

# Technological Advances for Weed Management

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## Introduction

Weeds are oftentimes the major pests of crop fields and farmers often utilize a variety of cultural, mechanical, and chemical methods to manage them. Herbicides have been the most preferred tool for weed management since the 1950s because these are significantly more efficient and economical over other weed management methods. The rapid growth of the herbicide industry and the industrial revolution in farm mechanization paved the way for large farm operations. Herbicides are indispensable for large farms.

The success of herbicide development and herbicide registration for crops hinges primarily on environmental safety and minimum off-target impact. Formulation and application technologies, coupled with sensible regulations, have mitigated the risks associated with herbicide use. The latent impact of herbicide use, with which we will continue to contend into the future, is ecological. It is inevitable that weeds will continue to adapt to selection pressure. This will be manifested as weed population shifts in the short-term and resistance evolution in the long-term. Resistance to herbicides will drive most of what we do henceforth for weed management and will also drive future discoveries of novel weed management tools. Breaking the yield ceiling, developing resilient varieties, efficient management of large farms, and improving the sustainability of crop production require a higher level of technological innovations. We are beginning to see such innovations today.

## New Generation of Herbicide-Resistant Crops

Herbicide-resistant (HR) crops are mainstays of modern agriculture (Duke, 2005). Adoption of HR crop technology has far outpaced insect-resistance technology in the USA. Historically, developing herbicide resistance in crops has required long-term and nontrivial investigation into identifying, selecting, and breeding/transforming the trait into a target crop line that is well suited for the goals of the technology developer and farmer. Recent advances in the fields of genomics, proteomics, biochemistry, and computational science have enhanced biotechnology and herbicide technology development.

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Herbicide resistance may be endowed by one or more mechanisms including enhanced herbicide detoxification, target enzyme modification, or reduction in the absorption/translocation of an herbicide in the plant (Anderson, 2007). To develop a herbicide-resistant and high-yielding crop, both traditional breeding and genetic engineering are employed. Traditional breeding using chemical mutagenesis or whole-plant selection from diverse germplasm (Anderson, 2007; Duke, 2005) has yielded crops with resistance traits to single sites of action including acetyl coenzyme-A carboxylase (ACCase)- or acetolactate synthase (ALS)-inhibitor herbicides. Biotechnology ushered in transgenic crops starting with single resistance traits such as bromoxynil-resistant cotton in 1995 (Stalker *et al.*, 1996), glyphosate-resistant (GR) soybean and cotton in the mid-1990s (Feng *et al.*, 2010) and later on, glufosinate-resistant crops (Duke, 2005). Resistance to bromoxynil and glufosinate is from bacterial transgenes that detoxify the respective herbicides. Resistance to glyphosate is from a bacterial transgene that is insensitive to glyphosate.

The impact of GR crops has been monumental across all aspects of the agriculture sector, resulting in the greatest advancement in modern agriculture, but also the biggest hurdles that farmers face at a global scale. It simplified weed control like never before, with a single herbicide that is very cheap and most environmentally friendly among all other herbicides, but eventually also resulted in the most widespread and difficult herbicide-resistant weed problems to date (Green, 2012; Heap, 2017; Vencill *et al.*, 2012). Approximately 40% of producers in the US have adopted no-till or conservation tillage systems after the commercialization of GR crops, which has helped to improve soil properties (Wade *et al.*, 2015). The evolution of resistance to glyphosate has forced many farmers to return to conventional tillage, and had increased the volume of herbicide use instead, in an effort to manage resistance reactively and proactively (Benbrook, 2012, Fernandez-Cornejo *et al.*, 2014).

Investment into HR crop technology and herbicide discovery declined steeply following the release of GR crops in 1996 (Dill *et al.*, 2008). However, due to increasing evolution of herbicide resistance and the need for diversification in agricultural production, new technologies have been, or are about to, be released. Acetolactate synthase-resistant soybean is not a new technology, but an enhanced trait (Bolt®) was released in 2015 with higher tolerance to ALS herbicides. This technology expands the registration of older herbicides and provides better options for management of problematic species. For the 2017 season, Inzen® *Sorghum bicolor* (L.) Moench ssp. *bicolor* (grain sorghum) was commercialized in the US, with traits endowing resistance to ALS inhibitors, specifically nicosulfuron and rimsulfuron. Nicosulfuron has been a standard post emergence grass herbicide for corn in the US due to its good control of *Sorghum halpence* (L.) Pers. (Johnsongrass) and *S. bicolor* (L.) Moench ssp. *drummondii* (shattercane), but it could not be used on grain sorghum. The new technology provides growers with an option for controlling weedy *Sorghum* spp. in grain sorghum.

A new non-transgenic rice with resistance to ACCase inhibitors (Provisia®) will be commercialized in 2018. The target of this technology is weedy rice and other difficult-to-control annual grass species, such as barnyard grass (*Echinochloa* spp.). This technology will complement the current ALS-inhibitor-resistant rice technology, Clearfield® rice. These technologies take advantage of mutant target enzymes (ACCase and ALS), with reduced herbicide affinity. All HR traits will require good stewardship including crop rotation, to ensure long-term utility. The somewhat specific spectrum of these herbicides also leave room for other weed problems to arise.

## Herbicide Trait Stacking

Stacked, or multiple-resistant, herbicide technology will become the key trait of new HR crops (Green, 2011, Green *et al.*, 2008). Stacked resistance traits expand the herbicide options for in-crop application and ensures complete weed control. Tank-mixtures and premixed herbicides will increase the weed spectrum of a single application and reduce the costs associated with multiple herbicide applications. This approach is touted to reduce the rate of resistance evolution and improve the long-term efficacy of herbicides (Norsworthy *et al.*, 2012). These technologies include dual-stacked traits such as resistance to glyphosate or glufosinate + ALS inhibitor, glyphosate + glufosinate, 2, 4-D + ACCase inhibitor, and glyphosate + dicamba or 2, 4-D. Multi-stacked traits of three sites of action are also close to registration, or already registered, including resistance traits to: glyphosate + glufosinate + 2, 4-D, glyphosate + glufosinate + dicamba, and glyphosate + glufosinate + HPPD inhibitor resistance in major agronomic crops. Most of these products have been released, or will be released within the next several years.

## Next Generation Herbicide Resistance Trait Development

Biotechnology and the production of new herbicide traits have expanded beyond selection of modified herbicide targets from bacteria or diverse germplasms. New strategies are being developed, and used, to assess novel mechanisms for HR crops. The development of dicamba and 2, 4-D resistance traits arose from investigations of the degradative processes used by bacteria to metabolize these compounds (Behrens *et al.*, 2007; Wright *et al.*, 2010). For dicamba, a soil bacterium was used to identify an enzyme responsible for degrading dicamba into non-toxic metabolites (Behrens *et al.*, 2007). TfdA was a known catalyst for 2, 4-D metabolism in bacteria, but was not efficient to use in HR crop development. Bioinformatics tools allowed mining similar genes in other organisms, leading to the identification of AAD-12 from *Delftia acidivorans*, which was used to transform crops for resistance to 2, 4-D (Wright *et al.*, 2010).

This enzyme, which catalyzes the cleavage of the oxygenolytic bridge of most auxinic herbicides, also cleaves a similar link in aryloxy-phenoxy-propionates endowing multiple-resistance to ACCase herbicides in monocot species. This is the first HR crop technology involving a single gene that endows resistance to more than one mode of action. Modified herbicide targets have been discussed previously. However, a novel approach for identifying, isolating, and ‘building’ an enzyme cassette was developed to achieve resistance to 4-hydroxy-phenyl-pyruvate dioxygenase (HPPD) inhibitors in soybean (Siehl *et al.*, 2014).

Most recently, BASF Corporation disclosed a novel protoporphyrinogen oxidase (PPO) enzyme that is insensitive to PPO inhibitors, which was developed utilizing protein chemistry and structural biology (Aponte *et al.*, 2017). The 3-D structure of the PPO enzyme was resolved and used as a model to design new herbicides targeting this protein. The novel mutant enzyme could tolerate all known PPO herbicides, but functions normally in corn and soybean. Advances in the molecular biology, genomics, proteomics, and bioinformatics have been very beneficial to weed science and the development of HR crops.

## Herbicide-Resistant, Resilient Crops, and the Future of Agriculture

Agricultural scientists and practitioners generally believe that herbicide-resistant (HR) crops have been important in advancing agriculture in the last 20 years. This technology is likely to dominate food production into the next 50 years. Recent discoveries in plant genomics and advancements in plant biology have led to significant changes in the methods used to develop HR crops.

These techniques could improve weed management and crop adaptation to abiotic and biotic stresses. Weeds have an inherent biological diversity and genetic plasticity that crop species lack, giving them a more strategic advantage under adverse conditions. The importance of HR crops will only increase with time, as weeds continue to evolve and adapt. Global climate change, resulting in increased atmospheric temperatures and CO<sub>2</sub> concentrations, will inevitably lead to several weedy species acquiring increased competitive advantages. Losses of prime agricultural land to industrial development and urban sprawl will push agriculture into marginal lands, where weed impacts are likely to be severe, and diminish the total production area altogether. These scenarios require tougher, resource-efficient, high-yielding crops.

New herbicides alone will not solve food shortage nor sustain sufficient food production. Producers will need to use these technologies as one tool for weed management, together with other tools. It is important that herbicide and HR crop technologies continue to advance; however, it is also equally important that tolerance to abiotic and biotic stress and competitive traits be incorporated into HR crops. Thus, future crop varieties will contain improved agronomic traits in addition to stacked herbicide resistance traits. Implementation of stewardship and best management practices aimed at disrupting the biology of weedy species will be necessary to keep in step with evolving weed problems.

### **Crops Resistant to Parasitic Weeds**

Parasitic weeds can cause up to 90% crop yield loss and are arguably the most-difficult-to-control among weedy plants because of their physical, biochemical, physiological, and genetic connection with their host plants (Aly, 2007). The most notorious parasitic plants include numerous species of *Orobanche* spp. (broomrape), *Striga* spp. (witchweed), and *Cuscuta* spp. (dodder). Conventional weed control strategies, whether cultural or chemical, do not work well on these weeds because their seeds germinate only upon exposure to certain compounds (collectively known as strigolactones) exuded from roots of host plants such as strigol from various species including *Gossypium hirsutum* L. (cotton), or sorgolactone from *S. bicolor* (Yoneyama *et al.*, 2010). Developing a parasite-resistant crop is being pursued. Traditional breeding for parasite-resistant crops generally has not been successful, or severely limited by the few number of resistance genes identified such as in sunflower and sorghum (Rispaill *et al.*, 2007).

Biotechnology had been used previously to generate a transgenic maize that exudes minimal amount of germination stimulant by inhibiting the terpenoid biosynthesis pathway (Matusova *et al.*, 2005). Advances in the next-generation-sequencing (NGS) technology, genomics, proteomics, and metabolomics coupled with advanced softwares for bioinformatics, will allow a comprehensive understanding of the molecular basis of host-parasite interaction. Besides the identification of host-parasite signaling genes, there is increasing evidence of horizontal gene transfer between host and parasitic weed (Kim and Westwood, 2015; Yoshida *et al.*, 2010). These will reveal novel techniques for developing parasite-resistant plants with new gene targets.

### **RNA Interference (RNAi) Technology**

Advancing technology is necessary to improve plant physiology to better adapt to abiotic and biotic stressors including herbicide application. Plant cells have intrinsic mechanisms to turn off expression of harmful genes, modulate responses to abiotic and biotic stressors, maintain genome integrity, adapt, or regulate developmental processes through non-coding, small RNA molecules that differ in their biosynthesis (Khraiwesh *et al.*, 2012).

The process, collectively called RNA interference, is attained either through short, interfering RNA (siRNA) or microRNAs (miRNA) (Sanan-Mishra *et al.*, 2013). RNA interference represents a genetic process that occurs naturally in many eukaryotes and can be modified to attain genetic modification in a large variety of economically important plants (Rutz and Scheffold, 2004). This process can be done artificially to manipulate the expression of genes of interest. It has been discovered that naturally occurring micro-RNAs (miRNA) can effect complex gene regulation by binding to reverse complementary sequences, resulting in cleavage or translational inhibition of the target RNAs (Khraiwesh *et al.*, 2012). siRNAs have a similar structure, function, and biogenesis as miRNAs, but are derived from long dsRNAs and can often direct DNA methylation at target sequences (Khraiwesh *et al.*, 2012).

Some miRNAs increase the expression of certain genes in response to plant developmental needs, or to stress factors (positive trait regulators), while others suppress gene expression (negative trait regulators) to turn off gene products that are not needed (Zhou and Luo 2013). Thus, artificially modulating the expression of miRNA can alter plant traits. A promising application of this technology in weed management would be related to increasing the tolerance to oxidative stress in crops. For example, it has been shown that overexpression of a negative regulator, such as the miR398 form of *CSD2* (Cu/Zn SOD gene) by RNAi increased plant tolerance to oxidative stresses (Sunkar *et al.*, 2006). The resultant trait could lend crop tolerance to some herbicides.

In the same manner, the competitive ability of crops can be improved by modifying the expression of miRNAs that control nitrogen metabolism (Fischer *et al.*, 2013) and tolerance to drought (Ferdous *et al.*, 2017), salt stress (Zheng and Qu, 2015), or other stress factors. The critical first step governing all these is identifying the appropriate miRNA target, which entails understanding the impact of modulating the target gene expression on plant phenotype across key species and environments.

## Gene and Genome Editing

Genome editing allows precise manipulation in the genome of an organism using sequence-specific nucleases. Nucleases create specific double-strand breaks at desired locations in the genome. The most rapidly emerging genome editing tool is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system from *Streptococcus pyogenes*, which is based on RNA-guided engineered nucleases (Jinek *et al.*, 2012). There are other genomic editing tools such as zinc finger nucleases (ZFN) and transcription activator-like effectors nucleases (TALENs) but the CRISPR/Cas9 system is the fastest, cheapest, most versatile, and most reliable system for gene editing (Abdallah *et al.*, 2015; Bortesi and Fischer, 2015).

This tool can accelerate crop breeding by allowing the precise and predictable modifications of desired traits directly in an elite background. Modifying the native herbicide-target gene can endow resistance to a herbicide. Deletion of PPO Gly210 in weedy *Amaranthus* endows resistance to PPO herbicides (Patzoldt *et al.*, 2006; Salas *et al.*, 2016). Deleting this Gly<sub>210</sub> codon using genomic editing tool can provide tolerance to PPO herbicides in crops. The CRISPR/Cas9 system can be programmed to increase gene expression, silence an undesirable gene, or modify trait stacking (e.g. tolerance to disease + herbicide) (Li *et al.*, 2013) including tolerance to other stresses such as drought.

One of the consumer objections to GM crops is the presence of a selectable marker, usually a foreign gene associated with the introduced trait. Dual targeting by CRISPR/Cas9 achieved precise excision of transgenes from the rice genome (Srivastava *et al.*, 2017); thus, generating marker-free plants. This means

a resistance trait from a weedy relative (or a germplasm variant) of the same species can be placed into a crop cultivar through genetic engineering, and the external genes (i.e. selectable markers) associated with the modification can be removed from the transformed cultivar line. Complete removal of the marker genes by genomic editing should alleviate the regulatory burden associated with transgenic plants, which until today, has hampered the development of HR rice.

Off-target activity is a major concern in genome editing. The specificity of CRISPR/Cas9 is determined by technology, the sequence of the guide RNA and the DNA target. A perfect match between the last 8-12 bases of the guide RNA sequence is needed for target site recognition and cleavage (Cong *et al.*, 2013; Jinek *et al.*, 2012). Careful selection of specific guide RNA sequences should minimize the risk of unwanted genomic modifications (Bortesi and Fischer, 2015). New techniques have been developed to avoid or minimize off-target mutations by Cas9 (Bortesi and Fischer, 2015; Fu *et al.*, 2014; Shen *et al.*, 2014). While off-target activity varies among cell types and species, whole genome sequencing analysis in *Arabidopsis* and rice revealed almost negligible mutations at off-target sites (Feng *et al.*, 2014; Zhang *et al.*, 2014). Substantially higher mutation rates, 117 indels and 1,397 single nucleotide variants, were observed in non-homologous gene sequences in CRISPR-treated mice. These rates are much higher than observed in spontaneous germline mutations, raising concern by the researchers and warranting further investigation of CRISPR-based research.

## **Genomics, Bioinformatics, and the 'Next-Generation' Tools**

Weed Science has long been a bystander in the advances of basic science due to a lack of funding to develop resources for non-model organisms and from a deficiency in cross-disciplinary research that can expand into new research areas. Recent advances in next-generation-sequencing (NGS) technology and computational science have made not only the investigation of genomes of organisms less expensive, but also introduced new low-barrier tools for researchers. Utilization of these technologies in research has expanded globally in the fields of weed biology, invasiveness, stress adaptation, and more importantly- evolution of herbicide resistance.

## **Advancing to the Next Generation**

Genomics in Weed Science is used in two broad areas: (1) classical molecular biology and (2) functional genomics (for comprehensive review, see Bodo Slotta, (2008). Classical molecular biology approaches involve sequencing and characterizing the genome of an organism. Genomic sequence and structure information as well as parameters associated with sequence diversity are used for phylogenetic assessments, gene flow studies, species relationships and population genetic studies, species identification, evolution of weedy traits, evolution of resistance to herbicides, and others. These classical approaches have been critically important in the early development of genomics tools in Weed Science, facilitating the identification of genes involved in herbicide modes of action. Using some of the principles developed in classical molecular biology, advances have been made into next generation technology that expand on our knowledge and understanding of weed biology.

Functional genomics informs us on the function of genes and in the process, enables understanding what controls phenotypic traits, such as weediness. Expressed sequence tag (EST) libraries (Basu and Zwenger, 2009) are among the earliest tools used for comparative gene expression and comparative genetic analysis of some weed species. This was followed by the development of DNA microarrays (Eisen *et al.*, 1998), which allow genome-wide analysis of differential gene expression in response to



biotic or abiotic stressors. This technique allows identification of expressed genes, which are quantified relative to a reference constitutively expressed gene. While this technique was used for a variety of research in weed science, its cost and complexity limit the identification of every potential gene of interest and the broader analysis of gene networks (Lee and Tranel, 2008). Its main limitation is the lack of available GeneChips for weedy species.

The most recent advancements in DNA sequencing and functional genomics are facilitated by NGS. NGS technology encompasses methods by which the genome of an organism is sequenced in a high throughput manner using a DNA template from short pieces of cDNA that are sequenced, imaged, aligned, and assembled into a contiguous genome (Metzker, 2010). Several NGS methodologies have been developed, including 454 pyrosequencing and genotyping by sequencing (GBS). Both DNA and RNA can be sequenced using NGS technology, allowing the assembly of an organism's genome, transcriptome, or proteome. It enables the study of non-model species because it does not require complete reference genome.

For Weed Science, the transcriptome produced with RNA-sequencing (RNAseq) provides an unprecedented global view of how weedy species modify gene expression in response to agro-ecological conditions, including management interventions (Wang *et al.*, 2009). The genome, transcriptome, or proteome for an organism can be assembled from NGS data using bioinformatics tools. Weed scientists are now using this technology to investigate genomic assembly (Yang *et al.*, 2013), herbicide resistance evolution and stress adaptation (Gaines *et al.*, 2014; Nah *et al.*, 2015; Yang *et al.*, 2013), and novel herbicide target genes (Riggins *et al.* 2010). This enabled identification of target-site and non-target-site gene groups associated with herbicide resistance. Investigation into non-target-site resistance mechanisms of diclofop-resistant *Lolium rigidum* using RNAseq identified four metabolism-related transcripts associated with herbicide detoxification (Gaines *et al.* 2014).

Advancing the discipline of Weed Science and discovering novel tools for weed management requires critical evaluation of the biology and physiology of weedy species using genomic methods. By integrating classical and next-generation technologies we can identify novel herbicide sites of action or herbicide targets, novel (perhaps nonchemical) means of weed control, and novel genes to use for improvement of crop competitive traits, weed-suppressive traits, and tolerance to stress.

## **Remote Sensing, Robotics and Drones**

Weed scouting to assess dominant weed species and their distribution within a field is necessary to guide the selection of suitable herbicide options. Weed scouting is expensive, time consuming, and weather-dependent. Remote sensing has the potential to provide an efficient and convenient alternative for weed scouting to optimize crop management. Remote sensing utilizes satellite or manned/unmanned aircrafts for capturing data. Satellite-based remote sensing is well suited for surveying a large area and large-scale crop yield monitoring (Zhang *et al.*, 2005). Satellite imagery lacks precision in assessing small areas, especially for weed detection, spatial distribution, and herbicide injury evaluations. These tasks require high- resolution imagery, which is typically achieved by closer observations using manned/unmanned aircrafts or ground vehicles.

Ground-based sensors have been used in the past to investigate the use of machine vision for weed detection and precision herbicide delivery (Hagger *et al.*, 1983). Machine vision technologies can facilitate site-specific, or spot applications of herbicides and reduce the amount of herbicide used (Medlin

*et al.*, 2000). Ground-based optical sensors (such as GreenSeeker<sup>®</sup>, WeedSeeker<sup>®</sup>) have been used to identify plants based on the surface reflectance of green tissues in agricultural fields (Andújar *et al.*, 2011; Franz *et al.*, 1991). Hagger *et al.* (1983) tested the first reflectance-based plant sensor for spraying weeds. This sensor measured the radiance ratio of red (R) and near infrared (NIR), which is typically higher for green surfaces, compared to bare soil. Hummel and Stoller (2002) evaluated a commercial weed sensing system (WeedSeeker<sup>®</sup>) attached to a sprayer and reported an average 45% saving on glyphosate use in corn and soybean in the US Corn Belt.

Utilization of optical sensors, or reflectance-based sensors for herbicide applications has been studied globally (Lamm *et al.*, 2002; Midtby *et al.*, 2011). However, optoelectronic sensors are not capable of discriminating between weed species (Sui *et al.*, 2008) and can be used only in specific situations where broad-spectrum herbicides (such as glyphosate, paraquat) are to be applied. Site-specific herbicide applications require a more strategic approach to use selective herbicides for specific weed situations, instead of a simple broad-spectrum control. More recently, Fennimore *et al.* (2016) developed a commercial ground-based machine vision system that can remove intra-row weeds, as well as thin crops to desired stands based on row crop patterns. However, this system cannot differentiate weeds from crops if weeds are large or dense and ground-based weed detection systems lack broad spatial scale information.

Integration of unmanned aerial systems (UAS) platforms (fixed-wing as well as rotary-wing) offer solutions to some limitations of satellite- or manned-aircraft-based remote sensing as well as ground-vehicle-based sensing. The applications of UAS have been increasing in forestry (Wallace *et al.*, 2012), rangeland ecology (Laliberte *et al.*, 2010), and agronomic cropping systems (Lelong *et al.*, 2008), among several other fields. Multispectral and hyperspectral imaging sensors mounted on unmanned aerial vehicles (UAVs) have been used successfully to detect weeds and distinguish species (Peña *et al.*, 2013). Multispectral and hyperspectral imaging can provide valuable information that is not obtained by RGB cameras or not visible to the naked eye. In particular, hyperspectral imaging has been used more often to classify agricultural systems and vegetation because it has more bands compared to that of multispectral sensors.

Hyperspectral remote sensing collects reflectance data over a wide spectral range through narrow bands (10 nm) (Mulla, 2013) and is advantageous over other sensors when small differences are to be detected. Hyperspectral imagery analysis carried out at College Station, TX indicated that *Amaranthus palmeri* (S.) Wats. (Palmer amaranth), *A. tuberculatus* (Moq.) Sauer (waterhemp), and *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass) can be distinguished at 700 – 1000 nm wavelength (Bishop *et al.*, unpublished data). Similar studies have distinguished *T. aestivum* from broadleaf weeds; *Glycine max* (L.) Merr. (soybean), *Sida spinosa* L. (prickly sida), *Senna obtusifolia* (L.) Irwin & Barneby (sicklepod), and *Ipomoea lacunosa* L. (pitted morningglory) (Gray *et al.*, 2009); *S. bicolor*, *Amaranthus* spp., *Echinochloa* spp., and *Cyperus rotundus* L. (purple nutsedge) (Che'Ya, 2016); and several other weeds (Yang and Everitt, 2010).

Hyperspectral imagery also has the potential to differentiate herbicide-resistant and susceptible weed biotypes. Jha *et al.* (2017) utilized hyperspectral imagery to differentiate *Kochia scoparia* (L.) Schrad. (kochia) biotypes resistant to glyphosate or dicamba from susceptible biotypes. Differential reflectance values were observed near 720 nm for susceptible and dicamba-resistant kochia. The management of herbicide-resistant weeds will be easier if resistant plants could be detected in a field during early stages of evolution. More research is necessary in this area to develop robust hyperspectral imagery-based early detection systems for herbicide-resistant weeds.



## Nanotechnology

Nanomaterials have found unprecedented utility in various fields of science including medicine, manufacturing and material construction, chemical formulation, delivery of molecules into cells, sensors, and others. It has opened uncharted territories of scientific investigations in agriculture (Kanjana, 2015). Being 100 nm or less in size, nanoparticles have large surface area:volume ratio enabling applications that were formerly untenable (NSTC 2017). Nanoparticles exhibit unique properties that have been found useful, for instance, in producing slow-release fertilizer and pesticide formulations, or formulations that offer protection from rapid biotic or abiotic degradation and reduced run-off because of increased sorption properties (Ghormade *et al.*, 2011; Gogos *et al.*, 2012). These would be among the most immediately recognizable applications. Despite its exciting potential for generating novel tools for weed management, nanomaterials and nanotechnology should be used with caution. Its risk to humans, flora and fauna, and the environment should be assessed thoroughly.

## Herbicide formulations

The use of nanomaterials in herbicide formulations is deemed to increase herbicide efficacy by improved absorption and translocation of herbicide in the plant, increase the adsorption of herbicide onto clay particles thereby reducing runoff or potential contamination of ground water, or protect the herbicide from microbial or UV degradation thereby increasing the residual activity. Nano formulations that have been commercialized so far are micro emulsions (ME). These are typically 10 - 50 nm in size, demonstrating one type of nanotechnology application in pesticide formulation (Gogos *et al.*, 2012).

Some specific examples in this category are fomesafen 1.88SL or 2SL (by Syngenta Crop Protection), a premix of fluroxypyr + clopyralid + MCPA (Greenor<sup>®</sup>, by Dow AgroScience) and a premix of S-metolachlor + metribuzin (Tailwind<sup>®</sup>, by MANA Crop Protection). The adjuvant business is also working on developing novel formulations with nanotechnology. One example is the adjuvant marketed as Nano Excel for Herbicide developed by Enviro Science Technologies (<http://estchemicals.com/nano-tech/nano-excel-for-herbicide/>) specifically for glyphosate and 2, 4-D for use in turfgrass.

## Herbicide Sensors and Tracers

Now, more than ever, agriculture practitioners (specifically those using herbicides or other pesticides) need to be more vigilant in minimizing potential negative impact on the environment. Significant resources have been devoted to monitoring the dissipation and off-target movement of pesticides. The commercialization of HR crops containing dicamba- or 2, 4-D-resistance traits underscores the need for highly sensitive, cost-effective, high-throughput methods to monitor these compounds (or other pesticides) in the air, plant, and water. Traditional analytical methods involving liquid or gas chromatography are complex and costly (Jankowska *et al.*, 2004). Recently, Rahemi *et al.*, (2015) simultaneously modified a glassy carbon electrode with a novel polyaniline (PANI)-carbon nanotube (CNT) cyclodextrin matrix. The modified electrode was used to analyze MCPA and its metabolite 4-chloro-2-methylphenol, as precisely and accurately as the HPLC method, and was simpler and cheaper to prepare.

Different sizes, and metals, of semiconductor nanocrystals (quantum dots, QDs) emit different colors of light when exposed to UV radiation as a manifestation of quantum confinement effect (Saleh and Teich, 2013). QDs are both photo-luminescent and electro-luminescent. Coupled with their ability to penetrate cell walls and cell membranes, QDs can be effective tracers of chemicals or biomolecules in

plants; thus, presenting the possibility of replacing the use of radioactive tracers in studying herbicide entry, movement, and metabolism in plants. Such studies are conducted traditionally with radio-labelled herbicides (Nandula and Vencill, 2015), which is a lengthy, laborious, costly process that comes with high potential hazard. As with radio-labelled tracers, QDs can help locate the organelle and molecular target of a herbicide or the destination metabolites. Gold nanostructures, through two-photon microscopy, have been effective as imaging platforms in plants, because of their low cytotoxicity (Jia *et al.*, 2016). Recently, Jia *et al.* (2016) demonstrated for the first time that gold nanorods (39.4 nm x 11.3 nm) can be used as fluorescent tracer for 2,4-D herbicide using tobacco (*Nicotiana tabacum* L.) as test plant.

## Herbicide Delivery

Among the earliest commercial applications of nanotechnology in agriculture is the development of controlled release formulations of agrochemicals (Loha *et al.*, 2012; Grillo *et al.*, 2012). Gradual release of a herbicide in soil translates to beneficial effects including prolonged effective activity and reduced losses via leaching, runoff, volatilization, or UV degradation (Pereira *et al.*, 2014). Many soil-applied herbicides have short half-lives. For example, flumioxazin, imazethapyr, *S*-metolachlor, and thiobencarb have half-lives of 12–18 d, 60–90 d, 15–50 d, and 30–90 d in soil under aerobic conditions, respectively (Shaner, 2014). Thus, follow-up application of other herbicides are needed generally within one to two months of applying residual herbicides.

If the herbicide molecule is released gradually, and distributed evenly in the root zone by the nanocarriers, it allows for better interaction with plant roots resulting in increased absorption and less probability of being moved off-site by runoff or leaching. Pereira *et al.*, (2014) reported higher activity of atrazine nanocapsules in the 0- to 4-cm soil depth than that of conventional atrazine formulation. Further, the atrazine nanocapsules had more activity on the sensitive species at lower quantity than conventional atrazine. The nano formulated atrazine did not injure corn. Thus, they demonstrated that the herbicide load in the environmental can be reduced by using nanoparticles to deliver herbicides, without sacrificing the level of weed control. The ability of nanocarriers to penetrate cell walls and cell membranes has sparked hopes for the development of systemic herbicide formulations (Aly, 2007), or even peptides (Perez-de-Luque and Rubiales, 2009) or RNAi agents that would be effective on parasitic weeds.

Just as the utility of nanotechnology in generating novel tools for weed management bears promise, the uncertainties are equally high. There is so much that we do not know yet about the fate of nanomaterials in the environment and plants and its safety on humans (and animals in general). When it pertains to food, feed, and fiber, consumers are averse to technologies they do not understand. It is logical to expect that nanotechnology in agriculture will run into the same road blocks as GM crops. The idea of applying tons of nano-carriers with fertilizer or spraying large volumes of nano-pesticide mixtures across vast agricultural lands is unnerving.

## Concluding Remarks

The study of genomics, proteomics, metabolomics, bioinformatics, systems biology, molecular biology, and physiology and their applications into biotechnology, crop improvement, weed management, plant identification tools, molecular assays, and others will have the broadest impact on weed science and agriculture. Stacking of traits in crops will become the norm. miRNAs will be utilized more in developing crop traits. Advances in miRNA technology is most limited by the discovery of appropriate targets, which is now starting to be overcome by NGS technology.

NGS allows us to address questions in weed science that we could not investigate before, such as the molecular mechanisms driving weed evolution and adaptation as well as the intricate interaction between crops and weeds. The simplicity, accessibility, versatility, and robustness of CRISPR/Cas9 technology will facilitate forward and reverse genetics, enhance research in model organisms, and speed up gene discovery and trait improvement in plants. This technology has vast applications, but we should be mindful of potential consequences. The extent of off-target modification needs to be investigated thoroughly.

Control of parasitic weeds is another area where novel tools are much needed. There is hope that various ‘OMICS’ tools will accelerate development in this area where traditional plant breeding has failed thus far in producing varieties resistant to infection by parasitic plant (Aly, 2007). Applications of nanotechnology in agriculture will increase and would likely continue to facilitate the delivery of RNAi agents for nonchemical weed control technology and deliver novel herbicide formulations. Remote sensing and robotics will most likely become a more practical, user-friendly, and affordable tool. Integration of color, shape, and textural features will improve the effectiveness of weed species detection with high-resolution images produced using UAS-based sensors (e.g. Downey *et al.*, 2004; Zhang and Chaisattapagon, 1995). The future holds great promise for developing novel tools for weed management in particular and producing crops efficiently in general. Technology is advancing fast; we are limited only by our imagination.

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