1996 followed shortly thereafter by RR cotton and RR corn with additional crops (including canola and sugar beet) also being released (Johnson et al. 2012a). After countless glyphosate applications over many years and millions of hectares, the widespread evolution of weed populations resistant to glyphosate has occured (Johnson et al. 2012a). Glyphosate-resistant weeds, such as Palmer amaranth (*Amaranthus palmeri* S. Wats.), horseweed (*Conyza canadensis* (L.) Cronq.), common ragweed (*Ambrosia artemisiifolia* L.), and giant ragweed (*Ambrosia trifida* L.) (Heap 2013), are examples of difficult to control weeds that have driven development of plants resistant to plant-growth-regulating (PGR) herbicides such as 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 2,4-Dichlorophenoxyacetic acid (2,4-D). In response to the evolution of glyphosate-resistant weeds, chemical companies have been investing in new methods of weed control. Companies are searching for new active ingredients and modes of action, but the cost of

developing them and the limited potential for economic return has made it difficult to bring new products to market (Johnson et al. 2012a). These companies have been on the forefront of genetically engineered crops which are resistant to herbicides other than glyphosate. The dicamba and 2,4-D-resistant crops were developed because these herbicides have few herbicide-resistant weeds occurring after more than 50 years of use (Johnson et al. 2012a). Secondly, 2,4-D and dicamba provide excellent control of glyphosate-resistant broadleaf weeds such as horseweed, giant ragweed, and common waterhemp (*Amaranthus rudis* Sauer) (Johnson et al. 2012b).

Auxinic herbicides, such as 2,4-D and dicamba, have little soil residual activity (Senseman 2007). These herbicides have been extensively used for weed control for over 60 years primarily due to their selectivity, wide spectrum of control, efficacy, and low costs (Mithila et al. 2011). Auxinic herbicides mimic natural occurring auxin, which is a plant growth hormone central to regulating plant growth and development (Abel and Theologis 1996). Auxinic herbicides, also commonly known as synthetic auxins, mimic the plant growth hormone indole-3-acetic acid (IAA); mimicking IAA disrupts growth and development processes, eventually causing plant death (Senseman 2007). Auxinic herbicides are readily taken up by the roots and foliage and are translocated in the both the phloem and xylem. Symptomology observed from auxinic herbicides include: swelling of the stems, cupping of the leaves, epinastic twisting of the stems and petioles of plants, chlorosis, and/or necrosis (Senseman 2007; Wax et al. 1969; Robinson et al. 2013; Egan et al. 2014).

In 2013, the state of Mississippi harvested 0.8 million hectares of soybeans averaging 2,825 kg per hectare with the value of production at $1.2 billion (USDA-NASS

2012). Soybean growth is split into two stages, vegetative and reproductive, and within each stage there are more specific subcategories. Soybean reproductive growth stages are the stages that are most important for soybean yield determination; the reproductive growth stages are when the seed number and size are determined (Pederson 2004). Reproductive growth stages begin when the first flower on the stem is present and is referred to as the (R1) growth stage, which is where the first pod will eventually form on the plant. The reproductive growth stage (R2) will form when there is an open flower at one of the two uppermost nodes on the main stem with a fully developed leaf. Reproductive growth stage (R3) occurs when the pod reaches a length of 0.5 cm long in the upper four nodes (Koger et al. 2013). The typical PGR injury symptoms in soybeans can be identified by the characteristic cupping of leaves with dicamba and injury can range from cosmetic leaf injury to 80 percent yield loss, depending on the amount of PGR residue left in the tank and the crop growth stage at application (Steckel et al. 2005). Soybeans exposed to 2,4-D or dicamba can develop vegetative malformations and produce a lower yield; however, damage is dependent upon rate and application timing (Andersen et al. 2004). Wax et al. (1969) found that soybean is susceptible to dicamba application at vegetative and reproduction stages. Injury due to herbicide does not always lead to yield loss (Al-Khatib and Peterson 1999); soybean has the ability to recover from early season injury depending on rate of herbicide exposure and soybean growth stage (Weidenhamer et al. 1989). Reduced soybean yield from dicamba exposure has been reported when dicamba caused severe injury and stunting. Yield reductions greater than 10% coincided with severe visual injury (Al-Khatib and Peterson 1999), such as terminal

bud kill, splitting of the stem, swollen petioles, and curled malformed pods (Weidenhamer et al. 1989).

Anderson (2004) concluded that soybean sprayed with 0.0056 kg ae ha^{-1} of dicamba at V3 resulted in at least 40% visual estimation 48 DAT and 14% yield reduction. Dicamba applied at 0.0112 and 0.056 kg ae ha^{-1} resulted in 13.8 and 71.5% yield reduction, respectively. Applications of 2,4-D at V3 with the same rates as dicamba showed yield reduction at 0, 7.2, and 31.7% of soybean; visual estimations of injury (VEOI) ranged from 5, 10, and 30% 48 days after treatment. The study concluded that visual injury and yield reduction were greater from dicamba applications versus 2,4-D.

Cotton (*Gossypium hirsutum* L.), is also an important crop for the economy in Mississippi. Cotton was ranked as the fourth most valuable agricultural commodity to the state of Mississippi in 2011 with a $563 million value of production and in 2013 with $271 million respectively (MDAC 2012). Cotton is a perennial shrub, but has been domesticated throughout the centuries as a pseudo annual shrub (Chaudlgry and Guitchounts, 2003). Through the use of plant growth regulators, harvest aids, and specialized management practices cotton can produce like an annual crop (Chaudlgry and Guitchounts 2003).

Damage to cotton by 2,4-D has been reported since 2,4-D was first commercially introduced (Staten 1946). Cotton is considered one of the most susceptible agricultural crops to 2,4-D (Bayley et al. 1992). Hamilton and Arle (1979) found that dicamba applied over the top of cotton had less effect on cotton foliage, yield, boll components, and fiber properties when applied before bloom than when applied later in the season. Previous research in cotton has indicated that a yield loss can occur due to exposure of

2,4-D or dicamba (Smith et al. 2010). Smith et al. (2010) found that yield reductions were observed from both 2,4-D and dicamba. The results of this study and similar research (Smith et al. 2010; Marple et al. 2008; Everitt et al. 2009) show that cotton is more sensitive to 2,4-D than dicamba, whereas other studies (Andersen et al. 2004; Johnson et al. 2012b) show that soybeans are more sensitive to dicamba versus 2,4-D. Cotton yield losses were observed where minimal VEOI from exposure to 2,4-D (Smith et al. 2010). Marple et al. (2007) reported greater cotton injury and yield reductions from titrated rates of 2,4-D than clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) or triclopyr (3,5,6-Trichloro-2-pyridinyloxyacetic acid).

Dow AgroSciences calls their 2,4-D-resistant technology the Enlist™ Weed Control System in corn, soybean, and cotton (Dow AgroSciences 2013). This technology became possible when the company inserted genes into broad-hectare agronomic crops that allow the plants to metabolize 2,4-D (Johnson et al. 2012b). Dow AgroSciences has introduced the Enlist Duo™ formulation that contains glyphosate and 2,4-D: choline (Dow AgroSciences 2013). The herbicide features what Dow AgroSciences calls Colex-D™ Technology (Dow AgroSciences 2013), which provides ultra-low volatility, minimized potential for drift, lower odor, and better handling characteristics than commercially available 2,4-D amine or ester formulations (Johnson et al. 2012b). Enlist™ soybean, cotton, and corn will have traits that make them tolerant to 2,4-D as well as glyphosate and glufosinate (Dow AgroSciences 2013).

Monsanto has introduced MON 87708 soybean, which was genetically engineered from A3525, a high-yielding soybean variety to be resistant to dicamba by expressing a mono-oxygenase gene (DMO) from *Strenotrophomonas maltophilia* that rapidly

demethylates dicamba, rendering it inactive (Johnson et al. 2012a; Behrens et al. 2007; USDA 2014). Their Roundup Ready Plus 2 Xtend System® will contain the Genuity® Roundup Ready 2 Yield® trait technology stacked with a trait enabling tolerance to dicamba (Monsanto 2013). By using an *agrobacterium* gene transfer, plants are inserted with genes that allows the breakdown of dicamba within the plant (Behrens et al. 2007).

Dicamba and 2,4-D have been widely used for over 60 decades and little evolution of auxin resistant weeds has been recorded (Nandula 2010). The introduction of new herbicide-tolerant crops may provide many benefits for producers such as alternative control options for resistant weed species, decreased costs, and different modes of action. Along with these benefits, the use of auxin containing herbicides also increases concern for issues such as herbicide drift, volatilization, and tank contamination. The adjuvant and solvent system utilized in several commercial herbicides often results in the release of herbicides which have been sequestered within the spray system thus resulting in injury to sensitive crops. Injury from plant growth regulator (PGR) herbicide tank residues on cotton and soybean is most prevalent in the first full tank of post applied herbicide (Steckel et al. 2005). Due to their chemical makeup, several herbicides most notably Roundup WeatherMax® (glyphosate) are very effective tank cleaners for PGR herbicides (Steckel et al. 2005).

With the new triple stacked gene technology (glyphosate + glufosinate + dicamba or glyphosate + glufosinate + 2,4-D) soon to penetrate the market, problems may arise from issues involving off-target movement from one producer's field to another because it is unlikely that everyone will immediately adopt the new technologies. Unlike glyphosate, which is very water soluble and can be easily cleaned out of a sprayer with

water, the PGR herbicides, although being highly water soluble, act as weak acids and take a lot more time, care and effort to be removed (Steckel et al. 2005). Considering that soybean and cotton are extremely sensitive to PGR herbicides, it is imperative that a quality clean-out technique become the standard adopted among producers. Kelley and Riechers (2003), found that as little as 1/10,000 of the 280 g ae ha^{-1} dicamba rate can produce injury symptoms on soybeans. Compounding this problem, is spray contamination caused by a failure to thoroughly clean a sprayer can cause crop injury up to several months after initial use, and following several subsequent applications (University of Illinois extension 2006). Boerboom (2004), showed that dicamba residues, even when an ammonia-water solution was used, had a subsequent percent use rate of 0.024% from the tank and 0.63% from the spray boom when refilled with water. If proper application practices are not performed by producers, there will likely be many incidents where injury to susceptible crops will occur due to tank contaminations (Johnson et al. 2012a). This justifies an investigation into cleaning methods and hose types used in pesticide applications on agronomic row crops and to quantify the lowest amount of auxinic herbicide residue that cause economic harm to the crop.

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CHAPTER II

THE EFFECT OF HOSE TYPE ON LEADOFF™ SEQUESTRATION IN SPRAYERS AND ITS EFFECT ON WHEAT YIELD

Abstract

Due to the number of herbicide active ingredients used in preemergence early season burndown applications, tank contamination issues have increased. Two experiments were conducted to evaluate the level of wheat injury from simulated tank contamination of LeadOff™ (rimsulfuron and thifensulfuron-methyl) + glyphosate + 2,4-D, while applying a labeled rate of Harmony® Extra SG (thifensulfuron-methyl and tribenuron-methyl). Treatments were applied to wheat at the elongation, pre-boot growth stage. Results indicate that injury from the 17.58 g ai ha^{-1} rate of LeadOff™ + 0.43 kg ae ha^{-1} glyphosate + 0.398 kg ae ha^{-1} 2,4-D increased incrementally on average 20% each week to total 84% injury in the final rating date when compared to 17.58 g ai ha^{-1} rate of LeadOff™ alone which had 45% injury based on visual estimations 28 days after treatment (DAT). The difference in visual estimations of injury (VEOI) is primarily contributed to glyphosate. The 40% VEOI decrease with 17.58 g ai ha^{-1} rate of LeadOff™ alone versus 17.58 g ai ha^{-1} rate of LeadOff™ + 0.43 kg ae ha^{-1} glyphosate + 0.398 kg ae ha^{-1} 2,4-D may also be observed in yield reduction where a 40% more yield loss occurred between treatments, when compared to the check. No differences based on injury, height reduction or yield reduction occurred with respect to rimsulfuron sequestration within

different agricultural hoses by standard cleanout procedures. When analytical analysis was performed rimsulfuron was not different among hose types and cleanout treatments. Analytical analysis showed differences among hose types and cleanout procedures where the polyethylene hose sequestered the least amount of the 2,4-D analyte.

Nomenclature: LeadOff™; Touchdown Total®; Weedar 64®; rimsulfuron; thifensulfuron-methyl; tribenuron-methyl, 2,4-D; glyphosate, wheat (*Triticum aestivum* L.)

Key words: Sequestration, tank contamination, interaction, crop oil concentrate, hose cleanout, agricultural hose types

Introduction

Wheat (*Triticum aestivum* L.) is classified as a winter or spring annual with flowering response to vernalization (Simmons et al. 1995). In 2015, 20 million hectares of wheat were harvested in the United States yielding roughly 43 million metric tons with an average metric ton per hectare of 2.37 (United States Department of Agriculture Economic Research Service 2015). In order to maximize wheat production pesticide applications must be utilized at specific growth stages. Ineffectiveness or possible injury may occur if chemical applications are applied at the wrong growth stage (Wise et al. 2011). Several growth scales are used to describe wheat growth including Feekes, Haun, BBCH, and Zadocks (Wise et al. 2011), with Feekes being the most commonly used in the United States. The Feekes scale numerically identifies growth stages of wheat such as tillering, jointing, and ripening but is not as detailed as the Zadocks or Haun (Simmons et al. 1995) systems until head emergence occurs.

In 2012, DuPont introduced LeadOff™ herbicide, which is comprised of rimsulfuron (16.7%) and thifensulfuron-methyl (16.7%), both belonging to the sulfonylurea family (Senseman 2007). LeadOff™ is used as a preplant, burn-down herbicide in early spring for corn (*Zea mayes L.*), cotton (*Gossypium hirsutum L.*), and peanut (*Arachis hypogaea L.*) throughout the South and Southeast regions of the United States. In certain geographies, a small number of sprayers were used to apply LeadOff™ herbicide on corn, cotton or soybean and then used to apply other crop protection products on winter wheat, including Harmony Extra™ which is comprised of thifensulfuron-methyl (33.33%) and tribenuron-methyl (16.67%) (Senseman 2007). Some of these winter wheat fields expressed varying levels of damage, which is believed to be due to cleanout issues. The material impacting wheat likely comes from somewhere within the boom section, although additional research needs to be conducted to be conclusive. Wheat yield loss due to misapplication of LeadOff™ may vary depending on rate and growth stage. Injury symtoms are initially chlorosis followed by stunting and necrosis.

The sulfonylurea and imidazolinone herbicide families are inhibitors of the branch chained amino acids valine, leucine and isoleucine; otherwise known as the acetolactate synthase/acetohydroxy acid synthase (ALS/AHAS) inhibitors. A major feature of the sulfonylurea herbicide family is the ability to be biologically active at extremely low use rates. LeadOff™ has provided excellent burndown control of many spring and winter weeds. However, due to the inability to control germinated horseweed (*Conzya Canadensis (L.)* Cronq.), tank-mixtures with glyphosate and 2,4-D are recommended. Glyphosate is a non-selective herbicide with excellent grass activity. Deeds et al. (2006)

and Roider et al. (2007) reported severe wheat injury and yield reductions with glyphosate aaplications. Glyphosate applications earlier in wheat development, i.e. tillering, are likely to have more injury than later growth stages (Orr et al. 1996). It was also reported that VEOI on wheat in response to glyphosate was an accurate indicator of yield loss (Orr et al. 1996).

The herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) can be found in numerous water-soluble amine salts and in the acid form, but also are produced with ester derivatives, which strongly enhance its diffusion properties (Chinalia et al. 2007). Phenoxy-herbicides are compounds used to control dicotyledonous weeds and have been produced and applied on a large scale since the 1940s (Hayward 1991). Commercially, 2,4-D has been formulated as either dimethylamine salt (DMA) and 2-ethylhexyl ester (EHE), which accounted for approximately 90% in global use in the last half of the twentieth century (Chinalia et al. 2007). In wheat, Feekes growth stage 6 is referred to as jointing, where the first node will become visible at the base of the shoot (Wise et al. 2011). The cutoff point for application of auxinic herbicides such as dicamba and 2,4-D in wheat is before the grain is in boot stage (Senseman 2007).

Harmony® Extra SG is a winter annual broadleaf prepackaged dry flowable premixture, which may be applied in wheat after the two leaf stage and before the flag leaf is visible (Smith and Smith 2012). Due to the increased number of herbicide active ingredients used in burndown applications, tank-contamination issues have become frequent over the past several growing seasons (Steckel et al. 2005; Boerboom 2004). Therefore, two experiments were conducted to evaluate the level of wheat injury from tank contamination; 1.Using titrated rates of LeadOff™ + glyphosate + 2,4-D, while

applying 39.21 g ai ha^{-1} rate of Harmony® Extra SG and 2. using five different common agricultural hose types to sequester LeadOff™ + glyphosate + 2,4-D, using common cleanout practices and then adding Harmony® Extra SG to the hoses for application in wheat. The objective was to simulate a range of contamination concentrations of a burndown treatment of glyphosate + LeadOff™ + 2,4-D that might still be present in a sprayer while applying a postemergence application of Harmony® Extra SG to winter wheat

Materials and Method

Titrated Rates of LeadOff, glyphosate and 2,4-D

Field studies were conducted in 2013, 2014 and 2015 to evaluate LeadOff™ (rimsulfuron and thifensulfuron) applied to winter wheat at various concentrations. Experiments were conducted at the Black Belt Branch Experiment Station in Brooksville, MS on a Brooksville silty clay (Fine, smectitic, thermic Aquic Hapluderts) with 7% sand, 48% silt, 45% clay, 2.3% organic matter and pH of 7.2. Winter wheat variety brand SS600 (Hurt Seed Company, INC. 1210 Industrial Rd. Halls TN 38040) was drilled at 101 kg ha^{-1} with a 18 cm row spacing. Concentrations of the burndown active ingredients were titrated while holding the Harmony® Extra SG concentrations constant. LeadOff™ concentrations were titrated alone as well as in combination with 2,4-D and glyphosate.

Herbicide treatments consisted of 17.58, 8.73, 2.195, 0.548, 0.136 and 0 g ai ha^{-1} of LeadOff™ product (Du Pont de Nemours and Co., 1007 Market Street, Wilmington, DE 19898). The constituents of LeadOff™ were rimsulfuron at 25% and thifensulfuron-methyl at 50%. Therefore, concentrations of 11.72, 5.82, 1.463, 0.365, 0.0906 and 0 g ai ha^{-1} of rimsulfuron plus 5.86, 2.910, 0.73, 0.1827, 0.045 and 0 g ai ha^{-1} of thifensulfuron-

methyl were used, respectively. Touchdown Total® (Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419) concentrations consisted of 0.43, 0.218, 0.0548, 0.0136, 0.0034 and 0 kg ae ha^{-1} of glyphosate. Weedar 64® (Nufarm Americas 11901 South Austin Avenue Alsip, IL 60803) concentrations consisted of 0.398, 0.199, 0.0498, 0.0124, 0.0031 and 0 kg ae ha^{-1} of 2,4-D. Harmony® Extra SG (Du Pont de Nemours and Co., 1007 Market Street, Wilmington, DE 19898) was applied in all treatments at a rate of 39.21 g ai ha^{-1}. The constituents of Harmony® Extra SG were thifensulfuron-methyl at 50% and tribenuron-methyl at 50%. Therefore, thifensulfuron-methyl was applied at 26.14 g ai ha^{-1} and tribenuron-methyl was applied at 13.07 g ha^{-1}. Non-ionic surfactant (Induce®, Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017) at 0.25% v v^{-1} was included.

Herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR80015 flat-fan nozzle (TeeJet Technologies, PO Box 7900, Wheaton, IL 60187) at an application volume of 140 l ha^{-1} and a pressure of 220 kPa. Herbicide treatments were applied when wheat plants were at Feekes 4 growth stage. Visual estimates of wheat injury were recorded 7, 14, 21, and 28 days after treatment (DAT), using a scale of 0 to 100%, where 0 = no injury and 100 = total plant death. Chlorosis, necrosis, height reductions and regrowth were visually evaluated to estimate injury. Plant height and plant height reduction from the check were collected 7, 14, 21 and 28 DAT. Wheat was machine harvested to determine yield and percent reductions were calculated.

The experiment was arranged as a split-plot arrangement of treatments in a randomized complete block with factor A consisting of LeadOff™ alone or LeadOff™ + 2,4-D + glyphosate. Factor B consisted of a rate titration of LeadOff™, 2,4-D and

glyphosate. Four replications for each treatment were used in the experiment with a plot size area of 2 by 9 m^{-1}. Data were pooled across years because experimental replication was considered a random variable. Untransformed and arcsine square root transformed data were subjected to analysis of variance, but interpretations were similar to untransformed data; therefore, untransformed data were used for analysis. Data were analyzed using PROC GLM in SAS 9.4 and means were separated using Fischer's protected LSD test at P = 0.05.

Hose Sequestration of LeadOff™, glyphosate and 2,4-D

Field studies were conducted in 2013, 2014 and 2015 to evaluate sequestration potential of five types of agricultural spray hoses. Experiments were conducted at the Black Belt Branch Experiment Station in Brooksville, MS. Winter wheat variety brand SS600 (Hurt Seed Company, INC. 1210 Industrial Rd. Halls TN 38040) was drilled at 101 kg ha^{-1} with a 18 cm row spacing.

Each hose was 1.5 m and had an inside diameter of 1.3 cm, which is enough carrying capacity to deliver a sufficient volume to treat a plot size area of 2 by 6 m. Length of the hoses were determined by figuring total volume needed to cover the plot area and using the formula $H=V/\pi r^2$. Where H is height, V is total volume and r is the radius of the hose opening. Hose types include **John Deere PMK 4131- 08** (Yellow/PVC-high tensile strength yarn-1 ply), **John Deere PMA 4086-08** (Blue/Linear/low-density polyethylene blend) (LLDPE), **John Deere PMA 1687-08** (Green/PVC/polyurethane-high tensile-strength yarn-2 ply), **John Deere PMA 1628-08** (Grey/PVC/polyurethane blend-high tensile-strength yarn-2 ply), and a **Goodyear hose** (Black/Versigard Synthetic Rubber). Each hose end was coupled with a female

pneumatic coupling to allow for sequestration of the solution within each hose and to prevent leakage.

For wheat analysis, spray lines were filled with LeadOff™ constituents totaling 35.17 g ai ha^{-1} using the same formulation as the previous study. Therefore, concentrations of 23.45 g ai ha^{-1} of rimsulfuron and 11.72 g ai ha^{-1} of thifensulfuron-methyl were used to constitute the LeadOff™ product. Touchdown Total® and Weedar 64® were added to the herbicide mixture along with LeadOff™ at rates of 0.87 and 0.79 kg ae ha^{-1}, respectively, and left to incubate for 48 hours. The spray solution was then flushed out of all lines and cleaned with one of three cleanout procedures: no-cleanout, water cleanout or ammonia cleanout at a rate of 5.67 l of water per line to simulate an actual in field cleanout procedure and then left to incubate in their designated cleaning solution for 24 hours. For the ammonia cleanout a 1% solution consisting of 10 ml of ammonia per l^{-1} of water was used. After 24 hours, lines were flushed of the designated cleaning solution and were left empty for 48 hours. The spray lines were then filled with Harmony® Extra SG at a rate of 39.21 g ai ha^{-1}. The same formulation of Harmony® Extra SG described in the previous experiment was used in this experiment. Therefore, thifensulfuron-methyl was applied at 26.137 g ai ha^{-1} and tribenuron-methyl was applied at 13.072 g ha^{-1} throughout the study. Non-ionic surfactant at 0.25% v v^{-1} was included with Harmony® Extra SG treatments. This solution was then incubated for 48 hours to aid in the release of any sequestered herbicides before collection. The solution from each hose type by cleanout procedure was then collected using CO_2 to push the solution from each hose to a collection bucket. A 10 ml aliquot was then taken from each collection bucket for analytical analysis performed by DuPont using High Performance Liquid

Chromatography (HPLC) to determine residual rimsulfuron and 2,4-D. The remaining solution was then placed in 355 ml bottles and applied to wheat before flag leaf emergence. Each hose type by cleanout combination was replicated three times; in essence, there was only one hose type per cleanout procedure per rep. Hoses were used for the same treatment from one year to the next in the entirety of the study.

Herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR80015 flat-fan nozzle at an application volume of 140 l ha^{-1} and a pressure of 220 kPa. Herbicide treatments were applied when wheat plants were at Feekes 4 growth stage. Visual estimates of wheat injury were recorded 7, 14, 21, and 28 days after treatment (DAT), using a scale of 0 to 100%, where 0 = no injury and 100 = total plant death. Chlorosis, necrosis, height reductions and regrowth were visually estimated. Plant height and plant height reduction from the check were collected 7, 14, 21 and 28 DAT. Wheat was machine harvested for yield and yield reductions were calculated.

The experiment was arranged as a factorial arrangement of treatments in a randomized complete block with factor A consisting of hose type and factor B consisting of cleanout procedure. Three replications for each treatment were used in the experiment with a plot size area of 2 by 6 m. Data were pooled across years because experimental replication was considered a random variable. Untransformed and arcsine square root transformed data were subjected to analysis of variance, but interpretations were similar to untransformed data; therefore, untransformed data were used for analysis. Data were subjected to ANOVA in SAS 9.4 and means were separated using Fischer's protected LSD test at P = 0.05.

Results and Discussion

Titrated Rates of LeadOff, glyphosate and 2,4-D

The interaction of herbicide and herbicide rate was significant for rating dates dealing with percent injury. Percent injury 7 DAT was different within the 1/2X and 1/4X rate when comparing LeadOff™ alone vs LeadOff™ + glyphosate + 2,4-D (Table 2.1). Wheat injury 14 DAT was different at the 1/2X, 1/4X and 1/16X rate when comparing LeadOff™ alone vs LeadOff™ + glyphosate + 2,4-D (Table 2.1). Percent VEOI at 21 and 28 DAT showed a difference at the 1/2X, 1/4X, 1/16X, and 1/64X rate when comparing LeadOff™ alone vs LeadOff™ + glyphosate + 2,4-D (Table 2.1). Injury from the 1/2X rate of LeadOff™ + glyphosate + 2,4-D increased incrementally from each observed week on average of 20% to total 84% injury in the final rating date when compared to LeadOff™ alone which had a 45% visual rating at 28 DAT (Table 2.1). The difference of percent VEOI observation is contributed to the addition of the glyphosate to the herbicide treatment. There was 40% reduction in injury based on visual estimations and percent yield loss with LeadOff™ alone versus LeadOff™ + glyphosate + 2,4-D at the 1/2X rate (Figure 2.1). It is the same for the 1/4X rate where a 38% decrease in the VEOI for LeadOff™ alone vs LeadOff™ + glyphosate + 2,4-D (Table 2.1) which is representative of the percent yield loss (Figure 2.1). These findings are similar to Deeds et al. (2006), Roider et al. (2007) and Orr et al. (1996) who stated that VEOI on wheat in response to glyphosate is an accurate indicator of yield loss. The 1/16X and 1/64X rate are differerent in percent VEOI with respect to herbicide treatment (Table 2.1) but do not differ within rate with respect to percent yield reduction (Figure 2.1). Visual observations 28 DAT with respect to LeadOff™ alone show no difference in VEOI with comparison of the

check once the rimsulfuron rate is reduced from 1.463 g to 0.365 g ai ha^{-1} (Table 2.1). Observations 28 DAT of VEOI is increased to the 1/64X rate with the addition of glyphosate and when the rate of glyphosate is reduced from 0.0136 kg to 0.0034 kg ae ha^{-1} (table 2.1). LeadOff™ concentrations of greater than 1/64X rate resulted in injury and reductions in yield; however, when glyphosate + 2,4-D were present, VEOI and yield were generally affected at concentrations above 1/256X.

Percent height reduction from the check due to rimsulfuron and glyphosate applications were observed at each rating date. LeadOff™ treatments resulted in a 28% height reduction at the 1/2X rate when compared to the check (Table 2.2). There is a 36% height reduction on average at the 1/2X rate when compared to the check. Rates of 1/16X or greater resulted in plant height reduction 14 DAT. The rate of 1/64X was 15% greater than that of the check with respect to height reduction 14 DAT (Table 2.2). Height reductions 21 DAT averaged over both LeadOff™ treatments resulted in a 50% height reduction on average at the 1/2X rate when compared to the check. All rates reduced plant heights 21 DAT when compared to the check. The rates of 1/2X, 1/4X, 1/16X and 1/64X showed a 73, 64, 50, and 31% reduction in height 28 DAT respectively when glyphosate is added (Table 2.2). When LeadOff™ alone was applied at the 1/2X, 1/4X, and 1/16X rate there was a 52, 47 and 34% reduction in height, respectively (Table 2.2). Height reductions are greatest at rates above 1/64X 28 DAT when comparing LeadOff™ alone vs LeadOff™ + glyphosate + 2,4-D (Table 2.2).

Total wheat yield from the check averaged 2100 kg ha^{-1} and showed a decrease in yield at the 1/2, 1/4, and 1/16X rate of LeadOff™ + glyphosate + 2,4-D, but does not completely explain the 31% reduction in height at the 1/64X rate needed to reduce yields

(Figure 2.1). When observing yields from LeadOff™ alone there is a reduction in yield at the 1/2 and 1/4X rates but this does not fully explain a 34% reduction in height at the 1/16X rate needed to coorespond to a yield reduction (Figure 2.2). These results are consistent with Protić et al. (2006), where a greater than 40% yield reduction from check was observed when rimsulfuron was added in the early leaf stage of wheat at a rate of 6.2 to 12.5 g ai ha^{-1}, as we observed a 48% reduction in yield with only 1.463 g ai ha^{-1} at the 1/16X rate of LeadOff™ alone.

Hose Sequestration of LeadOff™, glyphosate and 2,4-D

There were no differences in injury, plant height or height reduction, yield or yield reduction with respect to rimsulfuron sequestration within different agricultural hoses by standard cleanout procedures. When analytical analysis was performed, rimsulfuron was not different among hose type and cleanout treatments.

Analytical analysis showed differences among hose type (Figure 2.2) and cleanout procedures (Figure 2.3) with respect to 2,4-D ppm analyte retained. Data pooled across years and across cleanout procedures indicated that yellow, green and black hoses have greater sequestration potential than that of the blue hose and check respectively (Figure 2.2). Cleanout data pooled across years and hose type indicate no differences between water and ammonia cleanout with respect to 2,4-D ppm analyte retained (Figure 2.3). The lack of any cleanout procedure occurring with the no-cleanout treatment did result in an increase in the 2,4-D analyte when compared to the water and ammonia treatments (Figure 2.3).

Sulfonylurea herbicides, and in particular rimsulfuron, are residual herbicides that breakdown rapidly in soil and have a very short half-life in plant materials (Senseman

2007). The data suggests that tank contamination will occurr within a small number of sprayers used to apply LeadOff™ herbicide on corn, cotton or soybean and then used to apply other crop protection products on winter wheat. If the sprayer is used in an early season preemergent burndown application and not thoroughly cleaned, residual herbicide could persist in many parts of the sprayer (tank, pumps, valves, hoses, nozzles, screens, end caps etc.). Considering that LeadOff™ is a water soluble granule and agitation is needed for the granule to completely dissolve, agitation times are dependent on water temperature (Anonymous 2011). If the temperature of the water in the tank is less than 4.4 ºC and agitation is less than five minutes, the herbicide persistence could be a problem (Anonymous 2011). Compounding this problem is thorough dilution of the product and how many times the spray rig was used following the early season preemergent burn down. If the sprayer was not thoroughly cleaned prior to the application of Harmony® Extra SG and allowed to sit for months in the sprayer then residual rimsulfuron could be the problem and has been observed in certain sprayers with end caps (Figure 2.4).

Table 2.1 Visual estimation of injury from rimsulfuron and glyphosate 7, 14, 21 and 28 DAT at low rates to simulate tank contamination effects.

			Days after treatment[a]							
			7		14		21		28	
Relative rate	Rimsulfuron rate[b]	Glyphosate rate[b]	LeadOff alone[c]	LeadOff + glyphosate+ 2,4-D[d]	LeadOff alone	LeadOff + glyphosate+ 2,4-D	LeadOff alone	LeadOff + glyphosate+ 2,4-D	LeadOff alone	LeadOff + glyphosate+ 2,4-D
	--g ai ha^{-1}--	-kg ae ha^{-1}-	----------%----------							
1/2X	11.72	0.43	5cd	26a	17c	41a	27c	64a	45b	84a
1/4X	5.82	0.218	8c	14b	15c	33b	24c	48b	35b	73a
1/16X	1.463	0.0548	0e	2de	8d	13c	16d	27c	16c	44b
1/64X	0.365	0.0136	0e	0e	0e	3de	1e	12d	3cd	32b
1/256X	0.0906	0.0034	0e	0e	0e	0e	0e	0e	2cd	0d
0X[e]	0	0	0e	0e	0e	0e	0e	0e	0d	0d

[a] means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column.
[b]rimsulfuron and glyphosate are not labeled in wheat and cause injury potential
[c] a 1X rate of LeadOff alone consists of 23.44 g ai ha^{-1} rimsulfuron and 11.72 g ai ha^{-1} thifensulfuron-methyl
[d] a 1X rate of glyphosate and 2,4-D consists of 0.86 kg ae ha^{-1} and 0.56 kg ae ha^{-1}, respectively
[e] untreated check treatments

Table 2.2 Wheat height reduction from rimsulfuron and glyphosate 7, 14, 21 and 28 DAT.

Relative rate	Rimsulfuron rate[b]	Glyphosate rate[b]	Days after treatment[a]				
			7	14	21	28	
						LeadOff alone[c]	LeadOff + glyphosate + 2,4-D[d]
	--g ai ha^{-1}--	-kg ae ha^{-1}-	----------%----------				
1/2X	11.72	0.43	28a	36a	50a	52b	73a
1/4X	5.82	0.218	20ab	30a	49a	47b	64a
1/16X	1.463	0.0548	20b	27a	36b	34c	50b
1/64X	0.365	0.0136	12b	15b	19c	11de	31c
1/256X	0.0906	0.0034	14b	7bc	9d	9de	11de
0X[e]	0	0	0c	0c	0e	0e	0e
Herbicide							
LeadOff alone			13b		32a		
LeadOff + glyphosate + 2,4-D			19a		23b		

[a] means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column.
[b] rimsulfuron and glyphosate are not labeled in wheat and cause percent height reduction potential
[c] a 1X rate of LeadOff alone consists of 23.44 g ai ha^{-1} rimsulfuron and 11.72 g ai ha^{-1} thifensulfuron-methyl
[d] a 1X rate of glyphosate and 2,4-D consists of 0.86 kg ae ha^{-1} and 0.56 kg ae ha^{-1}, respectively
[e] untreated check treatments

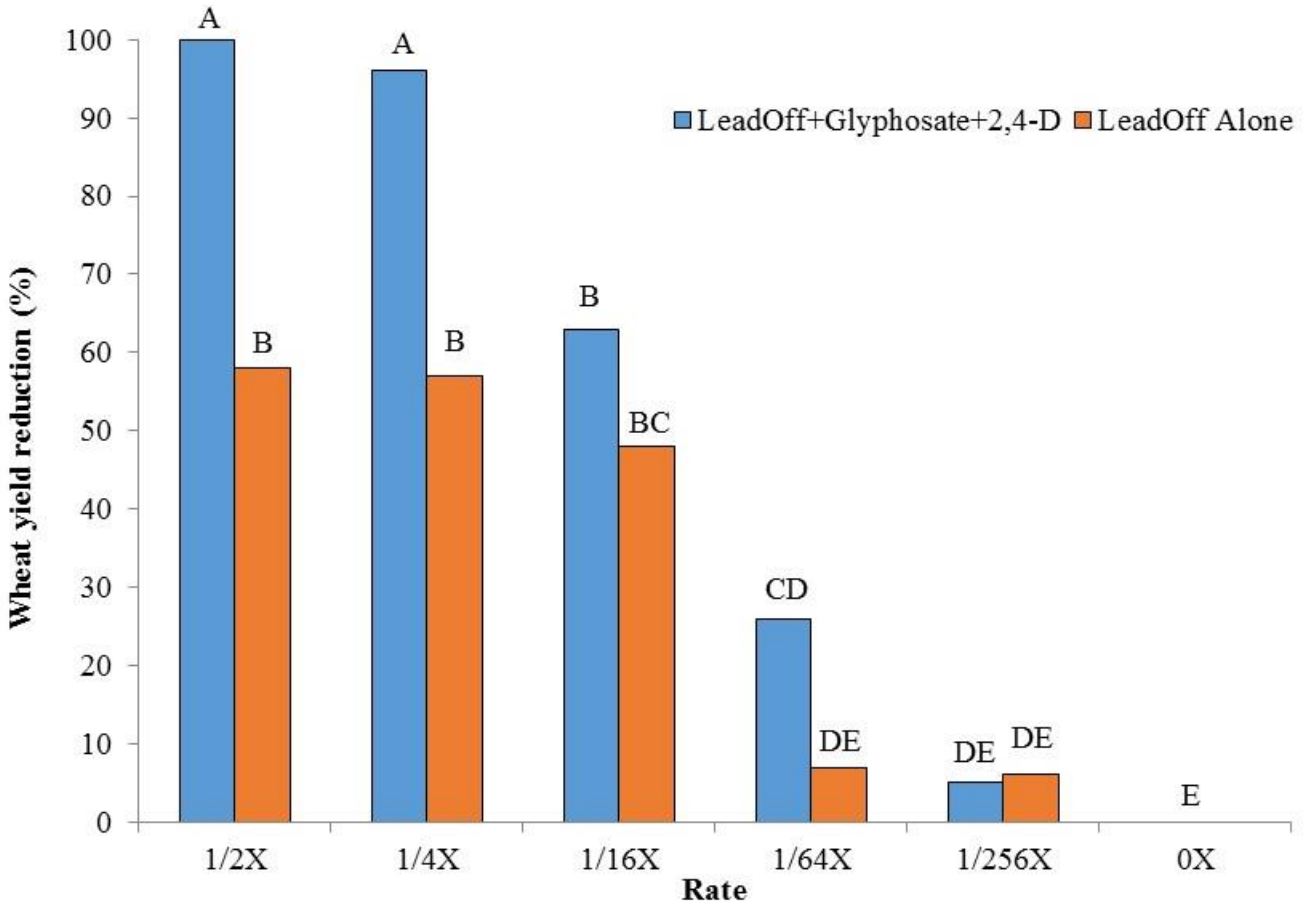

Figure 2.1 Wheat yield reduction from LeadOff™ (23.44 g ai ha^{-1} rimsulfuron and 11.72 g ai ha^{-1} thifensulfuron-methyl) alone and with glyphosate (0.86 kg ae ha^{-1}) + 2,4-D (0.56 kg ae ha^{-1}) at rates similar to what would occur from contamination.

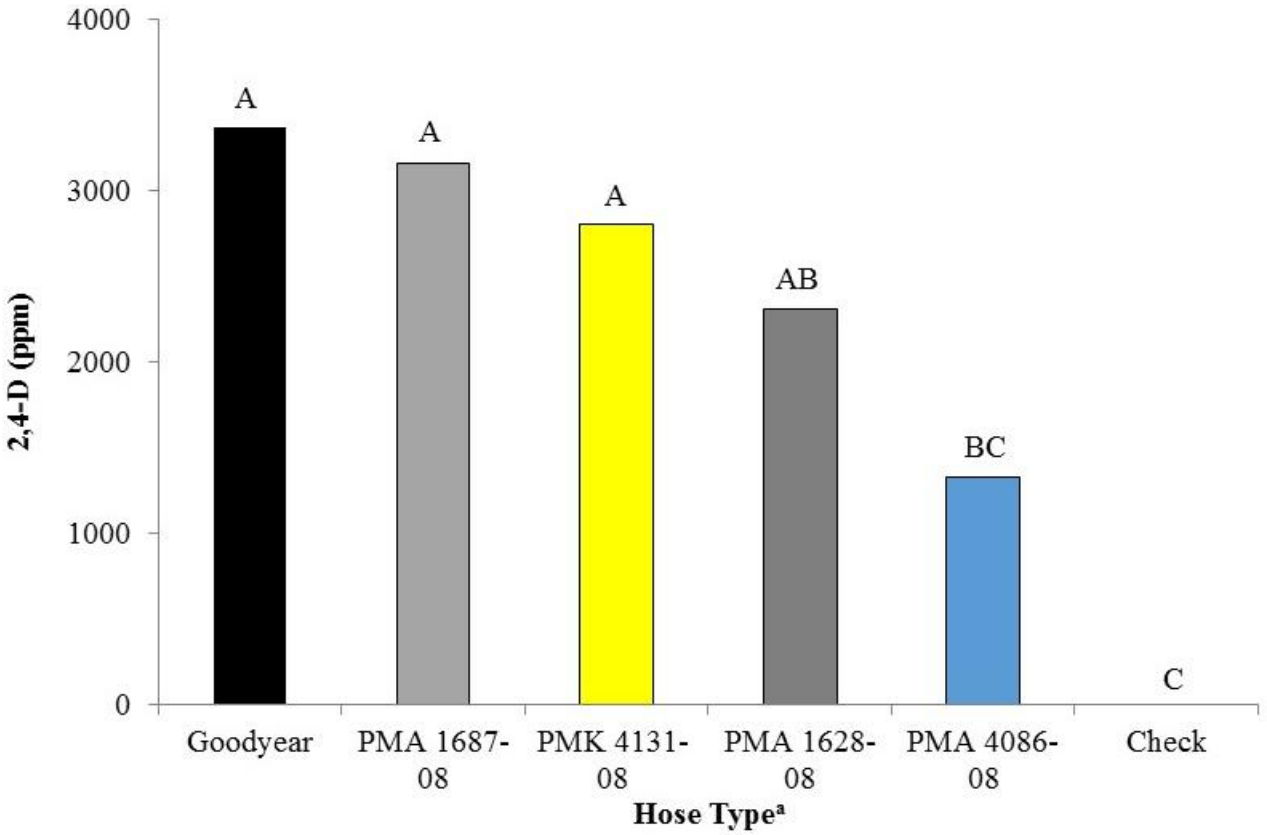

Figure 2.2 The effect of hose type on 2,4-D retention when averaged over cleanout procedures.

[a]John Deere PMK 4131- 08 (Yellow), John Deere PMA 4086-08 (Blue) (LLDPE), John Deere PMA 1687-08 (Green), John Deere PMA 1628-08 (Grey), and Goodyear (Black)

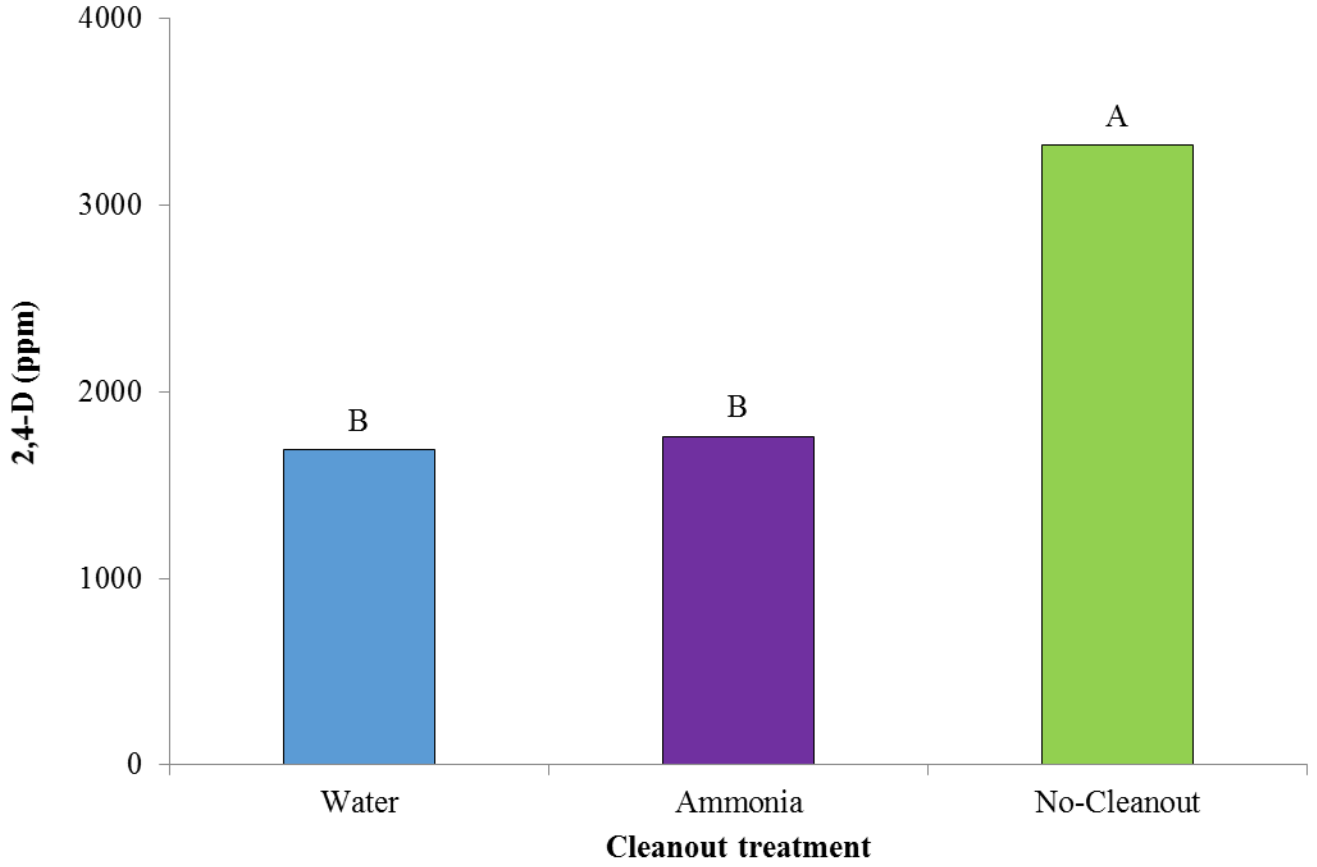

Figure 2.3 The effect of cleanout procedure on 2,4-D retention when averaged over hose type.

Figure 2.4 Visual observation of sprayer contamination with LeadOff™ + glyphosate + 2,4-D before and after initial cleanout.

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CHAPTER III

EVALUATION OF DICAMBA PERSISTANCE AMONG VARIOUS AGRICULTURAL HOSE TYPES AND CLEANOUT PROCEDURES USING SOYBEAN (*GLYCINE MAX* MERR.) AS A BIO-INDICATOR

Abstract

Synthetic rubbers, synthetic plastic polymers (Polyvinyl chlorides (PVC)), polyurethane blends and polyethylene blends comprise modern day agricultural spray hoses. The objective of this study was to determine if agricultural hose types would differ with respect to dicamba sequestration. Field and greenhouse studies were conducted to evaluate the sequestration-potential of dicamba within five agricultural hose types when cleaned with different cleanout procedures. Rinsate solutions were applied to soybean, which was used as a bio-indicator to test for cleanout efficiency. Differences among hose types and cleanout procedures exist with observations including visual estimations of injury (VEOI), height reduction, dry matter, yield, and ppm analyte retained. The makeup of PVC polyurethane blend and synthetic rubber blend hoses increased retention of dicamba analyte when compared to the polyethylene blend hose. No differences were observed by the addition of ammonia to the cleanout solution when compared to water alone. Differences in a hose type's ability to sequester the dicamba analyte may have

more to do with the hoses internal chemical composition and the manufacturing process rather than wear and tear. Scanning electron microscopy revealed imperfections in new PVC polyurethane and synthetic rubber hoses, which eventually lead to inner wall depletion of these hose types. This is in contrast to what was found in the polyethylene blend hose type, in which the inner wall is smooth and free of imperfections.

Nomenclature: Dicamba; 3,6-dichloro-2-methoxybenzoic acid; glyphosate; *Amaranthus palmeri*; soybean, *Glycine max* L.; Linear/low-density polyethylene blend (LLDPE); PVC-high tensile strength; PVC/polyurethane blend; Versigard Synthetic Rubber

Key words: Plant growth regulating herbicides, contamination, sequestration, tank contamination, drift, volitization, interaction

Introduction

Genetically modified Roundup Ready® (RR) crops have revolutionized weed control and no-till practices (Johnson et al. 2012a). Roundup Ready® crops are resistant to the herbicide glyphosate (N-(phosphonomethyl)glycine) (Senseman 2007), enabling producers to spray the herbicide postemergence throughout the growing season and achieve broad spectrum weed control. Roundup Ready® soybean was introduced in the United States in 1996 followed shortly thereafter by RR cotton and RR corn with additional crops (including canola and sugar beet) also being released (Johnson et al. 2012a). However, after glyphosate applications over many years and millions of hectares, the widespread evolution of weed populations resistant to glyphosate has occured (Johnson et al. 2012a). Glyphosate-resistant weeds such as Palmer amaranth (*Amaranthus palmeri* S. Wats.), horseweed (*Conyza canadensis* (L.) Cronq.), common ragweed

(*Ambrosia artemisiifolia* L.), and giant ragweed (*Ambrosia trifida* L.) (Heap 2013) are examples of difficult to control weeds that have forced producers to use other means of control. In response to the evolution of glyphosate-resistant weeds, companies have been developing new methods of weed control. Companies are searching for new active ingredients and modes of action, but the cost of development and the limited potential for economic return have made it difficult to bring new products to market (Johnson et al. 2012a). These companies have been on the forefront of genetically engineered crops, resistant to herbicides other than glyphosate. The introduction of dicamba and 2,4-D resistant crops was initiated because these herbicides have shown excellent resilience with few herbicide-resistant weeds occurring after greater than 50 years of use (Johnson et al. 2012a). Secondly, 2,4-D and dicamba provide excellent control of glyphosate-resistant broadleaf weeds such as horseweed, giant ragweed, common waterhemp (*Amaranthus rudis* Sauer), and other broadleaf weeds (Johnson et al. 2012b).

Auxin herbicides, such as 2,4-D and dicamba, have little soil residual activity (Senseman 2007). These herbicides have been extensively used for weed control primarily due to their selectivity, wide spectrum of control, efficacy, and low application costs (Mithila et al. 2011). Auxin herbicides mimic natural occurring auxin, which is a plant growth hormone central to regulating plant growth and development (Abel and Theologis 1996). Auxin herbicides, also commonly known as synthetic auxins, mimic the plant growth hormone indole-3-acetic acid (IAA); disrupting growth and development processes, eventually causing plant death (Senseman 2007). Auxin herbicides are readily taken up by the roots and foliage and are translocated in both the phloem and xylem. 2,4-D controls broadleaf species such as carpetweed (*Mollugo verticillata* L.), horseweed

(*Conzya canadensis* (L.) Cronq.), pigweed (*Amaranthus spp.*), and velvetleaf (*Abutilon theophrasti* Medik.), among many other problematic weed species found in cropping systems (Senseman 2007). Dicamba is most commonly used to control annual broadleaf weeds such as pigweed (*Amaranthus spp.*), wild buckwheat (*Polygonum convolvulus* L.), and lambsquarters (*Chenopodium album* L.); higher rates of dicamba are capable of controlling perennial broadleaf weeds such as field bindweed (*Convolvulus arvensis* L.) (Senseman 2007). Symptomology from auxin herbicides include: swollen stems, cupped leaves, epinastic twisting of stems and petioles, chlorosis, and/or necrosis (Senseman 2007; Wax et al. 1969; Robinson et al. 2013; Egan et al. 2014).

In 2013, the state of Mississippi harvested 0.8 million hectares of soybeans averaging 2,825 kg per hectare with the value of production at $1.2 billion (USDA-NASS 2012). Soybean growth is split into two stages, vegetative and reproductive, and within each stage there are more specific subcategories. Soybean reproductive growth stages are more important for soybean yield determination; the reproductive growth stages are when the seed number and size are determined (Pederson 2004). Reproductive growth stages begin when the first flower on the stem is present, referred to as the (R1) growth stage, which is where the first pod will eventually form on the plant. The reproductive growth stage (R2) will form when there is an open flower at one of the two uppermost nodes on the main stem with a fully developed leaf. Reproductive growth stage (R3) will be determined when the pod reaches a length of 0.5 cm long and will appear in the upper four nodes of the soybean plant (Koger et al. 2013). The typical PGR injury symptoms in soybeans can be identified by the characteristic cupping of leaves with dicamba and injury can range from cosmetic leaf injury to 80% yield loss, depending on the amount of

PGR residue left in the tank and the crop growth stage at application (Steckel et al. 2005). Soybeans exposed to 2,4-D or dicamba can develop vegetative malformations and produce a lower yield; however, the extent of that damage is dependent upon rate and application timing (Andersen et al. 2004). Wax et al. (1969) found that soybean is susceptible to dicamba application at both vegetative and reproduction stages. Injury due to herbicide does not always lead to yield loss (Al-Khatib and Peterson 1999); soybean has the ability to recover from early season injury depending on rate and application timing (Weidenhamer et al. 1989). Reduced soybean yield from dicamba exposure has been reported when dicamba caused severe injury and stunting, while yield reductions greater than 10% coincided with severe VEOI (Al-Khatib and Peterson 1999), such as terminal bud kill, splitting of the stem, swollen petioles, and curled malformed pods (Weidenhamer et al. 1989).

Anderson (2004) concluded that soybean sprayed with dicamba at V3 resulted in at least 40% VEOI 48 DAT at a rate of 0.0056 kg ae ha^{-1} with a 14 percent yield reduction. Dicamba was also applied at 0.0112 and 0.056 kg ae ha^{-1}, resulting in 13.8 and 71.5 percent yield reduction, respectively. Application of 2,4-D at V3 with the same application rates as dicamba showed yield reductions of 0, 7.2, and 31.7% respectively; VEOI ranged from 5 to 30% 48 DAT. The study concluded that VEOI and yield reduction were greater from dicamba applications versus 2,4-D.

Monsanto has introduced MON 87708 soybean, which was genetically engineered from A3525, a high-yielding soybean variety to be resistant to dicamba by expressing a dicamba mono-oxygenase gene (DMO) from *Strenotrophomonas maltophilia* that rapidly demethylates dicamba, rendering it inactive (Johnson et al. 2012a; Behrens et al. 2007;

USDA 2014). Their Roundup Ready Plus 2 Xtend System® will contain the Genuity® Roundup Ready 2 Yield® trait technology stacked with a trait enabling tolerance to dicamba (Monsanto 2013). By using an *agrobacterium* gene transfer, plants can be inserted with a gene that allow the breakdown of dicamba within the plant (Behrens et al. 2007).

The introduction of new herbicide tolerant crops may provide many benefits for producers such as alternative control options for resistant weed species, decreased costs, and different modes of action. Along with these benefits, the use of auxin containing herbicides may also increase concern for issues such as herbicide drift, volatilization, and tank contamination. The adjuvant and solvent system utilized in several commercial herbicides often result in the release of herbicides which have been sequestered within the spray system thus resulting in injury to sensitive crops. Injury from PGR herbicide tank residue most often occurs to cotton and soybean with the first tank of post applied herbicide (Steckel et al. 2005). Due to their chemical makeup, several herbicides, most notably Roundup WeatherMax® (glyphosate), are very effective tank cleaners for PGR herbicides (Steckel et al. 2005). Unlike glyphosate, which is very water soluble and can be easily cleaned out of a sprayer with water, the PGR herbicides, although being highly water soluble, act as weak acids and take a lot more time, care and effort to be removed (Steckel et al. 2005). Kelley and Riechers (2003), found that as little as 1/10,000 of the 280 g ae ha^{-1} dicamba rate can produce injury symptoms on soybeans. Compounding this problem, is spray contamination caused by a failure to thoroughly clean a sprayer can cause crop injury up to several months after initial use (University of Illinois extension 2006). Boerboom (2004), showed that dicamba residues, even when an ammonia-water

solution was used, had a subsequent use rate of 0.024% from the tank and 0.63% from the spray boom when refilled with water.

Broadleaf weed control is commonly accomplished by producers using dicamba and 2,4-D. With moderate volatility and high water solubility these compounds may exist in harvested food crops, ground water and eventually water ways. Historically, liquid chromatographic (LC) detectors available to regulatory laboratories lacked the capability to measure at trace levels, and interfering components were common (Takino et al. 2001). Gas chromatography (GC) became the instrument of choice (EPA 1986; EPA 1993). While the sensitivity was adequate, the GC technique lacked reliable selectivity and was prone to problematic data interpretation (Schaner et al. 2007). In addition, derivatization of the nonvolatile acid to the ester form for GC analysis caused difficulties, and the extra step reduced method efficiency (Takino et al. 2001; Hopper 1987; Lee et al. 1991).

Eun-Ho Shin et al. (2010) ran an analytical method using high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector for a simultaneous analysis of three different analytes which included dicamba, coming from samples of Chinese cabbage, apple and pepper fruits, soybeans and brown rice. Liquid-liquid partitioning and column cleanup procedures were used with residue confirmation coming from tandem mass spectrometry (MS/MS) in ion electrospray ionization (ESI) mode (Eun-Ho Shin et al., 2010). The extraction of residues from foods depends on the polarity of the herbicide as well as the sample matrix type (Tadeo et al. 2000).

Scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of a solid specimen. The signals from electron-sample interactions reveal information about the sample such as texture,

chemical composition, and structure of materials making up the sample (Goldstein 2003). Data are collected over a selected surface area, and a 2-dimensional image is generated that displaying spatial variation (Goldstein 2003). Areas ranging from approximately 5 microns to 1 cm in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm) (Goldstein 2003). The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (Reimer 1998).

Considering that soybean is extremely sensitive to PGR herbicides, it is imperative that a quality clean-out technique becomes the standard among producers. If producers do not perform proper application practices, there will likely be many incidents where injury to susceptible crops will occur due to tank contaminations and improper application practices (Johnson et al. 2012a). Therefore the objectives of this study were to investigate cleaning methods and hose types used in an agronomic row crop settings and to quantify the lowest amount of dicamba herbicide residue needed to cause economic injury to soybean.

Materials and Method

Field and Greenhouse Experiments

Field studies were conducted in 2012, 2013 and 2015 to evaluate the sequestration potential of five agricultural hose types and different cleanout procedures while using dicamba. In 2012, a preliminary study was conducted to determine whether the five hose type's lended to any differences with respect to injury in soybean. After preliminary results indicated differences among hose types, the experiment was replicated and data

were omitted from the preliminary trial. In 2013 and 2015, the experiment was conducted at the Black Belt Branch Experiment Station in Brooksville, MS on an Okolona silty clay (Fine, smectitic, thermic Oxyaquic Hapluderts) with 8% sand, 51% silt, 41% clay, 2% organic matter and pH of 6.8 and a Brooksville silty clay (Fine, smectitic, thermic Aquic Hapluderts) and the R. R. Foil plant research center in Starkville, MS on a Marietta fine sandy loam (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) with 71% sand, 17% silt, 13% clay and 1.03% organic matter and a pH of 5.9. Differences from 2012-2013 and 2015 involved the addition of an extra cleanout procedure and the addition of a rate titration followed by aqueous sample collection and analytical analysis. Planting date, planting populations, and seed variety varied among locations (Table 3.1).

Field studies conducted in 2012 and 2013 involved five different types of agricultural spray hoses by two cleanout procedures (water and ammonia). Each hose measures 3 m in length and had an inside diameter of 1.3 cm, which is enough carrying capacity to deliver a sufficient volume to treat a plot size area of 2 by 12 m. Hose types include **John Deere PMK 4131- 08** (Yellow/PVC-high tensile strength yarn-1 ply), **John Deere PMA 4086-08** (Blue/Linear/low-density polyethylene blend) (LLDPE), **John Deere PMA 1687-08** (Green/PVC/polyurethane-high tensile-strength yarn-2 ply), **John Deere PMA 1628-08** (Grey/PVC/polyurethane blend-high tensile-strength yarn-2 ply), and a **Goodyear hose** (Black/Versigard Synthetic Rubber). Each hose end was fitted with a female pneumatic coupling to allow for sequestration of the solution within each hose and to prevent leakage. Field studies in 2015, involved the same hose types previously mentioned and added a cleanout (water, ammonia, and no-cleanout) along with a rate titration of dicamba at 0.56, 0.140, 0.0087, and 0.0022 kg ae ha^{-1} to use for comparison.

Samples were collected from each hose type by cleanout procedure and rate titration. Analysis was performed on High Performance Liquid Chromatography to the Mass Spec (HPLC-MS).

In 2013 and 2015 herbicide treatments consisted of dicamba (Engenia®, 600 g l^{-1}, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) at 0.56 kg ae ha^{-1}. In all years, glyphosate (Roundup WeatherMax®, 540 g ae l^{-1}, Monsanto Company, St. Louis, Missouri, 63167) was applied at 1.1 kg ae ha^{-1}.

For soybean analysis, spray lines were filled with dicamba and glyphosate at a rate of 0.56 and 1.1 kg ae ha^{-1}, respectively and left to incubate for 48 hours. The spray solution was then flushed out of the lines and cleaned with one of three cleanout procedures: no-cleanout, water cleanout or ammonia cleanout at a rate of 11.35 l of water per line to simulate an actual in field cleanout procedure and then left to incubate in their designated cleaning solution for 24 hours. For the ammonia cleanout a 1% v/v solution was used. After 24 hours, lines were flushed of the designated cleaning solution and left empty for 48 hours. The spray lines were then filled with glyphosate at a rate of 1.1 kg ae ha^{-1}. This solution was then incubated for 48 hours to aid in the release of any sequestered herbicides before collection. The solution from each hose type by cleanout procedure was then collected using CO_2 to push the solution from each hose to a collection bucket. A 10 ml aliquot was then taken from each collection bucket for analytical analysis. The remaining solution was then applied to soybean at the R2 growth stage. Each hose type by cleanout combination was replicated three times; in essence, there was only one hose type per cleanout procedure per replication. Hoses were used for the same treatment from one year to the next throughout the entirety of the study.

Herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with TTI110015 wide angle, air induction, tapered flat spray tip (TeeJet Technologies, PO Box 7900, Wheaton, IL 60187) at an application volume of 140 l ha^{-1} and a pressure of 220 kPa. Visual estimates of soybean injury were recorded 7, 14, 21, and 28 days after treatment (DAT), using a scale of 0 to 100%, where 0 = no injury and 100 = total plant death. Chlorosis, necrosis, stunting, leaf cupping, epinasty, height reductions and regrowth were visually evaluated to estimate injury. Plant height and plant height reduction from the check were collected 7, 14, 21 and 28 DAT. Soybean was machine harvested where yield and yield reduction were calculated.

The experiment was arranged as a factorial arrangement of treatments in a randomized complete block with factor A consisting of hose type and factor B consisting of cleanout procedure. The rate titration is averaged separetly and used as a comparison. Three replications for each treatment were used in the experiment with a plot size area of 2 by 12 m.

Treatments described in the 2015 field studies were also evaluated in the greenhouse in 2014. The trial was replicated in the greenhouse in October and November of 2014. Soybean seeds were planted approximately 2.5 cm deep in 9.8 l plastic pots (RM3R RootMaker Pot, Stuewe and Sons, Inc., 2290 SE Kiger Island Dr., Corvallis, OR 97333) containing commercial potting soil mix (Metro-Mix 360, Sungro Horticulture, 770 Silver Street, Agawam, MA 01001). After planting, plastic containers were surface irrigated with tap water for the duration of the experiment. Plants were thinned to four plants per container within 1 week of emergence, and grown at 35/30° C day/night temperature. Natural light was supplemented with light from sodium vapor lamps

(General Electric Sodium Vapor Lamps, Lucalox LU 400, General Electric Consumer and Industrial Lighting, 1975 Noble Rd., Nela Park, Cleveland, OH 44112) to provide a 16-h photoperiod.

Approximately 2 weeks after thinning, plants had reached the V3 growth stage, herbicide treatments were initiated using a compressed air spray chamber equipped with a single 80015EVS flat-fan nozzle (TeeJet Technologies, PO Box 7900, Wheaton, IL 60187) at an application volume of 140 l ha^{-1} and a pressure of 220 kPa. Herbicide treatments consisted of dicamba at 0.56 kg ae ha^{-1} and glyphosate applied at 1.1 kg ae ha^{-1}. For methodology of greenhouse experiments, all spray lines were filled with dicamba and glyphosate at the same rate and cleaned in the same manor as the field experiments in 2015. The solution from each hose type by cleanout procedure was then collected using CO_2 to push the solution from each hose to a collection bucket. A 10 ml aliquot was then taken from each collection bucket for analytical analysis. The remaining solution was then added to 355 ml bottles and applied to soybean at the V3 growth stage in the spray chamber.

Visual estimates of soybean injury were recorded 3, 5, 7, and 14 days after treatment (DAT), using a scale of 0 to 100%, where 0 = no injury and 100 = total plant death. Chlorosis, necrosis, stunting, leaf cupping, epinasty and regrowth were visually evaluated to estimate injury. Plants were cut at the soil line 21 DAT, dried and weighed to calculate dry matter and dry matter reduction from the untreated check. Three replications for each treatment were used in the experiment with one pot representing one hose per hose type by cleanout procedure for each replication. Data were pooled across site years because experimental replication was considered a random variable.

Untransformed and arcsine square root transformed data were subjected to analysis of variance, but interpretations were similar to untransformed data; therefore, untransformed data were used for analysis. Data were analyzed using PROC GLIMMIX in SAS 9.4 and means were separated using Fischer's protected LSD test at P = 0.05.

Analytical Evaluation

Samples from field and greenhouse studies were collected in 2014 and 2015 in 20 ml liquid scintillation vials (Sigma-Aldrich Company, LLC, 3050 Spruce St., St. Louis, MO 63103). Rinsate from field and greenhouse samples were taken at the time of the experiment and frozen for analytical analysis. Samples were collected using a 50 ml silicone pipette filler, 3 way valve (Cole-Parmer instrument Company, LLC, 625 East Bunker Court, Vernon Hills, IL 60061) attached to a 10 ml serological, sterile, individually wrapped pipette (Cole-Parmer instrument Company, LLC, 625 East Bunker Court, Vernon Hills IL 60061). Samples were collected with one pipette per sample to eliminate cross contamination.

Analytical analysis was performed at the University of Tennessee (University of Tennessee, Knoxville TN, 37996). Instrumentation used in the analysis began with the Agilent 1100 Series HPLC System (Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051). The Agilent 1100 series included the G1379A degasser, G1311A quat pump, G1313A auto sampler (ALS), G1316A thermostatted column compartment (colcom), and the G1315B diode array and multiple wavelength detector (dad). Analysis was performed with the use of mass spec and included the Agilent 6120 quadrupole single quad LC/MS G1978B. The liquid phase of the analysis was acetonitrile

+ 0.1% formic acid and water + 0.1% formic acid. Agilent chemstation software was used for the data capture and integration

Samples collected from field and greenhouse studies were prepared by vortexing the aliquot solutions (Fisher Vortex Genie 2, Scientific Industries, INC., 80 Orville Dr., Suite 102, Bohemia, NY 11716) for 30 seconds. A 1 ml extraction from each of the 10 ml aliquot solutions collected from each treatment and rep was then extracted and added to 19 ml of methanol to constitute a 0.05 dilution rate. For the larger end rate titration of dicamba at 0.56 and 0.140 kg ae ha^{-1} a further dilution rate was conducted to 0.00063. This was obtained by adding 1 ml of the aliquot solution to 19 ml of methanol and then extracting 250 µl of that solution into 19.75 ml of methanol. For the lower end of the rate titration of dicamba at 0.0087 and 0.0022 kg ae ha^{-1} the dilution rate of 0.05 remained. After dilutions were made a final vortex of the solution was made for 30 seconds. A 2 ml extraction from each of the final dilutions was made with a BD 10 ml syringe with Luer-Lok™ (Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, New Jersey 07417-1880) and a 0.45 µm hydrophobic Polytetrafluoroethylene (PTFE) membrane filter (Thermo Fisher Scientific, INC. 09-719H. 300 Industry Drive, Pittsburgh, PA 15275) screwed to the end of the syringe. From this extraction, 1.5 ml were injected into a 12 x 32 mm target DP, clear glass vial, with a polypropylene open top cap, bounded PTFE/silicone septum (Thermo Fisher Scientific, INC. 300 Industry Drive, Pittsburgh, PA 15275).

The analysis began with an injection of methanol (to verify a lack of background carryover) followed by dicamba standards of 16.5, 30, 300 and 1000 ppb to establish linearity of MS response. A dicamba standard (30 ppb) was analyzed after every four

unknown samples, to verify consistency of MS detector response over time. The conservative lower limit of detection was 5 ppb, and all samples (with the exception of untreated samples) had dicamba concentrations above this amount.Three replications for each treatment were used in the experiment with one sample representing one hose per hose type by cleanout procedure for each replication.

Hose Analysis Using Scanning Electron Microscopy (SEM)

For hose analysis using SEM, subsamples of hoses used throughout experiments were derived by randomly selecting hose types used and comparing them to hoses of the same type that were never used and have never had solution within them. The used hoses were used a total of eight times in the previous experiments. Three subsamples were cut from each hose type into 7.6 cm samples using a ratcheting hose and PVC cutting tool (Professional ratcheting hose and PVC cutter 37100, Superior Tool Company, 100 Hayes Dr., Cleveland, OH 44131). Samples were then cut into smaller pieces roughly measuring 6.4 X 2.5 mm. Samples were then randomly chosen and glued to a 25.4 mm pin stub (Ted Pella INC. 16144, 4595 Mountain Lakes Blvd., Redding, CA 96003) using EPO-TEK® conductive Silver Epoxy and a liquid hardener (Ted Pella INC. H-22, 4595 Mountain Lakes Blvd., Redding, CA 96003) to affix four samples per pin stub with the outside of the hose attached to the stub for analysis of the inner tube. After 24 hours the samples were coated. A platinum coating was used on the sample necessary to create. The platinum coating was added with the use of an EMS 150T ES Coater (P.O. Box 550, 1560 Industry Road, Hatfield, PA 19440) using argon gas as the supply. Samples were coated in less than one minute with the platinum coating and left to cure for 24 hours.

Samples were then loaded to a Zeiss Evo 60 EP-SEM (Zeiss international, Carl-Zeiss-Strasse 22, 73447 Oberkochen, Germany) connected to a Bruker AXS Quantax 4010 energy dispersive X-ray spectrometer (EDS) (Bruker Corporation, Permoserstr. 15, 04318 Leipzig, Germany). The Bruker software was used for graphing the elemental make-up of the sample, as well as creating a color-coded map of the sample where different colors pertain to different elements. The Quantax 4010 was equipped with a Silicon Drift Detector (SDD) which provided a high resolution and accurate map and/or graph of the sample.

Results and Discussion

Field and Greenhouse Experiments

Experiments averaged over six locations from 2013, 2015 and the greenhouse study from 2014 showed an interaction of hose type by cleanout procedure with respect to VEOI at all rating dates (Table 3.2). Visual estimations of injury from 7 and 14 DAT are averaged over six experiments and show the black and green hose rinsates lending to greater injury than other hoses with respect to dicamba sequestration within all cleanouts (Table 3.2). At 7 DAT, the black and green hose rinsates showed approximately 20% VEOI in the no cleanout treatment, 13% in the ammonia treatment and 13% in the water treatment (Table 3.2). At 21 and 28 DAT averages are based on the 2013 and 2015 field trials alone as the greenhouse trials were terminated after 14 DAT. At 21 DAT, the black hose rinsate treatment had 28% VEOI and the green hose rinsate had 30% VEOI. They had a greater sequestration potential than the blue hose rinsate at 19% VEOI with respect to the no cleanout treatment (Table 3.2). Within both water and ammonia treatments 21 DAT, the black and green hose rinsates showed greater dicamba sequestration than other

hoses (Table 3.2). At 28 DAT, the black hose rinsate showed 29% VEOI and the green hose rinsate showed 31% VEOI, which was greater than the blue hose rinsate at 19% with respect to the no cleanout treatment (Table 3.2). Within the water and ammonia treatments 28 DAT, the black and green hose rinsates show greater VEOI than the other hose types (Table 3.2). Table 3.2 shows the rate titration used in field and greenhouse studies are averaged separately over four site years as a comparison to the amount of injury observed. Field and greenhouse trials from all dates show no indication that water or ammonia differ with respect to injury averaged within hose types. Results from field and greenhouse trials also indicate the blue hose shows the greatest potential to decrease sequestration of the dicamba analyte with respect to VEOI observations when compared to the check (Table 3.2). Height reduction from experiments in 2013 and 2015 show differences at 7, 14 and 21 DAT due to hose type (Table 3.3). Height reduction at 14 and 21 DAT was greatest with the black hose rinsate treatment (29% reduction from the check), which was greater than all other hose types (Table 3.3). At 28 DAT there was an interaction of hose type by cleanout procedure where height reduction was influenced by dicamba retention in the black hose (36%) when compared to the yellow hose (23%) and the blue hose (13%) with respect to no cleanout (Table 3.3). Within water and ammonia treatments 28 DAT, the black hose rinsate showed 29% plant height reduction from the check, which was greater than all other treatments (Table 3.3). At pre harvest, node reduction showed a hose type by cleanout procedure interaction, where the black and green hose rinsates were greater with (45 and 43%, respectively) node reduction when compared to the blue hose rinsate treatment (14%) with respect to the no cleanout treatment (Table 3.3). Within the water and ammonia treatments at pre harvest, the black

hose rinsate showed 33 and 32%, which was greater than all other treatments with the exception of the green hose rinsate with the water treatment that showed a 27% node reduction (Table 3.3). Percent yield reduction from field experiments in 2013 and 2015 showed differences based on hose type, where the black hose had the greatest amount of dicamba sequestration resulting in a yield reduction of 19%, which was greater compared to all treatments except the grey hose rinsate at 13% (Figure 3.1). Yield reduction observed from the black hose rinsate at 19% showed on average a comparison to the 1/256X rate of dicamba at 0.00218 kg ae ha^{-1}, which showed a 16% yield reduction (Figure 3.1).

Dicamba sequestration from black and green hoses produced less dry matter compared to the check (Figure 3.2). In comparison of the rate titration, the black and green hose rinsates produce the same amount of dry matter to the 1/64X rate of 0.0087 kg ae ha^{-1} of 9 g when averaged over cleanout treatments and site years (Figure 3.2).

Analytical Evaluation

The rate titration showed a 1X rate of dicamba yields roughly 3000 ppm of the dicamba analyte (Figure 3.3). This number decreases to 671, 55 and 16 ppm in relation with the 1/4, 1/64, and 1/256X rates, respectively. The black hose retains more of the dicamba analyte than any other hose regardless of cleanout and is comparable to the 1/256X rate of 0.0022 kg ae ha^{-1} in procedures tested (Figure 3.3). The green and grey hoses are comparable to the 1/256X rate of 0.0022 kg ae ha^{-1} with respect to no cleanout procedure (Figure 3.3). The blue hose showed less retention of dicamba when compared to all other hose types when the water and ammonia cleaning procedures were used (Figure 3.3). When averaging the water and ammonia cleanout over black hose, we

observed on average 16 ppm of dicamba analyte was retained which was comparable to that of the 1/256X rate (Figure 3.3). In 2013 and 2015 there was a 19% yield reduction with respect to dicamba sequestration of the black hose when compared to the check (Figure 3.1). This reduction would be comparable to that of a 1/256X rate, which had a 16% yield reduction from the check. During the cleanout process, whether it be water or ammonia, 12 l of water were passed through each hose separately. Each hose sequestered 392 ml of solution; when 12 l of clean water were passed through the hose, this is essentially 31X the amount of fluid that the hose actually retains. Analyte retention is based solely on hose type with respect to water and ammonia cleanout when observing ppm analyte retained (Figure 3.3). When averaging analyte retained with respect to the black hose, 16 ppm was an equivalent use rate of 0.5% when compared to the 1X rate. These data would agree with Boerboom (2004), who showed that dicamba residues, even when an ammonia-water solution was used, had a subsequent percent use rate of 0.63% from the spray boom when refilled with water. The blue hose showed retention capabilities of less than 1 ppm analyte of dicamba retained compared to other hose types with respect to water and ammonia cleanout with the exception of the yellow hose, which showed less than 3 ppm (Figure 3.3). Similarly, Kelly and Riechers (2003), found that as little as 1/10,000 use rate of dicamba may cause injury symptoms. In this research we observed injury symptoms with rinsates from all hose types to varying levels. When averaging over all cleanouts, even with the blue hose, injury was observed and yield reductions were significant with respect to the untreated check. In the field trials, yield reduction from the check is 7% with respect to the blue hose (Figure 3.1). Even when the

best hose was used, ppm analyte retained was 2.03, which was 0.06% of the 1X rate of dicamba and influences injury, height reduction, node reduction and t yield reduction.

Hose Analysis Using Scanning Electron Microscopy

Analysis using scanning electron microscopy may showed one reason behind the potential for certain hose types to have retention compared to that of another. The black hose that had never been used showed holes and retention potential at a magnification of 5.14 k (Figure 3.4). When compared to that of a used black hose, the inner lining had started to wear over time increasing the potential for analyte retention (Figure 3.5).

The visual examination of a new green hose showed imperfections in the manufacturing process of raised nodules (Figure 3.6) that have potential for breaking loose and creating pockets or increasing the occurrence of cracking as observed with a used green hose (Figure 3.7). A new blue hose showed a smooth almost pattern like structure throughout (Figure 3.8). Even after long term exposure to varying pressures and chemicals the blue hose still showed a smooth surface but not completely without the effects of wear (Figure 3.9). The inability of knowing what the manufacturing process is due to patent protected confidentiality makes determination difficult but the one main difference among all hose types is that the blue hose had a polyethylene core.

In conclusion, an increase in injury and height reduction will be observed when no-cleanout procedure is used regardless of the hose type. Both water and ammonia showed a decreased occurrence of injury, height reduction and yield reduction when compared to no cleanout of the hose. The determination of the reduction of the sequestration of the dicamba analyte within hose type was predicated on the chemical makeup of the hose itself, with the blue hose showing the least amount of dicamba

retention. Rinsates from the blue hose showed the least injury, height reduction, ppm analyte retained and yield reduction with respect to water and ammonia cleanout. The black hose showed the greatest potential for the sequestration of the dicamba analyte and also the greatest amount of injury, height reduction, ppm analyte retained and yield reduction when compared to the untreated check.

Table 3.1 Planting year, location, date, population, and seed variety information for dicamba hose sequestration trials.

Year	Location	Planting date	Variety[a]	Population
2012	Brooksville	August12	Asgrow 4932	345,000 seeds ha^{-1}
2013	Brooksville	May 1	Asgrow 4933	345,000 seeds ha^{-1}
2013	Starkville	May 30	Pioneer 95Y61	340,860 seeds ha^{-1}
2014[b]	Starkville	October 1	Pioneer 95Y71	345,000 seeds ha^{-1}
2014[b]	Starkville	October 15	Pioneer 95Y71	345,000 seeds ha^{-1}
2015	Starkville	May 4	Asgrow 4632	345,000 seeds ha^{-1}
2015	Brooksville	May 25	Asgrow 5332	326,040 seeds ha^{-1}

[a] Asgrow Soybean (Monsanto Agrochemical Company, 800 North Lindbergh Blvd., St. Louis, Missouri 63167)
Pioneer Soybean (Du Pont de Nemours and Co., 1007 Market Street, Wilmington, DE 19898)
[b] same variety used in both greenhouse runs.

Table 3.2 Visual estimation of injury on soybean due to dicamba rinsate from hose type by cleanout procedure with a rate titration of dicamba as comparison from field experiments in 2013 and 2015 and greenhouse 2014 at 7, 14, 21 and 28 DAT.

	Days after treatment[a]											
	7[b]			14[b]			21[c]			28[c]		
Hose type[d]	Water	Ammonia	None	Water	Ammonia	None	Water	Ammonia	None	Water	Ammonia	None
	----------%----------											
Black	12c	13c	21a	18d	19cd	26a	22d	23d	28ab	22d	25c	29ab
Yellow	5e	6e	17b	8f	8f	21bc	11gh	10h	26bc	11f	10f	26bc
Green	14c	13c	20a	19cd	19cd	25a	24cd	24cd	30a	25c	24c	31a
Grey	8d	6e	18b	12e	10e	21b	14f	13fg	26bc	14e	14e	26bc
Blue	1f	1f	9d	1g	2g	12e	0i	0i	19e	0g	0g	19d
Check[e]	0f	0f	0f	0g	0g	0g	0i	0i	0i	0g	0g	0g
Rate titration[f]												
0.56 kg ae ha^{-1}		100a			100a			100a			100a	
0.14 kg ae ha^{-1}		80b			85b			88b			88b	
0.0087 kg ae ha^{-1}		40c			40c			46c			46c	
0.0022 kg ae ha^{-1}		25d			25d			30d			31d	

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column group

[b]averaged over six site years (four field + two greenhouse)

[c]averaged over four site years (greenhouse trials were terminated after 14 DAT)

[d]John Deere PMK 4131- 08 (Yellow), John Deere PMA 4086-08 (Blue) (LLDPE), John Deere PMA 1687-08 (Green), John Deere PMA 1628-08 (Grey), and Goodyear (Black)

[f]rate titration averaged separately as comparison

[e]untreated check treatment

Table 3.3 Soybean height reduction and node reduction due to dicamba rinsate from hose type by cleanout procedure with a rate titration of dicamba as comparison from field experiments in 2013 and 2015 at 7, 14, 21 and 28 DAT and pre harvest

	Days after treatment[a]						Pre harvest[a]		
	7	14	21	28			Percent node reduction		
Hose type[b]				Water	Ammonia	None	Water	Ammonia	None
	----------			%					
Green	19a	21b	17b	21cd	16cde	31ab	27b	14cd	43a
Black	22a	29a	29a	29ab	29ab	36a	33ab	32b	45a
Grey	10b	17b	13bc	11ef	15cde	30ab	2f	17c	35ab
Yellow	14ab	15bc	9cd	7fg	15cde	23bc	6df	5f	33ab
Blue	7bc	10c	5de	6fg	4g	13def	7df	11c-f	14cde
Check[c]	0c	0d	0e	0g	0g	0g	0f	0f	0f
Rate Titration[d]									
0.56 kg ae ha^{-1}	44a	64a	67a	72a			87a		
0.14 kg ae ha^{-1}	45a	59a	68a	66a			68b		
0.0087 kg ae ha^{-1}	28ab	38b	38b	45b			44c		
0.0022 kg ae ha^{-1}	18bcd	18c-g	14d-h	20def			31cde		

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column group

[b]John Deere PMK 4131- 08 (Yellow), John Deere PMA 4086-08 (Blue) (LLDPE), John Deere PMA 1687-08 (Green), John Deere PMA 1628-08 (Grey), and Goodyear (Black)

[c]untreated check treatments

[d]rate titration averaged separately as comparison

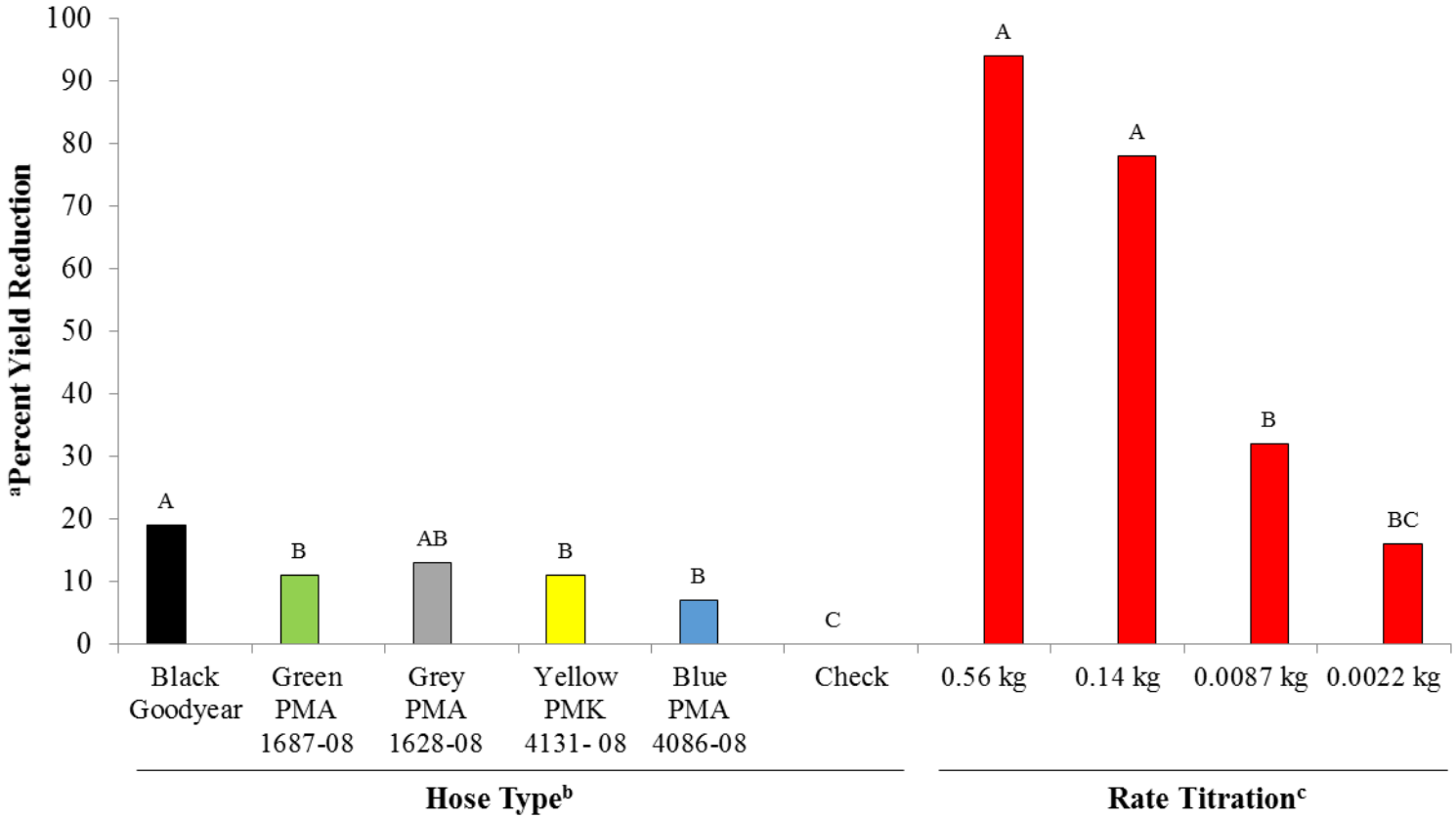

Figure 3.1 The effect of hose type on percent soybean yield reduction when averaged over all cleanouts in four site years of 2013 and 2015 and showing rate titration as comparison

[a]averaged over four site years
[b]John Deere PMK 4131- 08 (Yellow), John Deere PMA 4086-08 (Blue) (LLDPE), John Deere PMA 1687-08 (Green), John Deere PMA 1628-08 (Grey), and Goodyear (Black)
[c]rate titration averaged separately as comparison

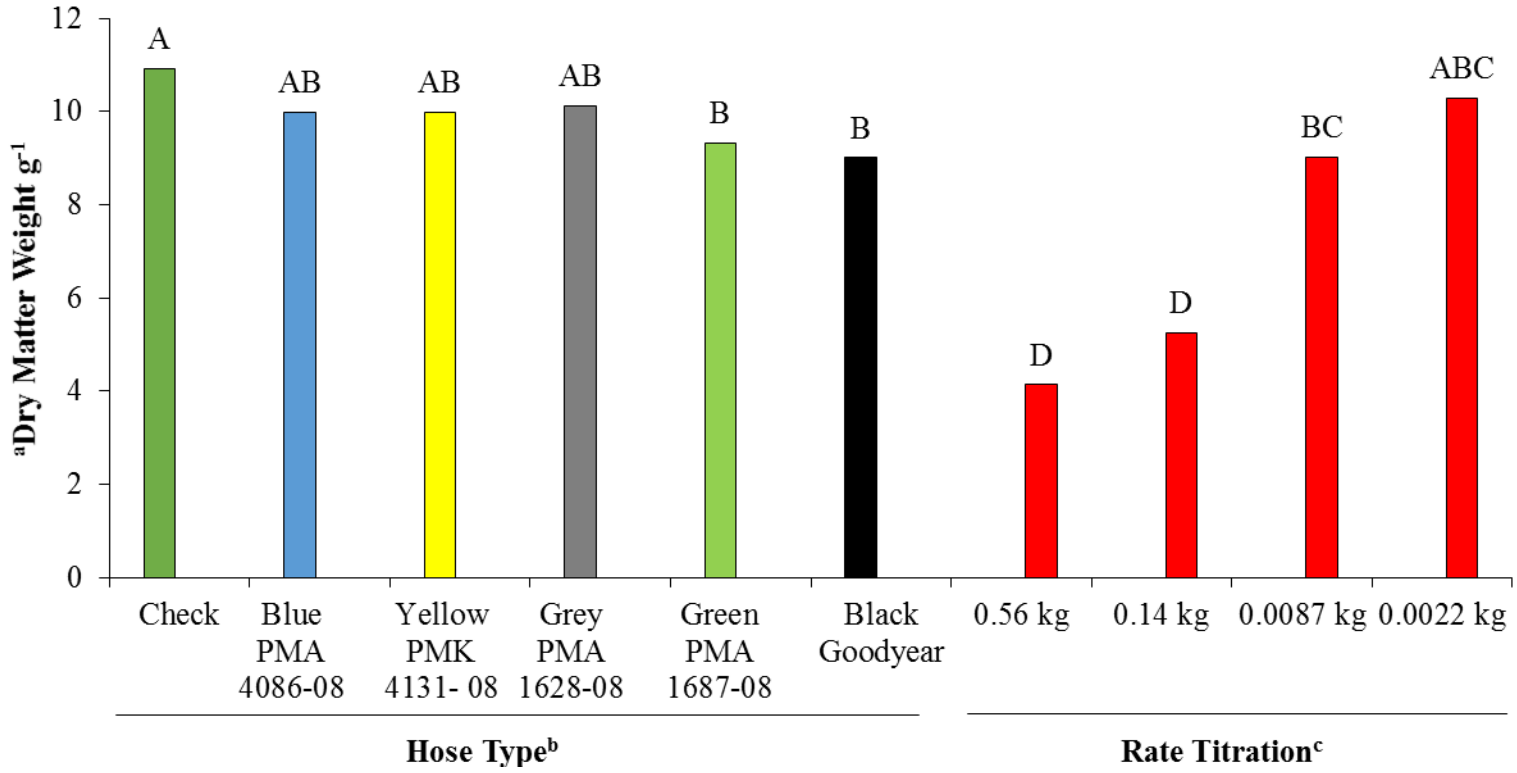

Figure 3.2 Soybean Dry matter weight from greenhouse experiments 2014 averaged over cleanout procedures and showing rate titration as comparison

[a]averaged over two site years
[b]John Deere PMK 4131- 08 (Yellow), John Deere PMA 4086-08 (Blue) (LLDPE), John Deere PMA 1687-08 (Green), John Deere PMA 1628-08 (Grey), and Goodyear (Black)
[c]rate titration averaged separately as comparison

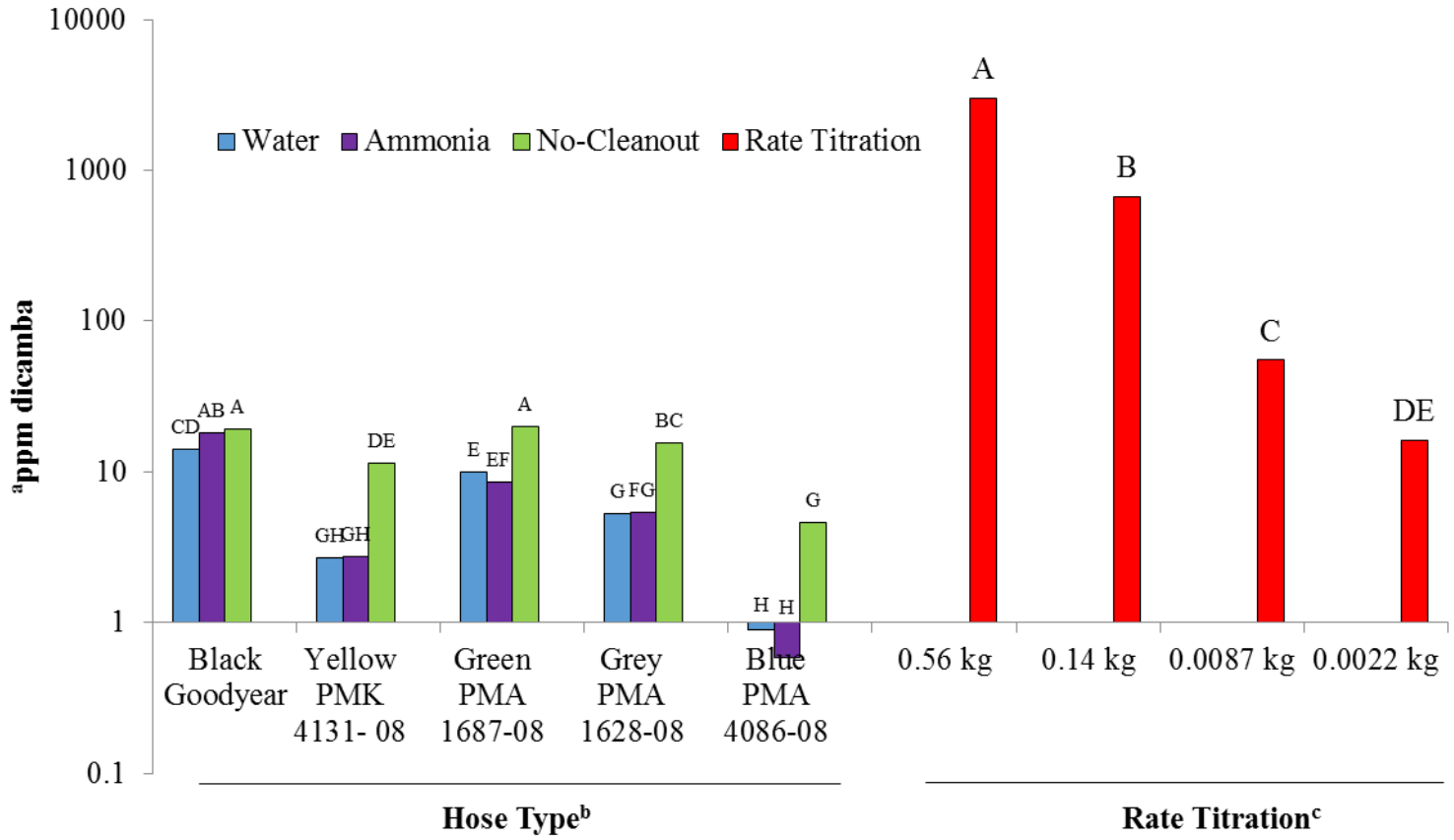

Figure 3.3 Hose sequestration of dicamba (ppm) showing all hose type by cleanout procedures and rate titration as comparison

[a]averaged over four site years
[b]John Deere PMK 4131- 08 (Yellow), John Deere PMA 4086-08 (Blue) (LLDPE), John Deere PMA 1687-08 (Green), John Deere PMA 1628-08 (Grey), and Goodyear (Black)
[c]rate titration averaged separately as comparison

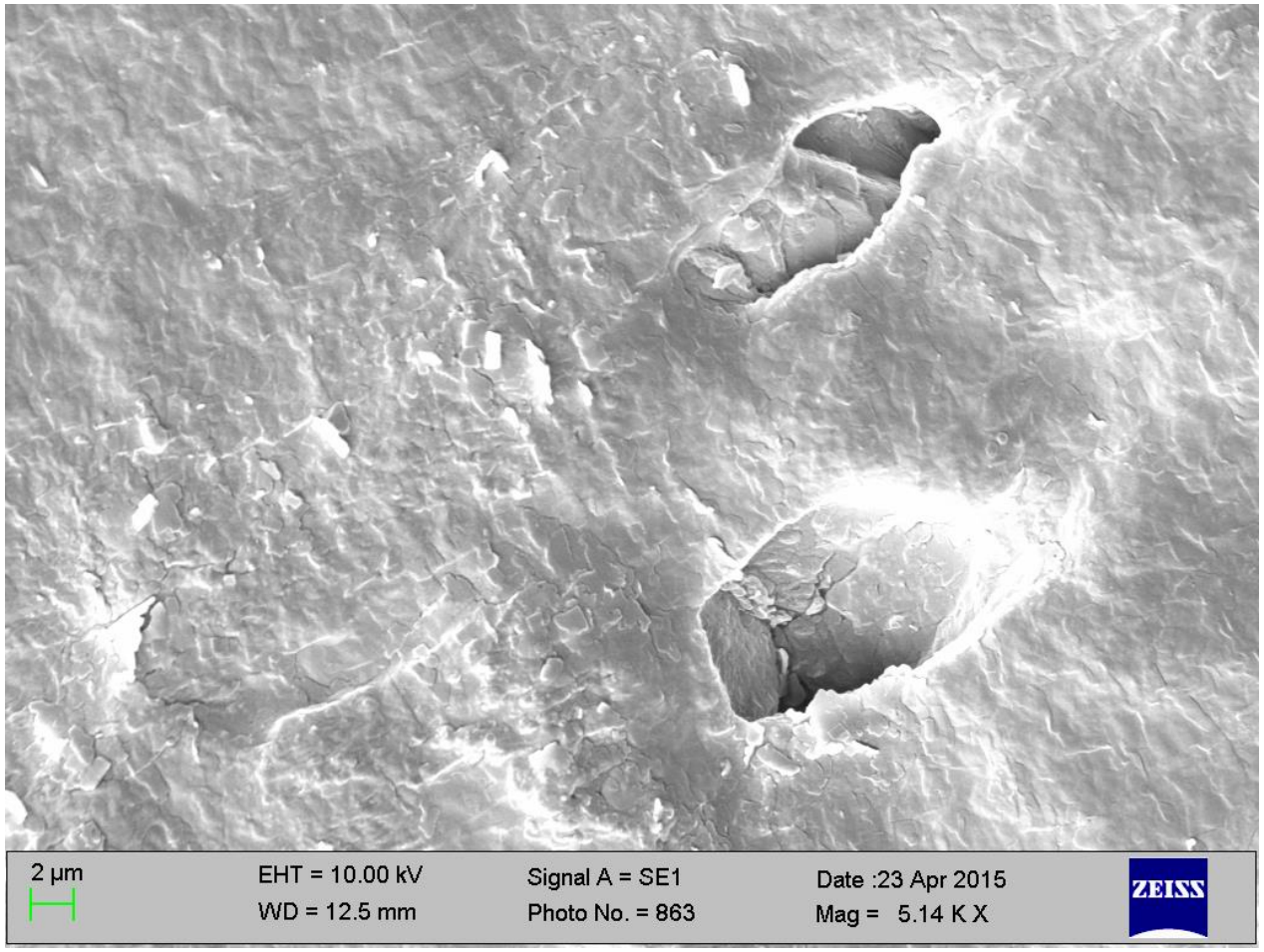

Figure 3.4 Scanning electron micrograph of a new **Goodyear** (Black/Versigard Synthetic Rubber) hose

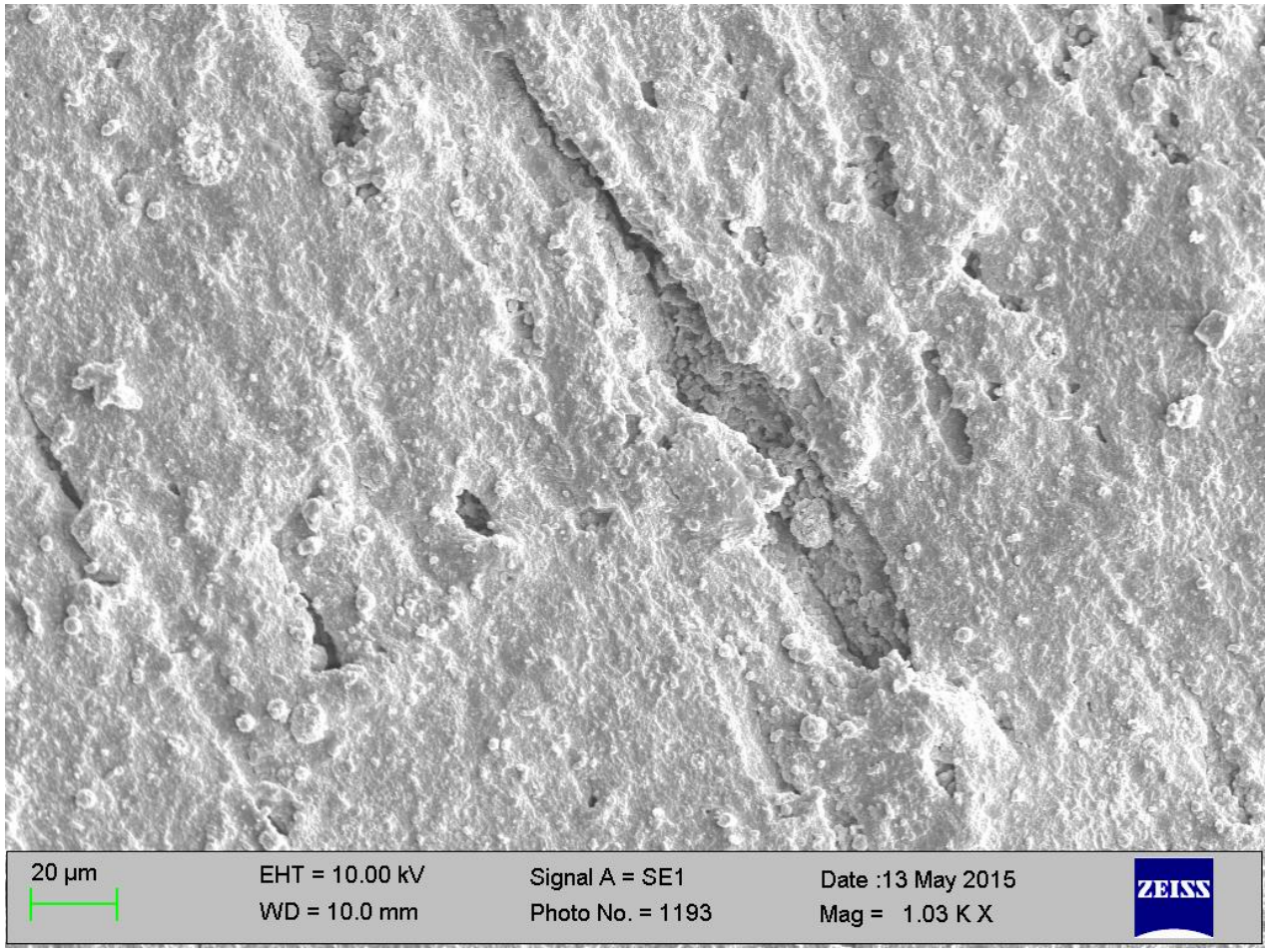

Figure 3.5 Scanning electron micrograph of a **Goodyear** (Black/Versigard Synthetic Rubber) hose used eight times

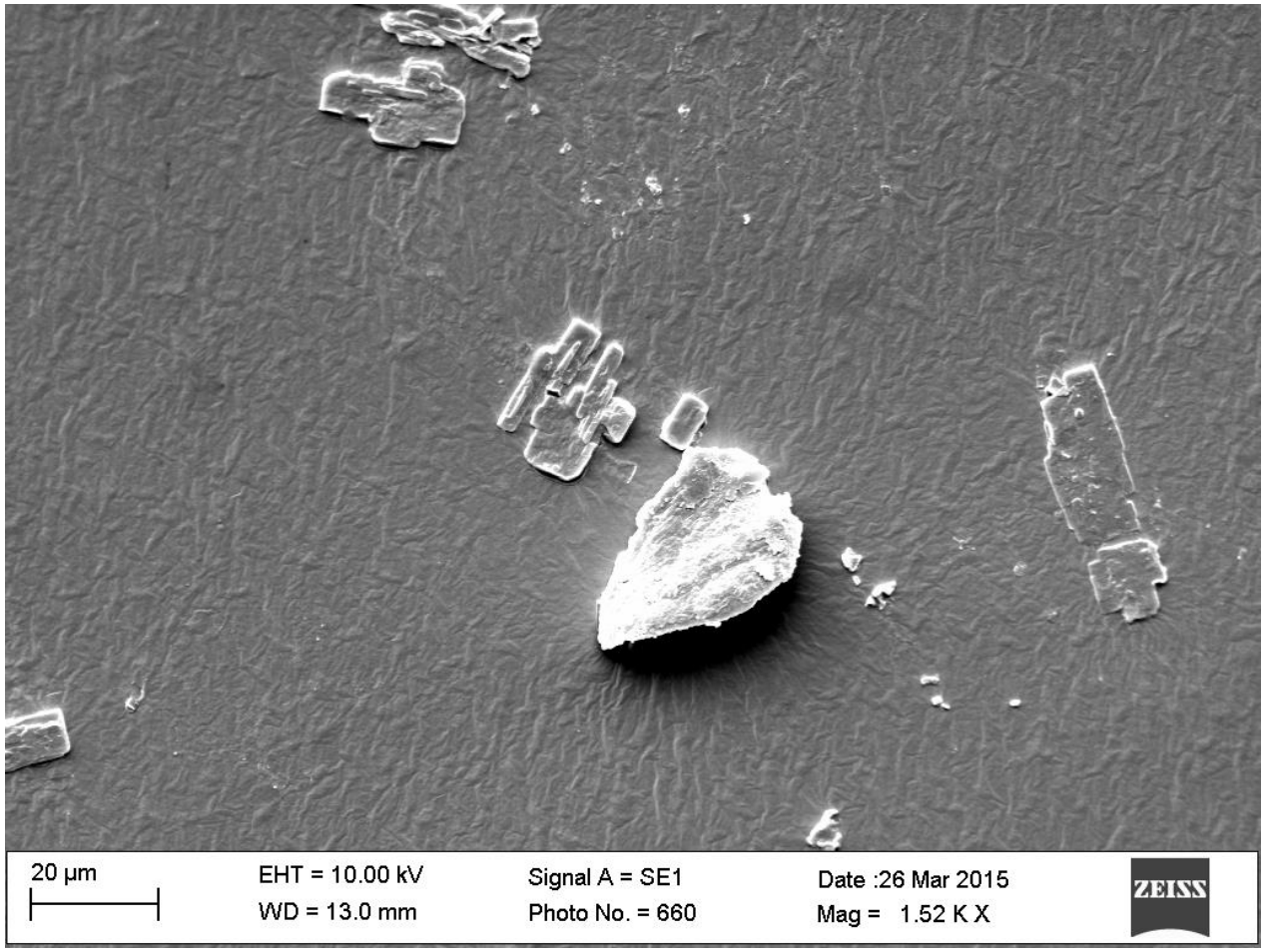

Figure 3.6 Scanning electron micrograph of a new **John Deere PMA 1687-08** (Green/PVC/polyurethane-high tensile-strength yarn-2 ply) hose

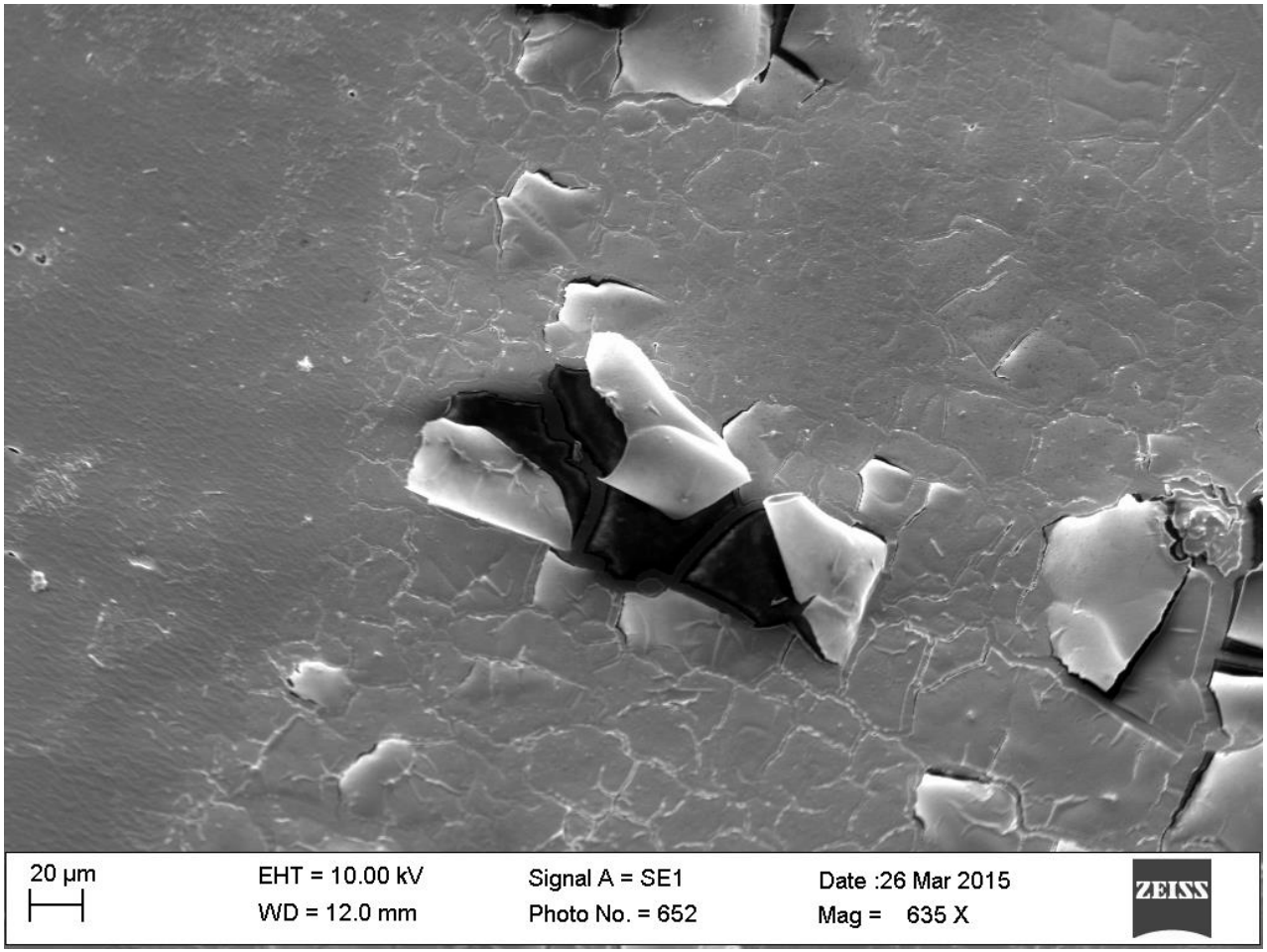

Figure 3.7 Scanning electron micrograph of a **John Deere PMA 1687-08** (Green/PVC/polyurethane-high tensile-strength yarn-2 ply) hose used eight times

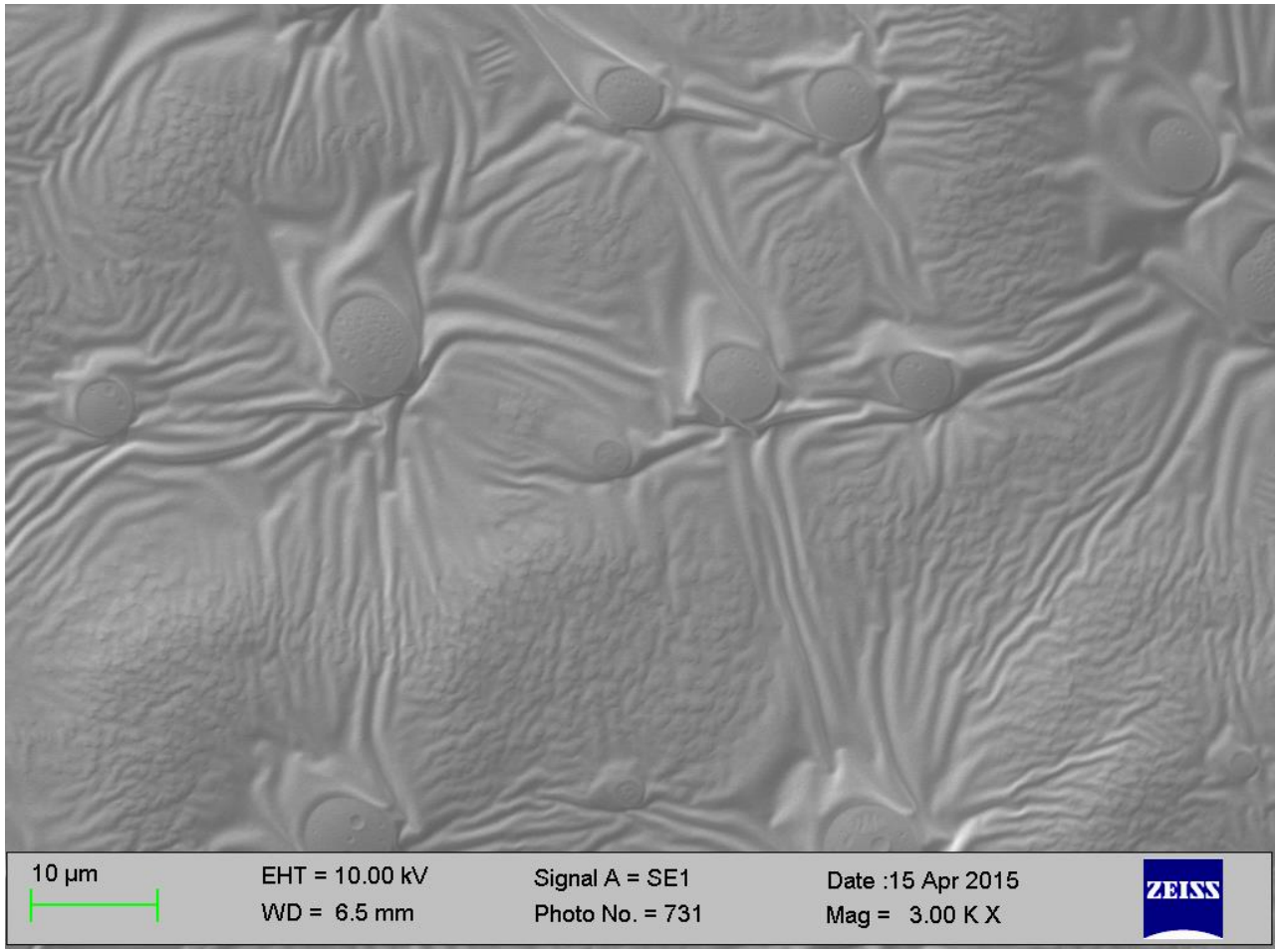

Figure 3.8 Scanning electron micrograph of a new **John Deere PMA 4086-08** (Blue/Linear/low-density polyethylene blend) (LLDPE) hose

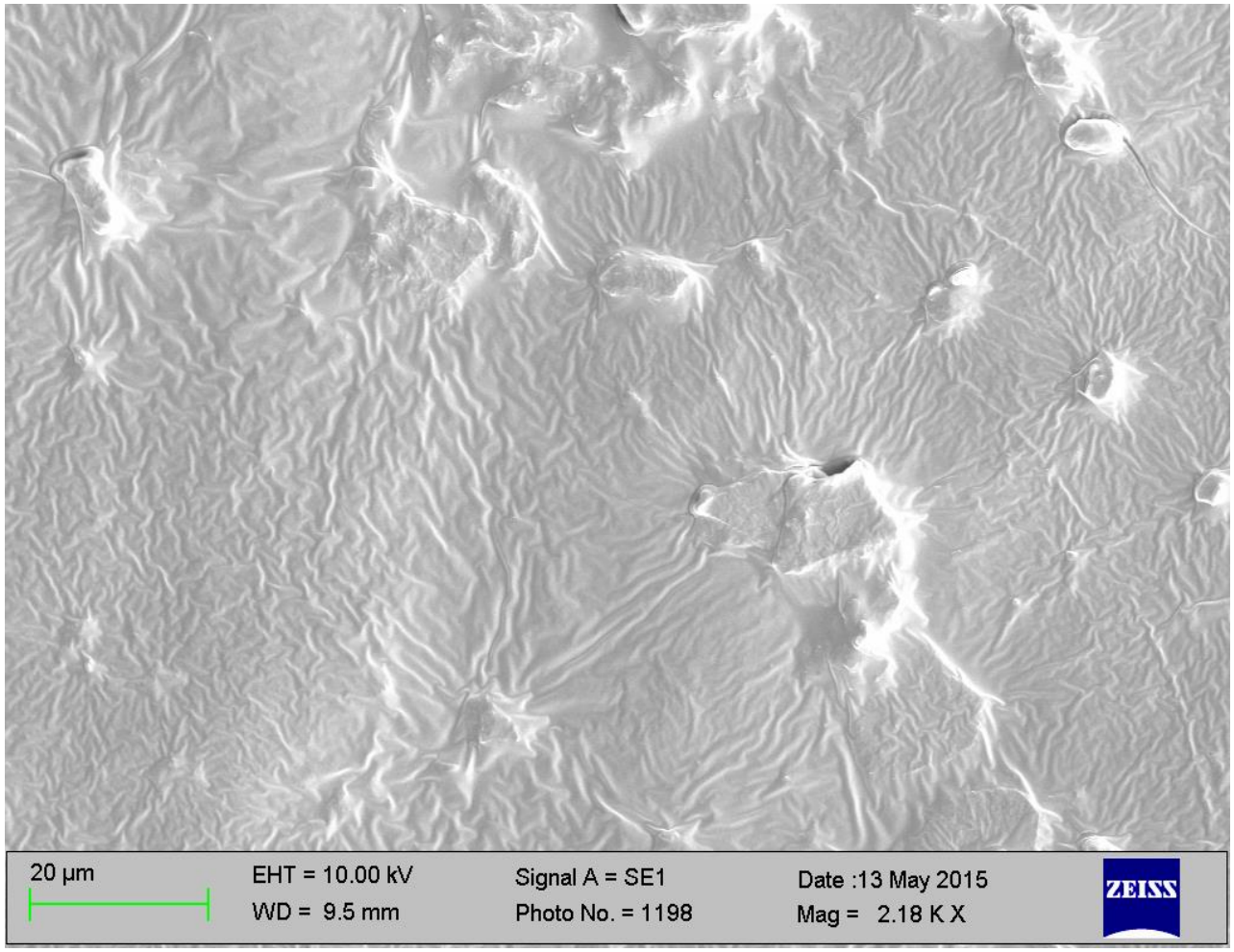

Figure 3.9 Scanning electron micrograph of a used **John Deere PMA 4086-08** (Blue/Linear/low-density polyethylene blend) (LLDPE) hose used eight times

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CHAPTER IV

EFFECT OF FORMULATION AND CLEANOUT PROCEDURE ON 2,4-D RETENTION IN A SPRAYER HOSE

Abstract

Field studies were conducted in 2014 and 2015 to evaluate the effect of spray equipment cleanout procedure with a new formulation of 2,4-D. Two, valved, manifold systems were created to simulate tank and hose sequestration of 2,4-D within a spray system. Two standard titrated rates of 2,4-D were used to compare cleanout efficiency with field observations of percent injury, nodes above cracked boll (NACB), height reduction and yield reduction. Visual estimation of injury (VEOI) observations from field studies did not lead to differences based on NACB, height reduction, or yield reduction. These observations would indicate that the titrated rates of 3.74 and 0.374 g ae ha^{-1}, which would be similar to that of a 1/128X and 1/1280X rate of 2,4-D, do not influence the quantitative data when cotton is used as a bio-indicator. Analytical analysis for the titrated standards of 2,4-D at a rate of 3.74 and 0.374 g ae ha^{-1} yielded 22 and 2 ppm analyte retained, respectively. Although not conclusive, considering that this was a preliminary analytical evaluation, a plant response with respect to injury was observed in field experiments due to the titrated standards. These standards are at a rate of 1/128X and 1/1024X and show a plant response with VEOI; but this response was not observed with respect to the quantitative data of NACB, plant height reduction and yield reduction.

Nomenclature: 2,4-Dichlorophenoxyacetic acid; 2,4-D; glyphosate; Roundup WeatherMax®

Key words: Plant growth regulating herbicides, contamination, sequestration, tank contamination, drift, volitization, interaction

Introduction

In response to the evolution of glyphosate resistant weeds, companies have been investing in new methods of weed control. Companies are searching for new active ingredients, but the cost of developing them and the limited potential for economic return has made it difficult to bring new products to market (Johnson et al. 2012a). These companies have been on the forefront of genetically engineered crops which are resistant to herbicides other than glyphosate. The dicamba and 2,4-D resistant crops were developed because these herbicides have shown excellent resilience with few herbicide-resistant weeds occurring after more than 50 years of use (Johnson et al. 2012a). Secondly, 2,4-D and dicamba provide excellent control of glyphosate-resistant broadleaf weeds such as horseweed (*Conyza canadensis* (L.) Cronq.), giant ragweed (*Ambrosia trifida* L.), common waterhemp (*Amaranthus rudis* Sauer), and other broadleaf weeds (Johnson et al. 2012a).

Dow AgroSciences calls their 2,4-D-resistant technology the Enlist™ Weed Control System in corn, soybean, and cotton (Dow AgroSciences 2013). This technology became possible when the company inserted genes into broad-hectare agronomic crops that allow the plants to metabolize 2,4-D (Johnson et al. 2012b). Dow AgroSciences has introduced the Enlist Duo™ formulation that contains glyphosate and 2,4-D: choline (Dow AgroSciences 2013). The herbicide features what Dow AgroSciences calls Colex-

D™ Technology (Dow AgroSciences 2013), which provides ultra-low volatility, minimized potential for drift, lower odor, and better handling characteristics than commercially available 2,4-D amine or ester formulations (Johnson et al. 2012b). Enlist™ soybean, cotton, and corn will have traits that make them tolerant to 2,4-D as well as glyphosate and glufosinate (Dow AgroSciences 2013).

With the new triple stacked gene technology (glyphosate + glufosinate + dicamba or glyphosate + glufosinate + 2,4-D) soon to penetrate the market, problems may arise from issues involving off-target movement from one producer's field to another because it is unlikely that everyone will immediately adopt the new technologies. Unlike glyphosate, which is very water-soluble and can be easily cleaned out of a sprayer with water, the PGR herbicides take a lot more time, care and effort to be removed (Steckel et al. 2005). Considering that soybeans and cotton are extremely sensitive to PGR herbicides, it is imperative that a quality clean-out technique become the standard and adopted among producers.

The herbicide 2,4-D can be found in a variety of water-soluble amine salts and in the acid form, but may also be produced with ester derivatives which strongly enhance its diffusion properties (Chinalia et al. 2007). Phenoxy-herbicides are xenobiotic compounds used to control dicotyledonous weeds and have been produced and applied on a large scale since the 1940s (Hayward 1991). Commercially, 2,4-D has been formulated as either dimethylamine salt (DMA) and 2-ethylhexyl ester (EHE), which accounted for approximately 90% global use in the last half of the twentieth century (Chinalia et al. 2007). The acid dissociation constant (pK_a) for 2,4-D is 2.8 and it acts as a weak acid meaning that translocation within the plant is generally phloem mobile due to ion

trapping (Senseman 2007), but translocation will also occur within the xylem. In general 2,4-D causes increased DNA, RNA and protein synthesis in plants, especially in the meristematic tissues of broadleaf weeds, with some indications of affecting lipid metabolism (Moreland 1980; Hangarter et al. 1980). The common symptoms of plants are accelerated foliar senescence, chloroplast damage and chlorosis following vascular damage (Chinalia et al. 2007). Grossman (2000), reported that auxin-like herbicides such as 2,4-D, induce 1-aminocyclopropane-1-carboxylic acid synthase (ACC-synthase), which is a key enzyme during the production of ethylene. Considering that cyanide is a co-product of ethylene biosynthesis in higher plants via the ACC pathway, and cyanide is toxic if it accumulates in plant tissues, Chinalia et al. (2007), suggests that it is the cyanide that causes the phytotoxic effects on plants that have been subjected to auxin type herbicide treatments.

Cotton (*Gossypium hirsutum L.*), is an important crop for the Mississippi economy. Cotton was ranked as the fourth most valuable agricultural commodity to the state of Mississippi in 2011 with a $563 million value of production and in 2013 with $271 million respectively (MDAC 2012). Cotton is a perennial shrub, but has been domesticated throughout the centuries as a pseudo annual shrub (Chaudlgry and Guitchounts 2003). Although cotton is a perennial shrub, it is grown as an annual through the use of plant growth regulators, harvest aids, and specialized management practices (Chaudlgry and Guitchounts 2003).

Cotton growth stages are defined in many ways, from plant heights to total plant nodes, nodes above white flower, formation of fruiting structures and even days after planting. Accumulated heat units ($DD_{60}s$) are a major component in the growth cycle of

cotton; the DD_{60} is an estimation of accumulated units during any given day and are based on the average of the maximum and minimum daily temperatures (Kerby et al. 1998). Approximately 4-14 days after planting, emergence will occur and within 40 days after planting on nodes 5-7 the "pinhead squares" will become visible (Bednarz and Nichols 2005). Squaring is actually the term associated with the development of fruiting structures prior to bloom, with the period from square to bloom lasting approximately 21 days. The general fruiting pattern for cotton is to have three day and six day vertical and horizontal fruiting interval, respectively (Jenkins et al. 1990). Following pinhead square is "match head square" or "one-third grown" square (Kerby et al. 1998). Once blooming or "flowering" begins it lasts for approximately 6 weeks (Kerby et al. 1998). When a flower first opens it is typically white and within a few hours of opening, it is pollinated. Flowers typically turn pink the second day after opening and within 5 to 7 days the flower itself dries, turns red in color, and falls off with a formed boll left in its place (Kerby et al. 1998). From plant to harvest takes approximately 140 days under optimum growing conditions and the plant has approximately 20-24 vertical nodes during a growing season (Jenkins et al. 1990).

Fruit shed is common during the life cycle of a cotton plant and may be caused by several factors including water stress, shading, high temperatures, high fruit set, insect damage, and nutrient deficiency. First position bolls have a higher chance of being retained than more distal bolls on the same branch (Chaudlgry and Guitchounts 2003.) Although fruit shed is undesirable, cotton has a high compensatory ability and once a fruiting form is shed, the plant quickly attempts to compensate for the loss through the production of fruiting forms on vertical and distal fruiting positions (Chaudlgry and

Guitchounts 2003). Harvest aids are used to remove leaves from the cotton plant as well as retard new growth and can be classified as herbicidal or hormonal. Some examples of hormonal harvest aids include thidiazuron, dimethipin, and ethephon. Herbicidal harvest aids include carfentrazone, pyraflufen ethyl, paraquat, chlorates and glyphosate. Boll openers are harvest aid chemicals that cause bolls to open and leaves to abscise from the plant by increasing ethylene synthesis (Jones 1997). A cotton crop may be harvested as quickly as seven days following harvest aid application; however, temperature reduction can result in delayed defoliation and harvest (Kerby et al. 1998).

Damage to cotton by 2,4-D has been reported since it was first commercially introduced (Staten 1946). Cotton is considered one of the most susceptible agricultural crops to 2,4-D (Bayley et al. 1992). Hamilton and Arle (1979) found that dicamba applied over the top of cotton had less effect on cotton foliage, yield, boll components, and fiber properties when applied before bloom than when applied later in the season. When cotton is in the reproductive phase of growth, these systemic herbicides reduced cotton yield more than contact herbicides (Snipes et al. 1992). Marple et al. (2007) reported greater cotton injury and yield reductions from simulated drift rates of 2,4-D than clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) or triclopyr (3,5,6-Trichloro-2-pyridinyloxyacetic acid). Cotton, is extremely sensitive to injury from dicamba. Marple et al. (2007) found that cotton was visibly injured by as little as 1/400th the labeled rate 561 g ae ha^{-1}, however cotton was not as sensitive to dicamba as compared to other auxin growth regulator herbicides such as 2,4-D, picloram (4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid), or fluroxypyr (4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid).

Studies have been conducted at Mississippi State in the past where a titration application of both dicamba and 2,4-D was applied to cotton (Smith et al. 2010). Smith et al. (2010) found yield reductions were observed from this study in both the 2,4-D and dicamba experiments, however 2,4-D was more injurious than dicamba to the cotton. The results of this study suggest that cotton is more susceptible to 2,4-D than dicamba. Yield losses were observed where minimal visual injury was present.

Auxin herbicides such as 2,4-D and dicamba can be extremely difficult to clean from spray equipment including nozzles, booms, tanks and pump systems. The normal course of action is to triple rinse with water or ammonia. In a 1955 University of California study, best procedures for removing 2,4-D residues from spray tanks was examined (Vargas et al. 2001). Several metals (zinc, copper, tin, iron and aluminum) and glass were soaked in 2,4-D solutions and then rinsed by various procedures to try to remove the residue. After these materials had soaked in the 2,4-D solution for 24 hours, the solution was poured off and the materials were then rinsed and subsequently analyzed for 2,4-D. In these early studies and subsequent investigations, nearly all the 2,4-D appeared to be rinsed from the metals and glass by the first of four rinses. However, subsequent rinse water that was used to soak the metal and glass for 24 hours showed varying amounts of absorbed 2,4-D were slowly released from the materials. The iron and zinc materials (galvanized iron) showed the greatest additional capacity to continue release of residual chemical, copper and glass trace residues and tin appeared to be free of contamination. Even rapid rinses in ammonia water did not remove the absorbed 2,4-D, but the use of ammonia for prolonged (3 days) soaking appeared to increase the release of the absorbed 2,4-D, with the conclusion of the initial study stating that the only safe way

to avoid 2,4-D contamination is to maintain separate sprayers for sensitive plants (Vargas et al. 2001). With this in mind and the introduction of old chemistries, in the form of auxin herbicides 2,4-D and dicamba, it is worth an investigation into a reevaluation of cleaning procedures for spray equipment. In this initial study conducted in 1955, it was even stated that ammonia does not work when attempting to clean auxin herbicide residues from surfaces. The way in which ammonia works as a cleaning agent, is that it will increase the pH to a point that will make compounds more water soluble. Keeping this in mind, dicamba and 2,4-D have pKa's of 1.7 and 2.8 respectively (Senseman 2007); they both act as weak acids and are already deprotonated when mixed in the spray tank with water. Using ammonia to increase the water solubility of an already deprotonated compound doesn't work in the case of auxin herbicides. With larger spray machines than ever before in the history of agriculture, these chemicals will have more places to sequester and will eventually become harder to clean or eradicate from the system. Considering that the new technologies coming to market will use auxin herbicides and that there are different genetically engineered crops imploring the use of both auxin herbicides in conjunction with glyphosate and glufosinate, the risk for contamination may be extremely high.

Materials and Method

Field Experiments

Field studies were conducted in 2014 and 2015 to evaluate the effect of the 2,4-D formulation GF2726 (Enlist Duo™, The Dow Chemical Company, 9330 Zionsville Road, Indianapolis, IN 46268) in conjunction with two cleanout procedures using either water or ammonia to clean four hoses in two valve banks (8 hoses total) while using cotton as a

bio-indicator. Experiments were conducted at the Black Belt Branch Experiment Station in Brooksville, MS on an Okolona silty clay (Fine, smectitic, thermic Oxyaquic Hapluderts) with 8% sand, 51% silt, 41% clay, 2% organic matter and pH of 6.8 and the R. R. Foil plant research center in Starkville, MS on a Marietta fine sandy loam (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) with 71% sand, 17% silt, 13% clay and 1.03% organic matter and a pH of 5.9. Planting date, planting populations, and seed variety varied among locations (Table 4.1).

Herbicide treatments consisted of GF2726 at 593 g ae ha^{-1} and glyphosate (Roundup WeatherMax®, 540 g ae l^{-1}, Monsanto Company, St. Louis, Missouri, 63167) was applied at 1.1 kg ae ha^{-1}. Valve banks were constructed using (5) 3.8 cm inside diameter PVC schedule 80 tee fittings equally spaced and glued together. Tee openings were fitted with 3.8 cm reducer bushings to allow for the addition of 1.3 cm ball valves to allow for the sequestration of the herbicide solution within the valve bank (Figure 4.1). Hoses were cut to a length of 3 m. Four **Goodyear hoses** (Versigard Synthetic Rubber) were attached to the ball valves at the valve bank with an additional valve attached to the out-end of the hose to allow for sequestration.

Sequestration experiments were conducted using several steps in a two-day period. Two 12 l stainless steel cans were filled with the herbicide solution of GF2726 at 593.21 g ae ha^{-1} and glyphosate at 1.1 kg ae ha^{-1} respectively. After thoroughly agitating the herbicide spray solution a 10 ml aliquot sample was taken from each can and would establish the first step in the analytical analysis. Valve bank and hose systems were then filled with the herbicide solution and allowed to incubate for 12 hours. After the system was filled, the valve bank was flushed of the herbicide solution only leaving the hoses

full of the herbicide mixture. After the 12-hour sequestration period, herbicide solutions were removed from each hose to allow sampling for analytical analysis. A 10 ml aliquot sample was then taken from each hose and would complement the second step in the analytical analysis. Each of the valve banks were then cleaned using 3 l of water while catching a minimum of 500 ml from each hose. A 10 ml aliquot was taken from each hose and would become the third step in the analytical analysis. Each can was then filled with 5.7 l of water with one can constituting the water cleanout and the other the ammonia cleanout. For the ammonia cleanout solution a 1% v/v of ammonia was used at a rate of 56.8 ml in 5.7 l of water. The cleaning solution was flushed through the valve bank and each hose releasing 500 ml at a time; the valves on each hose and at the valve bank were then closed to allow for the cleaning solution to incubate within. The valve bank was then flushed of all solution to allow sequestration within hoses only. The cleaning solution remained in the system for one hour. The cleaning solution was then removed from each hose separately which allowed for sample collection for analytical analysis. A 10 ml aliquot was collected from each hose and constituted the cleanout step in the analytical analysis. Twelve liters of clean water were then added to each spray can and flushed through each valve bank. Each hose in each valve bank was opened separately and 1500 ml of water were then flushed through each hose while closing the valve to allow for sequestration for fifteen minutes. This sequestration step would constitute the first water rinse and would become the solution applied within the field using cotton as a bio-indicator. Each hose in each cleanout step would essentially become a treatment within a rep (i.e. four hoses per valve bank and four reps within the field with four rinses per cleanout procedure). The solution was then removed one hose at a time

to allow sampling for analytical analysis. A 10 ml aliquot sample was collected from each hose and would become the first rinse in the analytical analysis. This cleanout step was replicated four times to constitute four rinses for each cleanout procedure. All solutions for field applications were then added to 591 ml bottles and applied to cotton at the pinhead square growth stage. Three standard treatments were added to the field trial along with all rinsates. Herbicide treatments consisted of one untreated check and two titrated GF2726 herbicide treatment standards used as field comparisons to rinsate treatments. The GF2726 treatments consisted of 3.74 and 0.374 g ae ha^{-1} respectively. A non-ionic surfactant (Induce®, Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017) was mixed with each field treatment at 0.25% v/v. For the three standard treatments a 10 ml aliquot was collected for analytical analysis.

Herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with TTI110015 (TeeJet Technologies, PO Box 7900, Wheaton, IL 60187) at an application volume of 140 l ha^{-1} and a pressure of 220 kPa. Herbicide treatments were applied when cotton plants were at the pinhead square growth stage. Visual estimates of cotton injury were recorded 7, 14, 21, and 28 days after treatment (DAT), using a scale of 0 to 100%, where 0 = no injury and 100 = total plant death. Chlorosis, necrosis, stunting, leaf cupping, epinasty, height reductions and regrowth were visually evaluated to estimate injury. Plant height and plant height reduction from the check were collected 7, 14, 21 and 28 DAT. Nodes above cracked boll (NACB) were collected before harvest. Cotton was machine picked where yield and yield reduction were calculated.

Analytical Evaluation

Samples from field studies were collected in 2014 and 2015 in 20 ml liquid scintillation vials (Sigma-Aldrich Company, LLC, 3050 Spruce St., St. Louis, MO 63103). Field samples were frozen for analytical analysis in 2014 and 2015. Samples were collected using a 50 ml silicone pipette filler, 3 way valve (Cole-Parmer instrument Company, LLC, 625 East Bunker Court, Vernon Hills, IL 60061) attached to a 10 ml serological, sterile, individually wrapped pipette (Cole-Parmer instrument Company, LLC, 625 East Bunker Court, Vernon Hills IL 60061). Samples were collected with one pipette per sample to eliminate potential for cross contamination.

Analytical analysis was performed at the University of Tennessee (University of Tennessee, Knoxville TN, 37996). Instrumentation used in the analysis began with the Agilent 1100 Series HPLC System (Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051). The Agilent 1100 series included the G1379A degasser, G1311A quat pump, G1313A auto sampler (ALS), G1316A thermostatted column compartment (colcom), and the G1315B diode array and multiple wavelength detector (dad). Analysis was performed with the use of mass spec and included the Agilent 6120 quadrupole single quad LC/MS G1978B. The liquid phase of the analysis was acetonitrile + 0.1% formic acid and water + 0.1% formic acid. Agilent chemstation software was used for the data capture and integration.

Samples collected were prepared by vortexing the solutions (Fisher Vortex Genie 2, Scientific Industries, INC., 80 Orville Dr., Suite 102, Bohemia, NY 11716) for 30 seconds. A 1 ml extraction from each of the 10 ml solutions collected from each treatment and rep was then extracted and added to 19 ml of methanol to constitute a 0.05

dilution rate. For the highest rates of 2,4-D in the initial three analytical steps and the cleanout step a further dilution rate was conducted to 0.00063. This was obtained by adding 1 ml of the solution to 19 ml of methanol and then extracting 250 µl of that solution into 19.75 ml of methanol. After all dilutions were made a final vortex of the solution was made for 30 seconds. A 2 ml extraction from each of the final dilutions was obtained with a BD 10 ml syringe with Luer-Lok™ (Becton, Dickinson and Company, 1 Becton Drive Franklin Lakes, New Jersey 07417-1880) and a 0.45 µm hydrophobic Polytetrafluoroethylene (PTFE) membrane filter (Thermo Fisher Scientific, INC., 09-719H, 300 Industry Drive, Pittsburgh, PA 15275) screwed to the end of the syringe. From this extraction, 1.5 ml were injected into a 12 x 32 mm target DP, clear glass vial, with a polypropylene open top cap, bounded PTFE/silicone septum (Thermo Fisher Scientific, INC. 300 Industry Drive, Pittsburgh, PA 15275).

The analysis began with an injection of methanol (to verify a lack of background carryover) followed by 2,4-D standards of 16.5, 30, 300 and 1000 ppb to establish linearity of MS response. A 2,4-D standard (30 ppb) was analyzed after every four unknown samples, to verify consistency of MS detector response over time. The conservative lower limit of detection was 5 ppb, and samples (with the exception of untreated samples) had 2,4-D concentrations above this amount. The initial three analytical samples, the cleanout step sample, the initial two rinse steps and the GF2726 standard samples were analyzed to determine a preliminary reading and if subsequent analysis was needed. Samples analyzed were those samples collected from the water only cleanout. Samples were selected based on visual observations in field.

These experiments were arranged as a factorial arrangement of treatments in a randomized complete block with factor A being rinse sequence and factor B consisting of cleanout procedure. The standards used in this experiment were averaged separetly and used as comparisons of the factorial. Four replications for each treatment were used in the experiment with a plot size area of 2 by 12 m. Data were pooled across site years because experimental replication was considered a random variable. Untransformed and arcsine square root transformed data were subjected to analysis of variance, but interpretations were similar to untransformed data; therefore, untransformed data were used for analysis. Data were analyzed using PROC GLIMMIX in SAS 9.4 and means were separated using Fischer's protected LSD test at P = 0.05.

Results and Discussion

Field Experiments

Results from field studies in 2014 and 2015 indicated differences with respect to injury 21 and 28 DAT (Table 4.2). At 21 and 28 DAT, the titrated standards of GF2726 were analyzed separately and used as comparisons. Both showed more injury than the rinse treatments of either water or ammonia (Table 4.2). Visual estimation of injury observations from the two rates of GF2726 did not influence NACB, height reduction, or yield reduction. These observations indicate 3.74 and 0.374 g ae ha^{-1}, which would be similar to that of a 1/128X and 1/1280X rate of 2,4-D, do not differ from the check with respect to the quantitative data when cotton is used as a bio-indicator.

Analytical Evaluation

Preliminary results from the analytical analysis indicate a reduction of the 2,4-D analyte from the initial analytical step through each subsequent step and ending with the first and second rinse step (Figure 4.2). A 1X use rate of 2,4-D yields roughly 2800 ppm analyte within the herbicide solution. This number is slightly increased to roughly 3000 ppm in the second analytical step once the herbicide solution is released after the 12 hour sequestration period. Once the initial water flush of the valve and hose system takes place in analytical step three, the analyte drops to 267 ppm (Figure 4.2). When the initial cleanout solution is added to the valve system, the analyte drops to 4 ppm (Figure 4.2). By time the rinse stages take place in rinse step one and two, ppm analyte is reduced to less than 1. For the titrated standards of GF2726, the rate of 3.74 and 0.374 g ae ha^{-1} yielded 22 and 2 ppm analyte retained, respectively (Figure 4.2). A plant response based on VEOI was observed in field experiments every year due to the GF2726 standards. These standards are at a rate of 1/128 and 1/1280X. It may be assumed that at these rates and with the ppm analyte retained within each rate a plant response is noted; but this response did not lead to differences with respect to the quantitative data of NACB, plant height reduction and yield reduction. For each step in the methodology a predetermined amount of water or cleanout solution was passed through the valve system and out of each hose separately. In the analytical step three and the cleanout step, 500 ml are run through each hose separately while sequestering 392 ml within the hose itself for analytical analysis. This totals 892 ml per step. Once the rinse steps begin in rinse steps 1, 2, 3 and 4, 1500 ml are passed through the hoses before sequestration of 392 ml takes place. This totals 1892 ml per step. A total of 9.352 l of water are passed through each

hose in each valve per replication of this study. This number is roughly 24X the amount of water retained within the hose itself. If the 1X use rate of GF2726 yields between 2800 and 3000 ppm and the 1/128X rate 22 ppm of the 2,4-D analyte, this shows a 0.7% use rate when compared to the 1X rate and did not reduce height or yield in any year of the study. Further dilution of the GF2726 analyte in the rinse stages of this study yielded less than 1 ppm, which was a subsequent use rate of less than 0.025% of the 1X rate. Dilution is the primary source of analyte reduction as opposed to cleanout procedure with respect to this study.

Table 4.1 Planting year, location, date, population, and seed variety information for Enlist hose contamination study.

Year	Location	Planting date	Variety[a]	Population
2014	Brooksville	May 20	Stoneville 4747	128,440 seeds ha^{-1}
2014	Starkville	May 6	Delta Pine 1321	123,500 seeds ha^{-1}
2015	Starkville	May 4	Delta Pine 1321	123,500 seeds ha^{-1}
2015	Brooksville	May 21	Delta Pine 1321	123,500 seeds ha^{-1}

[a] Stoneville 4747 (Bayer CropScience, 2 T.W. Alexander Dr., Research Triangle Park, NC 27709) Delta Pine 1321 (Monsanto Agrochemical Company, 800 North Lindbergh Blvd., St. Louis, Missouri 63167)

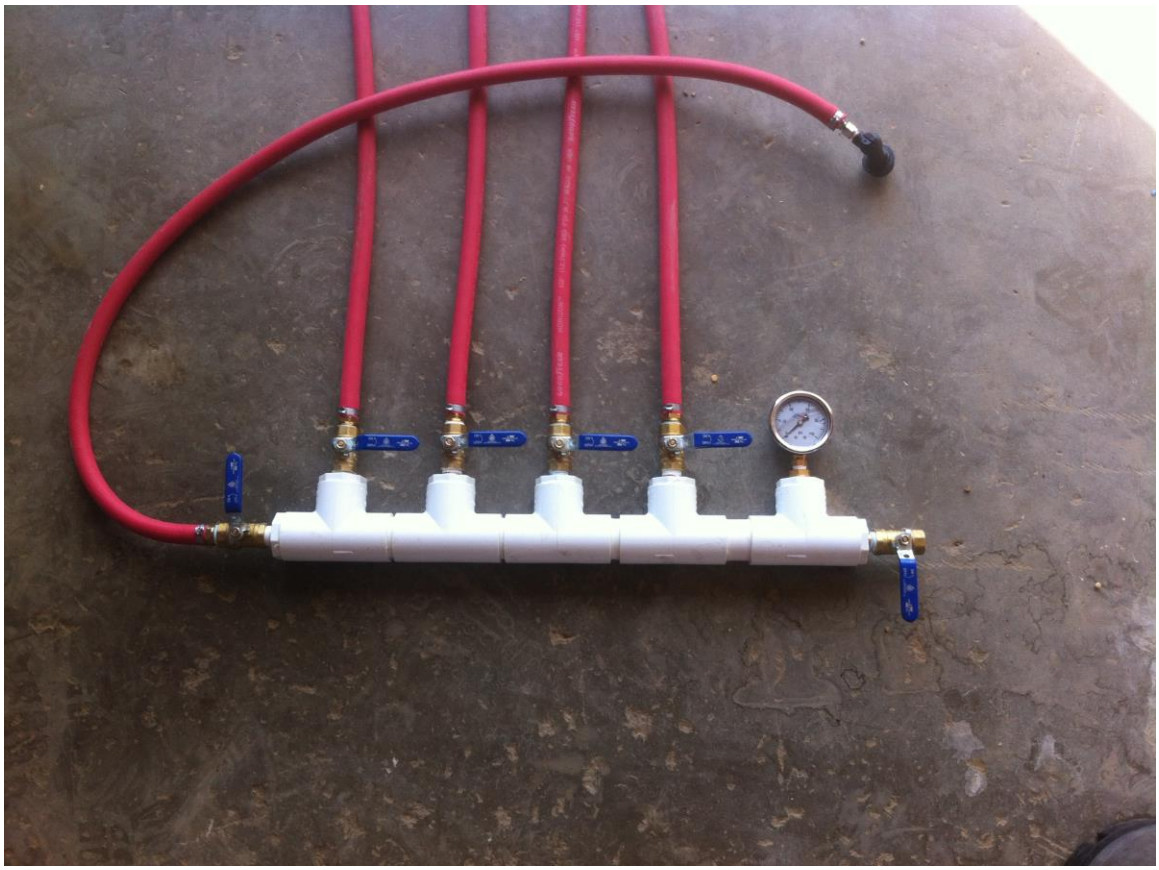

Figure 4.1 Finished valve bank and hose construction utilizing ball valves to allow for sequestration of GF2726 herbicide

Table 4.2 Visual estimation of injury on cotton from 2,4-D in hose rinsates with water and ammonia 21 and 28 DAT.

	Days After Treatment[a]	
Rate Titration[b]	21	28
----g ae ha^{-1}----	----------%----------	
3.74	5a	10a
0.374	0b	5b
0.0[c]	0b	0c

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LSD test at P = 0.05. A numerical LSD is given for each column group
[b]1X rate of GF2726 equals 0.59 kg ae ha^{-1}
[c]untreated check treatment

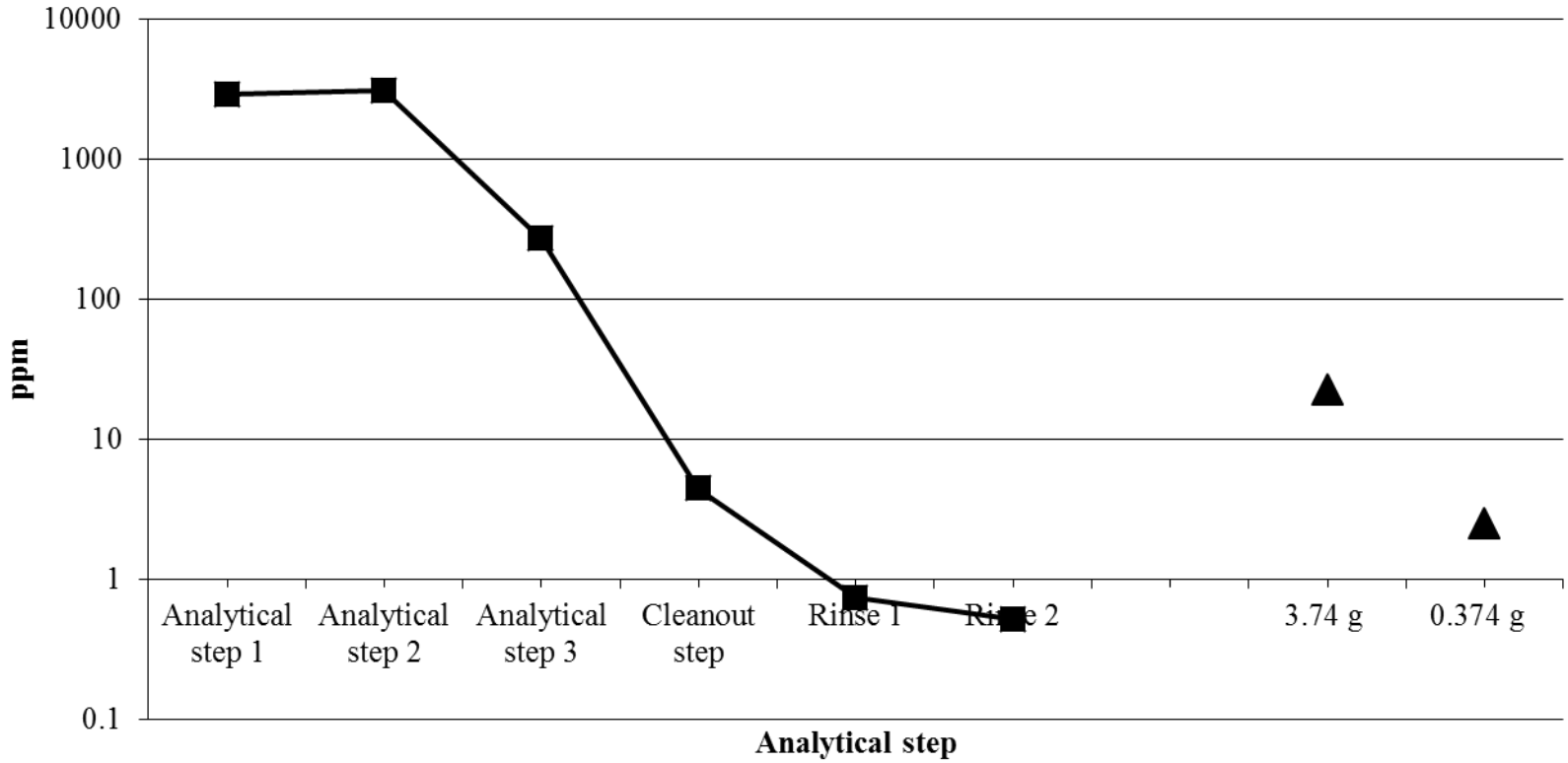

Figure 4.2 Analytical analysis indicating a reduction from initial analytical steps through rinsate steps and for the standard GF2726 treatments.

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CHAPTER V

DEACTIVATION OF 2,4-D AND DICAMBA RESIDUES WITH THE FENTON REACTION

Abstract

Field studies were conducted to evaluate the effect of the Fenton Reaction on various rates of dicamba and 2,4-D while using soybean and cotton as a bio-indicator. Soybean experiments from 2014 and 2015 showed an interaction with cleanout procedure and rate with respect to percent visual estimation of injury (VEOI), height reduction, yield reduction and ppm analyte retained. At 28 DAT, VEOI at the 1X (0.56 kg ha^{-1}) and 1/4X rate of dicamba alone showed 100 and 84% compared to 89 and 58% injury when the Fenton Reaction was applied, respectively. Soybean yield reduction at the 1/4X rate was 94% with dicamba alone and showed a 56% reduction from the check when the Fenton Reaction was applied. At the 1/16X rate, dicamba alone showed a 62% yield reduction from the check when compared to 32% with the Fenton Reaction. Cotton experiments from 2014 and 2015 showed an interaction with cleanout procedure and rate with respect to VEOI, height reduction, yield reduction and ppm 2,4-D analyte retained. At 28 DAT, the rates of 1X (0.56 kg ha^{-1}), 1/4X, 1/16X, 1/64X, and 1/256X of 2,4-D alone are significantly greater at 89, 57, 37, 27, and 13% visual injury when compared to the Fenton Reaction of 49, 31, 21, 14 and 4%. At the 1X, 1/4X, 1/16X, and 1/256X rates of 2,4-D alone yield reductions were 95, 83, 61, and 39% when compared to the Fenton

Reaction of 77, 53, 31, and 8%. These data show that the Fenton Reaction coupled with a dilution process reduced the occurance of tank contamination.

Nomenclature: Dicamba; 3,6-dichloro-2-methoxybenzoic acid; glyphosate; *Amaranthus palmeri*; soybean, *Glycine max* L.; 2,4-Dichlorophenoxyacetic acid; 2,4-D; Fenton Chemistry

Key words: Plant growth regulating herbicides, contamination, sequestration, tank contamination, drift, volitization, interaction

Introduction

Auxinic herbicides, such as 2,4-D and dicamba, have little soil residual activity (Senseman 2007) and have been extensively used for weed control for over 60 years primarily due to their selectivity, wide spectrum of weed control, efficacy, and low application costs (Mithila et al. 2011). Auxinic herbicides mimic natural occurring auxin, which is a plant growth hormone central to regulating plant growth and development (Abel and Theologis 1996). Auxinic herbicides, also commonly known as synthetic auxins, mimic the plant growth hormone indole-3-acetic acid (IAA); mimicking IAA disrupts growth and development processes, eventually causing plant death (Senseman 2007). Auxinic herbicides are readily taken up by the roots and foliage and are translocated in the both the phloem and xylem. 2,4-D controls broadleaf species such as carpetweed (*Mollugo verticillata* L.), horseweed (*Conzya canadensis* (L.) Cronq.), pigweed (*Amaranthus spp*.), and velvetleaf (*Abutilon theophrasti* Medik.), among many other problematic weed species that can be found in a cropping system. Dicamba is most commonly used to control annual broadleaf weeds such as pigweed (*Amaranthus spp*.), wild buckwheat (*Polygonum convolvulus* L.), and lambsquarters (*Chenopodium album*

L.); higher rates of dicamba are capable of controlling perennial broadleaf weeds such as field bindweed (*Convolvulus arvensis* L.) (Senseman 2007). Symptomology observed from auxin herbicides include: swelling of the stems, cupping of the leaves, epinastic twisting, chlorosis, and/or necrosis (Senseman 2007; Wax et al. 1969; Robinson et al. 2013; Egan et al. 2014).

Roundup Ready® soybean was introduced in the United States in 1996 followed shortly thereafter by RR cotton and RR corn with additional crops (including canola and sugar beet) also being released (Johnson et al. 2012a). However, after repeated glyphosate applications over many years and millions of hectares, the widespread evolution of weed populations resistant to glyphosate have become common (Johnson et al. 2012a). Glyphosate resistant weeds such as Palmer amaranth (*Amaranthus palmeri* S. Wats.), horseweed, common ragweed (*Ambrosia artemisiifolia* L.), and giant ragweed (*Ambrosia trifida* L.) (Heap 2013) are examples of difficult to control weeds that have driven a reevaluation of plant-growth-regulating (PGR) herbicides such as 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 2,4-Dichlorophenoxyacetic acid (2,4-D) for weed control. In response to the evolution of glyphosate resistant weeds, companies have been investing in new methods of weed control. Companies are searching for new active ingredients and modes of action, but the cost of development and the limited potential for economic return has made it difficult to bring new products to market (Johnson et al. 2012a). These companies have been on the forefront of genetically engineered crops, resistant to herbicides other than glyphosate. The introduction of dicamba and 2,4-D resistant crops was initiated because these herbicides have shown excellent resilience with few herbicide-resistant weeds occurring after more than 50 years of use (Johnson et

al. 2012a). Secondly, 2,4-D and dicamba provide excellent control of glyphosate-resistant broadleaf weeds such as horseweed, giant ragweed, common waterhemp (*Amaranthus rudis* Sauer), and other broadleaf weeds (Johnson et al. 2012b).

In 2013, the state of Mississippi harvested 0.8 million hectares of soybeans averaging 2,825 kg per hectare with the value of production at $1.2 billion (USDA-NASS 2012). Soybean growth is split into two stages, vegetative and reproductive, and within each stage there are more specific subcategories. Soybean reproductive growth stages are more important for soybean yield determination; the reproductive growth stages are when the seed number and size are determined (Pederson 2004). Reproductive growth stages begin when the first flower on the stem is present and is referred to as the (R1) growth stage, which is where the first pod will eventually form on the plant. The reproductive growth stage (R2) will form when there is an open flower at one of the two uppermost nodes on the main stem with a fully developed leaf. Reproductive growth stage (R3) will be determined when the pod reaches a length of 0.5 cm long and will appear in the upper four nodes of the soybean plant (Koger et al. 2013). The typical PGR injury symptoms in soybeans can be identified by the characteristic cupping of leaves with dicamba and injury can range from cosmetic leaf injury to 80% yield loss, depending on the amount of PGR residue left in the tank and the crop growth stage at application (Steckel et al. 2005). Soybeans exposed to 2,4-D or dicamba can develop vegetative malformations and produce a lower yield; however, the extent of that damage is dependent upon rate and application timing (Andersen et al. 2004). Wax et al. (1969) found that soybean is susceptible to dicamba application at vegetative and reproduction stages. Injury due to herbicide does not always lead to yield loss (Al-Khatib and Peterson 1999); soybean has

the ability to recover from early season injury depending on rate and application timing (Weidenhamer et al. 1989). Reduced soybean yield from dicamba exposure has been reported when dicamba caused severe injury and stunting, while yield reductions greater than 10% coincided with severe VEOI (Al-Khatib and Peterson 1999), such as terminal bud kill, splitting of the stem, swollen petioles, and curled malformed pods (Weidenhamer et al. 1989). Anderson (2004) concluded that V3 soybean sprayed with dicamba (0.0056 kg ha^{-1}) resulted in at least 40% visual injury 48 DAT with a 14% yield reduction. Dicamba was applied at 0.0112 and .056 kg ae ha^{-1} with these rates resulting in 13.8 and 71.5% yield reduction, respectively.

Monsanto has introduced MON 87708 soybean, which was genetically engineered from A3525, a high-yielding soybean variety genetically engineered to be resistant to dicamba by expressing a mono-oxygenase gene (DMO) from *Strenotrophomonas maltophilia* that rapidly demethylates dicamba, rendering it inactive (Johnson et al. 2012a; Behrens et al. 2007; USDA 2014). Their Roundup Ready Plus 2 Xtend System® will contain the Genuity® Roundup Ready 2 Yield® trait technology stacked with a trait enabling tolerance to dicamba (Monsanto 2013). By using an *agrobacterium* gene transfer, plants are inserted with genes that allow dicamba breakdown within the plant (Behrens et al. 2007).

Damage to cotton by 2,4-D has been reported since 2,4-D was first commercially introduced (Staten 1946). Cotton is considered one of the most susceptible agricultural crops to 2,4-D (Bayley et al. 1992). Hamilton and Arle (1979) found that dicamba applied over the top of cotton had less effect on cotton foliage, yield, boll components, and fiber properties when applied before bloom than when applied later in the season.

When cotton is in the reproductive phase of growth, these systemic herbicides reduced cotton yield more than contact herbicides (Snipes et al. 1992). Marple et al. (2007) reported greater cotton injury and yield reductions from simulated drift rates of 2,4-D than clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) or triclopyr (3,5,6-Trichloro-2-pyridinyloxyacetic acid). Cotton, in addition to any other broadleaved plant that does not possess resistance, is extremely sensitive to injury from dicamba. Marple et al. (2007) found that cotton was visibly injured by as little as 1/400th the labeled rate of 561 g ae ha^{-1}, however cotton was not as sensitive to dicamba as other auxin growth regulator herbicides such as 2,4-D, picloram (4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid), or fluroxypyr (4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid). Studies have been conducted at Mississippi State in the past where a rate titration of both dicamba and 2,4-D was applied to cotton (Smith et al. 2010). Smith et al. (2010) found yield reductions in both 2,4-D and dicamba experiments, however 2,4-D was more injurious to cotton than dicamba. The results of these studies suggest that cotton is more susceptible to 2,4-D than dicamba. Yield losses were observed where minimal visual injury was present.

Cotton growth stages are defined in many ways, from plant heights to total plant nodes, nodes above white flower, formation of fruiting structures and even days after planting. Accumulated heat units (DD_{60}s) are a major component in the growth cycle of cotton; the DD_{60} is an estimation of accumulated units during any given day and are based on the average of the maximum and minimum daily temperatures (Kerby et al. 1998). Approximately 4 to 14 days after planting emergence will occur and within 40 days after planting on nodes 5 to 7 the “pinhead squares” will become visible (Bednarz and Nichols 2005). Squaring is actually the term associated with the development of

fruiting structures prior to bloom, with the period from square to bloom lasting approximately 21 days. The general fruiting pattern for cotton is to have three day and six day vertical and horizontal fruiting interval, respectively (Jenkins et al. 1990). Following pinhead square is "match head square" or "one-third grown" square (Kerby et al. 1998). Once blooming or "flowering" begins it lasts for approximately 6 weeks (Kerby et al. 1998). When a flower first opens it is typically white and within a few hours of opening, it is pollinated. Flowers typically turn pink the second day after opening and within 5 to 7 days the flower itself dries, turns red in color, and falls off with a formed boll left in its place (Kerby et al. 1998). From plant to harvest takes approximately 140 days under optimum growing conditions and the plant has approximately 20 to 24 vertical nodes during a growing season (Jenkins et al. 1990).

Dow AgroSciences calls their 2,4-D-resistant technology the Enlist™ Weed Control System in corn, soybean, and cotton (Dow AgroSciences 2013). This technology became possible when the company inserted genes into broad-hectare agronomic crops that allow plants to metabolize 2,4-D (Johnson et al. 2012b). Dow AgroSciences has introduced the Enlist Duo™ formulation that contains glyphosate and 2,4-D: choline (Dow AgroSciences 2013). The herbicide features what Dow AgroSciences calls Colex-D™ Technology (Dow AgroSciences 2013), which provides ultra-low volatility, minimized potential for drift, lower odor, and better handling characteristics than commercially available 2,4-D amine or ester formulations (Johnson et al. 2012b). Enlist™ soybean, cotton, and corn will have traits that make them tolerant to 2,4-D as well as glyphosate and glufosinate (Dow AgroSciences 2013).

With the new triple stacked gene technology (glyphosate + glufosinate + dicamba or glyphosate + glufosinate + 2,4-D) soon to overwhelm the market, problems may arise from issues involving off-target movement from one producer's field to another, especially considering that not everyone will be so quick to adopt the new technologies. Unlike glyphosate, which is very water-soluble and can be easily cleaned out of a sprayer with water, the PGR herbicides take a lot more time, care and effort to be removed (Steckel et al. 2005). Considering that soybeans and cotton are extremely sensitive to PGR herbicides, it is imperative that a quality clean-out technique becomes the standard and adopted among producers. Auxin herbicides such as 2,4-D and dicamba can be extremely difficult to clean from spray equipment including nozzles, booms, tanks and pump systems. The normal course of action is to triple rinse with water or ammonia. In a 1955 University of California study best procedures for removing 2,4-D residues from spray tanks was examined (Vargas et al. 2001). Several metals (zinc, copper, tin, iron and aluminum) and glass were soaked in 2,4-D solutions and then rinsed by various procedures to remove the residue. After these materials had soaked in 2,4-D for 24 hours, the solution was poured off and the materials were then rinsed and subsequently analyzed for 2,4-D. In these early studies and subsequent investigations, nearly all the 2,4-D appeared to be rinsed from the metals and glass by the first of four rinses. However, subsequent rinse water used to soak the metal and glass for 24 hours showed varying amounts of absorbed 2,4-D were slowly released from the materials. The iron and zinc materials (galvanized iron) showed the greatest additional capacity to continue release of residual chemical, copper and glass trace residues and tin appeared to be free of contamination. Even rapid rinses in ammonia water did not remove the absorbed 2,4-D,

but the use of ammonia for prolonged (3 days) soaking appeared to increase the release of the absorbed 2,4-D, with the conclusion of the initial study stating that the only safe way to avoid 2,4-D contamination is to maintain separate sprayers for sensitive plants (Vargas et al. 2001). With this in mind and the introduction of old chemistries, in the form of auxin herbicides 2,4-D and dicamba, soon to overwhelm the markets it is worth an investigation into a re-evaluation of cleaning procedures for spray equipment. In this initial study conducted in 1955, it was even stated that ammonia does not work when attempting to clean auxin herbicide residues from surfaces. The way in which ammonia works as a cleaning agent, is that it will increase the pH to make compounds more water soluble. Keeping this in mind, dicamba and 2,4-D have pKa's of 1.7 and 2.8 respectively (Senseman 2007); they both act as weak acids and are already deprotonated when mixed in the spray tank with water. Using ammonia to increase the water solubility of an already deprotonated compound doesn't work in the case of auxin herbicides. With larger spray machines than ever before in the history of agriculture, these chemicals will have more places to hide and will eventually become harder to clean or eradicate from the system. Considering that the new technologies will use auxin herbicides and that there are different genetically engineered crops imploring the use of both auxin herbicides in conjunction with glyphosate and glufosinate, the risk for contamination may be extremely high.

The Fenton Reaction, also known as the Fenton chemistry, was described by H.J.H. Fenton who first described the oxidation process in 1894 while oxidizing tartaric acid by hydrogen peroxide (H_2O_2) in the presence of ferrous iron ions (Barbusinski, 2009). The Fenton reagent has been known for more than a century but its application as

an oxidizing process for destroying hazardous organics was not applied until the late 1960s (Barbusinski, 2009). Advanced oxidation processes (AOPs) employ chemical, photochemical, sonochemical or radiolytic techniques to bring about chemical degradation of pollutants. The most commonly used AOPs use H_2O_2, O_3 or O_2 as the bulk oxidant (Legrini et al., 1993). The principal active species in such systems is the hydroxyl radical (OH·). Second order rate constants of OH with compounds containing C-H or unsaturated C-C bonds typically are of the order 10^7 to 10^{10} l mol^{-1} s^{-1} (Buxton et al., 1988). This means that many compounds are potentially mineralized to CO_2, H_2O and inorganic ions (Barbusinski, 2009). Therefore, the objective of this research was to determine if the Fenton Reaction will deactivate dicamba and 2,4-D as a function of concentration and whether it will significantly influence percent visual injury, percent height reduction, percent yield reduction, and ppm analyte while utilizing soybean and cotton as a bio-indicator.

Materials and Method

Deactivation of dicamba and 2,4-D in Soybean and Cotton

Field studies were conducted in 2014 and 2015 to evaluate the deactivation of dicamba and 2,4-D using the Fenton Reaction while utilizing soybean and cotton as a bio-indicator. Experiments were conducted at the Black Belt Branch Experiment Station in Brooksville, MS on an Okolona silty clay (Fine, smectitic, thermic Oxyaquic Hapluderts) with 8% sand, 51% silt, 41% clay, 2% organic matter and pH of 6.8 and a Brooksville silty clay (Fine, smectitic, thermic Aquic Hapluderts) and the R. R. Foil plant research center in Starkville, MS on a Marietta fine sandy loam (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) with 71% sand, 17% silt, 13% clay and 1.03%

organic matter and a pH of 5.9. Planting date, planting populations, and seed variety varied among locations (Table 5.1).

For Soybean analysis, herbicide treatments consisted of dicamba (Engenia®, 600 g l^{-1}, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) at rates of 0.56, 0.14, 0.035, 0.009, 0.00218, 0.000549, and 0 kg ae ha^{-1}. For cotton analysis, herbicide treatments consisted of 2,4-D (GF2726, Enlist Duo™, The Dow Chemical Company, 9330 Zionsville Road, Indianapolis, IN 46268) at rates of 0.56, 0.14, 0.035, 0.009, 0.00218 and 0 kg ae ha^{-1}. Within all dicamba and 2,4-D rates, glyphosate (Roundup WeatherMax®, 540 g ae l^{-1}, Monsanto Company, St. Louis, Missouri, 63167) was applied at 1.1 kg ae ha^{-1}.

For field analysis, each rate of dicamba and glyphosate or 2,4-D and glyphosate were mixed in a solution volume of 1.9 l and applied to each experimental unit. Analytical samples were taken before application from each rate. Each rate of dicamba and glyphosate or 2,4-D and glyphosate was then mixed in a solution volume of 1.9 l where iron sulfate heptahydrate (F7002-1KG, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103) was added at a rate of 110 g per 1.9 l solution and agitated for one minute. After agitation, 30% hydrogen peroxide (216763-500ML, Sigma Aldrich, 3050 Spruce St., St. Louis, MO 63103) was added at a rate of 130 ml per 1.9 l solution and allowed to react for twenty minutes. Each dicamba and glyphosate or 2,4-D and glyphosate solution treated with the iron and peroxide was then applied to plots adjacent to experimental units previously sprayed with the corresponding rates. Analytical samples were taken before application of each rate.

Samples from field studies were collected in 2014 and 2015 in 20 ml liquid scintillation vials (Sigma-Aldrich Company, LLC, 3050 Spruce St., St. Louis, MO 63103). Samples were taken at the time of the experiment and frozen for analytical analysis. Samples were collected using a 50 ml silicone pipette filler, 3 way valve (Cole-Parmer instrument Company, LLC, 625 East Bunker Court, Vernon Hills, IL 60061) attached to a 10 ml serological, sterile, individually wrapped pipette (Cole-Parmer instrument Company, LLC, 625 East Bunker Court, Vernon Hills IL 60061). All samples were collected with one pipette per sample to eliminate potential for cross contamination.

Herbicide treatments were applied with a CO_2-pressurized backpack sprayer equipped with TTI110015 wide angle, air induction, tapered flat spray tip (TeeJet Technologies, PO Box 7900, Wheaton, IL 60187) at an application volume of 140 l ha^{-1} and a pressure of 220 kPa with a plot size area of 2 by 122 m. Soybean was sprayed at the R2 growth stage, while cotton was sprayed at the pinhead square growth stage. Visual estimates of soybean and cotton injury were recorded 7, 14, 21, and 28 days after treatment (DAT), using a scale of 0 to 100%, where 0 = no injury and 100 = total plant death. Chlorosis, necrosis, stunting, leaf cupping, epinasty, height reductions and regrowth were visually evaluated to estimate injury. Plant height and plant height reduction from the check were collected 7, 14, 21 and 28 DAT. Soybean and cotton were machine harvested and picked where yield and yield reduction were calculated.

The experiment was arranged as a split-plot arrangement of treatments in a randomized complete block with factor A consisting of the Fenton Reaction either occurring or not. Factor B consisted of a rate titration of dicamba and 2,4-D at various rates. Four replications for each treatment were used in the experiment. Data were pooled

across years because experimental replication was considered a random variable. Untransformed and arcsine square root transformed data were subjected to analysis of variance, but interpretations were similar to untransformed data; therefore, untransformed data were used for analysis. For analytical analysis, log transformed data were used because of the high variability due to rate comparisons. Data were analyzed using PROC GLM in SAS 9.4 and means were separated using Fischer's protected LSD test at P = 0.05.

Analytical Evaluation

Analytical analysis was performed at the University of Tennessee (University of Tennessee, Knoxville TN, 37996). Instrumentation used in the analysis began with the Agilent 1100 Series HPLC System (Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051). The Agilent 1100 series included the G1379A degasser, G1311A quat pump, G1313A auto sampler (ALS), G1316A thermostatted column compartment (colcom), and the G1315B diode array and multiple wavelength detector (dad). Analysis was performed with the use of mass spec and included the Agilent 6120 quadrupole single quad LC/MS G1978B. The liquid phase of the analysis was acetonitrile + 0.1% formic acid and water + 0.1% formic acid. Agilent chemstation software was used for the data capture and integration

Samples collected from field studies were prepared by vortexing (Fisher Vortex Genie 2, Scientific Industries, INC., 80 Orville Dr., Suite 102, Bohemia, NY 11716) the 10 ml solutions for 30 seconds then adding to 50 ml centrifuge tubes and spinning solutions at 5000 rpm for five minutes to pelletize the remaining iron flock. Samples were then extracted and added to 20 ml vials. A 1 ml extraction from each of the 10 ml

solutions collected from each treatment was then extracted and added to 19 ml of methanol to constitute a 0.05 dilution rate. For the higher rate titration of dicamba and 2,4-D at 0.56 and 0.140 kg ae ha^{-1} a further dilution rate was conducted to 0.00063. This was obtained by adding 1 ml of the aliquot solution to 19 ml of methanol and then extracting 250 µl of that solution into 19.75 ml of methanol. For the lower end of the rate titration of dicamba and 2,4-D, the dilution rate of 0.05 remained. After all dilutions were made a final vortex of the solution was made for 30 seconds. A 2 ml extraction from each of the final dilutions was made with a BD 10 ml syringe with Luer-Lok™ (Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, New Jersey 07417-1880) and a 0.45 µm hydrophobic Polytetrafluoroethylene (PTFE) membrane filter (Thermo Fisher Scientific, INC. 09-719H. 300 Industry Drive, Pittsburgh, PA 15275) screwed to the end of the syringe. From this extraction, 1.5 ml were injected into a 12 x 32 mm target DP, clear glass vial, with a polypropylene open top cap, bounded PTFE/silicone septum (Thermo Fisher Scientific, INC. 300 Industry Drive, Pittsburgh, PA 15275).

The analysis began with an injection of methanol (to verify a lack of background carryover) followed by dicamba or 2,4-D standards of 16.5, 30, 300 and 1000 ppb to establish linearity of MS response. A dicamba and 2,4-D standard (30 ppb) was analyzed after every four unknown samples, to verify consistency of MS detector response over time. The conservative lower limit of detection was 5 ppb, and all samples (with the exception of untreated samples) had dicamba or 2,4-D concentrations above this amount. Analysis was performed separetly between the two herbicides. Six replications of dicamba deactivation and four replications of 2,4-D deactivation were used in the analytical analysis. In essence fourteen samples of dicamba (seven rates activated and

seven rates deactivated) were used per replication. For 2,4-D, twelve samples (six rates activated and six rates deactivated) were used per replication. Reasoning for two extra replications of the analytical analysis with dicamba stems from an experiment performed within cotton in 2014 in which field data is omitted from these results.

Results and Discussion

Deactivation of dicamba and 2,4-D in Soybean and Cotton

At 7 DAT, VEOI in soybean was greater at rates of 1/64X or greater of dicamba when no Fenton Reaction occurs compared to the same rates when the Fenton Reaction was applied (Table 5.2). The 1X and 1/4X of dicamba alone showed 95 and 63% injury in soybean compared to 68 and 41% when the Fenton reaction was applied (Table 5.2). At 28 DAT, VEOI in soybean at the 1X and 1/4X of dicamba alone showed 100 and 84% compared to 89 and 58% injury when the Fenton Reaction was applied (Table 5.2). At the 1/1024X rate, there is are differences with respect to the check even with the Fenton reaction at 14, 21, and 28 DAT (Table 5.2). Although different from the check, the 1/1024X rate of dicamba with the Fenton Reaction showed 8% VEOI in soybean, while without the deactivation the same rate of dicamba showed 18% VEOI (Table 5.2). Soybean height reduction was affected by cleanout procedure and rate 7 DAT, but showed an interaction at 14, 21, and 28 DAT (Table 5.3). The 1X rate showed a 43% height reduction in soybean when compared to the check 7 DAT (Table 5.3). The dicamba alone treatment showed 21% height reduction when compared to 15% with the Fenton Reaction 7 DAT (Table 5.3). At 14 DAT, rates of 1/256X or greater of dicamba alone showed reductions in soybean height of 54, 42, 34, and 19% when compared to 38, 28, 25, and 8% with the Fenton Reaction (Table 5.3). Soybean height reduction 21 DAT

showed differences at the 1/4X and 1/256X rates of dicamba alone with 66 and 35% reduction when compared to the Fenton Reaction of the same rates of 55 and 17% reduction (Table 5.3). At 28 DAT, rates of 1/256X or greater of dicamba alone showed reductions in soybean height of 64, 55, 38, and 25% when compared to 49, 40, 30 and 13% with the Fenton Reaction (Table 5.3). Node reduction was greater with dicamba alone at the rates of 1X, 1/4X, and 1/256X of 79, 62, and 28% when compared to 64, 43, and 10% with the Fenton reaction (Table 5.3). Soybean yield reduction was different at the 1/4X and 1/16X rates of dicamba alone when compared to the same rates with the Fenton Reaction (Figure 5.1). Soybean yield reduction at the 1/4X rate was 94% with dicamba alone and showed a 56% reduction from the check with the Fenton Reaction (Figure 5.1). At the 1/16X rate, dicamba alone showed a 62% yield reduction from the check when compared to 32% with the Fenton Reaction (Figure 5.1). All rates were greater than the check with the exceptions of the 1/256X and the 1/1024X rates regardless of the treatment (Figure 5.1).

At every rating date, differences occur at each rate of 2,4-D alone compared to the Fenton Reaction occurring when compared to the check with respect to VEOI (Table 5.4). At 28 DAT, rates of 1/256X or greater of 2,4-D alone resulted in 89, 57, 37, 27, and 13% visual injury when compared to 49, 31, 21, 14 and 4% with the Fenton Reaction (Table 5.4). Cotton height reduction 14 DAT at the rates of 1X, 1/4X, and 1/16X of 2,4-D alone showed 47, 34, and 18% height reduction when compared to 30, 15, and -3% with the Fenton Reaction (Table 5.5). At 28 DAT, the 1X, 1/4X, and 1/16X concentrations of 2,4-D alone showed 60, 39, and 14% height reduction when compared to 32, 7, and -6% with the Fenton Reaction (Table 5.5). At rates of 1/256X or greater of 2,4-D alone cotton

yield reductions were 95, 83, 61, and 39% when compared to 77, 53, 31, and 8% with the Fenton Reaction (Figure 5.2). Yield reductions at the 1/64X rate was not different from the check when the Fenton Reaction was applied, but with 2,4-D alone at the same rate, yield reduction were different from the check (Figure 5.2).

Analytical Evaluation

There was a reduction of the dicamba analyte at the 1/16X, 1/64X, 1/256X, and 1/1024X rate when the Fenton Reaction was applied (Figure 5.3). These data do not fully explain why there is a decrease in yield reduction at the 1/4X rate (Figure 5.1) but yet, there is no difference in the dicamba analyte obtained from either treatment (Figure 5.3). If the solution is diluted to a 1/16X rate and the Fention Reaction is applied, a decrease in yield reduction occurs but is still greater than the check (Figure 5.1). It is not until the 1/256X rate in either dicamba alone or with the Fenton Reaction is yield reduction the same as the check (Figure 5.1).

There was a reduction in the 2,4-D analyte at every rate when the Fenton Reaction was applied (Figure 5.4). There is also a decrease in yield reduction at every rate when the Fenton Reaction was applied, with the 1/256X rate being the only exception (Figure 5.2). It is not until the 1/64X rate when the decrease in yield reduction due to the Fenton Reaction is the same as the untreated check (Figure 5.2). These concentrations will more likely approximate what might still be retained in a sprayer following an initial cleanout procedure. The Fenton reaction affectiveness is partially dependent upon moles of reactant available and is the probable reason it is not effective with the highest concentration of herbicides.

In conclusion, these data show that the Fenton Reaction coupled with a dilution process will reduce the occurance of tank contamination. With 2,4-D, there is a reduction in the analyte when the Fenton Reaction is applied which leads to a decrease in yield reduction at each rate. With dicamba, reductions in the analyte are not significant until the 1/16X rate. The Fenton Reaction proves that reduction in auxin analyte is possible but may be rate and molecule specific and may need a significant dilution process from the 1X rate before or after it is applied. More research is needed to determine at which rate the reaction is the most effective.

Table 5.1 Planting year, location, date, population, and seed variety information for Fenton Reaction studies in cotton and soybean.

Year	Location	Planting date	Variety[a]	Population
Soybean				
2014	Brooksville	May 1	Asgrow 5633	326,040 seeds ha^{-1}
2014	Starkville	May 30	Asgrow 4933	345,000 seeds ha^{-1}
2015	Starkville	May 4	Asgrow 4632	345,000 seeds ha^{-1}
2015	Brooksville	May 25	Asgrow 5332	326,040 seeds ha^{-1}
Cotton				
2014	Brooksville	May 20	Stoneville 4747	128,440 seeds ha^{-1}
2014	Starkville	May 6	Delta Pine 1321	123,500 seeds ha^{-1}
2015	Starkville	May 4	Delta Pine 1321	123,500 seeds ha^{-1}
2015	Brooksville	May 21	Delta Pine 1321	123,500 seeds ha^{-1}

[a]Asgrow Soybean (Monsanto Agrochemical Company, 800 North Lindbergh Blvd., St. Louis, Missouri 63167)
Stoneville 4747 (Bayer CropScience, 2 T.W. Alexander Dr., Research Triangle Park, NC 27709)
Delta Pine 1321 (Monsanto Agrochemical Company, 800 North Lindbergh Blvd., St. Louis, Missouri 63167)

Table 5.2 Visual estimation of injury in soybean from different rates of dicamba with and without the Fenton Reaction occurring 7, 14, 21 and 28 DAT.

		Days after treatment[a]							
		7		14		21		28	
Relative rate	Dicamba rate	No reaction	Fenton reaction[b]	No reaction	Fenton reaction	No reaction	Fenton reaction	No reaction	Fenton reaction
	kg ae ha^{-1}	----------	----------	----------	----------	----------	----------	----------	----------
					%-				
1X	0.56	95a	68b	100a	76b	100a	83b	100a	89b
1/4X	0.14	63b	41c	75b	47c	83b	55c	84c	58d
1/16X	0.035	44c	24de	48c	31de	52c	37d	54d	34e
1/64X	0.009	28d	18ef	35d	25ef	40d	29e	39e	28f
1/256X	0.00218	18ef	14fg	26e	18fg	28e	20f	28f	17g
1/1024X	0.000549	10gh	5hi	18g	8h	19f	8g	18g	8h
0X[c]	0	0i	0i	0i	0i	0h	0h	0i	0i

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column.
[b]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added
[c]untreated check treatments

Table 5.3 Soybean height reduction and node reduction from different rates of dicamba with and without the Fenton Reaction occurring 7, 14, 21, 28 DAT and at pre harvest.

		Days after treatment[a]								
		7	14		21		28		Node reduction	
Relative rate	Dicamba Rate		No reaction	Fenton reaction[b]	No reaction	Fenton reaction	No reaction	Fenton reaction	No reaction	Fenton reaction
	kg ae ha^{-1}	----------	----------	----------	----------	----------	%----------	----------	----------	----------
1X	0.56	43a	57a	54a	72a	68a	67a	64a	79a	64b
1/4X	0.14	32b	54a	38bc	66a	55b	64a	49c	62b	43c
1/16X	0.035	23c	42b	28de	52b	50b	55b	40d	47c	40cd
1/64X	0.009	18d	34cd	25fe	40c	39c	38d	30e	40cd	32de
1/256X	0.00218	6e	19f	8g	35c	17d	25e	13f	28e	10f
1/1024X	0.000549	1f	5gh	0h	8e	5ef	4g	2g	5fg	-2g
0X[c]	0	0i	0h	0h	0f	0f	0g	0g	0g	0g
Herbicide treatment										
No reaction		21a								
Fenton reaction		15b								

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column.
[b]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added
[c]untreated check treatments

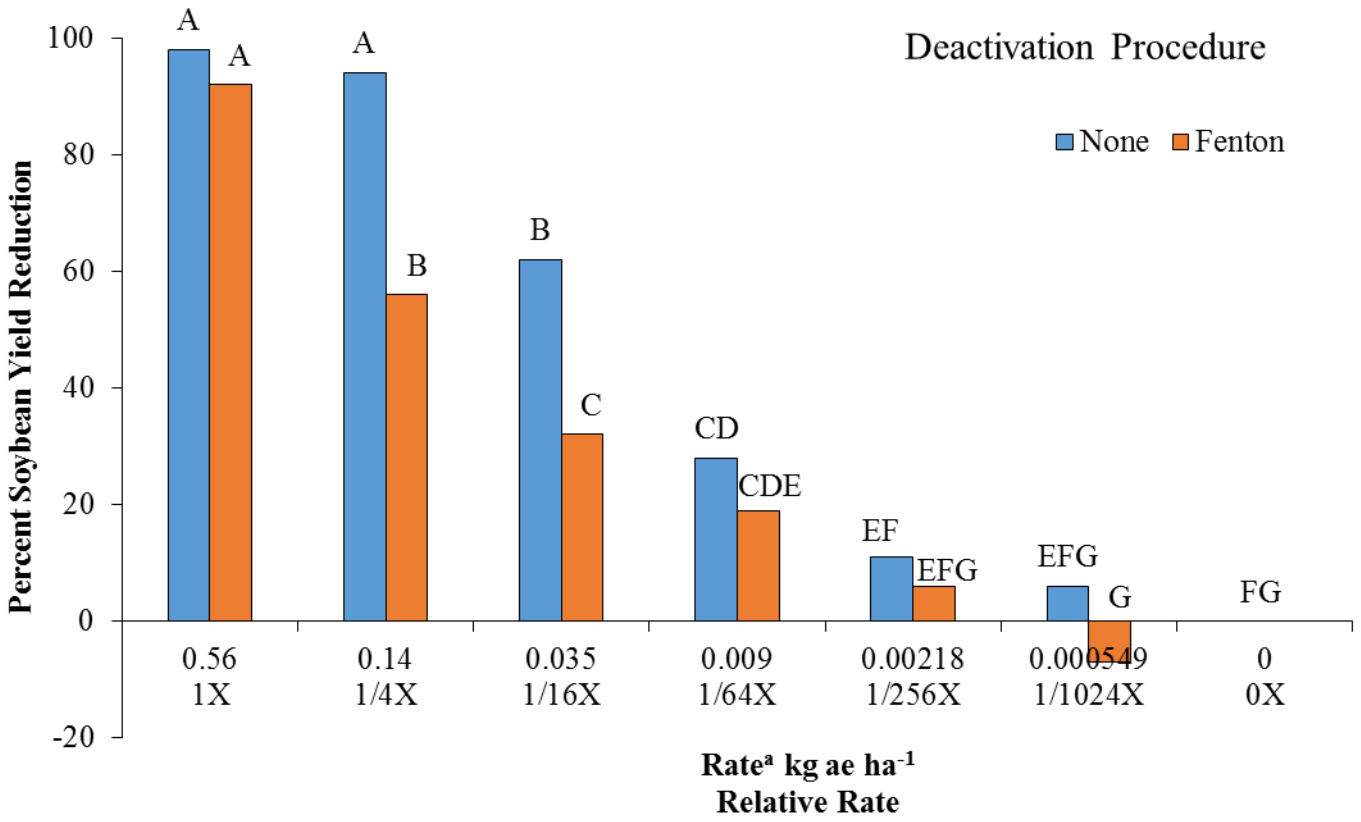

Figure 5.1 Soybean yield reduction from various rates of dicamba with and without the Fenton Reaction.

[a]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added

Table 5.4 Visual estimations of injury in cotton from various rates of 2,4-D with and without the Fenton Reaction occurring 7, 14, 21, 28 DAT.

		Days after treatment[a]							
		7		14		21		28	
Relative rate	2,4-D rate	No reaction	Fenton reaction[b]	No reaction	Fenton reaction	No reaction	Fenton reaction	No reaction	Fenton reaction
	kg ae ha^{-1}	----------%----------							
1X	0.56	62a	34b	73a	41b	86a	45c	89a	49c
1/4X	0.14	36b	19c	45b	24c	53b	28e	57b	31e
1/16X	0.035	21c	11d	26c	13d	34d	18f	37d	21g
1/64X	0.009	10d	5e	14d	6e	22f	11g	27f	14h
1/256X	0.00218	6e	1f	6e	0f	12g	2h	13h	4i
0X[c]	0	0f	0f	0f	0f	0h	0h	0i	0i

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column.
[b]110 g-1 iron sulfate heptahydrate and 130 ml-1 hydrogen peroxide added to the rate solution in 1.893 l-1 when Fenton Reaction is added
[c]untreated check treatments

Table 5.5 Cotton height reduction from various rates of 2,4-D with and without the Fenton Reaction occurring 7, 14, 21, 28 DAT.

		Days after treatment[a]							
		7		14		21		28	
Relative rate	2,4-D rate	No reaction	Fenton reaction[b]	No reaction	Fenton reaction	No reaction	Fenton reaction	No reaction	Fenton reaction
	kg ae ha^{-1}	----------%----------							
1X	0.56	29a	19b	47a	30b	55a	33b	60a	32c
1/4X	0.14	26ab	10c	34b	15c	35b	8c	39b	7e
1/16X	0.035	9c	-7e	18c	-3d	8c	-6d	14d	-6f
1/64X	0.009	1d	-5de	2d	-4d	2cd	-4d	-1f	-4f
1/256X	0.00218	1de	-2de	1d	-3d	1cd	-4d	-2f	-6f
0X[c]	0	0de	0de	0d	0d	0cd	0cd	0ef	0ef

[a]means within a rating date followed by a common letter are not different according to Fisher's Protected LD test at P = 0.05. A numerical LSD is given for each column.
[b]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added
[c]untreated check treatments

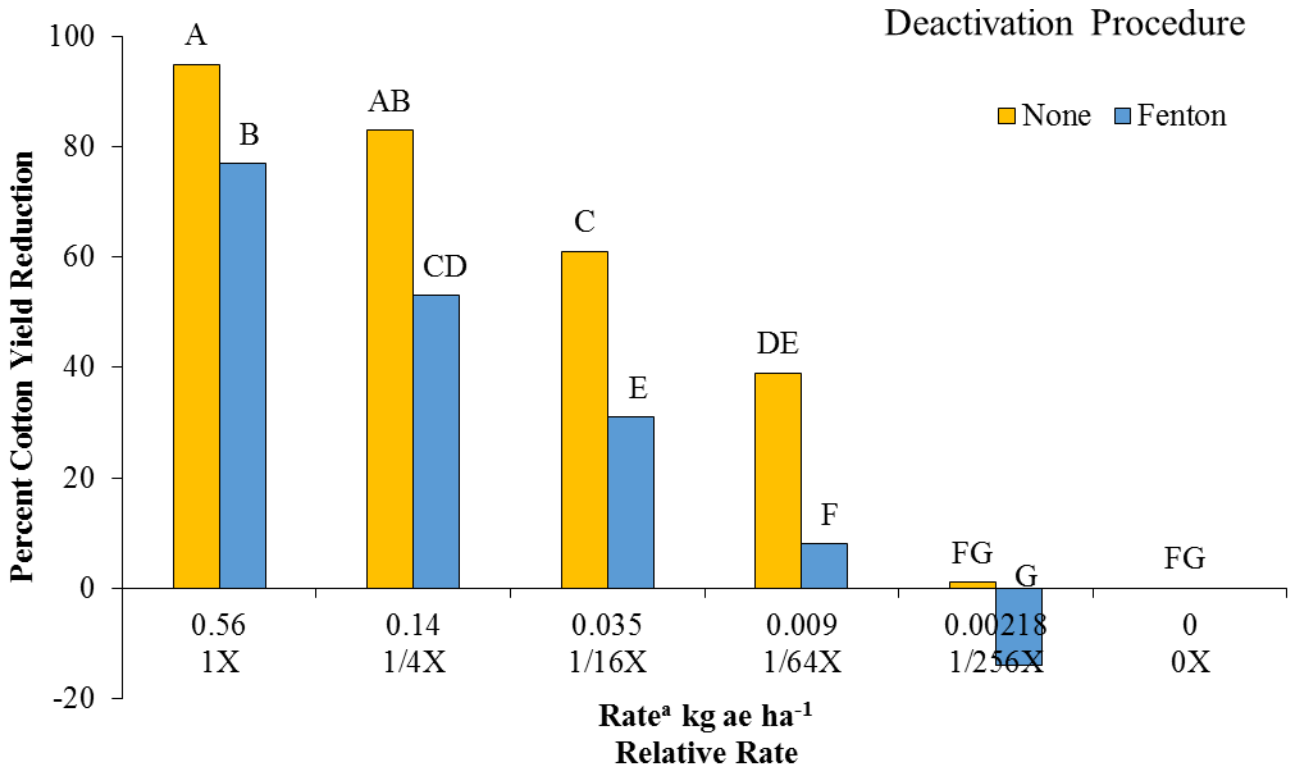

Figure 5.2 Percent cotton yield reduction from various rates of 2,4-D with and without the Fenton Reaction.

[a]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added

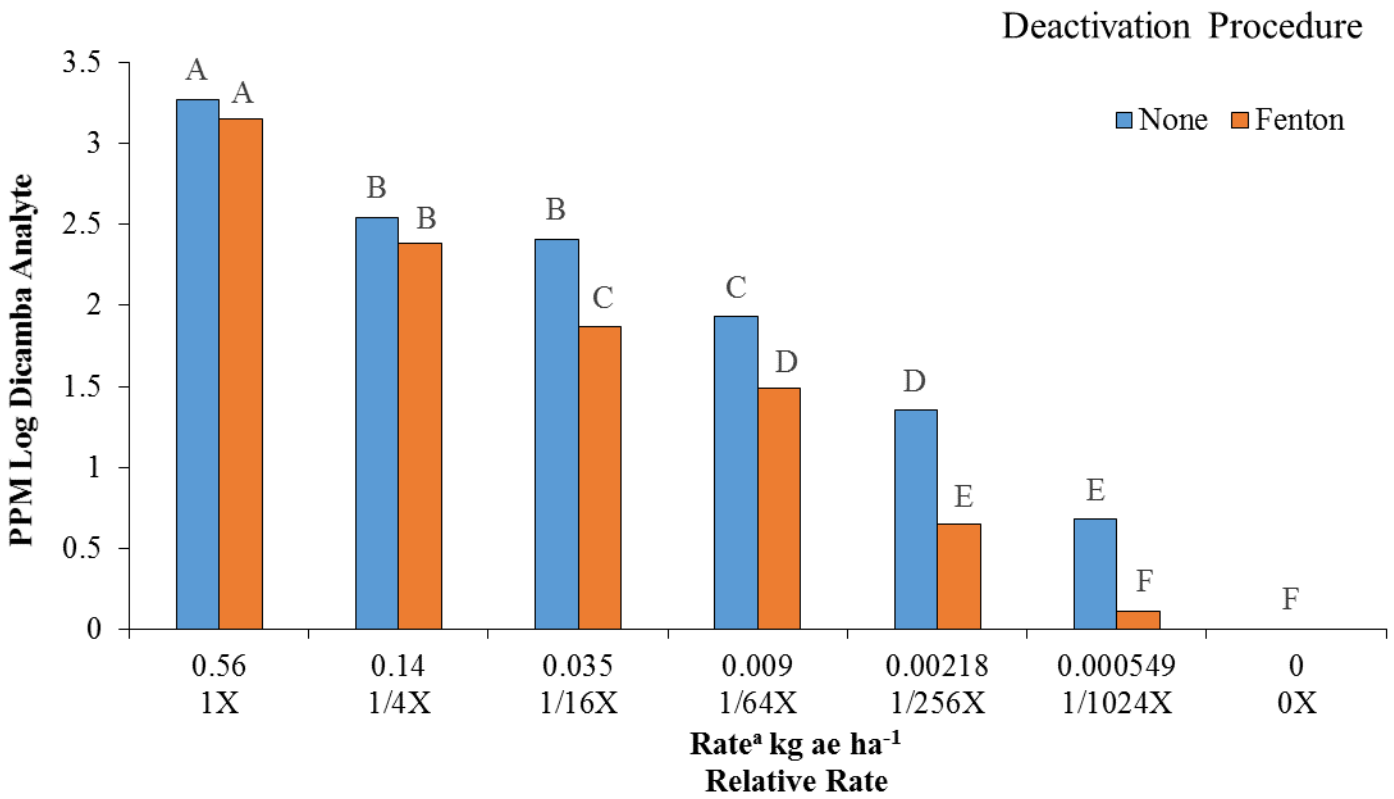

Figure 5.3 PPM log dicamba analyte from various rates of dicamba with and without the Fenton Reaction.

[a]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added

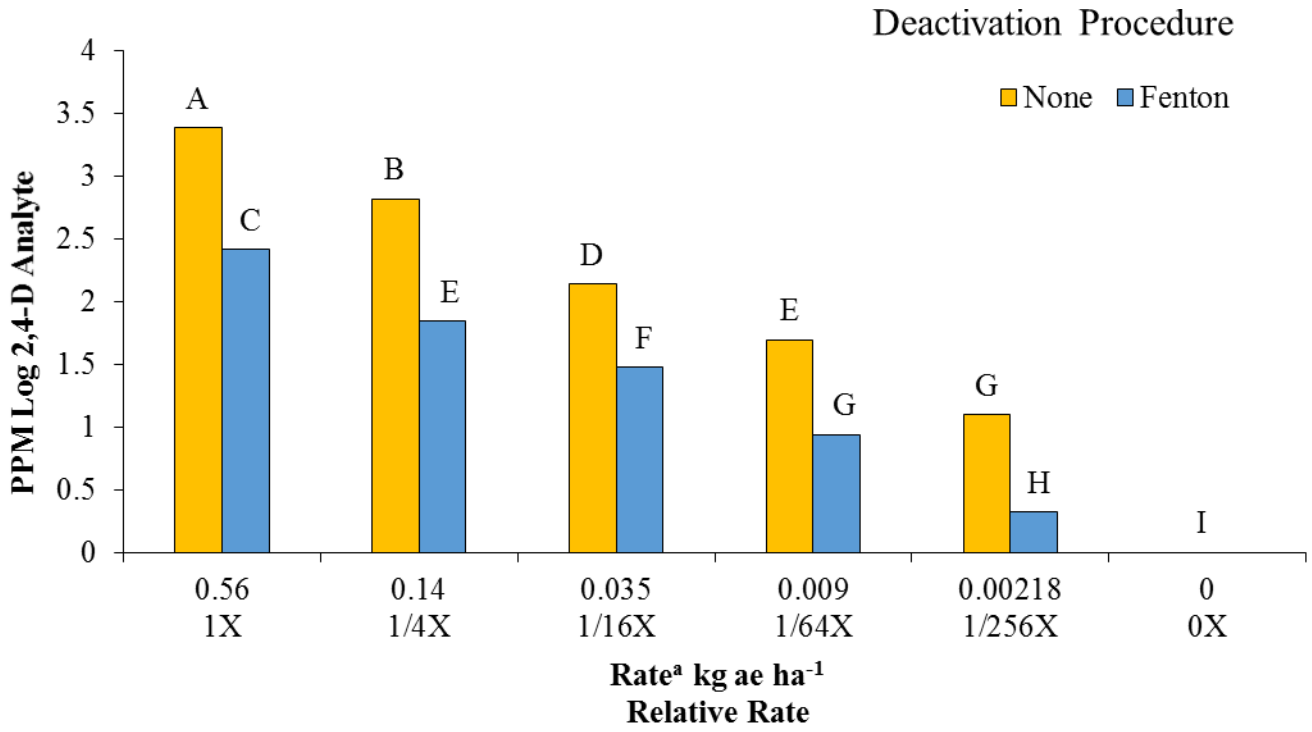

Figure 5.4 PPM log 2,4-D analyte from various rates of 2,4-D with and without the Fenton Reaction.

[a]110 g^{-1} iron sulfate heptahydrate and 130 ml^{-1} hydrogen peroxide added to the rate solution in 1.893 l^{-1} when Fenton Reaction is added

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