Herbicide-Resistant Crops: Utilities and Limitations for Herbicide-Resistant Weed Management

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ABSTRACT: Since 1996, genetically modified herbicide-resistant (HR) crops, particularly glyphosate-resistant (GR) crops, have transformed the tactics that corn, soybean, and cotton growers use to manage weeds. The use of GR crops continues to grow, but weeds are adapting to the common practice of using only glyphosate to control weeds. Growers using only a single mode of action to manage weeds need to change to a more diverse array of herbicidal, mechanical, and cultural practices to maintain the effectiveness of glyphosate. Unfortunately, the introduction of GR crops and the high initial efficacy of glyphosate often lead to a decline in the use of other herbicide options and less investment by industry to discover new herbicide active ingredients. With some exceptions, most growers can still manage their weed problems with currently available selective and HR crop-enabled herbicides. However, current crop management systems are in jeopardy given the pace at which weed populations are evolving glyphosate resistance. New HR crop technologies will expand the utility of currently available herbicides and enable new interim solutions for growers to manage HR weeds, but will not replace the long-term need to diversify weed management tactics and discover herbicides with new modes of action. This paper reviews the strengths and weaknesses of anticipated weed management options and the best management practices that growers need to implement in HR crops to maximize the long-term benefits of current technologies and reduce weed shifts to difficult-to-control and HR weeds.

KEYWORDS: corn, *Zea mays*, cotton, *Gossypium hirsutum*, soybean, *Glycine max*, crop, herbicide, resistance, tolerance, weed management

■ INTRODUCTION

Herbicide-resistant (HR) crops, particularly glyphosate-resistant (GR) crops, have transformed the way many growers manage weeds. However, after three decades and billions of dollars invested in research, only a few transgenic herbicide traits are commercially available.[1−3] Two transgenes code for a glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; EC 2.5.1.19), the *cp4 epsps* gene from *Agrobacterium tumefaciens* strain CP4 and the mutated *zm-2mepsps* from corn (*Zea mays* L.), and three transgenes code for metabolic inactivation. One gene from *Ochrobactrum anthropi* strain LBAA encodes for glyphosate oxidoreductase (GOX), and two homologous genes, *pat* and *bar* from *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus*, respectively, encode *N*-acetyltransferases that inactivate glufosinate. Today, HR traits are used on >80% of the estimated 134 million hectares of transgenic crops grown annually in 25 countries[3,4] with a single trait, CP4 EPSPS, being by far the most utilized.[5]

Growers rapidly adopted the first GR crops because the technology enabled a new weed control practice with glyphosate that was effective, easy-to-use, economical, safe, and novel. The novel attribute of the gene technology was essential to get patents that protected the large investment needed to develop the technology, whereas growers touted the simplicity and convenience of the glyphosate-based crop systems.[1−3] Initially, glyphosate was exceedingly effective in GR crops, and many growers relied only on glyphosate to control weeds. Some academic weed scientists were concerned about the sustainability of this approach and predicted the evolution of resistance. However, no cases of GR weeds had evolved after more than two decades of broad use in noncrop situations,[6] and some weed scientists and growers began to think that GR weeds would never be a problem.

Then the paradigm changed in 1996 with the discovery of GR rigid ryegrass (*Lolium rigidum* Gaudin) in Australia.[7,8]

Today, all accept the evolution of GR weeds is threatening the continued success of GR crops and the sustainability of glyphosate. Nineteen weeds have evolved resistance to glyphosate; about half evolved in GR crops.[9] The basis for resistance has been attributed to altered EPSPS target site,[10] reduced translocation or cellular transport to the plastid,[11] sequestration in the vacuole,[12] and gene amplification.[13] GR weeds increase the cost of weed control and diminish the benefits of glyphosate-based weed management systems. In retrospect, it was inevitable that GR weeds would evolve. Glyphosate was a victim of its own success. No matter how effective a herbicide is, weed management programs cannot rely so heavily on one tactic or weeds will ultimately adapt and survive in large numbers.

In essence, GR crops created the "perfect storm" for weeds to evolve resistance. Growers applied glyphosate alone over vast cropping areas to control genetically variable and prolific weeds year after year. Many of these weeds had already evolved resistance to other herbicide modes of action, so there was no good herbicide alternative when these weeds subsequently evolved resistance to glyphosate.[14] Of particular note is the case of the highly competitive and prolific Palmer amaranth (*Amaranthus palmeri* S. Wats.). The explosion of GR Palmer amaranth populations in the southeastern United States

Table 1. Herbicide Types Commonly Used in Corn, Soybeans, and Cotton and Their Application Method, Preemergence (PRE) or Postemergence (POST), with Respect to Crop

herbicide type (group[a])	corn	soybean	cotton
glyphosate (G)	PRE and POST	PRE and POST	PRE and POST
glufosinate (H)	POST	POST	POST
ALS inhibitor (B)	PRE and POST	PRE and POST	PRE and POST
synthetic auxin (O)	PRE and POST	PRE and POST	PRE and POST
HPPD inhibitor (F2)	PRE and POST	PRE	
PPO inhibitor (E)	PRE and POST	PRE and POST	PRE and POST
ACCase inhibitor (A)		POST	POST
photosystem inhibitor (C)	PRE and POST	PRE and POST	PRE and POST
cell division inhibitor (K2)	PRE	PRE	PRE
phytoene desaturase inhibitor (F3)		PRE	

[a] Herbicides grouped according to the Herbicide Resistance Action Committee http://www.plantprotection.org/hrac.

became known as the "pigweed disaster".[15] These GR populations are forcing growers to change their production practices and increase the costs for weed control, even to the extent of hand-weeding. Because of these shortsighted use practices, glyphosate is not as effective as it used to be and growers must supplement glyphosate with other herbicides.

Growers now need to diversify the herbicides they use to mitigate the spread of GR weeds.[16] Unfortunately, the chemical industry has not commercialized a herbicide with a new mode of action (MOA) for over two decades.[17] This is partly because the number of chemicals that must be tested to discover a new herbicide has increased from fewer than 1000 in 1950 to more than 500,000 today and partly because companies are investing less money to discover new herbicides as the widespread use of GR crops has reduced the market opportunity. To address the GR weed problem, the industry is now developing new herbicide resistance traits that will expand the utility of currently available herbicides. However, it is critically important to recognize that these traits represent interim solutions for current weed problems and do not replace the long-term need to discover herbicides with new modes of action and to diversify weed management tactics.

UTILITIES AND LIMITATIONS OF CURRENT HERBICIDE TECHNOLOGIES

Current Herbicide Use Practices. GR crops came at a time of great socioeconomic change in agriculture. Farm size was increasing, and the number of growers was declining; thus, growers had to become more efficient. Furthermore, weeds were rapidly evolving resistance to various herbicides, and growers perceived weed management as taking too much time. Growers wanted new weed management tactics, and GR crops enabled an economical, efficient, and simple solution. Once growers started using glyphosate, they overused it. The average rate and number of applications of glyphosate increased as its price declined, and the use of other herbicides decreased.[18,19] Competitors reacted by reducing the price of their herbicides, but those alternatives could not maintain their market presence.[20]

In retrospect, GR crops could have helped to increase the diversity of herbicides that growers used (Table 1). GR crops did not require that growers use only glyphosate and the added diversity of glyphosate combined with other herbicides would have mitigated the evolution of HR weeds. However, the use of tank mixtures and sequential application of different herbicides declined. In one year, from 1997 to 1998, the use of glyphosate increased 81% in parallel

with the increase of GR soybeans [*Glycine max* (L.) Merr.] from 13 to 36%.[21] The number of herbicide active ingredients used on at least 10% of the U.S. soybean area declined from 11 in 1995 to only 1, glyphosate, in 2002.[22] Even though the chance of weeds evolving resistance to glyphosate in a particular location is still predicted to be lower than that with other herbicides, weeds ultimately did evolve glyphosate resistance as a direct result of the lack of weed management diversification on incredibly large areas of GR crops.[23,24]

Interestingly, HR weeds often do not decrease the amount of herbicide used because growers make herbicide decisions based on weed complexes, not individual species or biotypes. If a weed evolves resistance to a herbicide, that herbicide has not lost all of its value as it still controls other weeds, and growers often continue to use the herbicide in a program with another herbicide to control the resistant weed. Furthermore, growers do not "recognize" the potential for weeds to evolve resistance to glyphosate until the biotypes appear in their fields.[25] Unfortunately, this can lead to the practice of sequentially using herbicides until they are no longer effective, which is the fastest way to evolve multiple HR weeds.[16] A combination of herbicides, cultural, and mechanical tactics provides the greatest protection from HR weeds.

Some weed species are particularly troublesome to control and in their propensity to evolve resistance (Table 2). Problematic weeds in glyphosate-based production systems that have evolved genetic mutations that confer glyphosate resistance include Palmer amaranth and waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer]. Other weeds such as velvetleaf [*Abutilon theophrasti* (L.) Medik.], morningglories (*Ipomoea* spp.), Asiatic dayflower (*Commelina communis* L.), tropical spiderwort (*Commelina benghalensis* L.), and field bindweed (*Convolvulus arvensis* L.) often survive because of naturally higher tolerance. Populations of tolerant weed species increase when growers use less than full-labeled rates.[26] Currently, at least seven GR weed species have evolved resistance to multiple herbicide MOAs, with one population of waterhemp in Illinois being resistant to four.[27] The rapid expansion of multiple HR weed populations threatens the sustainability of current crop production systems.[16]

The best weed management strategy is to control weeds prior to the loss of crop yield potential and proactively delay the evolution of weed resistance. Fortunately, most fields do not have GR weeds yet, and there is still time for many growers to implement diverse and proactive weed management practices (Table 3).[28] Generally, the basic management tactics are the same for both the prevention and control of HR weeds, that is, diversification of tactics to reduce selection pressure imposed by specific herbicides. The challenge is to implement these practices under prevailing economic constraints

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Table 2. Summary of Key Row Crop Weeds and Herbicide Efficacy

		control rating (0−10) and resistance status[b,c]						
	weed species[a]	gly-phosate	glu-fosinate	ALS inhibitors	synthetic auxins	HPPD inhibitors	PPO inhibitors	ACCase inhibitors
common name	scientific name							
Dicotyledons								
common lambs-quarters	*Chenopodium album* L.	8R	8	7R	9R	9	9	0
redroot pigweed	*Amaranthus retroflexus* L.	9	8	9R	9	9	9	0
waterhemp	*Amaranthus tuberculatus* (Moq.) Sauer	9R	8	9R	8	9	9R	0
Palmer amaranth	*Amaranthus palmeri* S. Wats.	9R	8	9R	9	9	9	0
velvetleaf	*Abutilon theophrasti* Medik.	8	8	8−9	8	9	8	0
common cocklebur	*Xanthium strumarium* L.	9	9	9R	9	8	8	0
common ragweed	*Ambrosia artemisiifolia* L.	8R	9	8R	9	7	9R	0
giant ragweed	*Ambrosia trifida* L.	7−8R	8	7−8R	9	8	8	0
horseweed	*Conyza canadensis* (L.) Cronq.	7−8R	8	7R	8	8	8	0
morningglories	*Ipomoea* spp.	7	8	7	9	7	8	0
kochia	*Kochia scoparia* (L.) Schrad.	9R	8	9R	9R	7	8	0
common sunflower	*Helianthus annuus* L.	9	9	9R	9	9	8	0
Monocotyledons								
giant foxtail	*Setaria faberi* Herrm.	9	9	8R	0	8	7	9R
green foxtail	*Setaria viridis* (L.) Beauv.	10	8	9R	0	4	5	9R
yellow foxtail	*Setaria pumila* (Poir.) Roemer & J.A. Schultes	9	8	9R	0	6	7	9
johnsongrass (rhizome)	*Sorghum halepense* (L.) Pers.	9R	6	8R	0	0	8	9R
shattercane	*Sorghum bicolor* (L.) Moench	10	9	10R	0	8	7	9R[d]
large crabgrass	*Digitaria sanguinalis* (L.) Scop.	9	8	9R	0	7	6	9R
barnyardgrass	*Echinochloa crus-galli* (L.) Beauv.	9	9	9R	0	7	6	9R
woolly cupgrass	*Eriochloa villosa* (Thunb.) Kunth.	9	9	9	0	7	5	8
fall panicum	*Panicum dichotomiflorum* Michx.	9	8	8	0	5	4	9R
Italian ryegrass	*Lolium multiflorum* L.	9R	8	8R	0	3	3	9R
feral corn	*Zea mays* L.	9R	7R	8R	0	0	6	9R[e]

[a] Weed selection determined by a market research survey of U.S. corn, soybean, and cotton growers by Gfk Kynetec, Inc., St. Louis, MO (used with permission). [b] Weed control ratings are summarized from U.S. extension guides with 0 being the lowest and 10 being the highest level of control. A rating of ≥7 indicates effective herbicide control. Weed ratings represent the highest observed for any active in that class. [c] An R next to herbicide efficacy rating indicates that this weed has developed resistance to herbicide mode of action (Heap 2010). [d] ACCase resistance has been confirmed but not listed at Heap 2010. [e] ACCase trait currently under development and anticipated to be an issue in feral corn after commercialization.

when growers are not convinced resistance management tactics will be effective or they believe industry will continue to deliver new solutions to manage weeds.[29] Many growers are reluctant to diversify weed management because they perceive alternative tactics as being less cost-effective despite growing evidence that such tactics can improve profitability as well as mitigate resistant weed issues.[30] More education will help overcome this perception as will the explosion of multiple HR weeds that emphatically persuades growers to diversify their weed management practices now or face serious long-term consequences.

Current Herbicide Technologies. Besides glyphosate, most current herbicides used for weed management in corn, soybean, and cotton are selective and typically used in mixtures to control a broad spectrum of weed species. The following section provides an overview of the utilities and limitations for various herbicide MOAs that have potential utility in HR crops.

Glyphosate. Glyphosate is a nonselective, broad-spectrum foliar herbicide with no soil residual activity that has been used for >30 years to manage annual, perennial, and biennial herbaceous grass, sedge, and broadleaf weeds as well as unwanted woody brush and trees. Glyphosate is labeled to control over 300 weed species. Many glyphosate formulations and salts are commercially available; the most common salts are the monopotassium and isopropylamine. The type and amount of adjuvant included in the various formulations differ greatly and strongly influence weed control. Glyphosate strongly competes with the substrate phosphoenolpyruvate (PEP) at the EPSPS enzyme-binding site in the chloroplast, resulting in the inhibition of the shikimate pathway. Products of the shikimate pathway include the essential aromatic amino acids tryptophan, tyrosine, and phenylalanine and other important plant metabolic products.[31] The relatively slow MOA and physicochemical characteristics result in glyphosate translocation throughout the plant and accumulation at the vital growing points before phytotoxicity occurs.

Favorable physicochemical characteristics, low cost, tight soil sorption, application flexibility, low mammalian toxicity, and availability of GR crops have helped make glyphosate the most widely used herbicide in the world.[32] A key advantage for glyphosate has been the consistent control of weeds almost without regard to size. However, the flexibility in glyphosate

Table 3. Assessment of Commonly Used Tactics for Herbicide-Resistant Weed Management (Adapted from Reference [28])

tactic	benefits	risks	potential impact
herbicide rotation	reduced selection pressure, control HR weeds	lack of different MOAs, phytotoxicity, cost, limited weed spectrum of alternatives	excellent
herbicide mixtures	reduced selection pressure, improved control, broader weed spectrum	poor activity on HR weed species, increased cost; potential phytotoxicity	excellent
variable application rate and timing	better control of HR species, more efficient herbicide use	lack of herbicide residual activity, timing may be too late to protect yield potential, more applications	good to excellent
adjusted herbicide rates	better control of target species	increased target-site selection pressure with higher rates, increased nontarget site with lower rates (polygenic resistance)	poor to fair
precision herbicide application	decreased herbicide use, reduced selection pressure	increased cost of application, unavailability of weed population maps; poor understanding of weed seedbank dynamics; increased variability of control	poor
primary tillage	decreased selection pressure, consistent efficacy; depletion of weed seedbank	increased time required, increased soil erosion, increased costs, additional tactics needed	good to excellent
mechanical weed control strategies	decreases selection pressure; consistent efficacy, relatively inexpensive	increased time required, high level of management skill needed, additional tactics needed, potential for crop injury	poor to fair
crop selection/rotation	changes agro-ecosystem, allows different herbicide tactics, reduced selection pressure	economic risk of alternative rotation crop, lack of adapted rotation crop, rotation crops similar and thus minimal impact on the weed community, herbicides, required, lack of research base, inconsistent impact on HR weed populations	fair to good
adjusted time of planting	potential improved efficacy on target weeds, reduced selection pressure	requires alternative strategies (tillage or herbicide), potential for yield loss, need for increased rotation diversity	poor to fair
adjusted seeding rate	reduced selection pressure, improved competitive ability for the crop	increased seed cost, potentially increased pest problems, increased intraspecific competition, reduced potential yields	fair
planting configuration	improved competitive ability for the crop, reduced selection pressure	unavailability of mechanical strategies, emphasis on herbicides, equipment limitations	good
cover crops, mulches, intercrop systems	improved competitive ability, reduced selection pressure, improved systems diversity, allelopathy	inconsistent effect on HR weeds, lack of understanding about systems, limited research base, potential crop yield loss, need for herbicide to manage the cover crop, lack of good cover crops	poor

Table 3. Continued

tactic	benefits	risks	potential impact
seedbank management	reduced HR weed pressure, reduced selection pressure	lack of understanding about seedbank dynamics, requires aggressive tillage, emphasis on late herbicide applications, high level of management skill needed	fair to good
adjustment of nutrient use	improved competitive ability for the crop, efficient use of nutrient	lack of research base, inconsistent results, potential crop yield loss	poor

application timing and lack of soil residual have often resulted in growers delaying applications to help ensure that all of the weeds have emerged. Unfortunately, such delay in application means that the weeds have begun to compete with the crop and thus reduced potential yield. The increased use of mixtures with herbicides that have soil residual activity will encourage growers to make earlier glyphosate applications and increase the likelihood that a single application gives season-long control. Other commonly noted weaknesses with glyphosate are higher rates needed to control the more tolerant broadleaf weeds, antagonism by hard water and tank mixture partners, slow speed of action, and poor rainfastness.

Glufosinate. Glufosinate is a nonselective, broad-spectrum foliar herbicide with no soil residual soil activity that inhibits glutamine synthetase [GS; EC 6.3.1.2], an enzyme that catalyzes the conversion of glutamate plus ammonium to glutamine as part of nitrogen metabolism.[31] Glufosinate is faster acting and controls key broadleaf weeds such as morningglories (*Ipomoea* spp.), hemp sesbania (*Sesbania herbacea* (P. Mill.) McVaugh), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), and yellow nutsedge (*Cyperus esculentus* L.) better than glyphosate. However, glufosinate is used at higher rates and has historically been more expensive than glyphosate. Cost and more restrictive application timing relative to weed size are probably its greatest disadvantages compared to glyphosate. Because glufosinate behaves as a contact herbicide, it must be applied to smaller plants than glyphosate and is not as effective on perennials that require significant translocation for complete control. Still, glufosinate is labeled to control >120 broadleaf weeds and grasses including key GR weeds. No weeds have been formally reported as glufosinate-resistant yet.[9]

Synthetic Auxins. Synthetic auxin herbicides act as auxin agonists by mimicking the plant growth hormone indole-3-acetic acid (IAA), disrupting growth and development processes, and eventually causing plant death, particularly in broadleaf species.[31] Growers have used auxin herbicides widely for over 60 years as selective herbicides in monocotyledonous crops. Auxins control a broad spectrum of broadleaf weeds, including key weeds that have evolved resistance to glyphosate. Some synthetic auxins such as dicamba have fair soil residual activity with a half-life from 7 to 21 days. Relatively few weeds have evolved resistance to auxin herbicides, which is noteworthy considering their long-term and widespread use. For example, only six weed species have evolved resistance to dicamba after 50 years of widespread use in cereal and noncrop environments.[9]

The increased use of dicamba and other auxin herbicides in auxin-resistant crops has the potential of injuring other broadleaf crops and reducing biodiversity in field edges and nearby noncrop habitat if unmanaged.[33] Off-target movement of auxin herbicides can occur via spray particle and vapor drift. Particle drift is more problematic than

vapor drift, but growers can manage with modified application techniques, drift control adjuvants, and correct decisions as to when, where, and how to apply. Particularly troublesome for auxin herbicides would be any movement onto highly sensitive crops such as soybeans, cotton (*Gossypium hirsutum* L.), or grapes (*Vitis vinifera* L.). Interestingly, 2,4-D is safer than dicamba on soybeans and dicamba is safer than 2,4-D on cotton.[34] As little as 0.01% of the labeled rate of dicamba can injure soybeans,[35] and 0.001% of the labeled rate of 2,4-D butyl ester formulation can injure tomatoes (*Lycopersicon esculentum* Mill.) and lettuce (*Lactuca sativa* L.).[36]

Some forms of dicamba and 2,4-D are highly volatile, especially at high temperatures. For example, the acid form of dicamba is more volatile than amine salt formulations, and some amine salts are more volatile than others. Considerable research is underway to minimize volatilization with new salts and formulations. The manufacturer can also reduce potential off-target movement with application restrictions based on temperature, droplet size, humidity, and wind speed. Because of their volatility and the sensitivity of nontarget crops, growers will probably not use auxin herbicides on vast areas during warm weather as is currently done with glyphosate.

HPPD Inhibitors. The enzyme 4-hydroxyphenyl pyruvate dioxygenase [HPPD; EC 1.13.11.27] converts 4-hydroxyphenyl pyruvate to homogentisate, a key step in plastoquinone biosynthesis. This is the most recently discovered herbicide MOA, and active analogue testing continues to generate new products.[37] Inhibition of HPPD causes bleaching symptoms on new growth by indirectly inhibiting carotenoid synthesis due to the requirement of plastoquinone as cofactor of phytoene desaturase [PDS; EC 1.14.99].[38] Visible injury depends on carotenoid turnover and thus is slower to appear on older tissues than young leaves.[31] HPPD-inhibiting herbicides control a number of important weed species and may have soil residual activity, and no weeds have been formally reported to be resistant to this MOA yet. Corn is naturally tolerant to key HPPD herbicides, but soybeans and cotton are generally sensitive.

ALS Inhibitors. Herbicides that inhibit acetolactate synthase (ALS; EC 2.2.1.6), also known as acetohydroxyacid synthase (AHAS), were discovered in the mid-1970s and are still widely used.[39,40] The ALS enzyme is a key step in the biosynthesis of the essential branched-chain amino acids valine, leucine, and isoleucine. ALS is a nuclear encoded enzyme that moves to the chloroplast via a transit peptide. More than 50 different ALS-inhibiting herbicides from five different chemical classes (sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinylthiobenzoates, and sulfonylamino-carbonyl-triazolinones) are commercially available. The characteristics of ALS herbicides vary in their soil residual properties, crop response, and types of weeds that are effectively controlled. ALS herbicides can provide foliar and soil residual activity on important grass and broadleaf weeds at low application rates. The tendency of weeds to evolve resistance to

Table 4. Summary of Commercial Herbicide-Resistant Crops in North America (Adapted from Reference 44)

herbicide type		crop	year available
bromoxynil		cotton	1995
		canola	2000
ACCase inhibitor	- sethoxydim	corn	1996
	- quizalofop-P	sorghum	2011
glufosinate		canola	1995
		corn	1997
		cotton	2004
glyphosate		soybean	1996
		canola	1996
		cotton	1997
		corn	1998
		alfalfa	2005
		sugar beets	2005
imidazolinones		corn	1993
		canola	1997
		wheat	2002
		rice	2002
		sunflower	2003
specific sulfonylureas		soybean	1994
		sunflower	2006
		sorghum	2011
triazines		canola	1984

Table 5. Summary of Nontransgenic Herbicide-Resistant Crops (Adapted from Reference 48)

selection method	herbicide type	crop
whole plant	triazine	canola
seed mutagenesis	terbutryne	wheat
	sulfonylurea	soybean
	imidazolinone	wheat
		rice
tissue culture	sulfonylurea	canola
	atrazine	soybean
	imidazolinone	corn
	sethoxydim	corn
cell selection	imidazolinone	sugar beet
pollen mutagenesis	imidazolinone	corn
microspore selection	imidazolinone	canola
transfer from weedy relative	ALS inhibitor	sunflower
		sorghum
	ACCase inhibitor	sorghum

ALS herbicides limits their utility,[9] and their use is now mainly in mixtures with other types of herbicides.

PPO Inhibitors. Protoporphyrinogen oxidase (PPO; EC 1.3.3.4) is an essential enzyme that catalyzes the last common step in the biosynthesis of heme and ultimately chlorophyll by the oxidation of protoporphyrinogen IX to protoporphyrin IX. PPO-inhibiting herbicides cause the accumulation of protoporphyrinogen IX, which is photoactive, and exposure to light causes the formation of singlet oxygen and other oxidative chemicals that cause rapid burning and desiccation of leaf tissue. The soil residual and fast action characteristics of PPO herbicides complement the lack of soil residual and the slow activity of glyphosate.

PPO enzyme mutations tend to reduce the enzymatic activity, which helps explain the relatively slow evolution of resistant weeds to this 40-year-old herbicide class.[41] Companies continue to synthesize analogues and commercialize new PPO-inhibiting herbicides. For example, saflufenacil was introduced in 2010 and is labeled for use in a wide variety of crops, including corn, soybeans, and cotton.[42] Its label describes burndown and residual control of 70 broadleaf weeds including key troublesome weeds in glyphosate-based systems such as common lambsquarters (*Chenopodium album* L.), horseweed [*Conyza canadensis* (L.) Cronq.], waterhemp, and common (*Ambrosia artemisiifolia* L.) and giant (*Ambrosia trifida* L.) ragweeds.

ACCase Inhibitors. Acetyl coenzyme A carboxylase [ACCase; EC 6.4.1.2] is the first step of fatty acid synthesis and catalyzes the adenosine triphosphate (ATP)-dependent carboxylation of malonyl-CoA to form acetyl-CoA in the cytoplasm, chloroplasts, mitochondria, and peroxisomes of cells.[43] ACCase-inhibiting herbicides generally inhibit the ACCase activity of monocot species and not dicots. The three chemical classes of ACCase inhibitors are cyclohexanediones (DIMs) (e.g., sethoxydim), aryloxyphenoxypropionates (FOPs) (e.g., quizalofop), and phenylpyrazolines (DENs) (e.g., pinoxaden). The ability to use ACCase herbicides selectively in corn would be useful, but the tendency of weeds to evolve resistance to this herbicide class would limit its utility to being part of a weed management system.[9]

Other Herbicide Types. Currently used selective and burndown herbicides will continue to play important roles in weed management in HR crop systems (Table 1). In addition to the herbicide types already discussed, photosystem II (PSII) inhibitors such as triazine and urea herbicides, lipid synthesis inhibitors such as *S*-metolachlor, and phytoene desaturase (PDS) inhibitors such as clomazone will continue to be used as crop-selective herbicides to provide soil residual activity on key weeds. Paraquat is a photosystem I (PSI) inhibiting herbicide typically used in conservation and no-tillage production systems for nonselective burndown control of emerged weeds or as a directed spray with specialized application equipment in crop. Paraquat controls a broad spectrum of weeds, and the lack of soil residual allows rotational crop flexibility similar to glyphosate and glufosinate. Paraquat rapidly desiccates leaf tissue and thus does not translocate well enough to control perennial weeds. Paraquat is relatively inexpensive, but its high mammalian toxicity imposes significant use and handling restrictions.

■ UTILITIES AND LIMITATIONS OF CURRENT AND FUTURE HERBICIDE-RESISTANT CROP TECHNOLOGIES

Current HR Crop Technologies. A large number of transgenic and nontransgenic HR crops have been commercialized

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Table 6. Summary of Currently Available Transgenic Herbicide-Resistant Corn, Soybeans, and Cotton

crop	resistance trait	trait gene	trait designation	first sales
cotton	glyphosate	cp4 epsps	MON1445	1996
		two cp4 epsps	MON88913	2006
		zm-2mepsps	GHB614	2009
	glufosinate	bar	LLCotton25	2005
corn	glyphosate	three modified zm-2mepsps	GA21	1998
		two cp4 epsps	NK603	2001
	glufosinate	pat	T14, T25	1996
soybean	glyphosate	cp4 epsps	GTS 40−3−2	1996
		cp4 epsps	MON89788	2009
	glufosinate	pat	A2704−12	2009

(Table 4). These HR crops generally eliminated all crop injury concerns and allowed the grower to select new herbicide options with improved weed activity and environmental safety. Before the advent of GR crops, most thought that the utility of HR crops would be limited to complementing selective herbicides.[45−47] The full impact of HR crops really started in 1996 with the sales of GR soybeans. Since then, the speed at which growers adopted GR crops has been unprecedented in corn, soybeans, and cotton.[4] Success came despite an unpopular "grower contract" and strong objections by biotechnology opponents to potential unknown effects on the environment and human health and the ethical question of interfering with the natural order.

Nontransgenic HR Crops. With the exception of Canada, nontransgenic HR traits are essentially unregulated. Scientists have used a wide range of nontransgenic techniques to create crops with resistance to a number of herbicide MOAs (Table 5). For example, the first commercial ACCase-resistant crop was a sethoxydim-resistant (SR) corn with an altered ACCase created using tissue culture selection.[49] A second ACCase trait is in the final stages of commercialization for use in sorghum. This trait was transferred with traditional breeding methods from feral sorghum (shattercane, *Sorghum bicolor* L. Moench) that had evolved ACCase herbicide resistance because of agronomic practices.[50]

Creating HR crops for ALS-inhibiting herbicides has been quite successful using tissue culture selection, pollen mutagenesis, microspore selection, seed mutagenesis, and gene transfer from close weedy relatives that had evolved herbicide resistance because of agronomic practices.[50−52] Today, at least seven different ALS-resistant crops are commercially available.[53] In all cases, resistance is due to an ALS mutation with three general crop phenotypes: broad resistance to ALS herbicides; resistance only to imidazolinone and pyrimidinylthiobenzoate herbicides; and resistance only to sulfonylurea and triazolopyrimidine herbicides.[54,55]

Glyphosate-Resistant Crops. Nontransgenic HR crops were only modestly successful; the big success with HR crops began with transgenic GR soybeans in 1996 (Table 6). Growers perceived glyphosate resistance as the ideal herbicide trait because glyphosate controls over 300 annual and perennial weeds, has flexible application timings, and does not have any rotational crop restrictions.[56] GR crops allowed growers to use glyphosate as an in-crop selective herbicide and replace more expensive, selective herbicides that controlled a narrower weed spectrum and had other issues (e.g., crop tolerance).

Within a decade after glyphosate became commercially available, the search began to find crop resistance to glyphosate. Nontransgenic approaches were not successful, and transgenic approaches were difficult and not initially successful.[57] Initial attempts to find any natural enzymes in crops that could metabolically inactivate or were insensitive at the target site failed. Eventually, a gene for a glyphosate insensitive EPSPS with enzymatic characteristics similar to plant EPSPS was isolated from a common soil bacterium, *Agrobacterium tumefaciens* strain CP4, which was surviving in a glyphosate manufacturing waste stream in Luling, LA.[57] This *cp4 epsps* gene has been used to develop GR soybeans, cotton, corn, canola, alfalfa (*Medicago sativa* L.), bentgrass (*Agrostis stolonifera* L.), and sugar beet (*Beta vulgaris* L.).[5]

Glyphosate resistance became the most rapidly adopted technology in the history of agriculture,[5] but the first GR crops were not perfect. The timing, rate, and number of glyphosate applications had to be restricted to ensure crop resistance,[5] and there were reports of a "yield drag".[58] A new generation of herbicide traits currently in development will be combined with current and new glyphosate traits to help continue to improve this technology and extend the transgenic weed management revolution.

Glufosinate-Resistant Crops. Glufosinate-resistant crops have been commercially available as long as GR crops (Table 6), but have not been as successful for a number of reasons, particularly because of the higher cost of glufosinate and its more restrictive application timings. Glufosinate resistance is widely available, not only because of its utility as a herbicide trait but also because it has been often used as a marker for other traits, particularly insect resistance traits. Resistance to glufosinate is due to metabolic inactivation of the parent molecule by either of two homologous enzymes, phosphinothricin *N*-acetyltransferase (PAT) or basta *N*-acetyltransferase (BAR), that catalyze the acetylation of glufosinate.[59] Both genes were isolated from soil microorganisms, *pat* from *Streptomyces viridochromogenes* and *bar* from *Streptomyces hygroscopicus*. Cotton and soybean growers who are troubled by difficult to control GR weeds such as Palmer amaranth and waterhemp may rapidly adopt glufosinate-resistant crops and the use of glufosinate. "Dual stack" crop cultivars that include resistance to both glufosinate and glyphosate are now commercially available in cotton, soybeans, and corn and provide growers a choice between two broad-spectrum herbicides as well as an array of naturally selective herbicides to diversify their weed management practices.

Future HR Crop Technologies. Whereas GR crops have been very successful, the evolution of GR weeds was faster and more widespread than many expected. This rapid evolution of GR weeds and the lack of any new selective herbicides with novel MOAs is encouraging HR crop technology to evolve again. The next wave of technologies will combine resistance to glyphosate and other herbicides to provide growers with more herbicide options with different MOAs as well as the possibility of using herbicides with both foliar and soil residual activity. Scientists have discovered a plethora of herbicide traits that can be combined with glyphosate resistance to make multiple HR crops (Table 7). If used correctly, multiple HR crops with these traits can sustain the usefulness of glyphosate.

Resistance to Synthetic Auxin Herbicides. Corn is relatively tolerant to most synthetic auxin herbicides, but soybeans and cotton are sensitive, and scientists have long sought a transgene to give these crops resistance and allow the use of auxin herbicides.[66] Auxin herbicides control a broad spectrum of broadleaf weeds, including most known GR broadleaf weeds. Because auxin herbicides act rapidly at multiple receptors and compete with an essential plant hormone pathway, making crops resistant by

Table 7. Publicly Disclosed Non-glyphosate Transgenic Herbicide-Resistant Traits with Significant Utility in Corn, Soybeans, and Cotton (Adapted from Reference 48)

herbicide/herbicide class	characteristics	reference
2,4-D	microbial degradation enzyme	60
ALS inhibitors	resistant ALS from many sources	61
ACCase inhibitors and synthetic auxins	microbial, aryloxyalkanoate dioxygenase	62
dicamba	*Pseudomonas maltophilia*, *O*-demethylase	63
HPPD inhibitors	overexpression, alternate pathway, and pathway flux	38
PPO inhibitors	resistant microbial and *Arabidopsis thaliana* PPO	41
multiple herbicide classes	glutathione *S*-transferase, *Escherichia coli*	64
	P450, *Zea mays*	65

modifying the site of auxin action is difficult. In addition, these receptors respond differently to different auxin herbicide classes, for example, phenoxyacetates (e.g., 2,4-D), pyridinyloxyacetates (e.g., fluoroxypyr), benzoates (e.g., dicamba), picolinates (e.g., picloram), and quinolinecarboxylates (e.g., quinclorac).[67] So far, metabolic inactivation has proven to be a more successful strategy.

A gene encoding for dicamba monooxygenase (DMO), an enzyme that deactivates dicamba, was cloned from a soil bacterium, *Stenotrophomonas maltophilla*, and used to make dicamba-resistant soybeans.[63,68] The DMO enzyme encodes a Rieske nonheme monooxygenase that metabolizes dicamba to 3,6-dichlorosalicylic acid (DCSA). The complete bacterial dicamba *O*-demethylase complex consists of the monooxygenase, a reductase, and a ferredoxin. Electrons are shuttled from reduced nicotinamide adenine dinucleotide (NADH) through the reductase to the ferredoxin and finally to the terminal component DMO. Researchers can successfully express DMO in the cell nucleus with or without a transit peptide as well as in the chloroplasts where the monooxygenase would have a source of electrons produced by photosynthesis and where transgenic proteins can often be expressed at higher levels. Commercialization of dicamba-resistant soybean and cotton is anticipated mid-decade.

A family of *aad* genes that code for aryloxyalkanoate dioxygenase provides resistance to certain auxin herbicide.[69,70] The *aad-12* gene was isolated from *Delftia acidovorans* and codes for a 2-ketoglutarate-dependent dioxygenase that inactivates phenoxyacetate auxins (e.g., 2,4-D) and pyridinyloxyacetate auxins (e.g., triclopyr and fluoroxypyr).[62] This trait, DHT2, is being developed in soybeans. A second gene known as *aad-1* was isolated from *Sphingomonas herbicideovarans* and inactivates auxins and ACCase-inhibiting herbicides in the class known as FOPs (e.g., fluazifop).[62] This trait, DHT1, is being developed in corn. Both traits are reported to provide resistance to high rates of 2,4-D with no adverse agronomic effects.

The 2,4-D and dicamba resistance traits will always be used in stacks with at least one other herbicide-resistance trait.[62,71] The expected increased use of auxin herbicides will increase the potential for off-target movement and injury to sensitive broadleaf plants. Due to this potential environmental problem, the herbicide and trait providers will likely introduce improved herbicide formulations with better use directions before the traits are commercialized mid-decade.[33,72] Ironically, this risk of off-target movement could drive more rapid adoption of auxin traits because growers will want to protect their soybean and cotton crops from nearby applications of auxin herbicides.

Resistance to HPPD Inhibitors. In some ways, HPPD-inhibiting herbicides are ideal to complement glyphosate. Many HPPD herbicides have soil residual activity and control key broadleaf

weeds that have already evolved resistance to glyphosate. Increased resistance mechanisms for HPPD herbicides include a less sensitive target site, overexpression of the enzyme, alternate pathway, increasing flux in the pathway, and metabolic inactivation.[38,48] Crops resistant to HPPD herbicides have been in field development tests since 1999, but there have been no technical disclosures of HPPD resistance traits under developments thus far. Bayer CropScience in collaboration with Mertec LLC (Adel, IA) and M.S. Technologies LLC (West Point, IA) and Syngenta (Basel, Switzerland) have independently announced plans to develop HPPD-resistant crops. Bayer CropScience recently disclosed that they were developing soybeans resistant to three herbicide types: glyphosate, glufosinate, and HPPD herbicides (e.g., isoxaflutole).[17] Isoxaflutole can provide pre-emergence (PRE) and postemergence (POST) control of a relatively broad spectrum of annual weeds with soil residual activity. The "triple stack" offers the advantage of enabling the use of two herbicide MOAs to which weeds have not yet evolved resistance.

Resistance to Other Herbicide Types. Resistance to other herbicide types could also have significant utility. For example, transgenic crops resistant to PPO-inhibiting herbicides have been developed, and the technology even received the trade name Acuron.[41] The first PPO-resistant corn used a double mutant PPO, PPO-1, from *A. thaliana*. Similarly, PPO-resistant rice used overexpression of the naturally resistant *Bacillus subtilis* PPO gene to confer resistance. Other approaches including increasing gene copy number and tissue culture to select for overexpression of wild type PPO genes have also been successful.[41] The broad-spectrum weed control and soil residual activity of PPO herbicides could be useful in corn, soybeans, and cotton, but the existing widespread resistance to this class among some *Amaranthus* species limits the value of the technology.

A transgenic DHT1 trait also gives resistance to ACCase-inhibiting herbicides by degrading the alkanoate side chains to a hydroxyl of the FOP class of ACCase herbicides (e.g., quizalofop).[62] DHT1 corn reportedly tolerates postemergence applications of quizalofop of up to 184 g/ha with no adverse agronomic effects. This trait has utility in corn where commercial ACCase herbicides are not naturally selective. In addition, the specificity of its inactivation could allow the use of other ACCase herbicide types for HR volunteer corn management in rotational crops.

Most herbicide traits only give resistance to herbicides with one MOA. Metabolic inactivation systems based on cytochrome P450 monooxygenases (P450) and glutathione transferase (GST) have the potential to inactivate a wide range of herbicide types (Table 7). For example, native P450 enzymes can metabolically inactivate acetanilides, bentazon, dicamba, some ALS-inhibiting herbicides, isoxazoles, and urea herbicides.[65,73] The chemical

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specificity of this metabolic system may offer the unique potential to allow growers to use herbicides in the same MOA to control weeds in one season and still manage any feral volunteers with a herbicide in the same MOA in the next year.

Multiple HR Crops. No single herbicide resistance trait will be sustainable if the grower uses only the single herbicide type that the trait enables recurrently. The weed problems and their technological resolution must evolve together. Multiple HR crops will help by allowing the use of new herbicide mixtures with multiple modes of action, but agriculture must manage this technology objectively and pragmatically, balancing short-term and long-term interests, so as not to create a "transgenic treadmill".[18]

The lack of soil residual activity has encouraged multiple in-crop applications glyphosate, as many as four or more applications per growing season. Some of the new, multiple HR crop technologies will enable herbicide applications with soil residual activity and thus help growers to reduce selection pressure on the weed community by glyphosate.[74] For example, the glyphosate and ALS trait stack that has recently been deregulated in the United States can allow the use of ALS-inhibiting herbicides with soil residual that are too phytotoxic to use on conventional crop cultivars.[75] This stack consists of a metabolic system to inactivate glyphosate based on an enhanced glyphosate acetyltransferase enzyme from the soil bacterium *Bacillus licheniformis* (Weigmann) Chester[76] and a highly resistant ALS allele (HRA) with two mutations, tryp574leu and pro197ala.[75]

A wide array of other combinations of current and new herbicide resistance traits is expected within the next decade. If used correctly, these multiple HR crops will provide new uses for existing herbicides to help growers better manage weeds and help sustain the utility of glyphosate and glyphosate resistance traits.

■ PATH FORWARD

Weed management dramatically changed with the widespread adoption of GR crops. Using glyphosate in GR crops made weed management too simple and convenient. Importantly, the high initial efficacy of glyphosate declined with repeated use, and current glyphosate-based weed management systems are in jeopardy as evidenced by the speed at which weed populations are evolving resistance. Still, glyphosate has not lost all utility; it controls more weeds more effectively than other herbicides, but it can no longer be applied alone anytime on any weed anywhere. Most growers still do not have any GR weeds in their fields and have time to implement proactive HR weed management practices to help sustain glyphosate. However, growers need to act now to diversify the herbicides and tactics they use, the crops they plant, their cultural practices, and field hygiene measures. The flexibility and range of alternative weed management practices will be narrow and require integration to replace glyphosate. These management practices will work better for the prevention rather than the control of GR weeds. Once present, GR weeds can be managed but are difficult if not impossible to eradicate.

Growers need new weed management options now. Current corn, soybean, and cotton cropping systems are based on a heavy reliance on glyphosate. Given the changes in weed populations that are being reported, it is of paramount importance that other weed management alternatives be identified and implemented quickly.[25,77] It is likely that no new herbicide or trait technology will match the impact of glyphosate and the first GR crops on agriculture. Growers will use

these new technologies in combinations to fill in efficacy gaps and diversify weed management practices. Initially, it may look like an attempt to make glyphosate look "as good as it used to be". Some traits such as glufosinate resistance will enable a broad-spectrum alternative to glyphosate. Others will enable options with soil residual activity or new MOAs to control key GR weeds. Some HR crop technologies may benefit from incremental improvements in efficacy and properties of herbicides within long-standing herbicide MOAs that companies are still commercializing.[37,42]

Growers must diversify their weed management practices now.[78] The more growers diversify, the less the risk that weeds will evolve herbicide resistance. Diversification may make weed management more complex, but growers must not use new HR crop systems in the same way that some used initial GR crops, as a means to rely only on one herbicide until it is no longer effective and then switch herbicides. If growers use the new HR crops and the herbicides that they enable properly, HR crops will expand the utility of currently available herbicides and provide long-term solutions to manage GR weeds.

HR crops will not replace the need for technical innovations, particularly the discovery of herbicides with new MOAs. Diversification will be much easier if growers can chose from among multiple effective and economical weed management options. In areas of the world that have not yet adopted GR crops, growers can learn from the experiences in North and South America. Growers must not wait, but implement best management practices as soon as new trait and herbicide technologies are available. By using diverse weed management practices, growers will preserve the utility of herbicide resistance traits and herbicide technologies and help maintain profitable and environmentally sustainable crop production systems for future generations.

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