Herbicide-Resistance
And
Weed-Resistance Management

(Chapter on Current Review of the Subject)

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1. Background

In the past two decades, evolution of newer herbicides provided wider user choice. Selection of most promising prudent products intensified in use followed by genetically induced herbicide resistant crops. This is how the broad-spectrum herbicides created a great deal of impact on the stakeholders. It has been an established fact that weeds reduce farm yields and farm income drastically. Among all other weed control practices, herbicides alone is easy prompt, most effective and economically acceptable mean; therefore, herbicides are overwhelmingly used by the farmers. Agriculture world over should have experienced a drastic shortfall in overall crop yields should herbicide availability be limited. Now, when the herbicide use gained a momentum and has been popularized as a formal input tool particularly in mechanized large holdings and commercial farming, the loss of herbicide effectiveness due to selection of herbicide-resistant weed populations has a negative impact on farmers. Herbicide resistance is the inability of a herbicide to effectively control a weed species that was previously controlled by the same herbicide. Herbicide resistance is detected when a biotype within a weed species possessing a resistant trait increases in abundance while susceptible biotypes are controlled by use of the same herbicide. The resistant trait is inheritable and therefore, is passed from one generation to the next. Once a herbicide-resistant population has been selected for, the likelihood of the weed population reverting back to a population dominated by the susceptible biotype is low. Resistant weed population becomes a serious constraint because it develops far faster (in 3 to 5 years) than the time and money investment on research, testing and registration for another newer chemical that meet modern environmental and health regulatory standards. As a result, herbicides with a new mode of action will not likely serve as a solution for herbicide-resistant weed populations. Therefore, it is absolute important that the herbicide options presently available be maintained through sound product stewardship. Stewardship implies that whoever produces, sells, or uses herbicide, exercises all precautions for minimizing any undesirable effects of the herbicide, including selection of resistant biotypes.

Agrochemical Industry has founded an international body, Herbicide Resistance Action Committee (HRAC) primarily aiming to collect information and prepare a database on resistant biotypes. It is a sort of cooperative project in collaboration with Weed Science Society of America (WSSA) lead by Dr. Ian Heap (WSSA.com). There is however, scant recognition by the largely land holding growers, the herbicide producers, the industry counterparts and the policy making partner i.e. the state and federal governments regulatory agencies. This reflection can be assessed from the fact

that there is no regulatory binding for posting remedy or precaution on the label that demonstrate any instruction on resistance management. Recognition of the resistance concern is absolutely non-existent in today's marketing plans of the herbicide-producing sector. In US Indiana, growers perspective study on herbicide resistance revealed that 65 percent respondents expressed moderate or low level of concern about weeds developing resistance to glyphosate. Only the growers who own 800 ha or more holding were the only willing to adopt resistance management strategies (Johnson and Gibson, 2006). In this scenario, one can imagine what will be response of developing and under developed countries where agriculture represent a large economic sector and is heavily input driven. Tons of herbicides are used beyond North America and Europe where absolutely meager or no awareness or resistance management actions are in place as a combat strategy in the years ahead.

In this chapter, emphasis is drawn on the overall review of the weed types and crops where mechanism of resistance imposed by either or in combination of genetic modification, environmental interaction and cultivation practices have been identified exerting pressure on plants for herbicide resistance. The contents of this chapter are segmented into different profiles to the extent of covering basic interest of stakeholders; growers, policy makers, professional scientists, farm technologists, and graduate students. Herbicide resistance is a growing concern that warrants efforts in the developing and implementing new input based cultivation strategies.

2. Introduction

For years farmers have been fighting against their farm pests: rodents, insects, micro-pathogen and weeds that tolled heavy crop yield losses dwelling a segment of world population from malnutrition to starvation. According to a recent Cornell University report, the overall effects of weeds and invasive plants on the US agriculture, water quality, wildlife and recreation have been estimated to cost U.S. $34.7 billion annually (WSSA.net). As research based knowledge accumulated, concepts of modern farm technologies shaped up and new choices replaced the older cost inefficient farm practices. Attempts were made to kill the pests by spraying pesticides and herbicides consequently, hundreds of pest species have become resistant to chemical pesticides. Although resistance of insects to chemicals was recognized as old as 78 years ago, the problem peaked up and was reported in several parts since 1940 after the use of synthetic organic pesticides was increased. Herbicide resistance was first reported against 2,4-D (Phenoxy group) in 1957 from Hawaii (Hilton, 1957). In 1968, first confirmed report of herbicide resistance in common groundsel *Senecio vulgaris* against triazine herbicide was documented (Ryan, 1970). Consequently, several other reports confirmed resistance developed against dozens of other herbicides in four decades (Table 1)

Glyphosate became a prominent herbicide in agriculture about 12 years ago when it was discovered that glyphosate resistance genes could be inserted into crops using biotechnology. Now, glyphosate resistant corn, cotton, soybeans, canola and sugar beets are common. Glyphosate being broad spectrum can kill most or all unwanted weeds while crops remain unharmed. Glyphosate thus became the dominant weed control method on many farms in North America and abroad, and quickly replaced other weed control practices. Glyphosate's effectiveness as a broad spectrum herbicide left many growers relying on it frequently and even exclusively in their battle to control weeds. Unfortunately, once a naturally resistant weed appears in a field, it can escape and multiply

into a serious problem in the next few years. In 2000, marestail (Horseweed) *Cornyza canadensis* surfaced resistance to glyphosate in soybean fields of Delware where glyphosate was consecutively used over 3 years. (Figure 1). Within 3 years the resistance spread in 100,000 of states 560,000 acres of cropland. Now nine other states including Indiana have either confirmed or suspect the presence of glyphosate resistance marestail. This rapid widespread has been due to the fast airborn property of its seeds i.e. the seeds can travel 1/4th mile per mild wind speed of 10 MPH (Barnes, J *et. al.* 2003).

Figure 1. Glyphosate resistant Marestail [Source: Barnes, J. *et al*. 2003)]

Over the past several years, we have seen the list of glyphosate resistant weeds grow to almost one dozen species (Table-1), which are scattered across at least 20 states (WSSA. net). A consultant's report from argentine endorsed that johnsongrass *Sorghum helepense* has shown resistance against glyphosate (Valverde & Gressel 2006). Farmers are being challenged to control glyphosate resistant weeds like Palmer amaranth, *Amaranthus palmeri* and giant ragweed *Ambrosia trifida* in certain crops. We urgently need to slow the development of resistance before glyphosate's value to farmers is diminished. Monsanto commercialized Glyphosate as RR (Roundup-Ready) with their respective genetically modified (GM) transgenic crop seed. This RR- seed package in soybean, corn, canola and cotton where offered a weed free high farm yield simultaneously, did evolve herbicide resistant biotypes, a challenge that has now become a key issue for all stakeholders. GM transgenic herbicide resistant crops are becoming volunteer weeds are also associated with

segregation and introgression of herbicide resistant traits in weed population that has ecological impact on plant communities (Owen and Zelaya, 2004).

Table 1: Worldwide Herbicide resistance against widely used types of herbicide groups.

Herbicide	Year of Resistance found	Year of Reporting
2,4-D	1945	1963
Dalapon	1953	1962
Atrazine	1958	1988
Picloram	1963	1973
Trifluralin	1963	1982
Diclofop	1977	1982
Trialate	1962	1987
Chlorsufuron	1982	1987
Glyphosate	2003	2006

Source: [LeBaron, 1991., Valverde & Gressel, 2006]

Most recent world wide updated *WSSA* inventory provides comprehensive information on herbicide resistant weeds by country and by mode of action is compiled by WSSA (Appendix-I).

Resistance is a phenomenon that develops rapidly. When a pest population starts showing resistance, it responds favorably to a change in tactics for only a small period of time after detection. Resistance progress within season(s) until it leads to its climax where change in control strategy becomes imperative. It has been generally observed that if a pest population is resistant to one or more pesticides, it will develop resistance to other compounds especially when the compounds have similarity in mode of action. Most pests can retain inherited resistance to pesticides for longer time therefore, it is logically attractive that different and newer compounds varying in their mode of action are used provided it comply with cost efficiency and regulatory approval. While developing new compounds, the application philosophy and principles of integrated pest management (IPM) can be a strength. As such delaying the duration of spread of resistance will help gain more time to monitor the resistance episode.

Pesticide resistance is solely a technical problem that can be readily overcome with the right type of new pesticide or by devising appropriate adjustments within the conventional use of pesticides. It is imperative because experience has revealed that resistance episode will enlarge unexpectedly. As research organizations and industrial groups concentrate on monitoring resistance issue, it is necessary that deeper understanding of resistance is taken collectively between applied and academic biology. The key idea to this concerted effort is to step forward and identify practical solution to pesticide resistance problem. It is however, understood that resistance is potentially a powerful pervasive natural phenomenon that can be minimized, eliminated or managed by human actions. Inadvertent neglect or intended defer in dealing with resistance can lead to explosions in weed pest population resulting crop in failure consequently driving nations in to grave food crisis that have already been escalated by global trade issues since 2007 (Ozair, 2007 & 2008).

3. Resistance verses Tolerance

Herbicide resistance is an induced inherent ability of some plant species to survive and reproduce after receiving a lethal dose of herbicide (Prather et. al. 2000). In contrast herbicide tolerance can be defined as the inherent ability of plant to survive and reproduce with a herbicide treatment at a normal use rate (Vargas and Wright 2004). In other words tolerance is the ability to compensate the damaging effects of the herbicide with no physiological mechanisms involved (Menalled and Dyer 2006)

In plants, herbicide resistance is developed either by random mutation or it is self-induced by genetic engineering. In nature, it is infrequent and there is no evidence of herbicide induced mutation at any point. Where herbicide cause **selection pressure** i.e. susceptible plants are killed whereas the resistant plants survive to reproduce without confronting any competition from the susceptible plants.

3.1. Types of Resistance

Herbicides target attack at one or more location in a weed plant. These locations can either be enzyme proteins, other non-enzyme proteins, cell division path etc. are called site of action. One such example is of ALS [acetolactosynthase also called AHAS acetohydroxy acid synthase] leads making branched chain amino acids in plants. Herbicides like; [sulfonylurea, imidazolinone and pyrimidinyloxybenzoate] bind to this enzyme. As such, when this enzyme is complexed with herbicide, it is no more available for the normal synthesis of certain amino acids and consequently, protein deficiency and ultimate death of plant result. The chemical structure of above set of herbicides is different but their target site is same. The plants that resist to ALS/or AHAS herbicides have altered the ALS enzyme in such a way that it does not bind with the herbicide, Now, the resistant weed biotype that has been evolved by selection pressure from one ALS attacking herbicide will be resistant to all herbicides that act on this particular site. Such resistance of plant where one class of herbicide within one group or to several herbicide classes within one group is called **Cross-Resistance**. A population of yellow starthistle, *Centaurea solstitialis* evolved resistance to synthetic auxin, picolinic acid that promoted Picloram herbicide in Washington State. When this population was tested with another picolinic acid herbicide, Clopyralid, the plant showed same resistance (Prather *et al*. 2000).

Another example of cross-resistance reported from Australia where a biotype of wild oats *Avena fatua* became resistant to fenoxiprop (an ACCase i.e. Acetyl Co Enzyme-A Carboxylase) also became resistant to several other ACCase inhibiting herbicides. (Powles and Holtum, 2008).

On the contrary, a **Multiple Resistance** is said to have occurred when resistance to several groups of herbicides with different biochemical target such as triazines acting on PS system as photosynthetic-e- inhibitor and sufonylure inhibit ALS i.e. Acetolactosynthase enzyme (Menalled & Dyer 2006). In southern Australia 3 herbicides, diclofopmethyl (postemergent), sulfonylurea such as Chlorsulfuron and Triasulfuron, had been used against annual ryegrass *Lolium rigidum* for almost 10 years 1978-89. The Diclofop-methyl resistant biotype revealed multiple cross-resistance to other groups as listed below (Powles and Holtum 1990):

Aryloxyphenoxypropionates:	Diclofop-methyl, Fluazifop-butyl, Fenoxyprop-ethyl, Haloxyprop-methyl, Quizalofop-ethyl.
Cyclohexanediones:	Alloxidim, Sethoxidm, Tralkoxydim
Sulfonylureas:	Chlorsulfuron, Metasulfuron-methyl, Triasulfuron
Dinitroanilines:	Trifluralin
Triazines:	Atrazine, simazine

The mechanism of multiple resistance caused in rye grass has been investigated not only due to change at the site of action of herbicide alone but the detoxification of herbicides by strong oxidising enzyme, cytochrome-P450 in plants. This enzyme is also called mixed-function oxidase (MFO). Similar enzyme develops resistance to insecticides in insects (Prather *et. al* 200). Oxidation rate of similar monooxygenase enzymes-P450 in mammal has been reported to show a broad specificity towards foreign lipophilic foreign chemicals including herbicides. A human P450 CYP1A1 metabolizes various herbicides with different structures and mode of action, was introduced in to rice plant. The transgenic rice showed broad cross-resistance towards various herbicides such as Atrazine, Chlortoluron, Norflurazon etc. (Kawahigashi *et. al.* 2006)

3.2. Gravity of Resistance

The herbicide resistance issue became a more serious issue when noxious weed plant species were identified emerging with resistance against selective herbicides. In recent years, the appearance of herbicide resistance in plants is increasing exponentially as compared with the case of other pests such as insects and fungi. Besides 61 species of triazine resistance, there are more than 200 species surfaced resistant to 16 other classes of herbicides. At least one weed species has emerged resistant to herbicide in each country of the world where herbicide is used as farm input. Country wise declared resistant weed species against site of action based classified herbicides are appended in Appendices-I. Resistance however, evolves rapidly where monoculture cropping is prevalent. Weeds belonged to one dozen genera have shown developed resistance at almost a dozen or more locations worldwide (Table-2)

Table 2. Most common genera of weeds developing resistance to herbicides worldwide.

Genus	Common Name	Number of documented Occurrence of herbicide resistance
Amaranthus	Pigweed	42
Chenopodium	Lambsquarters	25
Conyza	Fleabane or horseweed	22
Lolium	Ryegrass	21
Setaria	Foxtail	17
Avena	Wild oat	15
Echinochloa	Barnyardgrass or watergrass	15
Alopecurus	Blackgrass	13
Senecio	Groundsel	12
Polygonum	Knotweed or smartweed	12
Solanum	Nightshade	11

Source: [Vargas and Wright, 2004]

Most recently, in 2003-2006, Monsanto's very potent broad spectrum herbicide glyphosate has been reported resisted against some weeds such as grassy weed Johnson grass, *Sorghum helepense* in Argentina (Valverde & Gressel, 2006) and Rigid rye grass bio-type *Lolium rigidum* have exhibited resistance in almond orchard of northern California.(Vargas, 2001). Both hairy fleabane *Conyza bonariensis* and buckhorn plantain *Plantago lanceolata* have been reported glyphosate resistant in South Africa. Hairy fleabane has been difficult to control with glyphosate in California production system indicating possible resistance. Similarly, reports of poor or ineffective control of *Chenopodium Sp*. lambsquarters in Roundup Ready cotton system have surfaced in the last two years. Recently, Roundup resistance horseweed (marestail) *Conyza canadensis* has been confirmed in the eastern US. (Vargas & Wright, 2004). Until 2006, WSSA updated worldwide Roundup (glyphosate) resistant species enlarged to 11. (Figure 2).

Similarly, little seeded canary grass *Phalaris minor* and Jungle rice, *Echinochloa colona* have reportedly developed resistance against Isoproturon and propanil, respectively, within 3 years 1990-93. As a result, crop yield losses are reported in the tune of 40-60 percent in the affected areas of India (Malik and Sing, 1995). *P. minor* is steadily developing resistance against alternate used herbicides such as Clodinofop and Sulfosulfuron (Mahajan and Brar, 2001).

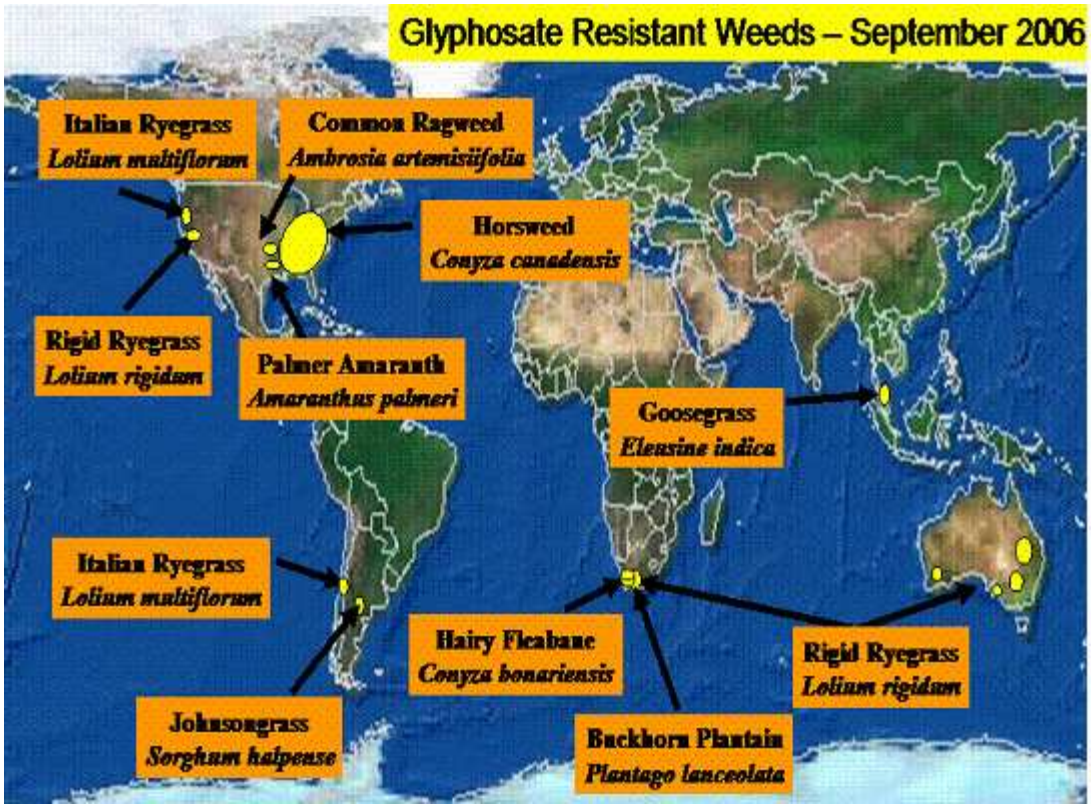

Figure 2. Worldwide reported glyphosate resistant dicot. and monocot. weeds.
[Source: WSSA.com]

A consortium of expert committee: Herbicide Resistance Action Committee (HRAC), the North American Herbicide Resistance Action Committee (NAHRAC) and the Weed Science Society of America (WSSA) founded by Agrochemical Industry, has jointly focused to monitor the evolution of herbicide-resistant weeds and assess their impact throughout the world (Heap, 2007). Global collaboration between weed scientists made the survey that claims dramatic number of weed species has developed resistance against variety of herbicides since 1980 (Figure 3). As to date, 319 resistant biotypes that belonged to 185 species (111 dicots. + 74 monocots.) are spread over internationally over 290, 000 fields (Appendix-I).

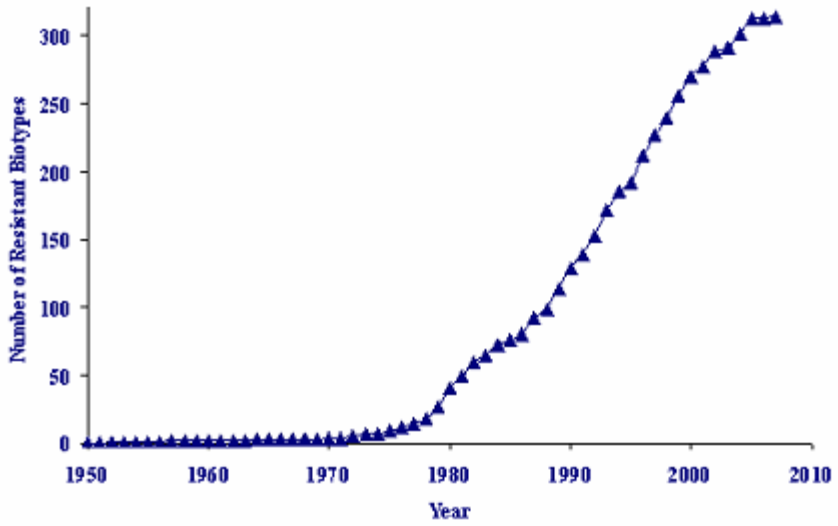

Figure 3. World wide chronological increase in the number of herbicide resistant weeds. [Source: Heap, I. M. 2007]

In two decades (1955-1975), the average annual rate in term of number of introducing herbicide in to the farm was six to seven and this number has now declined to as low as one to two herbicides. In the wake of introduction of high value transgenic crop seeds the package of crop selective herbicides gained commercial hegemony in the last 12 years. This high cost farm technology has further constrained the number of newer molecules. On the other hand, the requirement of re-registration of some herbicides in many crops reduced the number of choices for alternate use or rotation of chemicals. Repeated use of already dwindling types of molecules is itself becoming potential for promoting resistance in weeds. Also, it has been noticed that there are meager herbicides for minor crops that offer relatively less or no choice for different site of action herbicide use in rotation. Consequently, probability of herbicide resistance increases particularly, in countries where registration of herbicides for minor crops is further narrow. Therefore, it is not only the need to research newer herbicides, it is also vital to device a mechanism that ensure reduced repetition of the already available herbicides and switch over reliance on alternate weed control strategies so that selection pressure on resistance is minimized. Herbicides from 8 different groups that are most selectively target-acting in plants and have evolved resistance in weeds, have been gradually increasing resistance trend, most pertinent from 1990 onward (Figure 4).

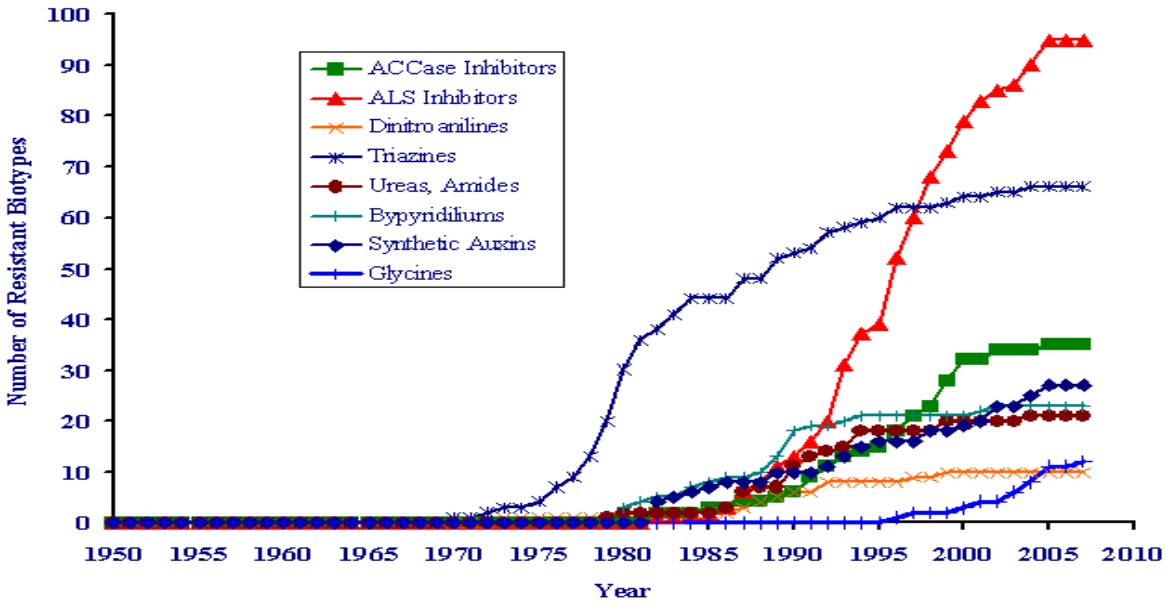

Figure 4. The Chronological increase in the number of herbicide resistant weeds for several herbicide classes.
[Source: Heap, I. M. 2007]

3.3. Sources of inducing Resistant Weeds

As evident from the definition of resistance, it is not due to the mutation caused by the herbicide as chemical, rather resistance appears from the selection of natural mutation that exist as small fraction of population of resistant plants. Herbicide-resistant plant biotypes are believed to be emerging from only one or a few plants that are already present in a population. It may be a single plant in a population of several millions. Although they look morphologically identical, minor invisible genetic differences do exist among them that confer inherent resistance against herbicides. Such a minute number of resistant plants continue grow and expand by generation over time in seasons. Generally, it is hard to predict resistance merely from visual inspection until at least 25 % or small patches of such resistant plants are observed. When we apply a herbicide continuously for consecutive seasons, the susceptible plants of a weed type decrease drastically and those resistant bio types increase gradually to the extent that we find that the herbicide appears to be ineffective at one point. At this stage we say that the weed has developed resistance against a herbicide or in other words called selection pressure of herbicides reached to maximum (Duke *et al*. 1991). This whole process is presented in figure 5.

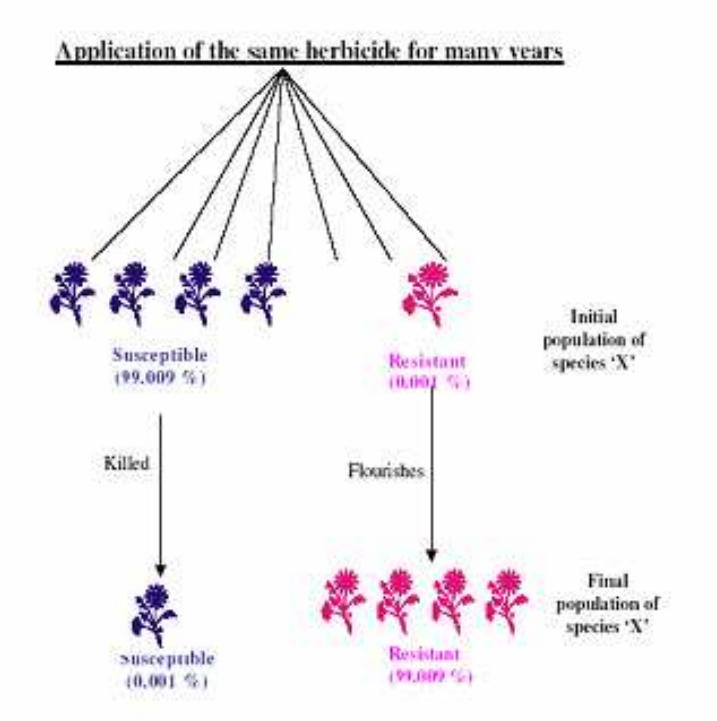

Figure 5. The evolution of herbicide resistance (percent values are arbitrary)
Source: [Tharayil-Santhakumar, N. 2003]

4. Factors Influencing Herbicide Resistance

Biodiversity is a product of evolution and natural selection. Plants being directly exposed to external environment are vulnerable to variety of stresses, therefore many plants, particularly weeds, contain enormous genetic potential to survive such variations. Both weed as well as the herbicide characteristics influence in the development of herbicide resistance in plants. Most weed species contain adequate genetic variations that allow them to survive under variety of environmental stresses. As discussed earlier, the selection pressure imposes resistance in plants. This selection pressure can be generated either by repeated use of one herbicide, or use of long residual soil applied pre-emergence herbicide or due to repeated application of same post emergence herbicide. Factors that stimulate the development of herbicide resistance are many folds; however, the key factors include weed characteristics, chemical properties of the herbicide and cultural practices that are discussed below briefly (Vargas & Wright 2004).

4.1. Weed Characteristics

The most likely weed characteristics that favor increase resistance against a particular herbicides can be as following:

- Initial frequency of resistant biotype. If in a particular population some species has relatively more individuals that have specific inherent resistance, then the chances for the resistance to surface are more.
- Annual growth habit. Annual plants complete their life cycle in a relatively shorter time and produce tremendous amounts of tiny seeds that have more rapid dissemination over perennials mode of growth. Perennials being proliferated vegetatively provide less chance of mutation and genetic variability.

- High seed production. Relatively rapid turnover of seed bank to high percentage of seed germination each year or in other words a little or low seed dormancy will lead to death of most susceptible plants by herbicide. Consequently the remainder resistant though fewer, will have brighter chance to grow with vigor and reproduce with increased resistant trait.

- Several reproductive generations in each growing season

- Extreme susceptibility to particular herbicide. It is also called hypersensitivity of weeds to a particular herbicide. Due to hypersensitivity, a single application of herbicide can eradicate most (90-95 %) population consequently, high selection pressure will allow the resistant biotypes to prevail and thrive best to stand fit in the field.

- Some weeds have high frequency of resistant gene that develop higher and rapid rate of resistance e.g. *Lolium rigidum*

4.2. Herbicide Characteristics

The following properties of herbicide molecule build the resistance in weeds to label them as different biotypes

- A herbicide that has single site of action favors resistance far more faster than the herbicide with several mode of actions. The resistant biotype can easily endorse resistant against the herbicide that has only one site of action rather than the herbicide has multiple sites of actions.

- Herbicides that are subjected to enhanced metabolism in weeds have least chance to endure resistance in plants than weeds expressing resistance due to change at site of action. However, metabolism-based resistance to ACC-inhibiting herbicides is much less known although this type of resistance seems to be wide-spread (Delye, 2005).

- Broad spectrum control

- Long residual activity in soil will keep susceptible biotypes eliminated or suppressed for longer time giving competition free growth autonomy to the resistant to flourish and reproduce.

4.3. Cultural Characteristics

Farm cultural Practices can also shift selection pressure on weeds. Because persistently using similar cultural practices such as below increase chances to develop herbicide resistant weed biotype are:

- A move from old multi-cropping pattern to monocrop culture where single crop is grown every season or where a fixed horticultural orchard is in place receiving similar input every season.
- Using reduced soil cultivation, or zero tillage system.
- Using single herbicide or combination (tank mix) that have same mode of action in every season persistently. This example refers to situations where transgenic herbicide resistant seeds are planted with a package of specific tolerant herbicide.
- Dose violation i.e. use of low or very high doses in relation to the optimum rates prescribed for a specific crop in a situation.

5. Mechanism of Herbicide Resistance

Dekker and Duke (1995) broadly grouped herbicide in to the following two categories:

5.1. Exclusionary Resistance

Resistance is caused in plants due to inaccessibility of the molecule at its site of toxic action. In other words, it is the inability of herbicide molecule to concentrate in right lethal amount at point of action within weed plant. This provides weed a blessed escape from death and avail a sort of herbicide resistance. Such exclusion of herbicide from the site of action can be due to several reasons. It can be differential herbicide uptake due to the morphological barrier on leaves such as extraordinarily increased waxy coating on the cuticle, hairy epidermis and low foliage number and size etc.

It can also be due to differential translocation whereby apoplastic (Xylem tubes) or symplastic path (Phloem cells) restrict or delay movement of right concentration of herbicide at the site of action. (Ozair *et. al.* 1987)

It is likely that herbicide undergoes a sequence of locations before reaching at the site of action e.g. some lipophilic herbicides may become immobilized by partitioning into lipid rich glands or oil bodies (Stegink and Vaughn, 1988).

It is also possible that herbicide is rapidly detoxified prior to its reaching at the site of action. The detoxification reaction can either be oxidation, reduction, hydrolysis or conjugation. Resistance gained due to metabolic detoxification involves the role of enzymes. In case of velvetleaf weed

Abutilion theophrasti, the enzyme Glutathione-s-transferase increases that detoxifies atrazine herbicide, hence the weed gains resistance. Similarly, in *Echinochloa colona* increased contents of enzyme aryl-acylamidase detoxify propanil herbicide. Herbicide metabolism can also be increased due to fast acting Cytochrome P450 monoxygenase with target enzymes such as; AACase (Acetyle CoenzymeA-Ccarboxylase), ALS (Acetolactosynthase) and PS II (Photsynthetic- e- transport pathway).

5.2. Site of Action Resistance

Site of action of herbicide in weed biotype is altered in such a way that it is no more vulnerable to be intoxicated by the herbicide. World over, many species were reported to have developed resistance against Sulfonylurea. In *Lactuca sative* biotype ALS enzyme, the site of action of sulfonylurea is modified in such a way that the herbicide can not bind with its site, hence the enzyme is not incapacitated and weed biotype skips the killing effect of herbicide (Eberlin *et.al;* 1999).
Target site-based resistance involves altered binding of herbicides to their target protein. How does the target protein change? A single nucleotide change or mutation in the gene encoding for the protein (mostly the enzyme to which herbicide binds) occurs. Change of one nucleotide changes the amino acid sequence of the protein, hereby destroying the ability of the herbicide to interact with the protein and at the same time do not incapacitate the normal functioning of the enzyme. As such, enzyme keeps normal function in the presence of the herbicide. Mutations leading to herbicide resistance may indirectly alter other unrelated physiological pathways affecting the otherwise growth and development of the resistant biotype. In kochia scopia, mutated resistance against sulfonylurea, reduces or diminishes the ALA (Acetolactate enzyme) sensitivity to normal feedback inhibition patterns. This generates excessive availability of amino acids for cell division and accelerated growth and development. Consequently, sulpfonyl urea resistant biotype of *K. scopia* dominates germination and establishment vigor as compared to its counterpart, susceptible biotype (Tharayil-Santhakumar, N. 2003).

It also happens in some cases that the site of action is enlarged or overproduced as a result dilution effect of herbicide occurs. The applied normal rate of herbicide is unable to inactivate the entire amount of enzyme protein produced. Therefore, the extra amount of enzyme produced by the plant biotype can allow it carry on its normal metabolic activities surmounting the lethal effect of the herbicide.

6. Assessing Herbicide Resistance

In case a herbicide treatment fails to control weeds at a situation, weed resistance may not necessarily be the cause. Before signing a positive statement for weed resistance to herbicide, the following parameters should be tested (Beckie, *et. al* 2000., Gunsolus, 2002., Menalled and Dyer 2007).

6.1. Visual Diagnosis

1. Read the herbicide label carefully. Are other weeds listed on the product label controlled satisfactorily? Chances are only one weed species will show herbicide resistance in any given field situation. Therefore, if several normally susceptible weed species are present, reconsider factors other than herbicide resistance as the cause of the lack of weed control.

2. Also observe, if the uncontrolled weeds exist in patches and each patch contains different species, this case is not of herbicide resistance because it is very unlikely that all species will develop resistance. There could be one of several other reasons.

3. Check if the herbicide is used repeatedly at the same field and is of the same one mode of action. If "yes" chances are that weed is likely evolving resistant biotype.

4. Further survey the area for any previous case of resistant weed reported. Interview growers for resistant-suspect to same herbicide. Did the same herbicide or herbicide with the same site of action fail in the same area of the field in the previous year?

5. Also, investigate if the level of weed control on suspected weed was declining in the past few years.

If the answer to some of the above questions is "yes", chances are that the weed species in question is leading to herbicide resistance.

6.2. Bio-Assay

If the above diagnostic survey support that a certain biotype has likely evolved herbicide resistance, adequate sample of seed/plant material may be collected from the suspect population for the subsequent confirmation tests.

6.3. Plant Assay/Seed Collection

For reliable germination and healthy growth of plants during the experiments, healthy plants and stage of seed harvest is essential Particularly the grass weed seeds the best timing to ensure ripened seeds is when plant has shed at least 20 % seeds. Collect adequate amount (1000 seeds) from an average area of 50-100 meters by gentle rubbing the inflorescence over paper bag or tray. Label species name, date and location on the bag.

6.4. Greenhouse / Plant-Pot Assay

This is preliminary bioassay on mortality, plant vigor check , measurement of whole or fractions of plant biomass. This test will reveal discriminatory spraying effect of herbicide. This test, however,

must include a treatment of susceptible set of pots for reference and not for herbicide sensitive or insensitive evaluation. Statistical advice must be sought for appropriate design and replications.

6.5. Dose Response Experiments

Use range of doses to formulate a standard response curve. This curve enables quantification of resistance by calculating the dose ratio required to produce the same effect in resistant and susceptible population. Usually the dose required to give a 50-70 reduction in the measured parameter (usually foliage weight or number of surviving plants) relative to the untreated control is determined (Figure 6). Ratios of these estimates (variously termed ED50, GR50, LD50 or 150), relative to that of a susceptible population, provide a resistance index (RI) which enables the degree of resistance to be described relatively simply. To obtain a good estimate of ED50 the dose range should be relatively wide and at least six doses are needed. It is usually best that each dose is twice the preceding dose in the range (e.g. 10, 20, 40, 80, 160, 320 g a. i./ha). The dose range used should include doses both below and above the field recommended rate as herbicides are normally more active under greenhouse conditions. (Moss, S.1999).

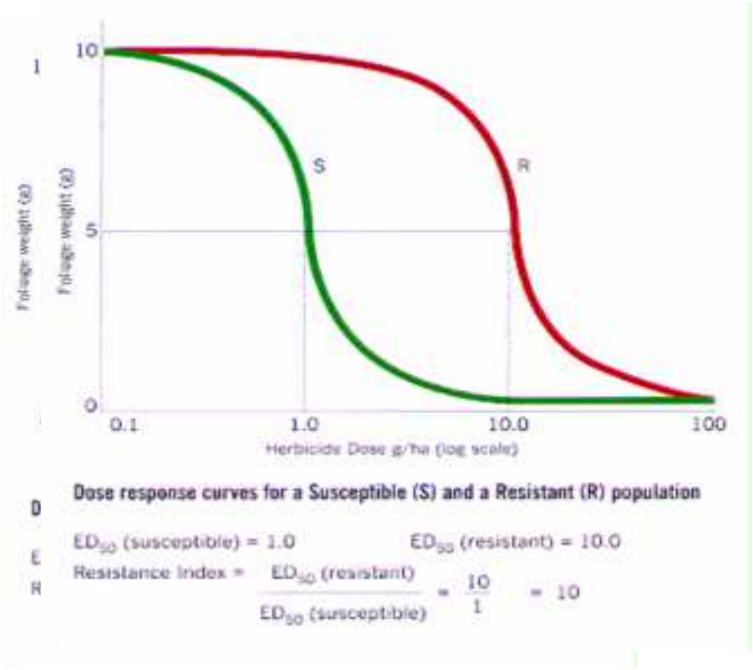

Figure 6. Dose response curve for susceptible (S) and Resistant (R) population
[Source: Moss, S 1999]

6.6. Single Dose Resistance Assay

After the dose response information is obtained from the above experiment, single or 2-3 discriminating doses can be used for the future screening assays conducted for more populations.

6.7. Specific Discreet Tests

Several other detailed confirmatory analytical tests can be used such as petri-dish germination assay, chlorophyll fluorescence, leaf disc floating and enzyme specificity/sensitivity assay etc.

7. Herbicide Resistance Management
(Prevention and Delaying Resistance)

Any weed management strategy applied to minimize selection pressure for resistance will block the emergence of resistance. All of the following or combination of some as applicable in a situation may prevent or at least delay the evolution of resistance in weeds.

7.1. Herbicide Rotation

As previously discussed the use of same herbicide or a different herbicide but similar mode of action in consecutive years increases evolution of resistance. Reverse action can be achieved by using different herbicides with different modes of action in the subsequent season(s); this process is called herbicide rotation. Herbicide resistant transgenic crops e.g. RR- soybean ready roundup are gradually increasing acreage beyond North America. In this package, single use of herbicide e.g. glyphosate in soybean or corn is indispensable. Such a practice will enhance weed resistant culture. Perhaps tank mix application of herbicides could prevent or delay resistance pressure. In Australia when annual rye grass along side railway lines evolved resistance against 10 years persistent use of amitrol and atrazine, a tank mix of glyphosate and sulfometuron-methyl controlled the resistant biotype effectively (Powles and Holtman 1990). However, this combination should subsequently be further rotated in order to delay resistance evolution. Additionally, inclusion of non-chemical control option such as IPM may reduce the potential threat of resistance evolution. Use of the least persistent herbicide reduces the resistance risk. If two herbicides in a tank mix have the same weed control spectrum, it is better to use each separately in seasonal rotation. Do not spray herbicide in one season, as it will allow the susceptible seeds to grow vigorously from the geo-seed-bank reservoir. Consequently, the non resistant biotype will out-compete the fewer resistant biotype. strong competition with the fewer resistant biotype. The resistant biotype will experience survival rejection due to natural rejection (survival of the fittest).

7.2. Crop Rotation

Growing the same crop every season will invite same inputs including herbicide because of the same ecological culture. Crop rotation allows manipulation of planting time, spectrum of weed infestation, cultivation techniques, choice of herbicide with different mode of action, different stage and different way of application. Using combination of weed control strategies offers a chance to eradicate the so-called resistant biotypes and reduces the chances of their establishment.

7.3. Post Treatment Monitoring

While monitoring visual rating for weed control, carefully watch if there is any pattern of weedy patches uncontrolled. Differentiate between resistance and spray application neglect. The inconsistent patches that do not seem to be a spray neglect should be eliminated manually or by superimposed herbicide application that leads to effective killing.

7.4. Integrated Cultural Practices

Inclusion of all possible non-chemical weed control methods help more effectively against the weed resistance evolution. In contrast to no-tillage, cultivation practice stirs the soil, buries the early emerged weed seedlings (both susceptible as well as the resistant one) and solairizes the soil. Hand weeding eliminates the weed plants before the seed set, discarding a biotype, 90-100 percent. Mulching for organic matter will simultaneously debris the weeds before seeding, offering reduced weed population with crop stand.

8. Herbicide Resistance Management

(Post-Evolution)

In order to maintain check over the herbicide resistant biotypes, integrated weed management approaches as discussed above must be incorporated as appropriate. Crop rotation or preferably fallow tillage followed by close cultivation will keep the resistant population down. Extensive manual weed control by effective crew will offer 100 percent eradication of the suspected resistant biotype ensuring leas emergence in the subsequent season. Ensure clean and certified seed is planted each season and clean farm machinery is driven in the farm. It is suggested that power washer be used to clean the machinery from the infested seeds of the obnoxious biotype.
By the use of these varied weed control practices, farmers have (unconsciously) acted to avoid or greatly delay the emergence of herbicide resistant weed biotypes. Farmers at risk of resistant biotype should be encouraged to maintain this diversity of operations in the control of weeds. The converse is represented by those relatively small numbers of farmers who rely heavily (or exclusively) on the use of selective and non-selective herbicides for weed control and who crop intensively or continuously on some fields. Our observations clearly identify that farmers who practice continuous cropping, or intensive cropping, run a much greater risk of developing resistance. Under such conditions, a consistent selection pressure is placed on the weed population and herbicide resistance is the inevitable result. Farmers who practice such cropping regimes need to be alerted to the probability of resistance appearing under these conditions and should consider modifications to their cropping practices.

8.1. Advanced Services for Resistance Management

In the popular scenario of mono-cropping pattern where intensified cropping includes single action broad spectrum ready herbicide with transgenic crop seed, gravity of herbicide resistance is likely

to aggravate, and the following extension services both in private and public sector may offer a prosperous check:

- Research facility must extend window option for DNA finger printing test for resistant and susceptible biotypes.
- Investigation for genetic resistance (one gene or additive gene effect) must be available.

- Development of technologies for remote aerial sensing of fields for clumps areas.

- Arrangement for establishing electronic data-base on regional basis, crop based or herbicide resistance species based for reference facility to the researchers.

9. Conclusion

It is important that resistance to herbicides is detected timely to implement resistance management strategies before situation grows in to gravity and requires higher cost and human endeavor for launching any extreme strategy. Currently, the progressive growers who grow crops extensively on commercial basis use herbicides as one of the input. There is however, scant recognition by the largely holding growers, the herbicide producers (industry counterparts) and policy making partner (the state or federal governments). This reflection can be assessed from the fact that there is no remedy or precaution on the label that demonstrate any instruction on resistance management. Recognition of the resistance concern is absolutely non-existent in today's marketing plans of the herbicide-producing sector. This is indeed a point to address this issue seriously if they have to keep herbicides as effective agricultural input. All stake holders, particularly the growers, the weed scientist/ farm and extension managers in the private and public sectors, and industrial unit (s) must jointly address herbicide resistance. Consorted efforts are required to invest on resistance research and promote action to reduce prolonged or eliminate resistance evolution. So far, as the review of literature reported in the text reveals, educated and professionally designed strategies can reverse or restrict the extent of resistance. It is, therefore, recommended that farming practices be rethought for actions such as crop rotation, tillage/cultivation practices, herbicide rotation for several factors prescribed in the text of this chapter, incorporation of integrated tools etc. While such reforms are accepted and practiced with the intention to delay or stop resistance management, professional vigilance for likely resistance followed by greenhouse/laboratory testing as discussed for the suspect areas should be monitored.

Appendix

Appendix-I **: Herbicide Resistant Weeds by Country and Mode of Action**

Sr. #	Country Click for details	Total	A ACCase Inhibitors	B ALS inhibitor	C1 Triazine	C2 Urea/ Amide	D Bypiri-dilium	K1 Dinitro-aniline	O Synthetic Auxin	Others
1	Argentina	3	0	1	0	0	0	0	0	2
2	Australia	51	9	20	5	0	5	2	1	9
3	Austria	2	0	0	2	0	0	0	0	0
4	Belgium	18	2	1	7	1	3	1	0	3
5	Bolivia	3	2	1	0	0	0	0	0	0
6	Brazil	19	3	8	0	0	0	0	2	6
7	Bulgaria	4	0	0	2	1	0	1	0	0
8	Canada	44	3	17	12	3	2	1	4	2
9	Chile	6	4	1	0	0	0	0	0	1
10	China	9	0	3	1	2	0	0	0	3
11	Colombia	4	1	0	0	1	0	0	1	1
12	Costa Rica	5	1	3	0	1	0	0	0	0
13	Czech Republic	16	0	2	13	0	0	0	0	1
14	Denmark	3	1	2	0	0	0	0	0	0
15	Ecuador	1	0	0	0	1	0	0	0	0
16	Egypt	1	0	0	0	0	1	0	0	0
17	El Salvador	1	0	0	0	1	0	0	0	0
18	Ethiopia	1	1	0	0	0	0	0	0	0
19	Fiji	1	0	0	0	0	1	0	0	0
20	France	32	5	2	22	1	0	0	1	1
21	Germany	19	1	2	13	3	0	0	0	0
22	Greece	5	1	1	2	1	0	0	0	0
23	Guatemala	1	0	0	0	1	0	0	0	0
24	Honduras	1	0	0	0	1	0	0	0	0
25	Hungary	1	0	0	0	0	0	0	1	0
26	India	3	1	1	0	1	0	0	0	0
27	Indonesia	1	0	0	0	0	0	0	1	0
28	Iran	5	5	0	0	0	0	0	0	0
29	Ireland	1	0	1	0	0	0	0	0	0
30	Israel	23	5	6	11	1	0	0	0	0
31	Italy	16	3	6	4	2	0	0	0	1
32	Japan	16	0	9	1	0	6	0	0	0
33	Kenya	1	0	0	0	0	1	0	0	0

34	Malaysia	16	1	4	0	0	6	0	4	1
35	Mexico	3	3	0	0	0	0	0	0	0
36	New Zealand	9	0	1	3	0	0	0	4	1
37	Nicaragua	1	1	0	0	0	0	0	0	0
38	Norway	5	0	1	3	1	0	0	0	0
39	Panama	1	0	0	0	1	0	0	0	0
40	Paraguay	2	0	1	0	0	0	0	0	1
41	Philippines	3	0	0	0	1	0	0	1	1
42	Poland	9	0	1	8	0	0	0	0	0
43	Portugal	2	0	1	1	0	0	0	0	0
44	Saudi Arabia	1	1	0	0	0	0	0	0	0
45	Slovenia	1	0	0	1	0	0	0	0	0
46	S. Africa	14	3	5	1	0	2	0	0	3
47	S. Korea	6	0	6	0	0	0	0	0	0
48	Spain	30	1	3	18	3	0	0	1	4
49	Sri Lanka	2	0	0	0	1	1	0	0	0
50	Sweden	2	0	1	0	0	0	0	1	0
51	Switzerland	14	0	0	11	3	0	0	0	0
52	Taiwan	1	0	0	0	0	1	0	0	0
53	Thailand	5	2	0	0	1	0	0	1	1
54	Netherlands	7	1	0	5	1	0	0	0	0
55	Tunisia	1	1	0	0	0	0	0	0	0
56	Turkey	3	2	1	0	0	0	0	0	0
57	United Kingdom	24	4	4	8	2	2	1	2	1
58	USA	122	15	38	23	7	4	6	8	21
59	Venezuela	1	0	0	0	1	0	0	0	0
60	Yugoslavia	6	0	2	4	0	0	0	0	0

Source: [Weedscience.org] http://www.weedscience.org/summary/countrySummary.asp

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