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Herbicide Resistant Weeds: The Technology and Weed Management

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Additional information is available at the end of the chapter

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1. Introduction

Pest resistance to control methods in general is not an isolated phenomenon but usually expected and well demonstrated when any method is repeatedly applied over a long period of time without being changed or modified in nature, structure, principals of application or formulation. All pests that growers must control in agricultural land have the capacity to become resistant to whatever tactic is used to control them [11]. It is usually expressed as a gradual adaptation or "fitness" of some individuals or populations of the targeted pest or organism to the frequently applied control methods and available conditions. This adaptation may be physical, morphological or phenological, physiological, anatomical or biochemical or could result from the interaction between any two or more of these. It may also be due to some genetic changes as mutations occur on the key site at which a specific method operates. These mutations are at least partially dominant and inherited. Traits are conferred by modifications to single nuclear genes. This indicates that the rate of resistance evolution will be driven by mutation, the intensity of selection, the dominance and relative fitness of mutations in presence or absence of the herbicide and by dispersal of resistance alleles within and between weed populations [28]. However, no proof that the herbicides cause the mutations leads to resistance [37]. However, most often resistance is controlled by a single, dominant or semi-dominant gene [38] although recessive genes control of herbicide resistant trait in natural weed populations has been also implicated in resistance to dintroaniline, while wild populations exposed to herbicide stresses for the first time may efficiently express herbicide-resistant genes.

Most weed modifications and adaptations, if not all, are advantageous to the pest, since allow its escape on time and/or place and thus avoid external hazard or threat to its existence and genetic line. Resistance therefore should not be confused with natural tolerance or low

susceptibility due to a normal physiological or behavioristic property of an unselected population [23].

Organisms are varied in sensitivity, responses and thus adaptability to such conditions and in responses to any treatment or imposed external factors. Tolerance and then gradual resistance of agricultural pests to any control method or environmental stress is thus a strategy through which organisms/ or pests encounter hazards and maintain life and therefore may be applied to any method of pest or weed control including prevention, mechanical, cultural, physical, biological and chemical [30]. For example, weeds resisting soil mulch cover show some morphological and/or physical characteristics that allow penetration of the mulch layer; also, flooding of resistant species possess water impermeable seed coat or generate O_2 and reduce CO_2 penetration. Firing or flaming is resisted through presence of a hard seed coat or deeply buried regenerative propagules; certain weed species show feedback mechanisms or luxury accumulation of mineral nutrients and thus avoid toxicity; high temperature and low soil moisture harmful effects are avoided by adoption of secondary or enforced seed dormancy, while harmful effects of excessive light is avoided by some morpho-physiological alterations. Soil acidity may be encountered in the microhabitat by root exudates or selective mineral absorption and salinity by excretion of salt through different mechanisms and formation of salt glands or vacuoles or shedding salt saturated organs; microbes attack is avoided by production of repellent allelochemicals, and pests through some morpho-chemical adaptations. However, the mechanism behind tolerance or resistance is different and based on the type of target pest or the hazard imposed.

Herbicides represent one of the external factors and form a group of synthetic- plus some bio-chemicals used to suppress or kill unwanted vegetation and are a major component of pesticides. They assist in management and restoration of areas invaded by invasive species. Herbicides are a major technological tool and responsible, in part, for an agricultural revolution and increase in food production in the last few decades. However, at present this technology faces radical changes in effectiveness under field conditions that lead in different cases to failure of weed control operation due to continued development of weed tolerance/resistance and evolution and limitations in the herbicide industry and development.

2. Agriculture practices and weed evolution

General weed control methods (tillage, hoeing, hand weeding, flooding, cuttings or mowing, flaming, use of general herbicides) are all nonselective and usually applied to a composite weed species or vegetation of inter and intra-specific variations in richness, morphology, growth habit and responses. Each species may adapt, or not, to any of these methods. Since weeds are widely different in mechanisms by which they encounter hazards they are exposed to, they are different in plasticity and responses. With continued use of a single control method for a long period of time, species migrate, flourish or die. Flourishing species gradually became better fit and adapted, and increase in number and population size in absence of others. The only surviving individuals are those possessing rare single gene mutations and evolved

resistance will be monogenic, resulting in a large change in the resistance phenotype. However, when doses are lower and selection acts within the range of standing genetic variation, polygenic responses will be possible and resistance will evolve by a gradual change in the mean susceptibility of the population [28]. On the other hand, population of not or less adapted individuals, decline in growth and number until greatly suppressed, limited and may become extinct. Therefore, with continuous dependence on a single method of weed control, a weed population is usually shifting toward better adapted species or individuals that cope well with existing control measures and new conditions. Self-thinning of a weed population is continued toward complete tolerance to employed control measures. Therefore, weeds adapted to mowing tend to grow short, in a rosette form, creeping above the soil surface or show high plasticity and softness of aerial parts and stems and become difficult to mow and also escape hand weeding. Deep rooted weed species are difficult to pull out even by soil tillers. Seasonal dormancy and shifts in the weed population in the growing season is well recognized for certain weed species such as *Senecio vulgaris* [29; 37], while physiological adaptation of *Echinochloa crusi-galli* and *Cyperus rotundus* to flooding conditions and the role of Alcohol dehydrogenase enzyme (Adh) in *E. crusi-galli* is well documented [5; 14]. Similar adaptations of *Cirsium arvense* ecotypes to temperature variations [43] and *Typha angustifolia* and *Typha latifolia* genetic and clonal variations [27; 40] have also been reported. In this regard, it is important to differentiate between tolerance and resistance of weeds to herbicides. Tolerance is the inherited ability of a species to survive and reproduce after herbicide treatment; it refers to the natural variability to herbicides and exists within individuals of a species and quickly evolves. It usually refers to relatively minor or gradual differences in intraspecific variability. Resistance is the inherited ability of a plant or a biotype to survive and reproduce following exposure to a dose of herbicide that is normally lethal to wild type [16; 23; 30; 37]. Therefore, it is a decreased response of a population of weed to herbicides as a result of their application. However, both terms sometimes are misused or used interchangeably.

Tolerant weed species are less harmed by herbicides; they exhibit a certain degree of avoidance or adaptation strategy that allows recovery and thus escape control measures. They may respond by timing stomata closure or having sunken pores or stomata, thick waxy cutical on upper leaf surface, encased growing points or some biochemical, physiological or anatomical properties better developed by time until they become best fit and adapted to applied herbicides and become thereafter resistant. This, however, leads to gradual but radical changes in the weed population composition and distribution spectrum at which resistant individuals or certain weed species increased and dominate and susceptible ones are reduced and replaced. Adaptation or exclusion of the less tolerant species depends on performance of these by time. Generally a weed population becomes rich in individuals and poor in species with the continuous use of the same herbicide or different herbicides of similar mode/mechanism of action. This shift does not however, reflect better competitiveness or higher regenerative ability but most likely due to absence of sensitive highly competing species or forms that allow resistant individuals to utilize more resources [9; 22].

In cultivated fields, associating weeds bear more resemblance to crop plants in morphology, physiology and responses to control measures and other agricultural practices in general. They

mimic crops from sowing and germination until harvest. Since herbicides used on crop plants are selective, weeds respond by exhibiting similar morphology, physiology and biochemistry as crop plants to avoid hazards. However, weeds derived from crop plants as hybrids, crop relatives or wild-weedy forms are better fit to such conditions than others. Weed-crop associations also exist between weed species of different taxa from crop plants. In this case, the longer the use of the same herbicide/s, the greater the close association between crops and certain well performed weed species that later transfer into adapted weed races. Crop relative weeds however, are of great potential to intra- and inter- gene exchange and efficient mating system among themselves and with crops, thus become best adapted and more difficult to control.

3. Selection pressure and weed races

With continuous use of the same agricultural practice/s, interspecies selection occurs and plant species are gradually purified (intraspecific selection) by time until they become best adapted. Since all control measures including herbicides aim to eliminate weeds without causing injury to crop plants, weeds respond by developing mechanism/s allowing escape of chemical hazards. Under such conditions, sensitive individuals are first limited or disappear. Tolerant individuals increase in number and accumulate tolerance until they become resistant. Therefore, a resistant population of any weed species is exposed to long-term selection pressure through which it is purified and performs well under prevailing conditions in absence of sensitive weed species. With continuous exposure to herbicide pressure, a population of resistance is usually developed.

Weeds tend to avoid herbicide toxicity by changing normal growth habits, or exhibiting some phenological (such as changes in germination patterns), physical and/or physiological changes through which they adjust emergence time, external appearance or physiology. These however, are inherited traits that allow plants to survive herbicide treatments. One best adaptation is that of weeds similar to crop plants in most or all growth aspects. These form weed races similar to crop plants and well adapted to their habitats. Among reported weed races are *Camelina sativa* to flax crop, *Echinochloa crus-galli* var. *Oryzicola* that associate with rice and the weedy wild rice or red rice in India and east-south Africa [8; 20]. All are genetically irrelevant to crop plants. However, in some cases weed races are of the same botanical family or belong to the same crop species. This kind of association leads to development of "crop-races" that possess weedy characters very well adapted to cultural practices; they are similar to crop plants in most growth aspects and difficult to control by herbicides or other control methods including hand weeding. They take an advantage from conditions under which crop plants are growing until they become difficult to leave their habitats or even become dependent on crop plants in their growth and environment. These weeds are specialized to certain crop plants or cultivars. Moreover, many genetically related species can exchange genes with crop individuals and mimic crops. It can be concluded that any agricultural practice exerts selection pressure and may become troublesome to farmers when repeatedly applied for a long period. Its positive impact on crop growth and productivity is usually negated with time until it

becomes a real trouble. Its residual negative effects may not possible to overcome for a long period after abandonment.

4. Field evidence of weed resistance and herbicide resistance protocol

In the field all growth patterns and distribution of weed species may be observed. Some species grow in colonies, in certain growth patterns, forming an ecological niche, sporadically distributed, or randomly scattered within crop plants. Certain species are dominant while others show moderate growth or are suppressed while some grow vigorous or have limited growth and short stature. This however, depends on the microhabitat and place they occupy in the field and their performance. Under intense cultivation and thick crop stands, individuals of certain weed species express phenotypic plasticity (phenotypes) at which they change/modify their appearance, reduce or drop lower branches and thus lateral growth, elongate and increase cell divisions, overtopping crop plants and trapping light, although some shade tolerant species perform better under such conditions. Phenotypic plasticity modifying the mode of growth and energy allocation in response to environmental changes is considered to be important adaptive mechanism. These phenological variations can be easily observed among different weed species. Uniform application of herbicides in the field should equally affect all individuals of a single weed species. When herbicides are best timed and properly applied they should yield similar mode of action on species individuals. While differences in influence of a herbicide on different weed species is expected, hence differences in taxonomy, morphology, physiology and biochemistry, but such differences among individuals of a single species should have resulted from some morphogenetic or other variations within the same or different populations of that species. Certain individuals are totally killed, others less injured and some escape control unharmed. When the same herbicide or herbicides of the same mechanism of action are used, it becomes clearer that previously less or unaffected individuals should exhibit similar responses as were first shown. Gradually these individuals increase in number and growth until they dominate the site with continuous use of the same herbicide or its analogues while sensitive individuals are suppressed or removed. This however, takes a relatively long time for the population to shift from susceptible to complete resistant and depends on herbicide, environment and plant factors. These are positive signs on possible herbicide-resistance development in the field. If less affected or unharmed individuals in the first herbicide application are killed or severely injured in repeated treatments then there should be another cause of escape or partial control at first application and herbicide resistance should be then excluded. On the other hand, unharmed individuals may also tolerate higher application rates. Therefore, farmers should keep observing changes in the weed population as long as the herbicides are in use. They must get familiarized with weed species, populations and densities at pre- and post- herbicide treatments, comparing weed growth, performance and densities and recording any changes in populations thereafter. Less or unharmed individuals of any species should be followed up throughout subsequent applications of the same herbicide or herbicides of similar mode of action.

Sometimes partial effect or failure of the applied herbicide to control certain weed species or individual weeds in the first application may be thought as due to wrong calibration, misapplication, incomplete coverage treatment by a general herbicide or unsprayed gaps resulting from low sprayer boom during spray, unfavorable weather conditions, improper timing of herbicide application, and weed flushes after application of a non-resisted herbicide [16]. This could be easily judged in the repeated application to these species or individuals. When the herbicide failed to control these for the second time or at higher rates then resistance may be underway. With continued use of the same herbicide for different times, resistant individuals aggregate forming irregular patches while other weeds are controlled. A patch of uncontrolled weeds starts spreading and healthy weeds are mixed with uncontrolled weeds of the same species (Fig. 1).

Therefore irregularly shaped patches of a single weed species in the field are an indicator of herbicide resistance, especially when:

- There are no other apparent application problems.
- Other weed species on the herbicide label are effectively controlled.
- Field history indicates extensive use of the same herbicide or herbicides of the same mechanism of action.
- No or minimal herbicide symptoms appear on the single uncontrolled weed species.
- There has been a previous failure to control the same species or population in the same field with the same herbicide or with herbicides of the same site of action.

However, the rate at which a resistant weed population is selected depends on the number and frequency of herbicide applications it receives, the size of the population and its genetic diversity, and characteristics of the herbicide target site. Resistance buildup is accelerated when the management of crops does not include different weed control methods that limit herbicide use. In addition, this may be greatly enhanced in conservation or zero tillage because weeds are not killed by mechanical disturbance and general herbicides.

5. Interaction between environment and genetics

Growth and productivity of any plant species are mainly influenced by genetics, ecology and their interactions. Weeds are different from crops in their responses to both factors. They are more flexible and thus better responsive and adapted to extremes in environmental conditions such as high temperature, freezing, excessive light, salinity, drought, etc. Tolerance of weeds and better responses are mainly due to better and rapid interaction between environment and genetics compared to crop plants. In addition, the long term breeding and selection pressure imposed on crop plants has led to selection of less adapted species or cultivars that are highly sensitive to ecological stresses and deficient in certain characteristics that offer protection or defense mechanisms against unfavorable environment. Weed fitness in natural habitats and their rapid responses to the changing environment allow evolution of weed

ecotypes, genotypes, biotypes or phenotypes. Some of the basic differences in the definitions of pest resistance depend on these terms. The basic unit of plant classifications is the “species” that is defined as a group of individuals displaying common characteristics and having the ability to mate and produce fully viable progeny. A species usually consists of several to many populations. A population is a group of organisms within a species that co-exist in time and space [35; 36] and share a distinct range of genetic variations. While a genotype is the sum of the genetic coding or the genome of an individual, a biotype may not be coincident with genotype as an individual has many genes. Certain genes may be expressed or unexpressed and not pertain to the phenotype associated with the biotype. A biotype is a phenotype that consistently expresses or exhibits a specific trait or set of traits; it represents a group of individuals or a population within a species with a distinctive genetic variation of biochemical or morphological traits. Phenotype refers to the physiological and morphological profile of the expressed gene in an individual [42]. A single genotype can produce different phenotypes in response to environmental conditions and the fundamental properties of organisms are known as phenotypic plasticity. The epigenetic change is thus reflecting the alteration of phenotype (morphological or biochemical) without change in either the coding sequence of a gene or the upstream promoter region. Therefore biotypes within the same species may be developed due to this interaction. On the other hand, ecotype is a population within a species that has developed distinctive morphological or physiological characters (herbicide resistance) in response to a specific environment and persists when individuals are moved to a different environment. Ecotypes are of different germination and growth optima for the same environmental factor and phenotypes may be emerged and observed in weed populations. These alter their morphological features in response to certain prevailing environmental conditions which aim at protection of their individuals against unfavorable ecological stresses. Somatic polymorphism of certain weed species is well recognized and expressed as seed polymorphism of different morphological or physiological requirements for germination on different parts of the same weed individual. These however, are somatic rather than genetically based differences.

6. Herbicide resistance and crop relative weeds

Crop relative weeds are usually derived from the same species of crop plants and thus are genetically related. Most crop species have wild relatives and can interact with them under field conditions. Examples are radish, carrots, vetch, celery, lettuce, fennel, eggplants, wheat, barley, oat, etc. In addition, crop plants which are domesticated from wild forms possess a high degree of compatibility with crops. These are referred to as wild and weedy relatives, in spite of the fact that all species are related because their cells can read a common genetic code [15]. Crop weedy relatives are genetically compatible with crop plants and easily exchange genes. The emerged hybrids may become noxious weeds with certain weedy characteristics derived from both crop plants and wild forms. They could exhibit a certain degree of dormancy that is usually weak or absent in its parents and possess other weed traits making them difficult to control. These new generations have the ability to resist environmental hazards much better than parents and can exist and dominate in both productive and unproductive habitats. These

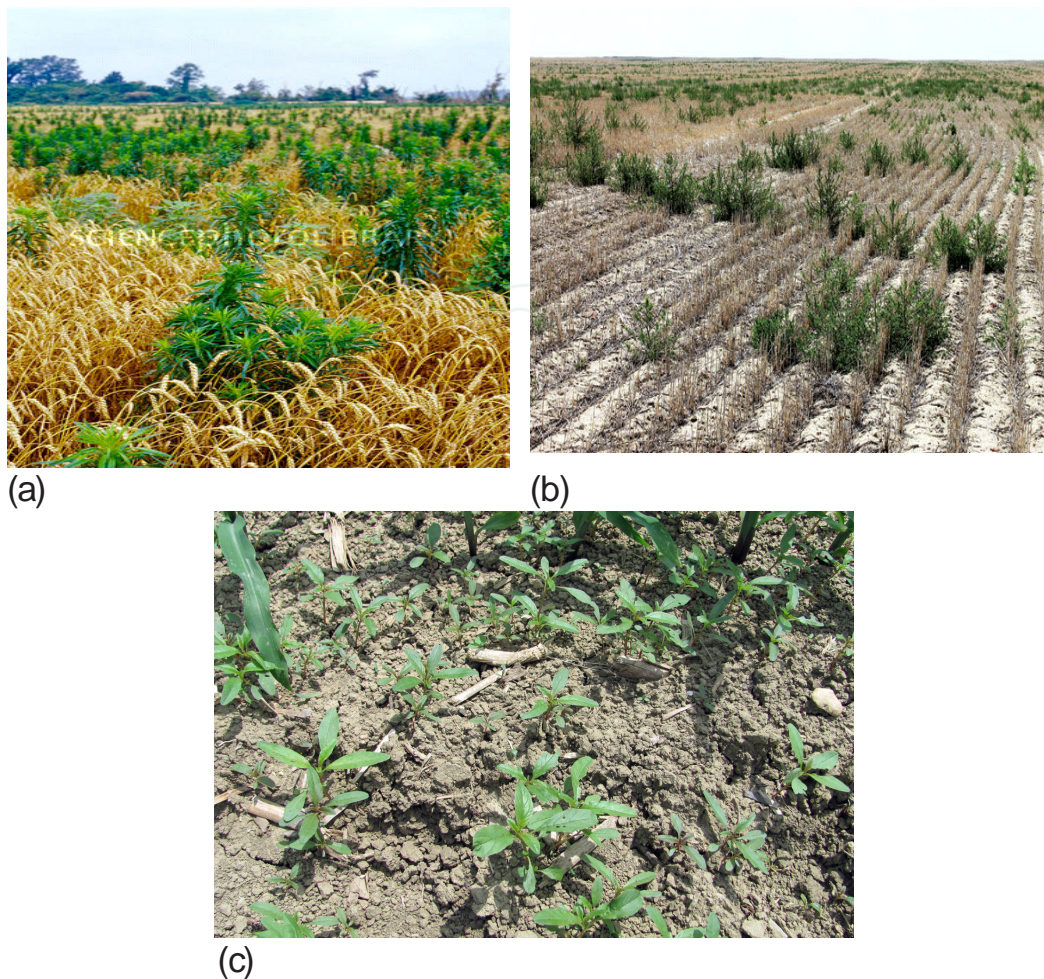


Figure 1. Three resistant weed species (a, b, c) to glyphosate herbicide at different growth stages and spray times. (a). *Conyza canadensis* resistant to glyphosate until harvest stage of wheat. Source <http://www.sciencephoto.com/media/> courtesy of the Montana State University (b). A field infested by suspected glyphosate- resistant *Kochia*, after the field was sprayed with three applications of glyphosate. Photo181407/enlarge Southern Agricultural Research Center. By Dillon Tabish, 08-11-12. Available at: http://www.flatheadbeacon.com/articles/article/scientists_discover_possible_herbicide_resistant_weed_in_montana/29184 (c). Palmer Amaranth (*Amaranthus palmeri*) resistance to glyphosate in corn at early growth. Source: E. Larson, April 21st, 2011. Available at: <http://www.mississippi-crops.com/2011/04/21/how-to-deal-with-glyphosate-resistance-and-weed-issues-in-corn/>.

are of a high genetic plasticity allowing their individuals to adapt to extensive herbicide applications and thus resist chemical treatments. Crop-weed crossed forms can easily exchange genes with crop plants as well as with weedy relatives and therefore are becoming troublesome weeds in fields with genetically modified crops.

7. Gene flow potential with wild/weedy relatives of world crops

In nature, genetic information is transferred between different individuals, populations, and generations (to progeny) and across spatial dimensions [2; 15]. This phenomenon, known as

gene flow, serves as a mechanism to maintain the biological diversity that helps to ensure long-term survival of populations and species in various environments.

Gene flow is a critical determinant of population genetic structure, playing an important role in both evolutionary and applied plant population genetics [12]. It is also known as 'migration' [13] or admixture [1] and can be defined as the movement of genes between populations of a species and between these populations and inter-fertile relatives [39; 41], conferring new traits, the biophysical characteristics of the organism to individuals of the recipient population [34].

Gene flow could occur through dispersal of pollen (via outcrossing between sexually compatible individuals within or among populations) or seeds (via seed dispersal), or vegetative parts capable of clonal propagation [34; 41]. Pollen dispersal is the typical method for such exchange of genetic information [15] and pollinating visitors or other agents including wind, animal, water current and other factors could play a significant role in this issue. This happens by cross-pollination (hybridization), that is, the pollination of members of one population or genetic pool with that of another [34]. These are natural and ordinary phenomena that occur in conventional as well as genetically modified crops.

Movement of pollen away from its site of production can result in true gene flow only if (1) the pollen first effects fertilization to form seeds, and (2) seeds germinate, produce plants that express the gene (i.e., are not silenced), and are able to reproduce [15]. Gene flow can be from crop to crop or landrace, from crop to wild relative, and even from wild relative to crop plant [34]. Spread of this phenomenon would lead to radical changes in vegetation composition and weed ecological distribution and their economic significance.

However, two types of gene flow are known; horizontal and vertical. Stewart [39] showed that 'horizontal' gene flow is the movement of genes between disparate, unrelated species, such as between plants and microbes while horizontal gene flow is more theoretic.

Among the world's 180 most damaging weeds, however, cause 90% of all crop losses, only five groups (related weeds of rice, sorghum, rape seed, sugarcane, and oats) are sexually compatible with the most important crops (Table 1). This fact emphasizes that the number of weed-crop crosses likely to lead to extremely troublesome or unmanageable problems is small.

Weed crosses with herbicide-tolerant biotech crops are likely to be favored in some agricultural fields where the herbicide is used. In areas where little or no herbicide is applied (e.g., native lands), the weed-biotech crop crosses will not be favored [15]. Self-pollinating crops are considered of low risk in terms of gene flow to weeds. Roundup Ready, Clearfield, or Liberty Link canola, in contrast, could pollinate nearby herbicide-susceptible canola as well as weedy canola relatives, resulting in volunteer canola plants and weeds that may be resistant to several herbicide families [38]. However, several pieces of evidence clearly show an escape of weedy transgene from fields via seed flow and this escape occurs via man-mediated long-distance dispersal events [4]. Other results revealed that development of weed resistance via selection pressure from repeated herbicide applications in herbicide resistant crops (in the absence of gene flow), often poses greater risks than that from gene flow to related weed species [15].

Rank	Crop	Scientific Name	Related weeds: sexually compatible with crops
1	Wheat	Triticum aestivum Triticum durum	T.aestivum Aegilops cylindrical A. tauschii A. triumcialis Agropyron spp
2	Rice	Oryza sativa Oryza glaberrima	O. sativa O. glaberrima O. barthii O. longistaminata O. rufipogon O. punctata
3	Maize	Zea mays	Z. mays ssp Mexicana
4	Soybean	Glycine max	G. soya
5	Barley	Hordeum vulgare	H. spontaneum
6	Sorghum	Sorghum bicolor	S. bicolor S. alnum S. halepense S. propinguum S. sudanense
7	Canola	Brassica napus, B. rapa, B. juncea	B. napus, B. rapa, B. nigra
8	Sunflower	Helianthus annus	Helianthus annus

Source: Different references

Table 1. Examples of some important food crops and their sexually compatible weed species

In this regard, biotech crops conferring stress tolerance (e.g., to water deficits, diseases, insects, salt stress, or nutritional deficiencies) may need more scrutiny because their crosses with weedy relatives may impart selective advantages in both agricultural and nonagricultural areas. Thus, some traits obtained from biotech crops could theoretically facilitate development into problematic weedy or wild species [15].

The economic consequences due to gene flow from biotech crops will primarily impact the agricultural fields in which those crops are grown, but potentially could impact natural areas given the proper rare combination of sexually compatible relatives, favorable environment, and reproductive/fitness advantages. As an example, rice grown in tropical countries may be relatively more prone to such processes because of the substantial populations of its wild/ weedy relatives that grow naturally in or adjacent to the rice-producing areas [8; 26].

Crop-wild hybridization may also create genotypes with the potential to displace parental taxa in new environments [7]. However, the most important variable affecting gene flow is the degree of relatedness and distance between the crop and the weed, because gene flow is only possible if close relatives are growing near the crop. As a result the possibility of gene flow

depends mainly on presence of wild or weedy relatives [11]. Transgene (s) transfer may have unpredictable and out of control ecological impacts under intensive cultivation of biotech crops [25]. While different crops can exchange genes with wild relatives, gene escape to wild or weedy relatives and its ecological impacts are outrated. The ecological consequences of gene flow however, depends on the amount of transgenes moved out to a wild population and the genetically modified traits and whether they have an evolutionary advantage under natural selection pressure or not and if enhanced fitness of wild and weedy relatives then the transgene followed by gene flow would persist and spread rapidly in the population of wild relatives through introgression, invade a new area and outcompete other individuals under natural conditions [24]. Weeds receiving transgenes will continue to evolve when exposed to selection pressure and it becomes nearly impossible to move them out from the environments if they can persist and spread in the populations.

8. Transgenic crops and weed evolution

The development of crops that are resistant to herbicides is a relatively new technology aimed to improve weed control in agricultural land. Herbicide-resistant crops can be created by standard methods of plant breeding, but the use of genetic engineering techniques is more usual. Herbicide-resistant crops are made resistant by either transgene technology or by selection in cell or tissue culture for mutations that confer herbicide resistance [10]. Glyphosate and glufosinate are herbicides most used in this regard. For example, soybean, corn, cotton, sugar beet, and canola are available as glyphosate- resistant cultivars and some are now widely planted in different countries. Importance of genetically engineered crops is to:

- Develop crops more tolerant/resistant to herbicides and thus increase herbicides uses and selectivity.
- Eliminate possible injury effects of soil persistent herbicides to crop plants.
- Increase options for weed control when the number of herbicides is limited, such as in minor crops.
- Effective control of certain difficult weed species and widening of weed control spectrum
- Achieve more effective weed control
- Increase bio-safety and enhance better eco-friendly use of new and less toxic herbicides
- May be more cost- effective weed control method

However, public concern about the impact of genetically modified crops on the natural environment encouraged more studies on this aspect in the last few years. Among the possible impacts, the 'escape' of the transgene, either through dispersal of the crop plant outside the agricultural area or through hybridization with wild relatives and thus increase the possibility of "weediness" [41].

In the majority of instances, there is a very low probability that an approved biotech crop introduction could create an environmental risk different from that of a nonbiotech version of

the same crop. This however, does not lessen the serious concerns about possible consequences of the escape of transgenes into the environment [41]. Examples of the risks mentioned in the context of gene flow from genetically modified plants are: i) new emerged weeds resulting from an escape by the crop itself; ii) super weeds resulted by hybridization of a (wild/weedy) species with the transgenic crop; iii) genetic erosion (loss of original diversity of wild relatives). To date, all instances of weeds becoming resistant have resulted from the weed evolving its own biochemical mechanism and not by acquiring genes for resistance from the crop. However, in some cases it would be possible for the herbicide resistance gene to flow from the crop to the weed [11].

Possible consequences of hybridization and introgression depend on the plant, gene, trait, and ecological factors [39]. In the case where transgenes might be introgressed into "weedy wild relatives", there are concerns about exacerbating "weediness" traits or even the disruption of natural ecosystems. Therefore, to assess the risk of gene flow it needs to be examined not only the probability of genes moving between plants, but how possible is it for the new plants to survive [39].

In general, people ideally would like to minimize or prevent gene flow from transgenic organisms to weedy wild relatives or to places where extensive crop breeding takes place [39]. Three approaches to gene flow mitigation are possible [3]. The first is by keeping the genetic modification out of the pollen, preventing the formation of pollen, and keeping the pollen inside the flower. It requires transplastomic plants hence the modified DNA is not situated in the cell's nucleus but is present in plastids, which are cellular compartments outside the nucleus. The second approach relies on male sterile plants unable to produce functioning flowers and therefore cannot release viable pollen. Cytoplasmic male sterile plants are known to produce higher yields. The third approach works by preventing the flowers from opening "cleistogamy" that occurs naturally in some plants. Cleistogamous plants produce flowers which either open only partly or not at all.

However, herbicide-resistant genes have no ecological significance in places where the corresponding herbicide is not used. When paired with a gene that might have an effect in a natural ecosystem, there is a potential problem with gene flow. Repeated application of the herbicide (especially general herbicides) would select for and protect crosses and backcrosses, increasing the possibility of successful gene flow to wild, related species [10].

9. Weed control spectrum of selective herbicides and population shifts

Some plants are genetically tolerant to certain herbicides while others have evolved resistance after repeated exposure to an herbicide. Tolerant and resistant plants usually degrade or metabolize the chemical to nonphytotoxic substances. In some cases of resistance, such as with triazine herbicides, the herbicide does not reach the key site in treated plants. Although tolerance and resistance are common, herbicide selectivity among plants is often conditional; thus it depends on plant, herbicide and environment factors.

Some of the factors that influence herbicide selectivity are as follows:

- Physiological or biochemical tolerance to the herbicide
- Herbicide application rate
- Time of application
- Herbicide formulations and surfactants used.
- Growth stage of weed and crop or other plant development
- Weather patterns (temperature, light, wind, rain, etc.)
- Variation in microenvironment or micro- topography
- Variation in resource level
- Soil type and pH

Many of the principles and practices of how herbicides used or applied to attain selective chemical and effective weed control are important. These involve the role of plant morphology and physiology, chemical properties, and environmental factors [31]. Herbicide selectivity in one way or another is in direct link with herbicide resistance. Crops are resistant to herbicides selectively used to kill weeds. Even with repeated treatment, crop plants can resist or tolerate higher rates of selective applied herbicide or repeated treatments. This depends on some level of tolerance/resistance higher in crop plants compared with weeds for that specific herbicide or herbicide group. For example, Syrian marjoram (*Origanum syriacum*) was found to withstand up to 4 times higher rates of oxadiazon and oxyfluorfen herbicides either applied on foliage parts or through the soil [32; 33]. Certainly many factors have an important role in giving a resistant value for crop plants. Some of these are listed below:

9.1. Plant factors and herbicide selectivity

Plant factors that influence the way weeds and crops respond to herbicides are genetic inheritance, age, growth rate, morphology, growth form and anatomy, and physiological and biochemical processes. The most effective use of herbicides results from considering these factors when selecting an herbicide or application method.

9.2. Plant age and growth rate

Weed seedlings or young plants are usually killed more easily than large or mature vegetation. In addition, some preemergence herbicides that suppress seed germination are often not effective when used to control larger, better established plants. Plants that are growing rapidly or in shaded places generally are more susceptible to herbicides than are plants of slow growth or unshaded.

9.3. Morphology

The morphology or growth habit of plants can determine the degree of sensitivity to some herbicides. Morphological differences in root structure, location of growing points, and leaf

properties between crops or other desirable plants and weeds can determine the selectivity pattern of some herbicides. Annual weeds in a perennial crop, meadow, or pasture usually can be controlled by herbicides because of their different root distribution and structure compared to those of perennial plants. For example, perennial crops such as alfalfa can recover from moderate contact herbicide injury to foliage whereas annual weeds, because of their small size and shallow root system, will be killed by the same herbicide application.

The meristematic regions of most grasses, such as cereal crops and grassy weeds, are located at the base of the plant or even below the soil surface. The growing points are protected from herbicide exposure by the foliage or soil that surrounds them. Thus, herbicide that contacts only foliage may injure some leaves but will not typically impair the ability of the plant to grow. In contrast, most dicot plants have their meristems exposed at shoot tips and leaf axils. For this reason, these plants are more susceptible than grasses to foliage-applied herbicides, especially of contact action.

Leaf properties of some plants can impart selectivity to certain herbicides, while other plants are effectively controlled. Spray droplets do not adhere well to the surfaces of narrow, upright, waxy leaves that characterize many monocot plants like cereals, onion, and most grasses. Thus, spray droplets do not adequately cover such leaves following herbicide application and the effect of the herbicide is reduced. In contrast, dicot plants have relatively wide leaves that are usually horizontal to the main stem. Leaves of dicot plants, therefore, intercept more spray solution than leaves of grasses and spray droplets spread more evenly over dicot foliage. Herbicide effectiveness is best when spray interception and coverage are greatest and with use of surfactants. However, ecological factors and geographical regions under which weeds are growing have significant influence on herbicide selectivity and rates of applications since they affect or modify weeds morphology and internal anatomy.

9.4. Physiological and biochemical processes

Plant physiology influences herbicide passage after its application. This process is called "absorption". The extent of herbicide movement in a plant- "translocation"- after it has been absorbed is also a physiological process. Both absorption and translocation are important processes governing herbicide activity and vary markedly among plant species. Generally, plant species that readily absorb and translocate herbicides are most easily killed.

Biochemical and biophysical processes are also important plant factors determining herbicide selectivity. Herbicide adsorption can be responsible for differential herbicide susceptibility among plant species. During this process an herbicide is bound so tightly by cellular constituents (usually cell walls) that it cannot be translocated readily and thus is inactivated. Membrane stability is another biochemical/biophysical process that results in herbicide selectivity among plants. In this case, the cell membranes of tolerant plants can withstand the disruptive action of the herbicide. The ability of carrot to withstand the toxicity of certain oils is an example of this form of herbicide selectivity.

9.5. Genetic inheritance

Plant species within a genus usually respond to herbicides in a similar manner, while responses to herbicides by plants in different genera often vary. The reason is that plants with similar taxonomic traits often have similar morphogenetic and enzymatic components. Thus, crops and weeds that belong to the same genera are usually susceptible to the same herbicides and are similarly affected since they have similar biochemistry. This rule is not absolute, however, because varieties of many crops are known to respond differently to the same herbicide and weeds usually adopt different mechanisms of herbicide resistance while crop plants have lost many of their traits in breeding programs that present in wild relatives.

10. Herbicides and edaphic factors

Soil factors affect herbicide performance and their effectiveness. These including soil-organic matter content, microorganism populations, soil water table and moisture content and soil pH. Organic matter acts through adsorption and release of chemical molecules. Certain herbicides are tightly adsorbed on soil particles and thus become unavailable to weeds. These molecules may be totally inactivated upon their release. Therefore weed control may be complete or not based on the amount of the herbicide adsorbed and whether the held amount on soil colloids is compensated or not before applied. The higher the percentage of organic matter and clay particles, the greater the adsorption in amount and time of herbicide molecules and the lower the herbicide activity and *vice versa*. This requires that some operations should be well managed when soil applied herbicides are used including their incorporation or placement in/on the soil.

Activity of soil microorganisms is another factor affecting activity of soil- applied herbicides and persistence. Microorganisms may degrade herbicide molecules and feed on organic herbicides. In general, favorable soil factors to microorganism populations stimulate their activity and thus rapid herbicide degradation. Therefore, soil-microbe population is an important factor in increasing or decreasing herbicide persistence and weed control duration.

Soil water also affects herbicide activity and performance. When high amounts of soil water are available or at high soil water levels, herbicide molecules may be hydrated. On the other hand, moisture is necessary to transfer herbicide molecules into the root system and then translocate these upward to vegetative parts through the xylem.

Soil pH affects cation exchange capacity of soil particles. Salt or mineral forms of certain herbicides may interact with soil particles under these conditions by exchanging cations or anions and thus lead to breakdown of herbicide molecules and inactivation.

All above soil factors and others such as soil- root temperature and soil mechanical properties can affect herbicide activity and performance and their effectiveness in controlling weed species and herbicide selectivity. Weeds may become adapted to certain soil

conditions, escape control operations and lead to dominance of well adapted species or populations.

11. Weed resistance and dormancy, avoidance and weed density

Dormancy is the state at which seeds in the soil or buds are not germinating or growing due to external conditions exert influences on physiological and biochemical internal processes including enzymes activities, food transport to embryo and metabolism. This state is keeping seeds or buds safe until the cause of dormancy is over. This behavior is important to maintain genetic line and continuity of the species in changeable environment. Under conditions of herbicide application, some of these chemicals are absorbed by seeds or dormant buds while others are not. These result differences in germination, emergence and growth patterns of different weed species. However, some herbicides may stimulate seed germination while others inhibit this process or even kill seed embryo. Differences also exist in hardness and permeability of seed coat of different weed species at which species of *Chenopodiaceae* and *Fabaceae* are good examples. These characters cause differences in germination and growth of seedlings and may confer another cause of herbicide resistance. Avoidance of herbicide toxicity may result from seed interring into dormancy and not further responding to the applied herbicide with no absorption or translocation of the herbicide into the embryo. In addition, herbicide molecules may be deactivated or degraded inside the seed itself by some oxidative enzymes or may bound into certain constituent inside the seed.

On the other hand, stimulation of weed seeds to germinate using certain herbicides also exist and allows higher seedlings emergence and partitioning of herbicide molecules among individuals of weed species. Division of herbicide molecules among high number of emerged seedlings would further diluted herbicide inside weed plants.

All above mentioned factors should be considered when herbicide-resistance is discussed. These may cause great differences in weed growth patterns and distribution in the field.

12. Weed resistance updates and resistance mechanisms

With continued dependence on herbicides for weed control and with the absence of other methods and herbicide rotation, the resistance problem is extenuated and the number of resistant weed species and biotypes is dramatically increased. At present, the reported herbicide resistant weeds are approaching 393 (species and their biotypes). These represent 211 species (124 dicots and 87 monocots) and detected from over 680,000 fields [21; 44] reported from 61 countries from all over the globe. However, the highest number of resistant species was reported from the advanced countries indicating efficient and rapid detection with available technology to diagnose, discover and deal with this issue. However, the highest number of weeds reported resist the main three groups of herbicides based on site of action including; the ALS (127 weeds), Photosystem II (69) and the ACCase (42) inhibitors. The

highest number of weed resistant species and biotypes came from the USA (141), Australia (61) and Canada (58). Most numbers of resistant species belong to the families Poaceae, Asteraceae and Amaranthaceae and most frequently mentioned are genera of *Amaranthus* (30 times and 11 species), *Echinochloa* (23 times and 6 species), *Lolium* (20 times and 4 species), *Alopecurus* (12 times and 3 species), *Avena* (11 times and 3 species), *Bromus* (11 times and 5 species), *Conyza* (10 times and 3 species), *Setaria* (9 times and 5 species), *Poa* (8 times and one species), *Ambrosia* (7 times and 2 species), *Digitaria* (6 times and 4 species), *Phalaris* (6 times and 3 species), *Hordeum* (5 times and 2 species) and *Sorghum* (6 times and 3 species). Most are of the grass family usually exhibiting distinct morphological features allowing wide dispersal and escape of herbicide treatment such as encased growing points, vertical leaf arrangement and thick waxy cuticle that reduce herbicide penetration and lead to herbicide droplets bouncing off leaves. Other genera reported are characterized by their prolific seed production and/or seed polymorphism. All above mentioned genera however, showed multiple resistance to different herbicides groups. Most resisted are herbicides widely and repeatedly used including: glyphosate, paraquat, atrazine and 2,4-D and others used in fields cultivated by genetically modified crops. Some recently developed herbicides are also resisted including chlorsulfuron and sulfonylurea group. This phenomenon demonstrates that the herbicide industry and development is far behind weed evolution. On the other hand, weed species and biotypes showing multiple resistance are most common and some are among the world's worst weeds [19] including: *Amaranthus* spp., *Echinochloa* spp., *Avena* spp. and *Chenopodium album* characterized by their polymorphic seed production and phenotypic plasticity. This reflects a great ability to maintain and exhibit high plasticity and possess various mechanisms of herbicide resistance.

The precise molecular mechanism of resistance varies with different plants, but in general plants resist herbicides in one of the following ways:

- Avoiding the herbicide by not absorbing it or, if absorbed, the weed compartmentalizing it away from its target site.
- Reducing the uptake or herbicide uptake is not enough to injure the weed or reach lethal level.
- Changing the structure of the target site of the herbicide so the plant is no longer sensitive
- Reduce herbicide translocation to the key site or binding it into certain plant constituent
- Sequestration by complete physical removal of the herbicide from the key site
- Target site mutation and changes in structure lead to insensitive plants and failure herbicide binding.
- Deactivating the herbicide by chemical alteration or herbicide metabolism before reaching target site

However, resistance mechanisms through which different weed species resist herbicide treatments are many and varied but most are physio-chemically based (Table 2).

Herbicide Group	Site of Action	HRAC Group
ALS inhibitors	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)	B
Photosystem II inhibitors	Inhibition of photosynthesis at photosystem II	C1
ACCase inhibitors	Inhibition of acetyl CoA carboxylase (ACCase)	A
Synthetic Auxins	Synthetic auxins (action like indoleacetic acid)	O
Bipyridiliums	Photosystem-I-electron diversion	D
Glycines	Inhibition of EPSP synthase	G
Ureas and amides	Inhibition of photosynthesis at photosystem II	C2
Dinitroanilines and others	Microtubule assembly inhibition	K1
Thiocarbamates and others	Inhibition of lipid synthesis - not ACCase inhibition	N
PPO inhibitors	Inhibition of protoporphyrinogen oxidase (PPO)	E
Triazoles, ureas, isoxazolidiones	Bleaching: Inhibition of carotenoid biosynthesis (unknown target)	F3
Nitriles and others	Inhibition of photosynthesis at photosystem II	C3
Chloroacetamides and others	Inhibition of cell division (Inhibition of very long chain fatty acids)	K3
Carotenoid biosynthesis inhibitors	Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS)	F1
Glutamine synthase inhibitors	Inhibition of glutamine synthetase	H
Arylamino propionic acids	Unknown	Z
Unknown	Unknown	Z
4-HPPD inhibitors	Bleaching: Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD)	F2
Mitosis inhibitors	Inhibition of mitosis / microtubule polymerization inhibitor	K2
Cellulose inhibitors	Inhibition of cell wall (cellulose) synthesis	L

Source: 21; Updated: November, 2012

Table 2. Herbicide resistant weeds summary table (Thursday, November 08, 2012)

13. Factors enhancing herbicide resistance

All natural weed populations, regardless of the application of any herbicide, probably contain biotypes that resist herbicides. Repeated application of an herbicide exposes the weed population to a selection pressure which may lead to an increase in the number of surviving resistant individuals in the population. As a consequence, the resistant weed population may increase to a level that adequate weed control cannot be achieved by the application of that herbicide [18]. Factors enhancing herbicide resistance include: the use of a single herbicide or herbicides of same mechanism of action, same formulation, same method of application, time of application, weather conditions during spraying, weed-density and application rate, surfactants, herbicide family and mechanism of action, crop rotation, and employed control methods.

Because weeds contain a tremendous amount of genetic variation that allows them to survive under a variety of environmental conditions, the development of a resistant species is brought about through selection pressure imposed by the continuous use of an herbicide or herbicides of similar mechanism of action. Long residual pre-emergence herbicides or repeated application of post-emergence herbicides will further increase selection pressure.

Factors in general that can lead to or accelerate the development of herbicide resistance include weed characteristics, chemical properties and cultural practices.

Weed characteristics conducive to rapid development of resistance to a particular herbicide include:

- Weeds having short life cycles (annuals).
- High seed production.
- Level of selection pressure imposed by the herbicide
- Relatively rapid turnover of the seed bank due to high percentage of seed germination each year (i.e., little seed dormancy).
- Several reproductive generations per growing season.
- Extreme susceptibility to a particular herbicide.
- One weed which would normally be controlled but not controlled while others were removed.
- High frequency of resistant gene (s).

Herbicide characteristics which lead to rapid development of herbicide resistance in weed biotypes include:

- A single site of action of the same herbicide continuously is used.
- Broad spectrum of weed control.
- Long residual activity in the soil.

Cultural practices can also increase the selection pressure for the development of herbicide-resistant biotypes. In general, complete reliance on herbicides for weed control can greatly enhance the occurrence of herbicide-resistant weeds. Other factors include:

- Shift from crop rotations towards mono cropping.
- Little cultivation or zero tillage for weed control or no elimination of weeds that escape herbicide control.
- Continuous or repeated use of a single herbicide or several herbicides that have the same mechanism of action.
- High herbicide use rate relative to the amount needed for weed control.
- Complete weed control

- Orchard and vineyard weeds.
- Roadside weeds.

14. Management of herbicide resistance

Herbicide-resistant weed populations can be managed following an integrated weed control program. The following practices are important for an effective management strategy:

- Herbicide rotation. Adopting this method, it should be known that herbicides of different chemical families may have the same site of action.
- Using mixtures of herbicides with different modes of action and overlapping weed spectrums. This would help in managing evolution of weed resistance.
- Crop rotation. Crops differ in their competitiveness against weeds. Plant crops having a different season of growth, different registered herbicides and crops for which there are alternate methods of weed control. Rotation breaks down weed population and prevents the build up of resistance to herbicides. In addition, different crops may require different types of herbicides and thus herbicides may be rotated as well. However, some herbicide groups include different chemicals that can be used in different crops; therefore crop rotation alone may not be enough to avoid resistance development in this case.
- Herbicides with the same site of action should not be applied or used in both fallow years and in the crop(s) planted within 3 years.
- Growers should keep rotating methods of weed control. Non-chemical control techniques including tillage, hand-weeding before flowering, mulching, soil solarization, prevention methods of weed dispersal (certified seed, clean equipments, use a power washer or compressed air to remove seeds).
- Herbicide-resistant weeds should be controlled before flowering and seed setting.
- Farmers should only use non- or short-residual herbicides and avoid using persistent chemicals and not applying them repeatedly within a growing season. This method would reduce the selection of herbicide-resistant weed biotypes. However, repeated applications within a single growing season of certain herbicides (paraquat, glyphosate) also lead to development of resistant weed populations.
- Where possible mechanical weed control such as rotary hoeing and cultivation is recommended to be combined with herbicide treatments.
- Weed escapes of resistant biotypes may be eliminated by cultivation in row crops. Fallow tillage can control herbicide-resistant and susceptible weed populations when they emerge at about the same time.
- Accurate record keeping. Farmers should be familiar with the history of herbicides use in their fields. Also keep tracking the weed species that have been present in a given field and

of how well particular herbicides have controlled them. Farmers should check for weedy patches in patterns consistent with application problems and hand-weeding these patches.

- Always weed free crop seeds should be used that greatly minimize introduction seeds of herbicide-resistant biotypes.
- Implementation of integrated weed management. This is important for effective control of all weeds including herbicide-resistance.
- Monitoring fields for weed escapes for resistant and susceptible biotypes. A resistance problem may not become visible until 30 percent or more of the weed population is no longer controlled. Check to see if the escapes are of one species or a mixture of species. If a mixture, the problem is more likely related to the environment or the herbicide application. If only one species was not controlled, the problem is likely to be resistance, especially if the species was controlled by the herbicide in the past and if the same herbicide has been used repeatedly in the field.
- Implementation of prevention methods of weed control. All measures aimed at prevention of weed introduction to fields and their dispersal should be strictly followed including governmental quarantine regulations.
- Alternating spring and winter crops, thus tillage and herbicides are used at different times in the different crops. Weed biotypes that survive in one crop could be killed in the other.
- Changing herbicide program, if weed resistance occurs, herbicides with other sites of action and other weed management practices must be used in an integrated management strategy. However, weed management strategies that discourage the evolution of herbicide resistance should include the following:
 - Use herbicide only when necessary and where possible herbicide application should be based on economic threshold.
 - Apply herbicides in tank mixed, pre-packed, or sequential mixtures of multiple site of action.
 - Never use unregistered mixtures, follow label recommendation at all times
 - Regularly monitor your crops so that resistant patches can be observed in time to be controlled with, for instance, spot spraying.
 - Apply the herbicide at the correct leaf stage of the weed and the crop.
 - Calibrate sprayer correctly before using herbicides
 - Planting new herbicide-resistant crop varieties should not result in more than two consecutive applications of herbicides with the same site of action against the same weed unless other effective control practices are also included in the management system.
 - Respond quickly to changes in weed populations to restrict spread of weeds that may have been selected for resistance.

- Encourage railroads, public utilities, highway departments and similar organizations that use total vegetation control programs and vegetation management systems that do not lead to selection of herbicide resistant weeds. Resistant weeds from total vegetation control areas frequently spread to cropland. Chemical companies, governmental agencies, and farm organizations can all help in this effort.
- To keep herbicide-resistant weeds under control, the following strategies should be also incorporated into a weed management plan:
 - Clean tillage and harvest equipments before moved from infested to clean fields from weed resistant species.
 - Total weed control in uncultivated places or sites
 - Close cultivation
 - Monitor hand weeding to insure more than 90% removal of weeds in the crop row.
 - Prevention of weed seed spread through:
 - Use of clean equipment.
 - Enter the field with resistant plants last.
 - Use a power washer or compressed air to remove seeds.
 - Recognizing patterns of weed escapes typical of resistant plants
 - Watch for small weed patches that appear in the same place in the next crop.
 - Watch for weed patches that do not have a regular shape that would indicate an herbicide application problem.

Herbicide resistance however, provides a basic understanding of the genetic basis of weedi-ness, while the development of weed genomics would provide three predictable and useful outcomes. The first is the identification of genes that could improve crop yields. The second is to improve our understanding of the evolution of herbicide resistance and the to aid in the identification of novel herbicide targets. Currently, there is little (if any) solid predictive capability of why some weeds develop resistance and others do not. Third, our understanding of weed biology would be exponentially expanded [6].

Research has recently been performed to assess the ability to cripple the effect of transgenes. The goal here is for the transgenic effect to not be as strong if it went to a wild relative. In one case, the genetic background of the crop weakened the weedy relative. In another case, the weakness was built into the genetic construct, called *transgenic mitigation*, in which an herbicide-resistant gene was paired with a dwarfing gene. In either case, transgenic weeds were less competitive than their non-transgenic parent weeds [39].

15. Conclusion

Weeds either leave (disappear), adapt, tolerate or resist any unfavorable environmental conditions that influence their normal growth and life strategies. Herbicide resistance is a complex phenomenon resulting from altered herbicide target enzyme, enhanced herbicide metabolism or reduced herbicide absorption and/or translocation. It is a survival strategy through which many successful weed species and/or biotypes counteract or escape chemical hazards. Weeds expressing this phenomenon have developed some morpho- (behaviorist), physio-, and/ or biochemical mechanism/s allowing existence. However, two theories are mainly considered: the mutation and the natural selection [17]. Colonizers, as well as some specialist weeds of high seed production and polymorphic characteristics, have rapid responses to prevailing environmental conditions and high ability to express herbicide-resistant genes and exhibit wide ecological variations [28]. This phenomenon is well documented in agricultural as well as other disturbed habitats while the list of weed resistant species gets longer with continued dependence on herbicides for weed control. From the information presented in this chapter, it is clearly demonstrated that herbicide resistance in weeds is far exceeding herbicide technology and industry. Most problematic weed species are genetically related to major food crops including wheat, rice and maize. This may pose another danger for the genetic industry and genetically engineered crops of wild relatives. Away from weed biology and resistance control, methods of weed control must be integrated and continuously rotated for effective weed control and prevention of weed resistance. This however, may not be achieved in absence of information and field data and well managed weed control strategies, considering all the factors that influence weed life and development.

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