THE INCIDENCE AND RAMIFICATIONS OF GLYPHOSATE RESISTANCE IN COTTON

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Keywords: cotton, economic impact, glyphosate, glyphosate-resistant, weed science

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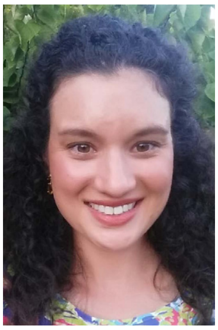

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Introduction

Herbicide-resistant cotton (*Gossypium hirsutum*) comprises one of the largest genetically modified crops within the United States (U.S.) along with corn (*Zea mays*) and soybean (*Glycine max*) (Figure 1). These genetically engineered (GE) crop hectares consisted primarily of the glyphosate-resistant trait shortly after commercialization of this technology in soybean, cotton, and corn in 1996, 1997, and 1998, respectively. Simplicity and increased grower revenues were prominent reasons cited for rapid adoption of glyphosate-resistant (GR) crops across vast areas. Ultimately, the overuse of glyphosate in Roundup Ready (RR) cotton in the U.S. quickly led to shifts in the weed spectrum and an increasing occurrence of GR weeds (Reddy & Norsworthy 2010; Riar *et al*., 2013). In 2012, it was estimated that more than 24.7 million ha had been affected by GR weeds (Owen *et al*., 2015) and that number has most likely increased. It has well been established that repeated use of any single management tactic, including cultural or nonchemical, across vast hectares will quickly select for resistance; hence, the occurrence of GR weeds across much of the U.S. Cotton Belt came as no surprise considering that RR cotton was planted on almost every cotton hectare, especially in the Midsouth and Southeast in early 2000s (Norsworthy *et al*., 2006; Nichols *et al*., 2009).

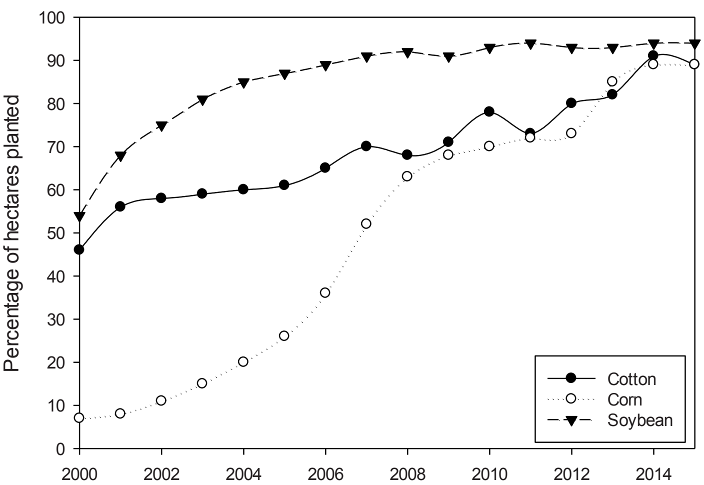

Figure 1. The percentage of US crop area planted in genetically engineered crops from 2000 to 2015. Adapted from USDA-ERS 2015.

Weed management in cotton

Herbicide resistance, particularly resistance of economically impactful weeds such as Palmer amaranth (*Amaranthus palmeri*) (pigweed) is one of the greatest threats to cotton production in the U.S. Glyphosate, which was very effective in controlling Palmer amaranth, was first reported to have herbicidal activity in 1971 (Baird *et al*., 1971) and was mainly used as a nonselective herbicide in non-cropped areas prior to becoming available for use in RR soybean in 1996 and then RR cotton in 1997 (Jones & Snipes, 1999). Weed management practices quickly changed when RR cotton was released (Norsworthy *et al*., 2007; Young, 2006).

Prior to GR Weeds. Weed management in cotton prior to the introduction of RR cotton relied heavily on preplant tillage, in-row cultivation, and post-directed herbicides in addition to use of residual herbicides throughout the growing season. Use of glyphosate in RR cotton was initially limited to applications from emergence through the four-leaf stage of the crop (Jones & Snipes, 1999). Thus, weed control continued to include the use of many herbicides, application timings, and application techniques (Table 1); albeit, use of preplant tillage and pendimethalin or trifluralin (HRAC Group K1) prior to planting cotton became less common in most cotton production regions. The complexity of this management tactic was due in part to the poor competitive ability of cotton with weeds and the multiple germination events of weed species in a growing season. The introduction of RR Flex (enhanced glyphosate resistance) cotton in 2006 allowed growers to apply as many as five glyphosate applications to cotton from

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cracking through 60% open cotton bowls safely (Owen *et al*., 2015; Norsworthy *et al.,* 2007). RR Flex cotton revolutionized and over simplified weed control in cotton because glyphosate (Roundup) controlled most economically important weeds and it was possible to obtain a high level of weed control in cotton with a single herbicide (Culpepper & York 1999). For example in 2006, more than half of the cotton crop consultants in Arkansas recommended four or more applications of glyphosate in RR Flex cotton (Norsworthy *et al*., 2006). The low cost of glyphosate coupled with the weed control spectrum and ease of glyphosate applications led to significant selection pressure on glyphosate. Due to this selection pressure and the lack of diversity in weed control programs, a large number of weeds evolved resistance to glyphosate in cotton producing regions of the U.S. during this period (Culpepper *et al*., 2010). Today, glyphosate-resistant (GR) weeds that can be found in cotton include Italian ryegrass (*Lolium multiflorum* ssp. *multiflorum*), Palmer amaranth, tall waterhemp (*Amaranthus tuberculatus*), horseweed (*Conyza canadensis*) (marestail), johnsongrass (*Sorghum halepense*), giant ragweed (*Ambrosia trifida*), and goosegrass (*Eleusine indica*) (Heap, 2015).

Following Widespread Glyphosate Resistance. The first glyphosate-resistant weed to impact U.S. cotton production was horseweed beginning in 2003 (Norsworthy *et al*., 2009). Programs were quickly developed and broadly implemented that included the use of dicamba plus residual herbicides for early spring burndown applications to manage and control GR horseweed populations (Norsworthy *et al*., 2006). Adoption of these additional herbicides has resulted in relatively no economic or yield loss to cotton in recent years.

Glyphosate-resistant Palmer amaranth has had the greatest deleterious impact on cotton by far and has caused the most notable changes in production practices than any other weed in the history of U.S. cotton production. GR Palmer amaranth was first documented in a Georgia cotton field in 2005 (Culpepper *et al*., 2006) and shortly thereafter in Arkansas and Tennessee (Norsworthy *et al*., 2008; Steckel *et al*., 2008). By 2008, GR Palmer amaranth had emerged as a threat to cotton production in the north Delta region of the Midsouth and much of the cotton production region across the southeastern Coastal Plains (Nichols *et al*., 2009). In cotton alone, it was estimated that 254,921 ha were infested with GR Palmer amaranth by 2008 in the states of Alabama, Arkansas, Georgia, Mississippi, North Carolina, South Carolina, and Tennessee. Within cotton fields, spread occurred rather quickly through seed movement by rain and irrigation along with tillage and harvest machinery, and in the absence of alternative modes of action, GR Palmer amaranth quickly overtook fields, resulting in complete crop loss (Figure 2) (Norsworthy *et al*., 2014). In addition to seed-mediated gene flow of GR Palmer amaranth, the high likelihood for pollen-mediated movement of glyphosate resistance was also demonstrated in laboratory and field experiments in Georgia (Sonsoskie *et al*., 2009, 2012).

GR Palmer amaranth completely changed cotton production, especially considering its multiple resistance to other herbicides. In several cotton producing states, Palmer amaranth is not only resistant to glyphosate, but also three other herbicide mechanisms of action that include acetolactate synthase (ALS) (HRAC Group B), microtubule assembly (HRAC Group K1), and most recently protoporphyrinogen oxidase (HRAC Group E) inhibitors (Heap, 2015). Therefore, most states have developed Integrated Weed Management (IWM) strategies to manage GR Palmer amaranth in cotton and other crops.

One of the most obvious impacts of GR weeds has been an increase in the number of herbicides needed to produce a cotton crop effectively (Table 1) along with a reduction in lint yield when these weeds are not adequately managed (Norsworthy *et al*., 2014). A once simple weed control program became very complex and expensive with the evolution of GR weeds. For instance, managing the soil seedbank became a critical component of a successful program for Palmer amaranth (Norsworthy *et al*., 2012). A single female plant can produce upwards of 500,000 seeds; therefore, the seedbank must be reduced to a level where Palmer amaranth management is possible. Several programs were developed to combat resistant Palmer amaranth. ‘Zero Tolerance’ was developed by University of Arkansas Extension faculty as an effort to remove Palmer amaranth plants from the field by any means necessary including: pulling, chopping, tillage, and spot spraying to prevent plants from producing seed and thus adding to the soil seedbank (Barber *et al*., 2015). By 2012, hand weeding occurred in more than 50% of cotton fields in several states as opposed to less than 5% in 2000 (Riar *et al*., 2013; Sosnoskie *et al*., 2014). One particular weakness of Palmer amaranth is that it is does not form a highly persistent seedbank; hence, the Zero Tolerance program was developed as a “community wide” approach to remove and manage Palmer amaranth, not only in the fields but also along field borders, ditches, equipment yards, and right-of-ways (Barber *et al*., 2015). Since introduction of this program in 2011, Arkansas’ producers who have implemented Zero Tolerance have significantly decreased the number of plants they have to manage on a field-wide basis.

Deep tillage involving a one-time moldboard plow was also found to be effective in burying the seed and reducing the number of seed that would germinate the next year in cotton (DeVore *et al*., 2012). Additionally, the use of cover crops seems to be one of the most effective cultural practices that can be implemented on-farm to reduce Palmer amaranth populations. Several studies at the University of Arkansas and other Midsouth universities have found that planting a cereal rye (*Secale cereale*) cover crop can greatly reduce the amount of Palmer amaranth seedlings that will germinate the following spring (DeVore *et al*., 2012). Today, the Natural Resource Conservation Service (NRCS) offers financial support to growers interested in using cover crops as a tool for managing resistant weeds, particularly those resistant to glyphosate.

In RR Flex cotton, use of protoporphrinogen oxidase (PPO) inhibitors applied preplant (Midsouth), preemergence (Southeast), and at layby (final directed application); photosystem II inhibitors (HRAC Groups C1, C2 and C3) applied preemergence and at layby; and very long chain fatty acid synthesis inhibitors (HRAC Group K3 – examples, metolachlor, acetochlor) make up the primary herbicide modes of action used to ensure a harvestable crop today. In addition to recommending glyphosate, dicamba, and flumioxazin for burndown weed control in late winter for GR horseweed and other winter vegetation, the University of Arkansas recommends fomesafen preplant followed by (fb) paraquat

Table 1. University of Arkansas recommended herbicide programs for Roundup Ready cotton in 2002, Roundup Ready cotton in 2006, and Glytol/Libery Link cotton in 2016. Adapted from UAEX 2015.

	Herbicide program		
Timing	**2002**	**2006**	**2016**
Burndown	Glyphosate	Glyphosate + Dicamba	Glyphosate + Dicamba
Preplant	Pendimethalin[a]	Paraquat	Glyphosate + Fomesafen
Preemergence	Fluometuron	–	Fluometuron + Paraquat
Early postemergence	Glyphosate	Glyphosate	Glufosinate + Metolachlor
Mid-postemergence	Glyphosate	Glyphosate + Metolachlor	Glufosinate + Metolachlor
Midseason directed	Glyphosate + Prometryn	Glyphosate + Prometryn	–
Layby directed	Glyphosate + Diuron	Glyphosate + Diuron	Glyphosate + Flumioxazin + MSMA

[a]Soil incorporation of pendimethalin was recommended

plus fluometuron at planting fb glufosinate plus *S*-metolachlor early postemergence. Another application of glufosinate plus *S*-metolachlor may be needed and should be followed by an application of MSMA plus flumioxazin, pyroxasulfone, or diuron post-directed at layby (Table 1; Scott *et al.,* 2015). This program may still require that some escapes be removed by hand. If producers have not planted cotton cultivars resistant to glufosinate, then applications of numerous residual herbicides must be made to overlap and maintain protection through layby (Whitaker *et al.,* 2011).

Since the appearance of GR Palmer amaranth, most producers have switched to planting Liberty Link (LL) cultivars that are resistant to glufosinate (HRAC Group H) or Widestrike cultivars containing the phosphinothricin aceytltransferase gene, a genetic marker that inactivates glufosinate and provides partial resistance to the herbicide. Cotton varieties that contain the LL or Widestrike trait have been planted on the majority of the cotton hectares in the Midsouth and Southeast from 2010 to 2015, simply because glufosinate is an effective option for controlling GR weeds, if applied correctly (Culpepper *et al*., 2009; Everman *et al*., 2007). Today, almost all herbicide transgenic cotton planted in the Midsouth or Southeast will be tolerant to over-the-top application of both glufosinate and glyphosate. Glufosinate is not as effective on Palmer amaranth or grasses as glyphosate once was; however, it is currently the only herbicide option producers have to manage GR Palmer amaranth after emergence in cotton due to its resistance to most other postemergence herbicides. Current extension recommendations for GR Palmer amaranth differ slightly by state, but in general, the use of residual herbicides is highly recommended and required in order to have a harvestable crop.

Economics. GR weeds have had a profound economic impact on cotton, most notably through increased input costs. Herbicide and seed costs differ by region, but by looking at changes in prices over time for a particular region, some general conclusions regarding the cost of glyphosate resistance can be drawn. For the purpose of this paper, one means of assessing the added costs of GR weeds was to compare enterprise budgets for seed and technology fees along with estimated herbicide expenditures prior to glyphosate resistance to those after resistance evolved. Additionally, published grower and consultant surveys were used to capture the added expense of GR weeds. Even with increased input costs, reduced cotton lint yields due to GR Palmer amaranth are known to occur, especially in the absence of hand weeding (Norsworthy *et al*., 2014), and no attempt was made to estimate monetary losses associated with lower yields or increased wear on equipment. Additionally, it is acknowledged that glyphosate resistance may have a negative environmental impact through greater herbicide use and tillage intensity; albeit, there was no effort to quantify the positive (increased cover crop use) or negative (increased herbicide or tillage use) environmental impact of glyphosate resistance. It is well documented that RR cotton did permit the wide-spread adoption of conservation tillage practices (Price *et al*., 2011); although, these conservation gains were rapidly negated by the evolution of GR weeds, mainly horseweed and Palmer amaranth, which drove producers to once again re-integrate tillage into their cotton production systems (Riar *et al*., 2013; Sosnoskie *et al*., 2014).

Based on University of Arkansas Enterprise Budgets for Northeast Arkansas in 2002, it was estimated that a grower would pay \$57.32/ha for cotton seed, including the RR technology fee, and an additional \$111.57/ha for herbicides, excluding application costs (UAEX 2015). Even with GR horseweed quickly spreading across much of the Midsouth Cotton Belt following its confirmation in Arkansas and Tennessee fields in 2003, the estimated herbicide costs for cotton producers in

Figure 2. Glyposate-resistant Palmer amaranth in cotton.

Northeast Arkansas actually decreased $32.93/ha from 2002 to 2006. GR horseweed brought forth a need for growers to use dicamba in their burndown programs (Table 1), but the increased cost of dicamba was offset by a reduction in the cost of glyphosate after patent protection was lost. Interestingly, the costs of cotton seed having tolerance to glyphosate, including the technology fee, more than doubled from 2002 to 2006, which more than offset the savings in herbicide costs associated with growing RR Flex cotton. Even though glyphosate resistance was mainly limited to horseweed in 2006, cotton consultants in Arkansas estimated each new resistant weed in cotton would require growers to add $62.84/ha to their weed management costs (Norsworthy *et al*., 2007).

As noted earlier, the rapid spread of GR Palmer amaranth after 2006 across the Midsouth and Southeast Cotton Belt caused growers to look closer at integrating a cotton trait into the system that could withstand over-the-top applications of glufosinate. The evolution of GR Palmer amaranth in cotton forced growers to integrate residual herbicides into their weed control programs, especially in Arkansas where furrow irrigation is widely used, because topical applications of glyphosate and ALS-inhibiting herbicides were virtually ineffective (Owen *et al*., 2015). In other states, growers had to integrate cotton varieties with tolerance to glufosinate into their weed control programs quickly. Widestrike cotton (Phytogen cultivars), having partial tolerance to glufosinate and complete tolerance to glyphosate, was widely grown in areas where irrigation was less prominent and herbicide activation was more dependent upon rainfall (Owen *et al*., 2015).

Initially, some growers opted to plant Phytogen Widestrike cotton and treat it with glufosinate whereas others quickly switched to LL cotton due to its higher level of tolerance to glufosinate. Once cotton became available with commercial tolerance to both glyphosate and glufosinate (Glytol/LL), growers quickly migrated to this technology because of inexpensive grass control with glyphosate along with the tank-mix option of glufosinate for controlling GR weeds. Current herbicide costs associated with the production of Glytol/LL Bollgard II cotton are estimated to be $300.48/ha along with seed costs of $296.85/ha, which includes all technology fees and a fungicide/insecticide seed treatment. Excluding herbicide application, cultivation, and hand-weeding costs, these budgets for cotton show that herbicide and seed costs more than tripled from 2002 to 2016 (UAEX 2015). Similarly in Georgia, cotton farmers reported that herbicides and expenditures for the control of Palmer amaranth from 2006 to 2010 were more than double those from 2000 to 2005 (Sosnoskie & Culpepper, 2014).

In addition to herbicide expenditures and those associated with the technology fee imposed on the purchase of transgenic cotton seed, hand weeding has been a common practice in much of the Midsouth and Southeast Cotton Belt. In Georgia, 52% of the cotton crop was hand-weeded from 2006 to 2010 at an average cost of $57 per hand-weeded ha compared to a cost of no more than $2/ha prior to GR Palmer amaranth (Sosnoskie & Culpepper, 2014). Similarly in Arkansas, Tennessee, and Mississippi, it was estimated that 49% of the cotton crop was hand-weeded for GR Palmer amaranth in 2011 at an average cost of $50 per hand-weeded ha (Riar *et al*., 2013). Additional expenses that occur but are not well captured include an increase in tillage for incorporation of preplant herbicides, row cultivation, and post-harvest deep turning of soil for weed control as noted earlier (Sosnoskie & Culpepper, 2014).

Future management of glyphosate-resistant weeds in cotton

New technologies that should aid in the management of GR Palmer amaranth and other difficult-to-control weeds are on the horizon. Roundup Ready Xtend cotton which is being developed by Monsanto will eventually allow for over-the-top applications of glyphosate, glufosinate, and dicamba herbicides, and the Enlist technology being developed by Dow AgroSciences will offer cotton producers the option of applying glyphosate, glufosinate, and 2,4-D to cotton. Both of these new technologies will offer two effective modes of action postemergence to manage GR broadleaves. While this is encouraging, it should be noted that none of these herbicides are as effective at managing Palmer amaranth as glyphosate once was. Both of these new technologies offer good tools for weed management, and, if used correctly in a program with diverse residual herbicides, may evade future resistance of Palmer amaranth and other broadleaves and provide options until new herbicides with unique modes of action are developed.

Conclusion

Glyphosate resistance in cotton is not unique to Palmer amaranth. Weed management with any technology requires an integrated approach utilizing cultural methods such as cover crops, hand weeding, tillage when necessary as well as herbicides to diversify management practices and delay further resistance development (Norsworthy *et al*., 2012). A diverse program that focuses on maintaining a low soil seedbank is crucial for long-term success of weed management programs in cotton.

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