Mini-review

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What have the mechanisms of resistance to glyphosate taught us?

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Abstract

The intensive use of glyphosate alone to manage weeds has selected populations that are glyphosate resistant. The three mechanisms of glyphosate resistance that have been elucidated are (1) target-site mutations, (2) gene amplification and (3) altered translocation due to sequestration. What have we learned from the selection of these mechanisms, and how can we apply those lessons to future herbicide-resistant crops and new mechanisms of action? First, the diversity of glyphosate resistance mechanisms has helped further our understanding of the mechanism of action of glyphosate and advanced our knowledge of plant physiology. Second, the relatively rapid evolution of glyphosate-resistant weed populations provides further evidence that no herbicide is invulnerable to resistance. Third, as new herbicide-resistant crops are developed and new mechanisms of action are discovered, the weed science community needs to ensure that we apply the lessons we have learned on resistance management from the experience with glyphosate. Every new weed management system must be evaluated during development for its potential to select for resistance, and stewardship programs should be in place when the new program is introduced.

Keywords: herbicide; glyphosate; resistance; mechanism of action

1 INTRODUCTION

Glyphosate is the most widely used herbicide in the world, owing, in part, to the introduction and widespread adoption of glyphosate-resistant crops.[1] Over 15 years ago there were no reported cases of glyphosate resistance, even though the herbicide had been in use for 20 years.[2] Several reviewers prematurely concluded that the evolution of glyphosate-resistant weeds was an unlikely event, based on the following observations: the two decades of glyphosate use without development of resistance; lack of known plant mechanisms to metabolize glyphosate; suboptimal enzyme kinetics of lab-created target-site mutants of 5-enolypyruvylshikimate-3-phosphate synthase (EPSPS); the fact that glyphosate-resistant crop development required the insertion of bacterial genes, a phenomenon not likely to occur in nature.[3–5]

A vast experiment was initiated with the introduction of glyphosate-resistant crops in 1996. For the first time, glyphosate was used as a stand-alone selective herbicide on millions of hectares of crop land both pre-emergence and multiple applications post-emergence. This unprecedented use pattern of a single herbicide imparted tremendous selection pressure on the weed populations and resulted in the selection of glyphosate-resistant weed populations in 12 species in glyphosate-resistant crops.[6]

Herbicide resistance can be due to at least three different mechanisms: (1) alterations of the target site; (2) changes in sequestration and/or translocation of the herbicide; (3) changes in rates of metabolism of the herbicide. Currently, two of these mechanisms have been identified as responsible for glyphosate resistance in weeds. Alterations of the target site via a mutation in the EPSPS gene so that it is no longer inhibited by glyphosate or overexpression of EPSPS have been documented in goosegrass [*Eleusine indica* (L.) Gaertn.], rigid ryegrass (*Lolium rigidum* Gaudin), Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.)], Palmer amaranth [*Amaranthus palmeri* (S. Wats.)] and tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer].[7] Reduced translocation of glyphosate to the meristems, presumably through sequestration at the site of application, has been identified in rigid ryegrass, hairy fleabane [*Conyza bonariensis* (L.) Cronq.] and horseweed [*Conyza canadensis* (L.) Cronq.].[7] To date, there have been no resistant weed populations identified with altered glyphosate metabolism.[7]

Ten years ago, Shaner[8] wrote a review on the impact of glyphosate-resistant crops on the use of other herbicides. In that review, he suggested that, if this new technology were part of an integrated system, then the selection of herbicide-resistant weeds could become a rarity. However, if glyphosate were used to replace most other herbicides, the value of this tool for resistance management would be diminished. Unfortunately, glyphosate-resistant crops were not integrated into a total weed management program but essentially replaced all of the other programs, particularly in soybeans and cotton. Today we are seeing the consequences of the overuse of glyphosate for weed management. However, glyphosate resistance has taught us a great deal about the biochemical mechanism of action of glyphosate and basic plant physiology and genetics. We can use the lessons learned from this experience to continue to use

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glyphosate effectively. In addition, this knowledge can be used to develop resistance stewardship programs for new herbicide-resistant crops, as well as herbicides with new mechanisms of action.

There have been a number of excellent reviews and books published on the mechanisms of glyphosate resistance.[7,9–13] and we do not want to repeat what has already been said. The objectives of this mini-review are to examine the different biochemical mechanisms of glyphosate resistance in terms of their diversity and breadth; how we might have anticipated these mechanisms on the basis of research that was done to develop glyphosate-resistant crops; how we might be able to use the knowledge gained by studying the biochemical mechanisms of glyphosate resistance to manage herbicide resistance in the future.

2 TARGET-SITE MUTATIONS

2.1 Development of glyphosate-resistant crops

Glyphosate kills plants by interfering with the shikimate pathway, which is responsible for biosynthesis of aromatic amino acids and many secondary aromatic products (e.g. auxins, lignin, phytoalexins, etc.).[14,15] Glyphosate does this by interfering with the binding of phosphoenol pyruvate (PEP) to EPSPS, mimicking the intermediate state of the enzyme reaction and leading to a dead end complex.[16] Inhibition of EPSPS leads to rapid accumulation of shikimate[10] and eventual death of the plant, although the exact cause of death still remains unclear.

EPSPS has been divided into two classes based on intrinsic sensitivity to glyphosate.[17] Class-I enzymes, which are found in all plants and many bacteria, are inhibited by glyphosate at micromolar concentrations. Class-II enzymes, which are found in bacterial species such as *Staphylococcus aureus* and *Agrobacterium* spp., are inhibited by glyphosate at millimolar concentrations and still retain efficient catalytic activity in the presence of high glyphosate concentrations. Most of the early attempts to select for glyphosate-resistant EPSPS were done on class-I EPSPS.

Comai *et al*.[18] isolated glyphosate-tolerant mutants in *Salmonella typhimurium*. The resistant mutants mapped to the aroA locus and the EPSPS activity of these mutants were less sensitive to inhibition by glyphosate than the wild-type EPSPS. The glyphosate-resistant *S. typhimurium* mutants contained a single amino acid change of a Pro to Ser at position 101 of the polypeptide sequence.[19] (To avoid confusion, we will use the *Escherichia coli* EPSPS numbering system for the amino acids in EPSPS.) Healy-Fried *et al*.[20] showed that, while Pro101 is not directly involved in glyphosate binding, replacing Pro with residues smaller than Leu results in a structural change in the glyphosate binding site without changing the catalytic efficiency of the enzyme. The structural change in the enzyme shifts Gly96 and Thr97 towards the glyphosate binding site and causes repulsive forces.[20] Although this mutated gene was inserted into tomato and a glyphosate-resistant line was regenerated,[21] this mutation was not commercialized.

Scientists at Monsanto successfully isolated other glyphosate-resistant mutants from petunia and *E. coli* EPSPS. These mutants contained a Gly-to-Ala substitution at position 97 that resulted in a 500-fold increase in the EPSPS IC_{50} for glyphosate.[22] Similar mutations were selected in tomato, canola, soybean, corn and arabidopsis [*Arabidopsis thaliana* (L.) Heynh.], where similar increases in glyphosate resistance were evident. Although the altered EPSPS had a higher apparent K_i for glyphosate, all of these mutations also had a many-fold increase in apparent K_m for PEP.[22] This much less efficient enzyme would have to be expressed at extremely high levels in plants to confer glyphosate resistance. It was concluded that this type of mutation could not be used to develop glyphosate-resistant crops.

Double mutations of EPSPS were made by Rhone-Poulenc scientists, and one was successfully introduced into field corn to produce the first commercial variety of glyphosate-resistant corn (*Zea mays* L.), which is still available today.[1] This double mutant converted Thr97 to Ile and Pro101 to Ser. Funke *et al*.[23] found that this double mutation caused a shift of Gly96 towards the glyphosate binding site, thus preventing glyphosate inhibition of the enzyme, but the mutant enzyme still retained high affinity for PEP because the Ile substitution for Thr allowed sufficient PEP binding to maintain the catalytic efficiency of EPSPS.[23] A single-site mutation of Thr 97 to Ile was still sensitive to relatively low levels of glyphosate and had substantially less affinity to PEP.

The successful development of glyphosate-resistant crops in cotton, soybeans and canola was due to the discovery of an EPSPS gene in *Agrobacterium* sp. strain CP4.[1] The class-II EPSPS enzyme encoded by this gene has an Ala at position 100 that corresponds to Gly96 in glyphosate-sensitive forms of EPSPS in *E. coli*.[23] The methyl group on the Ala residue clashes with one of the oxygen atoms of the glyphosate phosphonate group and thus interferes with binding. However, the active site of CP4 EPSPS can accommodate PEP more effectively than the glyphosate-sensitive forms of EPSPS, resulting in a more catalytically efficient enzyme.[23]

An early argument on why glyphosate would not select for EPSPS mutations was based on the observation that many of the mutations that provided glyphosate resistance also had lower affinity for PEP. The argument went that any such mutation selected in nature would be so unfit it would not survive. However, the P101S mutation had a twofold higher specific activity than the wild type.[10] In addition, the fact was ignored that the level of resistance that would allow a weed population to withstand glyphosate application and set seed in spite of severe injury would not have to meet the standards of resistance in a commercial crop.

2.2 Selection of altered target-site resistance

The discovery of the first glyphosate-resistant weed population containing an altered target site occurred in 2002. First reported by Lee and Ngim,[24] glyphosate-resistant populations of goosegrass were found in Malaysia. Reduction in the price of glyphosate herbicides, frequent applications (6–8 per year) and multiple goosegrass generations per year were factors contributing to a situation that led to the development of glyphosate resistance.[12,24] Baerson *et al*.[25,26] first showed that the resistant Malaysian goosegrass populations exhibited a reduced sensitivity of EPSPS to glyphosate, based on the finding that the glyphosate concentration required to inhibit EPSPS activity by 50% (I_{50}) in resistant populations was 3 times higher than in susceptible populations.

The molecular basis for this resistance was revealed to be a mutation in the EPSPS gene that caused a proline-to-serine substitution at amino acid Pro101 to Ser (P101S).[27] (Note that this is also referred to as P106S, using the plant enzyme numbering system.) A proline-to-threonine (P101T) substitution was also found to confer similar levels of glyphosate resistance in Malaysian goosegrass populations.[27–29] The result of these substitutions is a decrease in the affinity of EPSPS for glyphosate binding. These target-site mutations were subsequently linked to Australian[30] and Chilean[31] populations of glyphosate-resistant ryegrass (*Lolium* spp.). Kaundin *et al*.[32] showed that, although the resistance to

glyphosate in a goosegrass population from the Philippines was moderate, the P101S mutation was sufficient to provide glyphosate resistance at field use rates. The inheritance of the EPSPS P101S target-site mutation is through a single, nuclear-encoded gene that is incompletely dominant.[29]

A final issue in the target-site-based mechanism of glyphosate resistance that has yet to be resolved is the possible fitness penalties associated with these mutations. Few studies have been conducted, with limited evidence linking physiological differences between resistant and susceptible goosegrass populations to the target-site mutation.[24,33] However, several researchers contend that alterations of the EPSPS active site must also affect PEP binding, translating into significant fitness penalties.[34,35]

2.3 Lessons learned?

What has the discovery of EPSPS-based resistance in weed populations taught us? First, we now have a better understanding of how glyphosate and the substrates of EPSPS interact with the enzyme and the importance of various amino acid substitutions on those interactions. Second, we have a better understanding of the dominance of EPSPS-based glyphosate resistance and how the trait may spread through a population. It is interesting to note that, thus far, we have only found mutations at Pro101, although there are other mutations that could also provide resistance. It is even possible that, with continued selection pressure, a double mutation could occur in a population already containing the Pro101 mutation that could be analogous to the GA21 double mutant in corn. If this were to occur, the level of resistance would increase many-fold, based on the experience with the GA21 mutant corn lines.

Third, the selection of glyphosate resistance via mutations of EPSPS shows us that we should not ignore the potential of even weak resistance to be selected in weed populations. Although many of the mutations discovered in weed populations had previously been found during the development of glyphosate-resistant crops, none of them was commercialized because the level of resistance was deemed to be too weak. However, even a weak mutation can be very successful in a weed population under severe selection pressure if it allows the weed to set seed and reproduce. Such situations arise when low doses of herbicides are applied or herbicides are applied to plants above the recommended growth stage.[7]

3 AMPLIFIED EXPRESSION OF EPSPS

3.1 History

Another mechanism of glyphosate resistance was discovered in the laboratory in early attempts to develop resistant crops using tissue culture. Nafziger *et al*.[36] selected for glyphosate resistance in carrot (*Daucus carota* L.) cultures by a stepwise increase in glyphosate level, until they obtained cultures that could grow in 24–35 mM of glyphosate. Unselected cells died at 1 mM of glyphosate. The resistant cell line had a 12-fold increase in EPSPS protein levels, and the genome contained a 4–25-fold increase in EPSPS copy number.[36] Subsequently, glyphosate resistance was selected in cell cultures of petunia, tobacco, carrot, soybeans, chicory and alfalfa.[10] In all of these cases there was at least a 20-fold increase in the copies of EPSPS genes in the genome.

The stability of the increased EPSPS copies, particularly in the absence of glyphosate selection pressure, was tested in many cases. The results varied, depending on the cell culture. Stable resistance was achieved in cultures of chicory, tomato and tobacco.[10] However, resistance was slowly reduced or lost completely in other lines owing to the loss of the multiple copies of the gene.[10]

Attempts were made to regenerate plants with EPSPS gene amplification in tobacco, but the results were mixed. In some cases, regenerated plants maintained glyphosate resistance, but in others either the regenerated plants were not resistant or they lost the resistance over time.[10] Based on these results, researchers concluded that tissue culture selection of glyphosate-resistant cells was not likely to result in commercial levels of resistance.[10] In addition, it was argued that gene amplification could not be selected in the field because the ability to select for resistant lines in the laboratory was low and the selection pressure would not be duplicated in the field.[3] While there are many examples of insecticide resistance due to gene amplification,[37] there was little indication in the literature that gene amplification would become a herbicide resistance mechanism.

3.2 Selection of amplified copies of EPSPS genes

Increased (2–3-fold) levels of EPSPS mRNA have been found in several glyphosate-resistance species, including rigid ryegrass[25,26] and *Conyza* spp.,[38,39] but these increases were not thought to contribute greatly to glyphosate resistance. Glyphosate-resistant Palmer amaranth was selected in southern US cotton fields and first reported in 2004.[40] The resistance developed as a result of continuous cotton production for many years while relying on multiple, in-season applications of glyphosate. Gaines *et al*.[41] found that the gene encoding EPSPS was overexpressed in Palmer amaranth, thus providing additional active sites for PEP and shikimate-3-phosphate to bind normally and continue carbon flux through the shikimate pathway. Resistant individuals had, on average, 77-fold more copies of the EPSPS gene, 35-fold higher expression of EPSPS mRNA and approximately 20-fold higher expression of EPSPS protein. To rule out target-site mutation as the mechanism of resistance, EPSPS activity was measured for resistant and susceptible populations, and both enzymes were equally sensitive to glyphosate inhibition. Subsequent research has shown that glyphosate-resistant tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer.] also contains increased genomic copies of EPSPS, but it remains unclear whether this is the only mechanism in the population.[42]

The stability of inheritance of amplified EPSPS genes in the genome is not clear. The complication with this mechanism is the possible involvement of epigenetic stress-induced transposon activity.[43] F2 populations from crosses between resistant and susceptible Palmer amaranth lines show a range of EPSPS copy numbers. However, resistance is always associated with plants that have greater than 30 copies of EPSPS in the genome.[44] Additional research is needed to see whether this trait is stable in the absence of glyphosate use.

3.3 Lesson learned

The fact that glyphosate-resistant populations were found with amplified EPSPS genes was not expected by many weed scientists. However, the presence of gene amplification suggests that, with high selection pressure, this mechanism can be selected. We should learn from this discovery that no potential mechanism of resistance is impossible. Glufosinate-resistant crops are being offered as an alternative to control glyphosate-resistant weeds. However, it is known that glufosinate resistance can be selected in

tissue culture owing to overexpression of the target site, glutamine synthetase.[45] Based on the experience with glyphosate-resistant Palmer amaranth, it is highly possible that, if glufosinate were used in a similar manner to glyphosate, selection for resistance via overexpression of the glufosinate target-site gene would occur.

4 ALTERED TRANSLOCATION

4.1 History

One of the herbicidal strengths of glyphosate is how well it can translocate within the plant. As glyphosate kills plants by interfering with the shikimate pathway, which is most active in the growing points of the plant, translocation of the herbicide to the growing points is vital.[46] Glyphosate is translocated in the phloem from the source leaves to sink tissues following sucrose movement.[47,48] The phloem mobility of glyphosate is due to its unique combination of three acidic functions and one basic function.[49] Any change in the structure of glyphosate that affects its zwitterionic characteristics reduces its ability to move in the plant.

However, it is not known exactly how glyphosate accumulates into the phloem. The herbicide has to enter the phloem lumen, presumably through the cell symplasm. Glyphosate may do this either by mass diffusion into the mesophyll cells, followed by movement to the phloem through the plasmodesmata, or by being actively taken into the mesophyll and/or companion cells. Once glyphosate enters the sieve element, it is trapped owing to its hydrophilic properties and is transported to sink tissues. A mutation that would somehow interfere with the ability of glyphosate to translocate within the plant would also decrease its herbicidal activity.

4.2 Selection of reduced translocation

The first case of glyphosate resistance was reported in Australia in rigid ryegrass populations.[50,51] Much research was conducted to determine the mechanism of resistance, and it was concluded that it was not a result of reduced EPSPS affinity for glyphosate or glyphosate metabolism but, rather, reduced translocation out of the treated leaves to other plant tissues.[52] Shikimate accumulation in plant tissue was similar in both resistant and susceptible populations, indicating that EPSPS affinity for glyphosate had not been altered.[52] Additional studies with four different Australian glyphosate-resistant rigid ryegrass populations also indicated that reduced translocation was occurring.[53]

Subsequently, populations of glyphosate-resistant horseweed were discovered in Delaware soybean fields in 2000.[54] Many additional horseweed populations have been identified as being glyphosate resistant across the United States.[55,56] The primary mechanism of glyphosate resistance in these populations has been shown to be reduced translocation.[57,58] A similar mechanism of resistance has been identified in GR biotypes of hairy fleabane and Italian ryegrass.[38,59,60]

More recent studies have begun to determine the molecular cause of reduced translocation of glyphosate in resistant horseweed populations. Ge *et al*.[61] employed ^{31}P NMR techniques to investigate the intracellular distribution of glyphosate in resistant and susceptible horseweed populations. Results indicate that glyphosate is rapidly sequestered in the vacuole in resistant horseweed, thus reducing its availability for phloem transport throughout the plant.

There are a few cases of glyphosate resistance that are related to reduced movement of glyphosate to the growing points but are different from the cases described above. Glyphosate resistance has been correlated with reduced absorption of the herbicide into the leaves of resistant biotypes in ryegrass and Johnsongrass (*Sorghum halepense* L.).[7] The mechanism of this reduced absorption has not been elucidated. There are also glyphosate-resistant populations of giant ragweed (*Ambrosia trifida* L.) that have an unusual phenotype of rapid desiccation of treated leaves which then fall off the plant and reduce the translocation of glyphosate to the growing points (Sikkema P, private communication). The mechanism of this resistance is unknown.

Studies have also been conducted to elucidate the nature of the inheritance of this glyphosate resistance mechanism. Zelaya *et al*.[62] concluded that the reduced glyphosate translocation trait is semi-dominant and nuclear encoded in horseweed. F_2 and backcross patterns also indicate that the trait is inherited as a single gene. Lorraine-Colwill *et al*.[63] showed inheritance as a single nuclear gene with partial dominance in rigid ryegrass. Wakelin and Preston[64] looked at several rigid ryegrass populations and found that dominance ranged from full to partial among the populations. Reduced translocation results in a higher level of resistance relative to the target-site mutation mechanism.

4.3 Lessons learned?

What have we learned from this mechanism of glyphosate resistance? First, we now have a better understanding of how glyphosate may move in the plant. The selection of a mechanism that rapidly sequesters glyphosate into the vacuole supports earlier research on the role of an active glyphosate transporter in glyphosate movement in the plant.[46] Second, this mechanism of resistance was not discussed when scientists were assessing the probability of the selection of glyphosate resistance. However, resistance to other herbicides owing to reduced translocation had been documented prior to its occurrence in glyphosate-resistant weeds. Paraquat resistance in weeds occurred prior to glyphosate resistance, and was due to sequestration and reduced translocation of the herbicide in the plant.[65] The efficacy of glyphosate is dependent on its ability to move within the plant, and anything that interferes with that movement reduces activity. Intensive herbicide use patterns will select for any traits able to confer survival, including reduced translocation. These results suggest that we need to think broadly about mechanisms of resistance. While we cannot predict what mechanism of resistance will be selected in a specific population at a specific time, we can assume that the widespread use of a single mechanism of action over enough time will eventually select for resistance to that herbicide.

5 STEWARDSHIP

A number of stewardship practices have been advocated by weed researchers and implemented by growers over the years, with varying results. The major hurdle to this effort is the difficultly in empirically measuring the success of a preventive strategy. As more information regarding the genetic control of herbicide resistance becomes available, it becomes more apparent that a single preventive strategy may not be adequate. Glyphosate resistance mechanisms can appear as polygene and single-gene traits, as indicated above, or even epigenically controlled. Studies by Busi and Powles[66] and Gardner *et al*.[67] suggest that different types of traits can develop on the basis of the herbicide application

system employed (quantitative versus qualitative), with low doses selecting for multiple minor genes in a population, with a high likelihood of migrating to a single plant, and high doses selecting for single-gene traits.

The revolving dose strategy first suggested by Gardner *et al.*[67] holds some merit for delaying the development of resistant populations. While this may be theoretically effective, there is little applied research at this time to support this practice. The other issue is the complexity of making recommendations to applicators that are based on differing doses across years and across a multitude of weed species at different growth stages. Still, this line of thinking should be explored further if a long-term strategy is to be implemented.

Several management strategies are currently being employed either to respond to or to prevent the development of glyphosate-resistant weeds. As the evolution of resistance is a result of herbicidal selection pressure, rotating MOAs or tank-mixing herbicides is often one of the first strategies employed to manage resistance. The addition of another mode of action should negate the selection pressure placed on a population by one MOA or the other individually. This prevents the survival of resistant weeds from one season to another and their ability to pass on the genetics of herbicide resistance. Models have also been developed that suggest tank-mixing may reduce or delay the evolution of herbicide-resistant weed biotypes.[67–69] The addition of a residual herbicide in the MOA rotation has also been shown to manage glyphosate-resistant weed populations effectively by reducing the seedbank and by providing a longer duration of in-season control.[70] There have been additional studies done to evaluate the effectiveness of MOA rotation. A case study of Iowa corn and soybean production from 1990 to 1997 showed that multiple MOAs were used to control waterhemp, but ALS resistance was rapidly selected for during that period.[71]

Cultural practices should also be employed in an integrated fashion to complement chemical control measures. Glyphosate-resistant horseweed, an emerging problem in soybean production, has been found to be easily controlled using tillage.[72,73] However, tillage comes at the cost of additional production inputs and the loss of no-till benefits. Crop rotation has been shown to be effective in managing glyphosate-resistant horseweed.[70] Rotating corn with soybeans was found to reduce both in-field and seedbank horseweed densities in the third and fourth years of the study, but showed no effect in the first two years of the study. In fact, continuous soybean production has been found to be the second most descriptive factor in modeling the occurrence of late-season horseweed presence.[70] Crop rotation not only allows for MOA rotation but also presents different ecosystems to challenge weed growth, which reduces selection pressure. Cover crops have also been used to manage weeds and may have application in managing or preventing herbicide-resistant weed biotypes. A winter wheat cover crop was used in combination with glyphosate and non-glyphosate herbicides before planting to control horseweed.[70] Results show that horseweed densities were reduced by using the cover crop to levels equivalent to the application of residual herbicides. Cover crops are effective because they compete well with emerging seedlings in the spring and help deplete the seedbank. Cultural practices were in place well before the advent of herbicides and should continue to be a part of a comprehensive weed management plan.

In an effort to be good stewards of glyphosate and glyphosate-tolerant crops, a multifaceted and integrated approach is the most effective. Herbicide resistance is a situation that evolves out of selection pressures present under unique situations. Therefore, the development of weed resistance cannot be managed using one single strategy and is a complex process that will occur differently under varying conditions. The principles of weed management dictate that resistance is best combated with high levels of control as a result of multiple control strategies. The combination of chemical, mechanical and cultural methods of control prevents escapes that could reproduce and perpetuate the genetics of resistance. Several overviews of good sustainability and stewardship practices are available in the literature.[71,74] Ultimately, long-term sustainability of any herbicide technology is dependent on diffusing the effects of selection pressure before they can take hold in a weed population. Making the correct decisions is based on several factors, including the MOA target, chemical characteristics of the herbicide and the biology of the weed species.[71] In the end, the biggest hurdle to be overcome by weed scientists is education at the herbicide applicator level.

6 CONCLUSION

What can we learn from the mechanisms of resistance to glyphosate? First, the relatively rapid evolution of glyphosate-resistant weed populations shows that no herbicide is invulnerable to evolution of resistance. Any herbicide that becomes the sole basis for weed management, as occurred with glyphosate-resistant crops, will place a tremendous selective pressure on the weed populations, and inevitably resistance of one type or another will be selected. The selection of glyphosate resistance not only threatens the loss of this vital herbicide in many situations but could lead to the use of other weed management practices, such as extensive tillage, that have negative environmental consequences.

Second, if a new set of herbicide-resistant crops are developed to help manage glyphosate resistance, the weed science community needs to ensure that we do not make the same mistake as with glyphosate-resistant crops. New herbicide-resistant crops have been or will soon be developed for a number of mechanisms of action, including inhibitors of glutamine synthetase,[75] photosynthesis (bromoxynil),[76] auxenic mimics,[77] protoporphyrinogen oxidase (PPO)[78] and hydroxyphenyl pyruvate dioxygenase (HPPD).[79] Although there is no widespread resistance to glufosinate, bromoxynil, 2,4-D, dicamba, PPO or HPPD inhibitors, resistant populations do exist.[6] If we shift our dependence to crops with these mechanisms of resistance in the same manner as we did with glyphosate-resistant crops, we will eventually find ourselves in the same situation as we are currently in with glyphosate and glyphosate-resistant crops.

The same conclusions as made above can be drawn if a herbicide with a new mechanism of action is discovered. There is no silver bullet. All herbicides have their strengths and weaknesses. Every herbicide needs to be evaluated during development as to its potential for selecting for resistance, and stewardship programs should be in place when the chemical is introduced. We can no longer let strictly market forces determine the use pattern of herbicides, or we will continue to destroy the full utility of the herbicide through the selection of resistance.

To illustrate this point, in a recent paper on new 2,4-D-resistant crops, Wright *et al.*[77] stated that, in spite of the widespread use of 2,4-D, there were very few 2,4-D-resistant weed species, and suggested that this meant the frequency of 2,4-D-resistant weeds would be low. This article resulted in a letter from Egan *et al.*[80] disputing Wright *et al.*'s statement and warning that this type of mindset could lead to the further selection of herbicide-resistant

weed populations. Egan *et al*.[80] stated that herbicide overuse would not be solved just by adding new herbicide-resistant crops if they were not part of an integrated system. Wright *et al*.[81] wrote a rebuttal in which they defended their statement on the low frequency of resistance to 2,4-D, but they also stated that they do not advocate that the 2,4-D resistance trait be used as a stand-alone trait, but that it be stacked with other herbicide-resistant traits to ensure that multiple mechanisms of action of herbicides are used. In addition, they recommended that these new crops be used in an integrated weed management system. This exchange of papers is exactly what should be taking place as new herbicide-resistant crops and herbicides with new mechanisms of action are being developed. It is heartening to see this dialogue early in the development of a new herbicide-resistant trait. If the new herbicide-resistant crops are actually used as recommended, then hopefully the selection of a weed population resistant to these herbicides will be a rare event. Management strategies, such as those listed above, if implemented when a new herbicide mechanism of action or herbicide-resistant crop is introduced, will go a long way towards extending the effectiveness of these new tools.

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