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# Herbicide resistance in weeds: Survey, characterization and mechanisms

**V.K. Nandula***
*USDA Agricultural Research Service, Crop Production Systems Research Unit, Stoneville, Mississippi, USA*

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**ABSTRACT**

This paper presents a systematic diagnostic approach towards the characterization of herbicide resistance in a given weed population with regards to profile (single, multiple, cross resistance), magnitude (fold level), mechanism, and related bio-physiological aspects. Diagnosing herbicide-resistant weeds can be achieved by crafting robust procedures for seed sampling, survey protocol and seed collection, seed processing and storage, germination, emergence and growth (sufficient number of representative plants), treatment conditions (*i.e.,* discriminating dose, adjuvants, spray volume and parameters, water quality, and nutrient status), experimental design, appropriate controls including wild type/susceptible accessions, and biological parameters being measured. Understanding the processes and means by which weeds withstand labeled herbicide treatments is an important step, as well, towards devising effective herbicide resistance management strategies. Several physiological, biochemical, and molecular approaches for studying resistance mechanisms are available to researchers. The various omics approaches including genomics (DNA), transcriptomics (RNA), proteomics (proteins), and metabolomics (metabolites) will revolutionize herbicide resistance research.

**Key words:** Herbicide resistance, Mechanisms, Omics, Survey

Weeds have been in existence since before humans took up cultivation of plants for food, feed, fuel, and fiber. Before the advent of synthetic organic-based herbicides in the 1940s, weeds were controlled for thousands of years by mechanical, cultural, and biological means. 2,4-Dichlorophenoxy-acetic acid was the first herbicide to be used selectively to control weeds. Since then, several herbicides belonging to different chemical classes and possessing diverse modes of action have been synthesized and commercialized around the world. Herbicides have vastly contributed to increasing world food production in an efficient, economic, and environmentally sustainable manner. However, repeated application(s) of the same herbicide or a different herbicide with a similar mode of action on the same field, growing season after growing season, has contributed to the widespread occurrence of resistance to herbicides in several weed species. The goal of this paper is to present a systematic diagnostic approach towards the characterization of herbicide resistance in a given weed population with regards to profile (single, multiple, cross resistance), magnitude (fold level), mechanism, and related bio-physiological aspects.

## Herbicide tolerance versus resistance

The Weed Science Society of America (WSSA) defines herbicide tolerance as "the inherent ability of a species to survive and reproduce after herbicide treatment." This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant. Herbicide resistance is defined as "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis" (WSSA 1998). Herbicide resistance has also been defined as "the evolved capacity of a previously herbicide-susceptible weed population to withstand a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation" (Heap and Lebaron 2001).

## Definitions used in herbicide resistance literature

Discovery of herbicide resistance in weeds and subsequent research over the past decades has generated a wealth of information, which has contributed to a much better understanding of how plants function and respond to the environment in which they thrive. For example, triazine resistant weeds have served as an ideal model system to understand the mode of action of the photosystem II-inhibiting herbicides. The knowledge accumulated from this research has brought forth several concepts and expressions that are frequently used in herbicide resistance discourse. A non-exhaustive compendium

***Corresponding author:** vijay.nandula@ars.usda.gov

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of these terms is listed below [selected definitions adapted from Raven *et al.* (1992)].

*Accession*. A collection of individual plants of a weed species whose characteristics (genetic, physiological, biochemical, or biological) are yet to be determined.

*Allele*. An alternative form or copy of a gene.

*Biotype*. A plant selection that has a unique genotypic pedigree.

*Cross-Resistance*. The expression of a mechanism that endows the ability to withstand herbicides from the same or different chemical classes with similar mode of action (Hall *et al*. 1994). It can be target-site based or nontarget-site based (reduced uptake, translocation, activation; increased metabolism-deactivation; compartmentation/sequestration).

*Dominance*. State of an allele whose phenotypic expression is similar both in the homozygous and heterozygous stages.

*Ecotype*. A biotype that has adapted to a specific growing environment.

*Evolution*. Progressive change in the gene pool of a given weed (species) population in response to most recent growing conditions (herbicides in this context).

*Fitness*. Ability of a biotype to survive and reproduce in an environment that may or may not include herbicide treatment.

*Genotype*. The complement of a plant's complete hereditary information.

*Hormesis*. Stimulation of growth processes in plants treated with low doses of herbicide(s).

*Inheritance*. Process of transfer of a genetic trait from one generation to the next.

*Mating System*. System by which pollen moves from the anthers to the stigma of the same flower or different flowers on the same plant (self-pollination), or to stigma of flowers on a different plant (cross-pollination) of a weed species.

*Multiple Resistance*. The expression of more than one resistance mechanism endowing the ability to withstand herbicides from different chemical classes (Hall *et al*. 1994). Multiple-resistant plants may possess two or more distinct resistance mechanisms (Gunsolus 1993).

*(Gene) Mutation*. An inheritable change to genetic material or the process resulting in such a change.

*Negative Cross-Resistance*. An expression of mechanism that occurs when a resistant biotype is more susceptible to other classes of herbicides than the susceptible biotype (Gressel 1991).

*Population*. A group of plants of a single weed species with potential to interbreed and inhabit a specific geographic area.

*Recessive*. Condition of an allele whose expression is veiled by a dominant allele in the heterozygous stage.

*Selection Pressure*. The effectiveness of natural selection in altering the genetic composition of a population over a series of generations (King and Stansfield 2002).

*Target Site*. A gene or gene product (protein) on which a herbicide is potently inhibitory.

*Trait*. A genetic characteristic of interest.

**Diagnosis of herbicide resistance**

Diagnosing herbicide-resistant weeds is a first step in resistance management, and monitoring their nature, distribution, and abundance demands efficient and effective screening tests (Beckie *et al*. 2000). This can be achieved by crafting robust procedures for seed sampling, survey protocol and seed collection, seed processing and storage, germination, emergence and growth (sufficient number of representative plants), treatment conditions (*i.e.*, discriminating dose, adjuvants, spray volume and parameters, water quality, and nutrient status), experimental design, appropriate controls including wild type/susceptible accessions, and biological parameters being measured.

**Field survey and seed sampling**

An appropriate and unbiased sampling procedure is required to accurately detect or predict the occurrence of herbicide resistance in a weed population. Grower surveys, cropping and herbicide application history, on-site visual examination of fields, and data from grain elevators, seed cleaning facilities, or cotton gins are common sources of information to decide survey objectives, techniques, and extent of survey.

Selection of a field site for collecting suspect weeds depends upon the objective (Beckie *et al*. 2000). For example, investigation of poor herbicide performance in a particular field, the occurrence of resistance in one or more weed species to a particular herbicide or to herbicides with the same or different sites of action, grower suspicion of resistant weeds in a field, broad nonperformance of a particular herbicide or herbicide chemistry, or a roadside survey will determine the extent of the survey and techniques to be used.

A large field could be divided in to workable sub-units and each sub-unit may be sampled separately. A

larger geographic area could be divided in to sectors and each sector may be further categorized in to smaller sub-divisions for convenience and accuracy of sampling. Roadside surveys are convenient, rapid, and cover a large sampling area. Seed collected from individual plants must be kept separate if the sampling area is small or if suspect weed infestation is patchy. Samples from large fields or sampling areas may be bulked, but a few representative samples must be kept separate as a reference. Prior knowledge of biology of the weed species is advantageous to avoid unnecessary sampling of 'seed heads' from male plants in case of dioecious genera such as Palmer amaranth (*Amaranthus palmeri* S. Wats.) and waterhemp (*Amaranthus tuberculatus* Moq. Sauer) or nonsampling of seed-bearing nodes in monoecious ragweeds that are distal to the male flower-bearing terminal nodes.

As far as possible, detailed information on cropping and herbicide history must be acquired. Accurate records of site at time of sampling should be noted such as condition of field (dry or wet), weather conditions, date and time of the day during collection, global positioning system (GPS) location of site, crop and crop growth stage (if crop present), growth stage of weed, level of infestation, general weed control in the field (if crop present), and any other discernable information such as neighboring fields, etc. Seed samples collected from suspect fields must be dried in properly ventilated and dry areas to prevent microbial contamination and physiological deterioration.

**Dose response**

Typically, dose response experiments are performed on whole plants. Herbicide treatments are applied within a window of growth stages of the weed species based on the herbicide label. Further, additives/adjuvants and spray delivery volume are determined centered on label recommendations. Potentially resistant plants are compared with characterized susceptible/wild type plants of the same weed species. Herbicide dose range for the resistant biotype/population/accession should encompass the recommended label rate as well as rates above and below. Herbicide doses for the susceptible biotype/ accession must include the recommended rate as well as doses low enough to capture the lowest measurable phytotoxic symptomology.

The following criteria for dose response studies are adapted from Beckie *et al.* (2000). Six to eight herbicide doses are recommended for evaluation of potentially herbicide resistant weed populations. Herbicide injury is measured as a visual estimate or mortality or growth reduction. Resistance is determined by comparing the dose response of the resistant plants to the susceptible plants. A nonlinear regression model is fitted to the data to explain the response of measured biological data to the herbicide dose range. The herbicide dose required to cause a 50% inhibition of growth (% control – $ED_{50}$; shoot dry weight – $GR_{50}$; mortality – $LD_{50}$) is extrapolated from the regression equation based on parameters of the fitted model. Resistance index or the relative proportion of resistance if calculated by dividing the value for resistant plants by the value for susceptible plants.

Dose response experiments involving application of herbicides on whole plants require greenhouse/growth chamber space, access to a spray chamber or backpack sprayer, pots, trays, soil, fertilizer, and support personnel. All of these facilities require availability of adequate financial resources. Also, screening a large collection of putative resistant accessions is often time consuming and labor intensive. An alternative could be the utilization of other methods such as plant cuttings (Boutsalis 2001), germinating and growing seedlings in Petri plates or 24-cell culture cluster plate (Shaner 2010), or floating excised whole leaves or leaf discs (Koger *et al.* 2005). However, the level of variability in a weed population makes it difficult to obtain consistent measurements to accurately assess resistance in a population (Shaner 2010).

**Bioassays**

A biological assay, or bioassay in short, is a study or research project that investigates effect(s) of a treatment on a particular process in a living organism. Bioassays can play a major role in determining inherent differences between putative resistant and known susceptible biotypes of a weed species. Several biochemical and physiological processes in plants, based on response to herbicidal treatments, have been characterized via bioassays to test for herbicide resistance. These include photosynthesis, transpiration, chlorophyll biosynthesis, shikimate accumulation, *etc.* (briefly reviewed by Shaner 2010).

**Herbicide resistance mechanisms**

Understanding the processes and means by which weeds withstand labeled herbicide treatments is an important step, as well, towards devising effective herbicide resistance management strategies. In general, five modes of herbicide resistance have been identified in weeds: (1) altered target site due to a mutation at the site of herbicide action resulting in

complete or partial lack of inhibition; (2) metabolic deactivation, whereby the herbicide active ingredient is transformed to nonphytotoxic metabolites; (3) reduced absorption and/or translocation that results in restricted movement of lethal levels of herbicide to point/site of action; (4) sequestration/compartmentation by which a herbicide is immobilized away from the site of action in cell organelles such as vacuoles or cell walls; and (5) gene amplification/ over-expression of the target site with consequent dilution of the herbicide in relation to the target site.

**Physiological, biochemical, and molecular approaches for studying resistance mechanisms**

Current methodologies employed in herbicide resistance mechanisms research include: biochemical (enzyme kinetics and assays), physiological [photosynthesis, transpiration, respiration, chlorophyll biosynthesis, absorption and translocation using radioisotopes (Nandula and Vencill 2015)], and molecular [DNA/RNA-based: polymerase chain reaction (PCR) and single nucleotide polymorphisms (SNP), DNA sequencing, or quantitative PCR (qPCR)] techniques (Fig. 1). Newer mechanisms of herbicide resistance will most likely be discovered in the near future through the applications of 'omics' tools (Fig. 1).

Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms. The various omics approaches include genomics (DNA), transcriptomics (RNA), proteomics (proteins), and metabolomics (metabolites) (Délye 2013). Also, recent advances in molecular analysis such as next generation sequencing (NGS: RNA-Seq, and restriction site associated DNA sequencing (RAD-Seq)) are rapidly becoming routine.

## Conclusion

Accurate and timely diagnosis of the nature and level of herbicide resistance in a weed population and knowledge about the inherent resistance mechanism(s) involved will greatly strengthen efforts towards devising sound herbicide resistant weed management strategies. New technologies, especially, molecular tools such as NGS and 'omics' approaches, are revolutionizing herbicide resistance research.

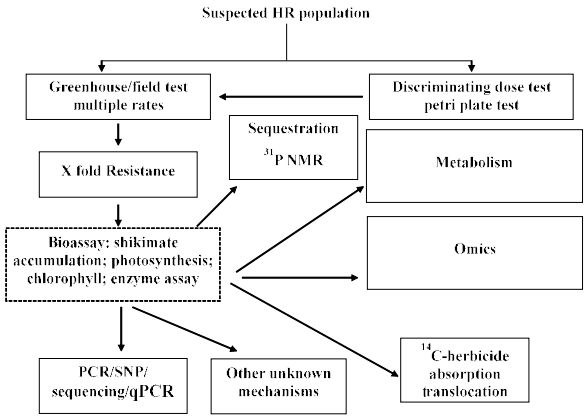


**Fig. 1. Sequence of methods for testing herbicide resistance in weeds (modified from Shaner 2010).**

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