The Rise and Future of Glyphosate and Glyphosate-Resistant Crops

Running Title: Glyphosate and glyphosate-resistant crops

Jerry M. Green*

*Correspondence to: Jerry M. Green, Green Ways Consulting LLC, Landenberg, PA 19350, USA

E-mail: jerry.m.green@greenwaysconsulting.com

Abstract

Glyphosate and glyphosate-resistant crops had a revolutionary impact on weed management practices, but the epidemic of glyphosate-resistant (GR) weeds is rapidly decreasing the value of these technologies. In areas that fully adopted glyphosate and GR crops, GR weeds evolved and glyphosate and glyphosate traits now must be combined with other technologies. The chemical company solution is to combine glyphosate with other chemicals, and the seed company solution is to combine glyphosate resistance with other traits. Unfortunately, companies have not discovered a new commercial herbicide mode-of-action for over 30 years and have already developed or are developing traits for all existing herbicide types with high utility. Glyphosate mixtures and glyphosate trait combinations will be the mainstays of weed management for many growers, but are not going to be enough to keep up with the capacity of weeds to evolve resistance. Glufosinate, auxin, HPPD-inhibiting and other herbicide traits, even when combined with glyphosate resistance, are incremental and temporary solutions. Herbicide and seed businesses are not going to be able to support what critics call the chemical and transgenic treadmills for much longer. The long time without the discovery of a new herbicide mode-of-action and the epidemic of resistant weeds is forcing many growers to spend much more to

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manage weeds and creating a worst of times, best of times predicament for the crop protection and seed industry.

Keywords: biotechnology, weed, tolerance, mixture, formulation, stacked traits, weed management

1 INTRODUCTION

Growers urgently needed glyphosate when glyphosate-resistant (GR) crops when they became available in 1996, making glyphosate the herbicide of choice in these major crops. Weeds were evolving resistance to most commonly used selective herbicides and making weed management too time consuming for both the increasingly large and more efficient farm operations as well as the small farms that lacked the hired expertise to determine the necessary herbicide prescriptions. The ability to use glyphosate in GR crops was revolutionary. Growers could have used glyphosate to increase the diversity of their weed management practices, but they did the reverse. Glyphosate was good enough to use alone, and many growers used it alone quite successfully for the first 15 years.

Using glyphosate in GR crops was easy, economical, effective, and environmentally compatible. Growers rapidly adopted GR crops wherever they became available and made GR crops the most rapidly adopted technology in the history of agriculture.[1,2] Today there are six main GR crops: soybeans [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), canola (*Brassica napa* L.), alfalfa (*Medicago sativa* L.) and sugar beet (*Beta vulgaris* L.). More than half of the glyphosate used in the world, 56 percent of an estimated 8.6 billion kg, is used on GR crops,[3] which account for more than half of the 180 million ha of the genetically modified crops planted in 2015.[1]

Glyphosate and GR crops transformed weed management practices in most of the largest agricultural markets in North and South America, but the revolutionary impact is over. GR crops will remain fully adopted where they have been accepted and their use with glyphosate will even expand into some new crops and geographies.

2 RISE OF GLYPHOSATE-RESISTANT CROPS

The genetically modified herbicide-resistant (HR) crop revolution has been based on a remarkably few genes (Figure 1).[2] Most of the impact was due to just one gene, *cp4 epsps* that encodes for glyphosate-resistant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, EC 2.5.1.19). GR crops gave companies a new way to intellectually protect technology and make money. The success of GR crops brought huge investments to the seed industry.[4] Unfortunately, companies often just shifted funds from pesticide discovery budgets to transgenic seed development.

When GR crops were introduced, key glyphosate patents were expiring and generic manufacturers were selling glyphosate at low prices. A patent for using glyphosate on GR crops gave new intellectual property protection. Still, the price of glyphosate continued to decline and reduced the demand for more expensive selective herbicides. In the U.S., companies dramatically reduced the price of selective herbicides but sales still declined.[3] In 2002 after six years of sales, the number of herbicides used on 10 percent of the U.S. soybeans decreased from 11 to just one and that one was glyphosate.[5]

The GR crop systems were not perfect, but they were good enough.[6] Crop safety margins were often narrow and glyphosate application times restricted. Side-by-side comparisons of isogenic lines were generally not allowed by independent researchers, but it was clear that some initial varieties were lower yielding.[7] The issue is no longer as relevant because GR crops are so widely adopted that they are the 'conventional' crops now.

The process to commercialize new genetically modified crops was very expensive and slow, but having regulatory approval and patent protection allowed Monsanto to charge growers a technology fee and require them to sign an unprecedented contract that waived some of their rights including the ability to replant seed. The contract was a risky marketing innovation that growers strongly complained about but still signed. The contract dramatically reduced grower-saved seed, which was essential to maintain the value of the technology for the seed developers and suppliers.

The initial GR crops are now off-patent, and the value of glyphosate and GR crops is declining because of GR weeds. However, glyphosate continues to get less expensive, and GR traits are fully established in the germplasm of major crops. There is relatively little cost now to maintain the availability of the trait. GR seed is so common and generic glyphosate so inexpensive that some corn, soybean and cotton growers will likely just assume the seed they buy is GR and add glyphosate to every spray as they do surfactants.

3 PROBLEMS WITH RESISTANT WEEDS

Glyphosate was initially extremely effective, and many growers totally relied only on it. A key question always was whether such a widely used system that relied only on glyphosate would be sustainable. Before GR crops, glyphosate was already being used widely with few suspected cases of resistance, so it clearly was not as vulnerable to the evolution of resistant weeds as some other herbicide types. In addition, the development of GR crops had been very difficult, so difficult that some thought weeds would not naturally evolve resistance.[8] However, the unprecedented use of glyphosate over very wide areas and the concomitant decline in the use of other herbicides created such tremendous selection pressure that GR weeds eventually evolved.[9]

Looking back, growers should have used glyphosate as a new mode-of-action in combination with existing selective herbicides to diversify their weed management practices. Photosystem II (PSII) inhibitors such as triazine and urea herbicides, lipid synthesis inhibitors such as S-metolachlor, and inhibitors of phytoene desaturase (PDS), and protoporphyrinogen oxidase (PPO) are still very useful and provide soil residual activity on key GR weeds.[10] However, growers *en masse* switched to using only glyphosate in GR crops.

The overuse of glyphosate opened Pandora's Box of GR weeds. So far, weeds have evolved nine mechanisms of resistance to glyphosate and more are possible.[11] Unfortunately, most GR weeds are resistant to other herbicides. Currently, 18 GR weed species are reported to be resistant to other herbicides.[12] These multiple HR weeds threatening current crop production practices and left many growers almost out of weed management options.[2,10] GR weeds are forcing growers to use large volumes of partially effective herbicides that have hardly been used since the introduction of GR crops. Growers have lost the benefit of being able to use less herbicide because of GR weeds, e.g., U.S. soybean growers now use 28 percent or 0.30 kg/ha more herbicides.[13]

Some of the most problematic GR weeds are *Amaranthus palmeri* S. Wats., *A. tuberculatus* (Moquin-Tandon) J. D. Sauer, *Ambrosia artemisiifolia* L., *A. trifida* L., Conyza canadensis (L.), *Lolium rigidum* Gaud., and *Sorghum halepense* (L.) Pers.[12] Their spread has reached the tipping point and is finally forcing growers to change their weed management practices. So far, the first response of many growers to GR weeds has been chemical – reapply glyphosate, use higher rates, and then mixtures of glyphosate with other herbicides. That approach will not be effective much longer. New herbicide technologies are needed to sustain chemical herbicide systems, especially since some of the non-target site resistance mechanisms that have evolved can give cross-resistance to new herbicides before they are ever used.[14,15]

Synthetic herbicides have had a revolutionary impact on weed management, but no new modes-of-action have been discovered for over three decades and herbicide technology needs to be renewed.[16] Today, the commercialization of a new herbicide takes 11 years and US$286 million dollars.[17] Current estimates are that scientists must screen more than 200,000 chemicals to find one new product, one almost certainly that will not have a new mode of action. That is discouraging, and the number of chemical companies trying to discover herbicides continues to decline from about 45 in 1970 and soon will be down to less than six if the publicly announced mega-mergers are successful.[16]

The chance of finding another herbicide as good as glyphosate, a once in a century herbicide, is very small.[18] The increased use of glyphosate with selective herbicides with soil residual activity indicates that growers are making a positive change in their management practices, but going back to using such old herbicides is not a great step forward.

4 NEXT GENERATION OF GLYPHOSATE-RESISTANT CROPS

Patents were expiring on the "first generation" GR crops[19] and nobody knew what the process would be when traits became generic. A "next generation" of GR crops overcame some of the initial weaknesses of the first GR crops and gave new intellectual property protection. The new GR soybean crop claims a yield advantage, the new GR cotton has better crop safety and a wider application window, and the new GR canola improves crop safety without need for the *gox* gene that encodes an enzyme that degrades glyphosate.

The days of crops being resistant to just glyphosate are ending.[20,21] A "third generation" of GR crops are being developed with glyphosate traits combined with other herbicide traits (Table 1).

The leading approach is triple HR crops with resistance to glyphosate, glufosinate, and another herbicide type. These combination of traits will allow new herbicide combinations to combat weeds with combinations of resistance mechanisms – essentially to repair the GR crop systems. A top business leader is reported to have recently claimed the triple HR crop approach will be able to eliminate weed resistance by 2050.[22] Let's hope that leader knows about new broad spectrum herbicides with new modes-of-action that have not been publicly announced yet.

Unfortunately, the known pipeline of herbicide options with significant utility for the triple HR approach is exhausted and current options already have resistant weed problems that would not meet the overlapping weed spectrum with "effective" and "different" modes-of-action criteria.[23,24] What growers really need to manage the resistant weeds are new weed management technologies, either new selective herbicides or new non-selective herbicides with corresponding resistance traits.

Despite decades of safe use, the pipeline of new genetically modified HR crops could be coming to an end because the regulatory process has become too slow and costly. The current incremental improvement approach of adding additional resistance mechanism to GR crops does not give a very high return for a risky investment. A 2012 survey of key companies found that it costs $136 million over 13 years to bring a new biotech crop trait to the market.[25] Waiting 13 years uses much of the patent life and is twice as long as some experts now speculate as the likely life span of the utility of a new herbicide. These issues are forcing some companies to look for safer investments.

The next HR crop technologies will be resistant to synthetic auxin herbicides, 2,4-D and dicamba.[26,27] Auxin-resistant soybeans and cotton will enable new uses of these old herbicides and new formulations with less volatile salts and improved directions for use are being promoted as solutions to reduce off-target drift.[28] The issue of off-target movement and effects on non-target organisms is of such significance that the registration of the new 2,4-D and glyphosate formulation was seriously threatened when Dow claimed synergism in a patent application.[29,30] The new salt and formulation ingredients will be patented and mandated by government approved labelling. The new inert ingredients could greatly increase the cost of the 2,4-D product over currently available commercial offerings, so they need to work well to justify the extra expense.

Crops with resistance to 4-hydroxyphenyl pyruvate dioxygenase (HPPD)-inhibiting herbicides will follow auxin crops. HPPD-inhibiting herbicides control key weeds with some soil residual activity, but the spread of HPPD-resistant Palmer amaranth and waterhemp before market introduction will limit the value of this technology.[31] Two traits are currently under development that will enable new uses for some HPPD herbicides.[32,33] As with auxin herbicides, corn generally has some inherent tolerance to HPPD herbicides, so soybeans and cotton will be where the technology will have the most utility.

Monsanto and Sumitomo are cooperating to develop crops resistant to PPO-inhibiting herbicides.[34] Sumitomo Chemical has a new generation PPO chemistry that shows broader spectrum control with application flexibility with commercialization early next decade, pending regulatory approvals. The concept is not new as Syngenta had a similar effort more than a decade ago that even had a trade name.[2,35]

HR crops made resistant to acetyl coenzyme A carboxylase (ACCase) – and acetolactate synthase (ALA)-inhibiting herbicides created by both transgenic and non-transgenic means will also be commercially available. These modes of action still have some utility despite widespread weed resistance.[10,12] Other resistance mechanisms could be based on metabolic degradation by transgenic cytochrome P450 and glutathione-S-transferase (GST) enzymes, giving resistance to a wide range of herbicides'. However, weeds have already evolved analogous non-target site resistance mechanisms that would likely limit their utility.[14,15]

5 FUTURE FOR GLYPHOSATE AND GLYPHOSATE CROPS

The "Roundup Ready revolution" is over, but the use of glyphosate and glyphosate traits will continue to be the primary weed management system for many farmers and even expand into some new crops and areas. Regions of the world that do not have GR crops yet can learn from the American experience and maintain a diversity of weed management practices to better sustain the utility of GR crop systems. The use of glyphosate and its traits in combination with other herbicides and traits can provide near-term ways for growers to combat GR weeds.

Multiple HR crops are currently a strong enough business model to support incremental improvements in current GR crop systems. Stewardship programs could help sustain the utility of these technologies, but growers are often slow adopters of recommended programs and current business practices make it difficult for both growers and agribusinesses to delay near-term profits for uncertain long-term benefits. As the recent introduction of dicamba-resistant soybeans proved, many growers will not follow mandatory label directions, even when the risk is well known and they are given extensive training.[36,37]

Despite being widely approved by many government regulatory agencies, glyphosate and GR crops are still the targets of daily news stories about potential health effects. The health issue was reignited in 2015 when the International Agency for Research on Cancer (IARC) published a monograph concluding that glyphosate is "probably carcinogenic to humans."[38] The U.S. Environmental Protection Agency and others have since concluded that glyphosate is likely not a carcinogen,[39] but the firestorm over its safety continues in both popular press and scientific publications.[40-42] In addition, concerns are often raised about the safety of glyphosate formulations that contain the original tallow amine ethoxylate "Roundup surfactant." This surfactant type has known aquatic and eye toxicity issues,[43,44] that are commonly addressed with mandatory application restrictions and requirements for operators to wear personal protective equipment (PPE). The high level of public concern over glyphosate and GR crop safety is expected to continue and lead to glyphosate being banned in some areas and for much of Europe to continue to prohibit production of GR crops.[45] These restrictions are not expected to reduce the total amount of glyphosate used in GR crops globally.

Glyphosate will continue to be the backbone of weed management, even when the glyphosate trait is incorporated into multiple HR crops. Despite its flaws, glyphosate has not lost all its effectiveness on most weeds, and its sales are predicted to grow to US$10 billion by 2021.[46] The spread of GR weeds is creating more incentive for industry to renew herbicide discovery efforts. The payoff for the discovery of a new herbicide class and corresponding trait could be very high but would also be risky as non-target site resistance cross-resistance mechanisms could make the utility of a new herbicide short, too short for the expensive and decade long development process. Plus, no herbicide, no matter how effective, will be the total solution - weeds will evolve resistance.

The lack of success discovering a mode of action over the last three decades is a major concern. The proposed mergers of some of the largest pesticide companies should help ensure some herbicide discovery programs continue as the remaining mega-companies should be able to more easily fund a viable effort with research support based on percentage of sales. The lack of discovery success has not meant a chemical herbicide business decline yet. Ironically, the lack of new herbicides has often resulted in growers spending more and using older and almost forgotten herbicides to combat HR weeds, setting up what has been called a resurgence of crop protection.[47]

Co-developing a broad spectrum herbicide and corresponding HR crops could have very high value and could help rejuvenate the herbicide trait business model. However, that approach has very high risks with the time and cost to simultaneously commercialize both a synthetic chemical and a biotech trait likely to be over 12 years and US$500 million.[17,25] At the current rate of evolution of resistance in some weeds, a single new herbicide would not be enough and quickly lose its utility. If that is true, the economics to support these efforts will not work out and may be the explanation why companies have shifted resources away from transgenic HR crops to less regulated technologies such as gene editing and RNAi. Weed management solutions with new herbicides paired with genetically modified crops may have already become too slow and too expensive.

Traditional approaches by the herbicide and seed businesses are not going to be able to support the pesticide and transgenic treadmills for much longer.[48, 49] Still, the use of glyphosate will continue to grow despite GR weeds as it still provides weed management value, usually much than other herbicides. As an agribusiness manager once said, “It takes a long time for all the weeds to evolve resistance and a herbicide to lose all its effectiveness.” That type of thinking will lead to short-term business tactics and poor product stewardship practices, but is not totally incorrect. It takes a long time for all mutated resistance genes to combine together and disperse everywhere with factors such as reduced fitness from multiple non-wild type genes slowing the process. The status quo of using all available weed management tactics, chemical and non-chemical, “Throwing the kitchen sink” at HR weeds will work most of the time for most growers for a while, hopefully long enough to survive until researchers develop new innovations. In the interim, the over three decades without the discovery of a new chemical herbicide mode-of-action and the disastrous epidemic of resistant weeds is forcing growers to spend much more to manage weeds and creating a worst of times, best of times predicament for the crop protection and seed industry.

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Table 1. Key commercial and publicly announced genetically modified transgenic multiple herbicide-resistant crops.

Herbicide types	**Crops**
Glyphosate and glufosinate	Soybeans, corn and cotton
Glyphosate, glufosinate and 2,4-D	Soybeans and cotton
Glyphosate, glufosinate and dicamba	Soybeans, corn, cotton and wheat
Glyphosate, glufosinate and HPPD inhibitors	Soybeans and cotton
Glyphosate, glufosinate, 2,4-D and ACCase inhibitors	Corn

Figure 1. First 15 years of currently commercial transgenic herbicide trait introductions – crop, herbicide, and resistance gene(s).