



PERSPECTIVE ARTICLE

The scenario of herbicide-resistant weeds: Management challenges and perspectives

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ABSTRACT

The evolution of herbicide-resistant (HR) weeds is becoming a challenge for sustainable food production. As of March 2023, 267 weed species have been confirmed resistant to one or more herbicides globally. The objectives of this review were to summarize the status of herbicide-resistant weeds, their mechanisms of resistance, herbicide resistance dispersal mechanisms, management options, and future perspectives on herbicide-resistant weeds. Target site resistance (TSR) and non-target site resistance (NTSR) are two mechanisms for the evolution of HR weeds. TSR results from changes in the specific target site/enzyme for the herbicide, whereas NTSR includes physiological processes that reduce herbicide concentration at the target site. Once an individual weed evolves resistance, the resistance can disseminate through seed-mediated gene flow, pollen-mediated gene flow, or vegetative propagules. Widespread dispersion of HR weeds threatens crop production, and effective steps need to be taken to restrict this dispersion. A widespread occurrence of HR weeds, particularly in developed countries, requires a systemwide, integrated, holistic approach for their effective management that can reduce reliance on herbicides and integrate non-chemical control techniques, including cultural practices, cover crops, conservation tillage and residue retention, harvest weed seed control, and mechanical weed control. Multiple herbicide-resistant crops and herbicide premixes with multiple sites of action are widely used for HR weed management; however, their long-term sustainability is questionable. Moreover, new herbicides with novel site of action and other non-chemical weed management strategies need to be developed that can be adopted by growers. Thus, management of herbicide-resistant weeds is complex and requires a multidisciplinary approach.

Keywords: Conservation tillage, Herbicides, Herbicide-resistant, Weed control

INTRODUCTION

Although the first case of herbicide resistance was reported in Hawaii in 1957 (Hilton 1957), the first confirmed case of triazine resistance in *Senecio vulgaris* L. (common groundsel) was reported in 1960s in western Washington, USA (LeBaron 1989). As of March 2023, 267 weed species have evolved resistance to one or more herbicides (Heap 2023). Out of 267 weed species, 171 are resistant to acetolactate synthase (ALS) inhibitors, followed by photosystem-II (PS-II) inhibitor (87), atrazine (66), glyphosate (56), tribenuron-methyl (48), imazethapyr (44), imazamox (42), iodosulfuron-methyl-Na (40), metsulfuron-methyl (40), chlorsulfuron (38), fenoxaprop-ethyl (33), paraquat (31), simazine (31), thifensulfuron-methyl (31), bensulfuron-methyl (29), mesosulfuron-methyl (27) and nicosulfuron (27) (Heap 2023).

Wheat and maize are widely grown crops, and several herbicides are applied in different countries for weed control in these crops. A total of 83 weeds in wheat and 64 weeds in maize production fields have evolved resistance to at least one herbicide, followed by rice (54) and soybean (52) (Heap 2023). Weeds from the Poaceae and Asteraceae families have the highest instances of herbicide resistance, with more than 56 weed species showing resistance to multiple herbicides. In general, weeds with annual or biennial life cycles are more likely to evolve herbicide resistance compared with perennial weeds. *Lolium rigidum* Gaudin, *Poa annua* L., *Amaranthus palmeri* S. Watson, *Echinochloa crus-galli* (L.) P. Beauv., *Eleusine indica* (L.) Gaertn., *Lolium perenne* ssp. *Multiflorum* (Lam.) Husnot, *Amaranthus tuberculatus* (Moq.) J. D. Sauer, *Avena fatua* L., *Amaranthus hybridus* L., *Conyza sumatrensis* Retz., *Echinochloa colona* (L.) Link and *Raphanus raphanistrum* L. are examples of troublesome weed species that have evolved resistance to more than 6 herbicide sites of action (Heap 2023).

It has been estimated that more than 25 million hectares are infested with *L. rigidum*, *A. fatua*, *Phalaris*

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minor Retz., *Setaria* spp. and *Alopecurus myosuroides* Huds. in cereal crops globally. From an economic perspective, *A. palmeri* is the number-one herbicide-resistant weed in maize and soybean production systems and *Conyza* spp. are the most widespread (Heap 2023).

Herbicide-resistant weeds in India

In India, herbicides account for 16% of the total pesticide market (Bhullar *et al.* 2017). The first case of *P. minor* (little seed canarygrass) resistance to PS-II inhibitors and ACCase inhibitors was reported in 1991 and 1994, respectively, in wheat production fields in Haryana (Heap 2023). *P. minor* resistant to isoproturon in wheat in Haryana was first reported in 1995 (Malik and Singh 1995) due to an overreliance on substituting phenyl urea herbicides (chlortoluron, isoproturon, methabenzthiazuron and metoxuron). Thereafter, aryloxyphenoxypropionates (fop-herbicides) including clodinafop and fenoxaprop were used for control of isoproturon-resistant *P. minor*, but it soon developed resistance against clodinafop, fenoxaprop, pinoxaden, sulfosulfuron, and tralkoxydim (Bhullar *et al.* 2017). By 2006, *P. minor* resistance to ACCase, ALS, and PS-II inhibitors had been confirmed (Rao *et al.* 2020). There are reports of *Avena ludoviciana* Durieu resistance to ACCase inhibitors, including clodinafop in Haryana. Chhokar *et al.* (2017) confirmed *Rumex dentatus* L. and *Polypogon monspeliensis* L. Desf. resistance to ALS inhibitors. Similarly, *Chenopodium album* L. has evolved resistance to ALS inhibitors (metsulfuron) (Bhullar *et al.* 2017) and Choudhary *et al.* (2021) reported that *Cyperus difformis* L. (smallflower umbrella sedge) has evolved resistance to ALS inhibitors (bispyribac sodium) in Chattisgarh and Kerala.

Evolution of herbicide-resistant weeds

The evolution of HR weeds follows the basic principles of natural selection and selection pressure. Selection pressure of an herbicide in a particular weed species refers to the ratio of resistant plants to susceptible plants following exposure to that herbicide (Gressel and Segel 1982). Herbicides do not cause weed resistance by themselves; rather, they select for individual plants with herbicide-resistant traits (Hanson *et al.* 2013). Repeated use of the same or different herbicide with the same site of action results in the selection of individuals that are not killed by that herbicide (Bhullar *et al.* 2017). These individuals then reproduce and grow in number over the years, resulting in a build-up of the HR weed population. However, the build-up of HR populations

depends on numerous factors such as the initial frequency of resistant individuals, the reproductive ability of the respective weed species, and competition (Hanson *et al.* 2013).

The source of variation among individuals of a weed/plant species is mutation. Some mutations can be positive and may aid in the survivability of the plant, some may be lethal and lead to plant death and some may be neutral with no effect (Loewe and Hill 2010). Thus, mutations leading to the evolution of herbicide resistance can be considered positive mutations, as they aid in the survivability of the species following herbicide exposure. The rate of mutation is not constant within and between species; rather, it varies with plant age, the type of tissue involved, environmental factors, and the genome and locus involved (Christoffers 1999). Mutations can either affect the herbicide target site (target site herbicide resistance) or modify plant metabolic processes (non-target site herbicide resistance), resulting in either reduced herbicide uptake, reduced movement of the herbicide inside the plant system or increased herbicide detoxification (Hanson *et al.* 2013).

Mechanism of herbicide resistance

The mechanisms for herbicide resistance are primarily classified as target-site resistance (TSR) and non-target-site resistance (NTSR). In TSR, the target site of the herbicide is modified/mutated, causing the target enzyme or protein to become less sensitive to the herbicide, and requiring a higher concentration of herbicide to inhibit the enzyme activity (Jugulam and Godar 2013; Gaines *et al.* 2020). For this to occur in a weed plant, either change occurs in the sequence of amino acids or the overexpression of genes to produce a greater number of target enzymes that can be inhibited by the herbicide. Therefore, TSR occurs either due to mutation (point/double/deletion) or gene amplification. The target-site protein has a specific site for herbicide binding, and several amino acids exist nearby this site that, if substituted, can lead to TSR (Gaines *et al.* 2020). Hence, there are several possibilities for target-site mutations, though mutations usually occur in or near the binding site of the herbicide, with structural changes sometimes occurring at other places on the target protein (Délye *et al.* 2015; Gaines *et al.* 2020). A change in a single amino acid on the target-site protein can reduce the capacity of the herbicide to inhibit the enzyme without affecting the normal function of the protein (Gaines *et al.* 2020). The resulting resistance from amino acid substitution can vary from low to high

levels depending on the mutation and herbicide molecule (Délye *et al.* 2015; Gaines *et al.* 2020). Mostly, TSR occurs due to point mutations and is frequently controlled by a single gene (monogenic), as herbicides are mostly meant to target specific enzymes/proteins. This also creates a relative ease in understanding the molecular mechanisms underlying TSR (Yuan *et al.* 2007). Monogenic resistance can spread rapidly within a population, as observed in the widespread occurrence of resistance to ALS-inhibiting herbicides conferred by a single nuclear gene (Tranel and Wright 2002).

NTSR mechanisms encompass all the physiological processes that reduce the concentration of active herbicide ingredients at the target site. NTSR includes a decrease in the uptake/translocation of herbicides, and/or an increase in sequestration, degradation, metabolism, or breakdown of herbicides to lesser toxic compounds compared to the parent material (Jugulam and Godar 2013). The reduced absorption of the herbicide is uncommon in the NTSR mechanism; differential absorption of foliar-applied herbicides is usually credited to differences in leaf anatomy (Gaines *et al.* 2020). However, it has been implicated as a mechanism of resistance in some cases such as glyphosate (Michitte *et al.* 2007) and atrazine (Svyantek *et al.* 2016), though it is usually deemed to confer low levels of resistance (Délye *et al.* 2013; Gaines *et al.* 2020). Herbicide resistance is sometimes conferred through reduced translocation, as the herbicide is trapped within the plant leaves due to vacuolar sequestration or changes in the activity of the plasma membrane transporter (Goggin *et al.* 2016; Gaines *et al.* 2020). For example, reduced translocation has been well-documented for imparting resistance to glyphosate (Ge *et al.* 2010; Gaines *et al.* 2019) and paraquat (Yu *et al.* 2010; Hawkes 2014).

The most important and researched NTSR mechanism is metabolic resistance, or enhanced herbicide degradation (Délye *et al.* 2013). Herbicide degradation is usually a three-phase process. In Phase I, the herbicide molecule is oxidized, hydrolyzed or reduced to modify it to a more hydrophilic metabolite. While in Phase II, this metabolite is conjugated, and in Phase III, the conjugated molecule is exported to the vacuole or cell wall for further degradation (Délye *et al.* 2013; Gaines *et al.* 2020). During this process, some enzymes have gained particular attention for conferring resistance by rapidly metabolizing herbicide molecules in resistant weed biotypes (Yuan *et al.* 2007). These enzymes are cytochrome P450 monooxygenases (P450s), which mediate Phase I of

herbicide degradation, and glutathione S-transferases (GSTs) and glucosyltransferases (GTs), which mediate Phase II of herbicide degradation. Metabolic resistance can pose a challenge for weed management, as it can confer broad-spectrum resistance (cross-resistance) and even combine with reduced translocation and TSR mechanisms to provide a greater level of resistance (Gaines *et al.* 2020). Compared to TSR, NTSR mechanisms are more complex and difficult to understand (Délye *et al.* 2015). This is because NTSR mechanisms are usually controlled by many genes (polygenic) and can provide resistance to numerous herbicides with different sites of action, even herbicides that are not yet commercially available (Petit *et al.* 2010; Délye *et al.* 2013). Moreover, plants carry these genes as gene families, with P450s and GSTs being the most important. Hence, this involvement of gene families makes it more difficult to discover specific genes that are conferring resistance in a specific scenario (Gaines *et al.* 2020).

Discovering the exact mechanism(s) of herbicide resistance can be more complex, as TSR and NTSR can co-exist in the same population. For example, Nandula *et al.* (2013) reported both TSR (Pro-196 amino acid substitution) and NTSR (reduced translocation) mechanisms for glyphosate resistance in *A. tuberculatus* (waterhemp). Therefore, if herbicide resistance is suspected to be occurring due to one of the two mechanisms, it is important to test other mechanisms, because sometimes more than one mechanism might be conferring the resistance (Jugulam and Shyam 2019). As a result, genomics, metabolomics, proteomics, transcriptomics and next-generation sequencing technologies have become crucial for improved understanding of the biological, genetic, ecological and molecular basis of herbicide resistance during this critical time when weed populations are showing multiple-herbicide resistance (Gaines *et al.* 2020).

DISPERSAL OF HERBICIDE-RESISTANT WEEDS

When weed(s) evolve resistance to herbicide(s), the dispersal of the resistance plays an important role in the widespread occurrence of HR weeds across the region and at the landscape level. The importance of weed dispersal processes in shaping genetic structure has been difficult to evaluate because the relationship between genetic structure and gene flow is notably complicated in weed species due to the existence of three components of gene movement: (1) seed-mediated gene flow, (2) pollen-mediated gene flow and (3) gene flow through vegetative

multiplication (Jhala *et al.* 2021a). The main evolutionary processes underlying gene flow dynamics rely on at least one of these three components of dispersal mechanism (Mallory-Smith and Zapiola 2008). For instance, hybridization is the result of pollen-mediated gene flow, whereas colonization of a new environment primarily occurs through seed-mediated gene flow (Jhala *et al.* 2008). Hybridization and introgression, although considered a form of gene flow, may also increase differentiation if admixture levels vary across populations (Jhala *et al.* 2008; Jhala *et al.* 2021a). To infer the level of pollen- and seed-mediated gene flow from patterns of genetic structure, it is important to monitor molecular markers that allow differentiation between seed and pollen movement (Sarangi *et al.* 2017).

Seed-mediated gene flow

Seed-mediated gene flow is the dispersal of weed species through the activity of seed(s). A number of factors play a role in seed-mediated gene flow such as seed viability, persistence, longevity, seed size, and seed dispersal mechanism (Oddou-Muratorio *et al.* 2001). A number of weed species such as waterhemp and *A. palmeri* (Palmer amaranth) are small seeded and prolific seed producers (Jhala *et al.* 2021b). For example, a single female plant of *A. palmeri* in central Nebraska, USA under ideal conditions can produce 5,00,000 seeds (Figure 1). In contrast, large-seeded weed species such as *Ambrosia trifida* L. (giant ragweed) can produce about 10,000 seeds per plant. There are several ways that seeds can be disseminated from one field to another through equipment, transportation, water, animals or human activities. Seed-mediated gene flow is the most common type of dispersal mechanism once resistance has been evolved in weed species.

Pollen-mediated gene flow

Pollen-mediated gene flow (PMGF) is the dispersal of alleles through pollen via wind, insects, or other pollinators. Several factors influence the frequency and distance of pollen movement and gene flow, including reproductive biology of the weed species, the type and presence of pollination vectors, pollen viability and longevity, flowering synchrony and pollen production, wind speed and direction, and others. Pollen-mediated gene flow is a natural phenomenon not unique to weed species that has occurred since the existence of flowering plants. After the evolution of HR weeds, PMGF is believed to be an important avenue for the spread of resistance within and between weed species. The dissemination

of herbicide-resistance alleles through pollen is more common in weed species that are dioecious (male and female plants are separate), such as Palmer amaranth and waterhemp. Certain weed species such as giant ragweed are prolific pollen producers; therefore, although giant ragweed is a monoecious species, pollen-mediated gene flow has been reported from glyphosate-resistant to susceptible giant ragweed (Ganie and Jhala 2017). Research has been conducted in Georgia, USA to determine whether the glyphosate-resistance trait can be transferred via PMGF from a glyphosate-resistant Palmer amaranth biotype to a glyphosate-susceptible biotype (Sosnoskie *et al.* 2012). Results from this study demonstrated that glyphosate-resistant Palmer amaranth could be dispersed up to 300 m under natural field conditions, and that the widespread occurrence of glyphosate-resistant Palmer amaranth is due in part to the movement of pollen between spatially segregated populations (Sosnoskie *et al.* 2012). A similar study is being conducted in Nebraska, USA to determine PMGF from glyphosate-resistant common waterhemp to glyphosate-susceptible common waterhemp under field conditions, with transgene movement detected at up to 50 m from the pollen source. Studies on PMGF from HR broad-leaved weeds (Jhala *et al.* 2021a) and grass weeds (Jhala *et al.* 2021b) have been reviewed and provide additional literature on this topic.

MANAGEMENT OF HR WEEDS

Weed management strategies that can reduce dependency on herbicides and herbicide selection pressure can aid in the management of HR weeds. Integrated weed management (IWM) is one such approach that can reduce the risk of evolution of HR weeds. IWM is a weed management strategy that integrates different weed management tools to help achieve effective weed control. The IWM approach aims at discouraging the introduction, spread and adaptation of weeds, and helping the crop to outcompete weeds. Some of the tools that can be used in IWM are listed below:

Cultural practices

Cultural practices are normally low-cost decisions that can serve as efficient weed management tools when integrated with herbicides or other weed management strategies. Cultural practices such as planting crops at the optimal time, using improved crop varieties, good quality seed material, optimal seed rate and row spacing, diversified cropping systems, the correct time and rate of

nutrients and irrigation are very effective for weed management if integrated with other weed management practices (Kumar *et al.* 2021). Chhokar and Malik (1999) reported that wheat planted in October had a lower infestation of *P. minor* compared to wheat planted at later dates. Some weeds are prominent under cropping systems; for example, *P. minor* is common in rice-wheat cropping systems in the Trans Indo-Gangetic plains compared to other cropping systems because of favourable growing conditions, and crop rotation can help reduce infestation of this problematic weed (Rana *et al.* 2018). Reducing soybean row spacing from 76 cm to 38 cm also reduced weed biomass by three times (Harder *et al.* 2007). Similarly, a recent meta-analysis by Singh *et al.* (2023) concluded that narrow row spacing (< 76 cm) reduced weed biomass by 71% and improved weed control by 34%. Cultural practices can reduce the selection pressure of herbicides on weeds, but such practices alone cannot provide the desired level of weed control and need to be integrated with additional weed management practices.

Cover crops

Cover crops are generally planted in the off-season between two successive cash crops and are terminated/killed before (*i.e.*, planting brown) or after (*i.e.*, planting green) the planting of cash crops (Figure 2). Cover crops offer various advantages such as reducing soil erosion, improving soil organic matter, water infiltration, and many others. In addition, cover crops also help in suppressing weeds (Pittman 2020). During their active growth period, cover crops compete with weeds and thereby reduce their growth compared to growth occurring on bare ground (Smith *et al.* 2015). After termination, cover crop residue produces a mulching effect (Figure 2) and blocks sunlight from reaching the soil surface, discouraging the germination and growth of weeds (Huarte and Arnold 2003; Teasdale *et al.* 2007). Moreover, cover crop residue also has allelopathic effects on weeds (Kruidhof *et al.* 2009; Sias *et al.* 2021). Cornelius and Bradley (2017) observed a 68–72% reduction in density of *Stellaria media* (L.) Vill., *Thlaspi arvense* L. and *Lamium amplexicaule* L. by growing a cereal rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth) mixture as cover crops. Similarly, cereal rye and canola reduced total weed biomass by 91% and 74%, respectively, compared to no cover crop (DeSimini 2020). Therefore, if managed properly, cover crops have the potential to suppress HR weeds and reduce the HR weed seedbank (Bunck *et al.* 2020).

Conservation tillage and crop residue retention

Conservation tillage has been defined as: “any tillage and planting system that covers 30% or more of the soil surface with crop residue, after planting, to reduce soil erosion by water” (CTIC 2002). While shifting from a traditional to a conservation tillage system may increase weed infestations during the initial years (*i.e.*, the transition phase), the long-term adoption of conservation tillage practices can aid in weed management (Bhullar *et al.* 2016). The reduced disturbance of soil under conservation tillage prevents the mixing of weed seeds into the soil seed bank and reduces weed emergence (Nandan *et al.* 2020). Moreover, if weeds are managed effectively and discouraged from seed production during the initial years, the weed seed bank can be significantly reduced, as limited tillage reduces the movement of weed seed from the lower soil layer to the soil surface (Kumar *et al.* 2021). Furthermore, retaining residue from previous crops also helps improve weed control (Chauhan and Abugho 2013; Bana *et al.* 2020), soil health (Parihar 2020) and maintaining soil moisture (Jat *et al.* 2019). Retention of crop residue in direct-seeded rice (DSR) reduced weed biomass and weed density by 47% and 41%, respectively, compared to DSR without residue retention (Bana *et al.* 2020). Crop residue retention promotes the growth of soil micro-organisms, which can help in the predation of weed seeds (Nichols *et al.* 2015).

Harvest weed seed control (HWSC)

HWSC is the process/method of preventing the deposition of weed seed in the soil seedbank during crop harvesting, either by removing weed seeds along with crop residue, concentrating weed seeds in narrow lines or destroying weed seeds using impact mills (Walsh *et al.* 2017). Preventing weed seed deposition in the soil seed bank during crop harvest reduces dependency on herbicides. Somerville *et al.* (2018) reported that destroying 50% of seeds before their deposition in the soil seedbank can slow herbicide-resistance by approximately 10 years. HWSC can be achieved through several methods such as using chaff carts to collect chaff material and weed seeds, narrow window burning of chaff material containing weed seeds, bale direct systems, chaff tramlining and chaff lining, and the use of a weed seed destructor such as Harrington Seed Destructor (HSD) or Redekop™ seed control unit (Walsh *et al.* 2017; Shergill *et al.* 2020b). Several studies have been conducted on chaff carts and chaff lining indicating their success in controlling weeds; for example, the collection and removal of soybean residue after harvesting reduced Palmer amaranth

density by 41-70% over three years (Norsworthy *et al.* 2016). However, removing crop residue also removes the nutrients present in the residue (Spath *et al.* 2022), leaving the soil exposed to wind and water and resulting in soil erosion. Harrington Seed Destructor (HSD) and Redekop™ seed control unit are more advanced technologies that mechanically destroy weed seeds at the time of crop harvest without removing crop residue from the field (Walsh *et al.* 2017). It has been reported that HSD can destroy 85-100% of seeds from several weed species tested, including *A. artemisiifolia*, *A. hybridus*, *Abutilon theophrasti* Medik., *A. trifida*, *A. tuberculatus*, *C. album*, *Datura stramonium* L., *Ipomoea hederacea* Jacq., *Setaria faberi* Herrm., and *Xanthium strumarium* L. (Shergill *et al.* 2020a). Similarly, Walsh *et al.* (2012) recorded greater than 90% destruction of *Avena* spp., *Bromus* spp., *L. rigidum* and *R. raphanistrum* through the use of HSD.

Mechanical weed control

Mechanical weed control is an age-old practice. Before the discovery of herbicides, mechanical weed control was the most important weed management strategy, though with the increasing popularity of herbicides, mechanical weed control become obsolete, as it is a laborious and tedious task, as well as expensive due to the increased cost of fuel (Rueda-Ayala *et al.* 2010). Previously, mechanical weed control was performed by either tractor-driven cultivators or human managed tillers such as rototillers or handheld small equipment such as hoes. However, mechanical weed management has made numerous advances in recent years, with the development of robots that are capable of real time imaging and can identify and kill weeds using mechanical blades/cutters, laser lights, high temperatures or electric current (King 2017). Equipment such as the Weed Zapper™ has been developed in recent years to use electricity as a medium to control weeds. When the weeds come into contact with the electricity, the electric current travels through the plant system and to the soil, killing the plants (Moretti 2021). Electric weeders have the potential to be an effective mechanical weed management option if used at the correct crop and weed growth stage, especially under no-till organic crop production systems.

Stacked herbicide-tolerant crops and herbicide premixes

Stacking herbicide-tolerant crops refers to modifying a crop variety by breeding resistance to two or more herbicides; for example XtendFlex®

soybean is resistant to dicamba, glyphosate and glufosinate (Striegel and Jhala 2022). Stacking or combining resistant traits in a crop expands the available herbicide options, as it allows for the rotation of different herbicides and hence, reduces the selection pressure against a single herbicide chemistry. Similarly, herbicide pre-mixes have different active ingredients mixed in a single herbicide, which aids in broad-spectrum weed control. Using herbicide premixes with different sites of action decreases the selection pressure exerted by an individual herbicide or an herbicide with the same site of action, thereby delaying the evolution of HR weeds (Norsworthy *et al.* 2012). Therefore, herbicide premixes reduce the possibility of survival and fecundity of weed species with resistance to a particular site of action herbicide (Norsworthy *et al.* 2012). Moreover, herbicide premixes can reduce application cost and are easy to use for farmers, as they do not have to buy different herbicides and can avoid herbicide tank-mixing complexities such as compatibility issues and calculating the herbicide rate of an individual active ingredient.

FUTURE PERSPECTIVES

Although diverse weed management strategies are the path to long-term sustainable weed management, herbicides are the central pillar of most weed management plans. Therefore, it is concerning that herbicide options are limited in the era of multiple HR weed populations. The discovery of herbicides with new sites of action is urgently needed (Dayan and Duke 2020), though in recent years, several herbicides with new potential targets have been discovered. For instance, fatty acid thioesterase (FAT) has been discovered as a target of cinmethylin with the potential for use in wheat, homogentisate solanesyltransferase (HST) has been discovered as a target of cyclopyrimorate with the potential for use in rice and dihydroorotate dehydrogenase (DHODH) has been discovered as a target of tetflupyrolimet with the potential for use in rice (Qu *et al.* 2021). For the future of herbicide discovery, several approaches have been introduced that can speed up the discovery process:

- a. Develop active ingredients that inhibit dual or multiple target enzymes (Gressel 2020).
- b. Use metabolomics to identify target enzymes, which on inhibition will accumulate (*in vivo*) phytotoxic metabolites or use proteomics to identify target sites that have low molecular concentration, allowing the use of low herbicide doses for killing weeds (Dayan and Duke 2020).

- c. Develop molecules with binding sites on substrate recognition regions of the enzymes to create less frequent target site mutations or develop smart inhibitors that are self-adaptive and have conformational flexibility, which leads to lower vulnerability to resistance mutations (Qu *et al.* 2021).
- d. Use innovative technologies, artificial intelligence, big data, *in vivo* and target-based high-throughput screening to identify novel herbicide molecules with the desired activity (Dayan 2019).

Weed management has become complex as weeds have evolved resistance to herbicides with multiple sites of action. With the prevalence of herbicide pre-mixtures and crops with multi-stacked herbicide-tolerant traits, the choice of herbicide and knowing which post-emergence herbicide to apply is critical. Therefore, it has become necessary to educate and guide farmers to make better decisions so they can effectively use available technology for long-term sustainability. Moreover, agronomists, crop advisors, and seed retailers who influence farmers' decisions should be trained about available herbicide options and weed management plans for specific scenarios in each farmer's field (Beckie *et al.* 2019). Additionally, farmers' feedback is crucial to tracking the occurrence of localized HR weeds; therefore, two-way communication with the significant involvement of growers through weed surveys, questionnaires, and other platforms is necessary for the future of HR weed management plan. Researchers have shown that the early detection or screening of herbicide resistance is possible, in some cases even at a large scale. For example, Kutasy *et al.* (2021) showed the potential of targeted amplicon sequencing (TAS) that uses the next generation sequencing (NGS) approach. They successfully detected two evolved TSR mutations that provided resistance to imazethapyr and linuron in *Ambrosia artemisiifolia* L. (common ragweed) out of 16 specific point mutations that are identifiable with this approach. Similarly, Ma *et al.* (2015) demonstrated that an excised leaf assay can be used to detect NTSR due to enhanced herbicide metabolism in waterhemp (*A. tuberculatus*). Likewise, other screening tests such as leaf-disk assay using leaf disks (Wu *et al.* 2021), an agar-based assay using seeds (Perez *et al.* 2021), thermal infrared imagery (Shirzadifar *et al.* 2020a), spectral reflectance indices (Shirzadifar *et al.* 2020b) and Raman spectrometry using leaves (Singh *et al.* 2021) have been shown to screen putative resistance for

many herbicides such as glyphosate, dicamba, clethodim, fomesafen and pyroxasulfone in several weed species. Thus, these new approaches, methods, tests, and techniques are available for detecting putative resistance early on, and hence can aid farmers in choosing only the most effective herbicides, reducing the overall use of herbicides and improving weed management. Herbicide resistance is an evolutionary process, and therefore the agricultural community needs to be proactive and keep evolving, adapting and developing new and effective solutions to the challenges posed by HR weeds. In conclusion, the future of herbicide resistance management should involve fewer herbicides and more integrated HR management options.

In the era of modern genetics and omics, many novel technologies hold promising solutions for HR weed management. Targeted genome editing technologies such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) have the potential to develop non-transgenic crops with resistance to herbicides, which can bypass genetically modified organism (GMO) regulations in some countries such as Canada (Gosavi *et al.* 2022; Jhala *et al.* 2008). RNA interference (RNAi) is promising for restoring herbicide susceptibility; in RNAi, small RNAs (sRNAs) are sprayed on resistant weed populations, inducing gene silencing and ultimately herbicide sensitivity in the weed population (Zabala-Pardo *et al.* 2022). Similarly, gene-driving technology has the potential to restore weed susceptibility to herbicides that they had become resistant against (Perotti *et al.* 2020).



Figure 1. A female Palmer amaranth plant in a food-grade white corn field in southcentral Nebraska, USA with the potential to produce a significant number of seeds



Figure 2. Cereal rye cover crop residue acting as mulch and providing early season weed suppression in corn in eastern Virginia, USA

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