

Mechanism Of Herbicide Resistance In Weeds

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1. Introduction

Biological flexibility and ecological adaptability have been recognized as laws of nature for long time. The ability of living organism to compensate for or adapt to adverse or changing environment conditions is remarkable. Regardless of how and when various living species began, the survival of the fittest has been and still is going on.

In order to feed the ever-growing population researchers were always in the lookout of new technologies; technologies that will increase the food production manifolds and that are economically viable at the same time. So the introduction of pesticides in agriculture was a welcome move. It helped the farmers to control some of the noxious pests and thus reduced the yield loss caused by them at an affordable cost. But along with these advantages there came some inadvertent disadvantages; development of resistance against these pesticides in targeted organisms was the most prominent among them.

Insects were the first to develop resistance against pesticides. Sanjos scales resistant to lime sulphur were sited in the year 1908. Later, pathogens resistant to fungicides were reported in 1940. Owing to the late commencement of use of herbicides in agriculture and probably due to the long generation cycle in plants, the resistance against the herbicide was the last to surface. Although herbicide resistance was reported as early as 1957 against 2,4-D from Hawaii (Hilton, 1957), the first confirmed report of herbicide resistance was against triazine herbicide in common groundsel (*Senecio vulgaris*), and was reported in 1968 from U.S.A. (Ryan, 1970).

Since then, the number of resistant weed biotypes against various herbicides is on the rise (Fig. 1). Till recently, 254 biotypes belonging to 155 species (93 dicots and 62 monocots) have reported resistance against various herbicides (Heap, 2002) (Table 1&2).

Herbicide resistance is the inherent ability of a species to survive and reproduce following exposure to a dose of herbicide normally lethal to its wild type.

The gravity of the problem became obvious with the reporting of some of the crop-bound weeds like *Phalaris minor* and *Echinochloa colona* developing resistance against selective herbicides like isoproturon and propanil, respectively. Due to resistance the control of *Phalaris minor* dropped from an impressive 78 % to a bleak 27% within a time span of 3 years (1990-93) (Malik and Singh, 1995), causing yield loss to the tune of 40-60% in affected areas. The development of cross resistance in isoproturon resistant *Phalaris minor* to diclofop-methyl within 2 years of its employment, and some alarming reports that *P. minor* is slowly but steadily developing resistance against some of the alternate herbicides like clodinoxop and even to sulfosulfuron (Mahajan and Brar, 2001) are just a warning of the danger that lies ahead. It also underlines the fact that use of alternate herbicides will not preclude the problem of resistant weeds if not delay them. This necessitates the importance for a better understanding of the mechanism of herbicide resistance so that we can tackle this menace in a better way.

A sound knowledge about the mechanism of herbicide resistance is important for several reasons:

- i. The resistant trait can be used as a tool to understand basic plant biochemical processes and fundamental mechanisms by which plant defend themselves from the toxic xenobiotic chemicals.
- ii. New methods to overcome resistance and thus to control resistant weeds may be developed.
- iii. Genes of herbicide resistance once identified can be transferred to crops to produce herbicide resistance, thus allowing the use of alternative herbicides in crops.

2. How do herbicide resistance weeds evolve?

Resistance is not due to mutation caused by herbicides; rather it arises from the selection of natural mutation or small pre-existing population of resistant plants (selection pressure exerted by herbicides) (Duke *et al.*, 1991)

Biologists confirm that weeds do not change to become resistant; instead the population changes. Weed population is extremely diverse, even though they are similar in appearance minor differences exist at the genetic level. Sometimes, it so happens that this minor genetic variation confers some of these variants the inherent ability to resist some of the herbicides. However, frequency of such variants in a normal weed population is very less, one in a million or even one in a billion. But if we are applying an herbicide to this population, to which the naturally occurring variants are immune, the entire picture changes and majority of the susceptible species are killed. This provides the resistant species, which are normally less competitive than the susceptible species, with a unique opportunity to proliferate themselves. So if we are using the same herbicide continuously for many years, in the natural weed population the number of susceptible biotypes decreases drastically and resistant biotypes increase dramatically. Since it is difficult to distinguish susceptible from resistant biotypes morphologically, we will not notice any difference between the initial susceptible and final resistant population. But the only difference we notice is that a particular herbicide that was able to control a particular weed species is no more able to control it. So we say that the weed species have developed resistance against the particular herbicide (Fig. 2).

3. When and why is herbicide resistance more likely?

Both characteristics of the weeds and that of the herbicide influence this.

Weeds

- 1. Initial frequency of the resistant individuals:** If the initial frequency of the resistant individual is high in a natural weed population, then the resistance

will surface more quickly than in a population where the frequency of the resistant individual is low, provided we are continuously applying the herbicide to which the biotypes exhibit resistance.

2. **Weed seed residue in the soil seed bank:** For a species if the seed residue is more in the soil seed bank, appearance of resistance will be delayed due to continues recruitment of susceptible individual from soil seed bank. That is, Nature will allow the resistant species to flourish only after major portion of the susceptible weed seeds have been exhausted from the soil. For this very reason the species that germinate readily from its propagules will develop resistance more quickly than those species whose propagules remain dormant in the soil.
3. **Hypersensitivity of weeds to a particular herbicide:** Because of hypersensitivity, with a single application the herbicide about 90-95% of the susceptible type is killed. So selection pressure will be high and resistance species evolve rapidly.

Herbicides

1. **Lack of rotation of the herbicides:** Continues application of the same herbicide or different herbicide with the same mode of action will create selection pressure and will allow resistant population to flourish.
2. **Herbicides with long residue period:** This result in continues suppression of susceptible of biotypes for a longer period, thus allowing the resistant species to flourish.
3. **Herbicides with highly specific mode of action:** If a herbicide has only one site of action in weeds, then a biotype need to be different in that particular site to be resistant. So the evolution of resistance against such herbicides will be quicker than against herbicides having multiple site of action

4. Mechanism of Herbicide resistance

Mechanisms of herbicide resistance can be broadly grouped into two categories (Dekker and Duke, 1995)

4.1. Exclusionary resistance: Those that exclude the herbicide molecule from the site in plants where they induce toxic response.

In exclusionary resistance mechanism the herbicide is excluded from the site of action in many ways.

(a) Differential herbicide uptake: In resistant biotypes the herbicides are not taken up readily due to morphological uniqueness like overproduction of waxes, reduced leaf area etc.

(b) Differential translocation: In resistant biotypes the apoplastic (cell wall, xylem) and symplastic (plasma lemma, phloem) transport of herbicide is reduced due to different modifications.

(c) Compartmentation: Herbicides are sequestered in many locations before it reaches the site of action. e.g. some lipophilic herbicide may become immobilized by partitioning into lipid rich glands or oil bodies (Stegink and Vaughn, 1988).

(d) Metabolic detoxification: Herbicide is detoxified before it reaches the site of action at a rate sufficiently rapid that the plant is not killed. The biochemical that detoxifies herbicides can be grouped into four major categories: oxidation, reduction, hydrolysis, and conjugation.

Three enzyme systems are known to be involved in resistance due to increased herbicide detoxification.

- Resistance to atrazine in some population of *Abutilon theophrasti* is due to increased activity of glutathione-s-transferase that detoxifies atrazine.

- Resistance to propanil in *Echinochloa colona* is due to the increased activity of enzyme aryl-acylamidase that detoxifies propanil.
- Increased herbicide metabolism due to cytochrome P450 monooxygenase is responsible for resistance to inhibitors of ACCase, ALS and PSII in a number of grass weed species.

4.2. Site of action resistance

Those that render specific site of herbicide action resistant

(a) Altered site of action: Site of action is altered in such a way that it is no longer susceptible to the herbicide e.g. In *Lactuca sativa* biotypes which are resistant to sulfonylurea herbicides, the ALS enzyme which is the site of action of herbicide is modified in such a way that herbicide can no longer bind with the enzyme and inactivate it (Eberlein *et al.*, 1999)

This target site based resistance is usually associated with resistance involving altered binding of herbicide to their target protein. This results from a single nucleotide change (mutation) in the gene encoding the protein to which the herbicide normally binds. This change the amino acid sequence of the protein and reduces or destroys the ability of the herbicide to interact with the protein and at the same time do not incapacitate the normal functioning of the enzyme so that the enzyme functions normally in the presence of the herbicide.

However these mutations conferring herbicide resistance may cause changes in other seemingly unrelated physiological processes. Usually these changes adversely affect the biological fitness of the resistant biotypes. For example, in triazine resistant biotypes the mutation in the plastoquinone binding D₁ protein of PSII results in reduced photosynthetic efficiency (Radosevich and Holt, 1982); also seeds of some of these resistant weeds biotypes exhibit poor germination as compared to susceptible biotypes. But in some resistant biotypes, as found in *Kochia scoparia*, the mutation conferring resistance to sulfonylurea herbicides will concomitantly reduce or abolish acetolactate synthase sensitivity to normal feedback inhibition patterns, resulting in elevated levels of branch chain amino acids available for cell division and growth during early germination.

Hence sulfonylurea resistant biotypes of this species exhibit a rapid germination even at lower temperature compared to their susceptible counterpart.

(b)Site of action overproduction: This causes the dilution effect of the herbicide. Here the site of action is overproduced so that the herbicide at its normal rate of application will not be able to inactivate the entire enzyme produced. Thus the enzyme spared by the herbicide will carry on the normal plant metabolic activities.

5. Resistance mechanism against some important herbicide groups

5.1 Photosystem II (PSII)

Photosystem II is a part of photosynthetic electron transport complex which is located in chloroplast thylakoid membrane (Fig. 3)

PSII consist of light harvesting complex (LHC), a reaction center (P680), two proteins (D1 and D2) and two mobile electron carriers- plastoquinone-A (PQA) and plastoquinone-B (PQB). These PQA and PQB are attached to specialized niches in protein D2 and D1 respectively.

In the normal plant system when LHC transfers the excitation energy to P680, charge separation takes place and one electron is absorbed by pheophytin. From pheophytin electron moves first to PQA and then to PQB.

In the niches of D1 protein PQB is held by two hydrogen bonds; one with serine 264 and other with histidine 215 (Fig. 4A). After accepting two electrons from PQA, both the H bonds are broken and PQB leaves the site as reduced PQB. Now an unreduced PQB occupies this vacant niche in D1 protein and electron transport continues.

Photosystem II inhibitors

The chemical families and herbicides that inhibit photosystem II are

Chemical family	Herbicides
Triazines	Atrazine, Cynazine, Simazine, Propazine
Triazinones	Metribuzin
Uracils	Bromacil, Terbacil
Nitriles	Bromoxinil
Phenylureas	Diuron, Fenuron
Pyridazinones	Pyrazon
Benzothiadiazole	Bentazon

(Retzinger and Smith, 1997)

If a triazine herbicide is present in the system, the triazine molecule will act as non-reducible analog of PQB and will get itself attached to D1 niches by two hydrogen bonds- one with serine 264 and other with phenylalanine 265 (Fig. 4B). Because of their greater affinity to these niches, the herbicide molecule cannot be replaced by PQB. Since the herbicide molecule is non-reducible, they will not receive electron from PQA; as a result chlorophyll molecule will not be able to dissipate its excitation energy and so forms a high energy chlorophyll molecule –the triplet chlorophyll molecule. This triplet chlorophyll molecule reacts with oxygen resulting in the formation of singlet oxygen. The triplet chlorophyll molecule along with singlet oxygen will start lipid peroxidation. As a result integrity of cell membrane is lost and cell contents oozes out. Thus herbicide brings about the fatal effect (Fuerst and Norman, 1991).

Resistance to PSII herbicides

First triazine resistant biotype to be reported was *Senecio vulgaris* in 1968 from US (Ryan, 1970)

Till recently 55 weed species including 40 dicots and 15 grasses have reported resistance against triazines (Heap, 2002).

Some of the species in which resistant biotypes were sited are:

Amaranthus hybridus

Solanum nigrum

Chenopodium album

Phalaris paradoxa

Mechanism of resistance to PSII inhibitors

1. Point mutation in psbA gene

The psbA gene encodes for D1 protein of the PSII. Due to mutation the serine at 264th position is replaced by glycine in the mutant D1 protein. Because of this the herbicide molecule is deprived of one H bond as it cannot form H bond with glycine. So the affinity of herbicide molecule towards D1 niches is decreased considerably and now the normal PQB molecule easily replaces them from the niches thus the normal electron transport continues in the mutant even in the presence of herbicide (Hirschberg *et al.*, 1984).

This is the resistant mechanism in most of the triazine resistant weed species.

2. Glutathion conjugation

In velvet leaf resistance is conferred by the activity of glutathion-s-transferase enzyme in leaf and stem tissues. The result is enhanced capacity to detoxify the herbicide via glutathion conjugation (Anderson and Gronwald, 1991).

3. Oxidation of herbicide

In simazine resistant *Lolium rigidum* the resistance is conferred by increased metabolism of herbicide. Here the herbicide is acted upon by cytochrome P-450 monooxygenase enzyme and converted to herbicidally inactive de-ethyl simazine and di-de-ethyl simazine (Fig. 5, .Burnet et al., 1993).

5.2. Photosystem I (PS I)

PSI is a part of photosynthetic electron transport located in thylakoid membranes (Fig. 3)

In normal case when LHC I transfers excitation energy to P700 (chlorophyll a dimer) it undergoes charge separation and an excited electron is released. This e^- is received by A₀ (chlorophyll a monomer). From A₀ electron moves to Fe-S centers, F_x and F_a/F_b and finally to ferridoxin (F_d). F_d transfers electron to F_d-NADP⁺ oxido-reductase (FNR), which in turn catalyses the reduction of NADP to NADPH.

Photosystem I inhibitors

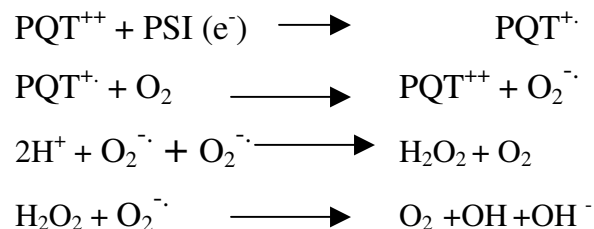
Chemical family

Bipyridillium

Herbicides

Paraquat and diquat

They are post-emergence non-selective contact herbicides. Paraquat is a cationic herbicide and is applied as divalent cationic solution (PQ⁺⁺). The redox potential of PQ⁺⁺ is -446 mv, that of F_a/F_b is -560 and that of F_d is higher than that of PQ⁺⁺. This enables PQ⁺⁺ to act as a competitor for electron flow from F_a/F_b. So F_a/F_b donates electron to PQ⁺⁺ instead of F_d (Fig. 6). After receiving the electron PQ⁺⁺ becomes intensely blue colored monovalent cation (PQ⁺). This PQ⁺ is very reactive and will reduce oxygen to superoxide and in the process PQ⁺⁺ is regenerated.



In the reaction that ensues, H₂O₂ and hydroxyl radical are produced. These are toxic products and they initiate lipid peroxidation. Thus, the cell membrane integrity is lost, cell contents leak out and subsequently desiccation takes place (Furest and Norman, 1991).

Resistance to PSI inhibitors

Tolerance to paraquat was first reported in *Lolium perenne* (Faulknes, 1982). Resistance was spotted in cases where paraquat had been applied 2-3 times for 5-11 years (Polos *et al.*, 1988). Till recently 21 weed species have reported resistance against bipyridiliums (Heap, 2002)

Resistance has been spotted in species like:

Amaranthus lividus (Livid amaranth)

Bidens pilosa (Hairy beggarticks)

Conyza spp.

Eleusine indica (Goosegrass)

Solanum nigrum (Black nightshade)

Mechanism of resistance

1. Detoxification of the toxic products formed

In resistant biotypes of *Conyza bonariensis* the superoxide radical, hydroxide radical, hydrogen peroxide, and singlet oxygen produced due to herbicide treatment are enzymatically detoxified before they could initiate lipid peroxidation. The detoxifying enzymes are superoxide dismutase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, catalase and peroxidase which are collectively called as protective enzymes. Of these enzymes all but catalase and peroxidase are present in chloroplast and this detoxification pathway is referred as 'Halliwell-Asda System' (Shaaltiel, 1988). The detoxification mechanism was also reported to exist in *Lolium perenne*.

2. Rapid sequestration of the herbicide

The mobility of paraquat is restricted in R biotypes since it is being rapidly sequestered. Autoradiogram studies indicated a striking difference in mobility of ¹⁴C-paraquat in R and S biotypes. Radiolabeling was uniformly distributed in S biotypes, but radiolabel movement was highly restricted in case of R biotypes, with most of the radiolabel present in lower half of the leaf and adjacent vascular tissues (Fuerst et al., 1985) (Fig. 7).

Leaf disc of *Conyza bonariensis* were incubated in paraquat solution for 24 hours. It was seen that bleaching had taken place entire disc of S biotype, but bleaching was restricted to some outer most patches in case of R biotypes (Fig. 8). It suggested that paraquat has been sequestered rapidly as it was absorbed through the edges of leaf disc (Vaughn and Fuerst, 1985).

Two potential mechanism of Paraquat sequestration was proposed (Fuerst and Vaughn, 1990).

(a) Paraquat is adsorbed to cellular component by ionic interaction:

Cell wall has cation exchange properties due to the presence of de-esterified galacturonase in pectin fraction. So the divalent paraquat cations are strongly adsorbed to these cation exchange sites in *Conyza bonariensis*

(b) Paraquat is sequestered in cell organelle:

Paraquat is actively transported to a membrane bound organelle such as vacuole and is sequestered as if in the case of calcium and manganese ions.

5.3. Mitotic Disrupter Herbicides

Chemical family	Herbicides
Dinitroanilines	Pendimethalin, trifluralin, oryzalin
Phosphoroamidates	Butamiphos, amiprofos-methyl
Pyridines	Dithiopyr, thiazopyr
Benzoic acid	DCPA
Benzamides	Pronamide, tebutam
Carbamates	Propham, cloroprotham

Most of the herbicides that affect mitosis do so by affecting the cellular structure known as microtubule (Vaughn and Lehnen, 1991)

Microtubules are hollow cylindrical structures which are primarily composed of dimeric protein tubulin, which in turn is composed of similar but distinct subunits of 55 kilodaltons each. Other proteins known as microtubule associated proteins (MAP) cross link microtubules to each other.

According to the theory of microtubule growth called dynamic instability, microtubules have two ends- a growing '+' or 'A' end where tubulin heterodimers are added and a depolymerising '-' or 'B' end where tubulin subunits are lost. This process is called treadmilling. The microtubules performs a number of vital cellular functions like organizing cellulose microfibril deposition, setting cell shape, setting plane for subsequent cell division, movement of chromosome during mitosis, and organizing new cell plate formation after mitosis.

The dinitroaniline herbicides are used primarily as a pre-emergence herbicide for grass control of dicot crops. When herbicide is present in the system of sensitive plants it binds to the tubulin heterodimer in the cytoplasm. As the herbicide-tubulin complex is added to the '+' end of growing microtubule, further growth of microtubule ceases. With depolymerization of microtubule continuing from the '-' end, the tubule become shorter and shorter, eventually resulting in the complete loss of microtubules. This results in uneven thickening of cell wall, isodiametric cells, absence of division plane, absence of chromosomal movement, tetraploid reformed nucleus, incomplete cytokinesis and abnormally oriented cell wall. These fatal irregularities manifest as club shaped roots, swollen bases, and arrest of growth and elongation of roots and shoots.

Some of the species in which resistance has been sited are:

<i>Eleusine indica</i>	(Goosegrass)
<i>Alopecurus myosuroides</i>	(Blackgrass)
<i>Echinochloa crus-galli</i>	(Barnyardgrass)
<i>Lolium rigidum</i>	(Rigid Ryegrass)
<i>Avena fatua</i>	(Wild Oat)

Mechanism of resistance

Altered site of action

Dinitroaniline resistant biotypes of *Eleusine indica* were sixty times more resistant to the herbicide than their susceptible biotypes. It was shown the major α -tubulin gene of resistant biotypes has three base changes within the coding sequence. These base changes swap cytosine and thymine, most likely as a result of the spontaneous deamination of methylated cytosine. One of these base changes causes an amino-acid change in the protein: normal threonine at position 239 is changed to isoleucine. (Anthony *et al.*, 1998)

EiStua1 cDNA/genomicDNA Protein	<p style="text-align: center;">715</p> <p>GTCATTTCATCACTGACAGCCTCTCTGAGGTTCT</p> <p>-V--I-- S--S-- L--T-- A--S--L- -R--F-</p> <p style="text-align: center;">239</p>
EiRuta1 cDNA/genomicDNA Protein	<p style="text-align: center;">715</p> <p>GTCATTTCATCACTGATAGCCTCTCTGAGGTTCT</p> <p>-V--I-- S--S-- L-- I-- A--S--L- -R--F-</p> <p style="text-align: center;">239</p>

A single base mutation results in an amino-acid difference between the goose grass major α -tubulin gene from the sensitive biotypes (EiStua1) and from the resistant biotype (EiRuta1).

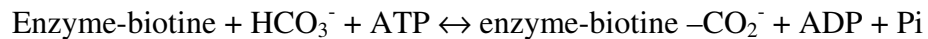
Normally the Thr239 in α -tubulin is positioned at the end of long central helix; thus it is close to the site that interacts with β -monomer of the next dimer in the microtubule protofilament. So replacing threonine with isoleucine either disturbs the herbicide binding site of tubulin so that the herbicide binds up to 60 times more weakly, or causes an increase in stability of the dimer-dimer interaction 60 folds.

5.4 Acetyl CoA carboxylase (ACCase)

ACCase is a multifunctional, biotinylated protein located in stroma of plastids. It catalyzes the ATP dependent carboxylation of Acetyl CoA to form malonyl CoA. Malonyl CoA is the precursor of fatty acids (Fig. 9).

ACCase catalyses two partial reaction occurring at two different sites.

(a) Reaction at carboxylation site



(b) Reaction at carboxytransferase site



Enzyme with biotine prosthetic group serves as a mobile carboxyl carrier between the two sites (Gronwald, 1991).

Acetyl CoA carboxylase inhibitors

Chemical families

Aryloxyphenoxypropionates (AOPP)

Cyclohexanediones (CHD)

Herbicides

Clodinafop, diclofop, fenoxaprop

Sethoxydim, cycloxadim, clethodim

AOPPs and CHDs are known as ‘fops’ and ‘dime’ respectively. Both are foliage active, systemic and used for the control of annual and perennial grasses in broadleaf crops and in certain cereals, hence known as graminicides.

The selectivity in case of dicots is based on low sensitivity of dicot ACCase, and in case of cereals selectivity could be attributed to an enhanced herbicide detoxification.

In susceptible grasses AOPPs and CHDs are linear, noncompetitive inhibitors of grass ACCase for all 3 substrates (Mg, ATP, HCO_3^- and acetyl CoA) (Burton *et al.*, 1991). As a result the carboxylation of acetyl COA is prevented and hence fatty acid synthesis is hampered.

Resistance to ACCase inhibitors

First reported case of resistance was in *Lolium rigidum* from Australia. Resistance was noted in fields where herbicide was used for 4 consecutive years (Heap and Knight, 1982). Till recently 27 weed species have reported resistance against these herbicide group (Heap, 2002)

Some resistant species are:

<i>Avena fatua</i>	-	(Wild oat)
<i>Digitaria sanguinalis</i>	-	(Large crabgrass)
<i>Echinochloa crusgali</i>	-	(Barnyard grass)
<i>Echinochloa colona</i>	-	(Jungle rice)
<i>Lolium spp.</i>	-	(Rye grass)

Mechanism of resistance of ACCase inhibitors

(a) Presence of tolerant form of ACCase (alteration of target site enzyme)

In majority of weed biotypes, resistance to ACCase inhibitors is conferred by reduced sensitivity to these herbicides.

In resistant biotypes of *Lolium multiflorum* resistance is conferred by tolerant form of ACCase. ACCase activity measured in extracts from etiolated shoots of the resistant biotype is 28 fold more tolerant to dicloflop than that from susceptible biotypes (Gronwald *et al.*, 1992). In the same experiment it is also shown that the resistant biotypes were approximately 130 times more tolerant than susceptible biotypes (Table 3).

This target site-based resistance is associated with a mutation of the nuclear gene encoding the ACCase I isoform (DePrado, 2000). (In grasses two isoforms of dimeric multifunctional ACCase is present- ACCase-I and ACCase-II. Of these, ACCase-I is the predominant isoform, it is plastid localized and is highly susceptible to graminicides. In contrast, the multifunctional ACCase-II isoform represents a smaller fraction of total ACCase, it is extra plastidic and is resistant to graminicides).

Based on I_{50} values (the amount of herbicide required to inactivate 50 % of an enzyme), ACCase of resistant accessions of *Setaria faberi* was 4.8, 10.6 and 319 fold resistant to clethodim, fluazifop and sethoxydim and *Digitaria sanguinalis* was 5.8, 10.3 and 66 fold resistant compared to susceptible accessions. This clearly

indicated that the resistance to ACCase inhibitors in these accessions resulted from an altered ACCase enzyme that confers a very high level of resistance to sethoxydim (Volenberg and Stoltengerg, 2002).

(b) Detoxification mechanism as in wheat

In resistant biotypes diclofop methyl is rapidly hydrolyzed to form toxic diclofop, it is then irreversibly detoxified by arylhydroxylation in presence of cytochrome P450 monooxygenase to form ring OH diclofop, which is in turn rapidly conjugated to form herbicidally inactive O-glucoside (Fig. 10; Romano *et al.*, 1993).

(c) Overproduction of ACCase

Though ACCase of both sensitive and resistant biotypes of Johnsongrass were having the same I_{50} value, the specific activity of ACCase in resistant biotypes was found to be 2 to 3 times greater than that of the susceptible biotypes which inturn confers them resistance (Bradely *et al.*, 2001).

5.5 Acetohydroxyacid synthase (AHAS)/ Acetolactosynthase (ALS)

AHAS/ALS is the first enzyme common to biosynthesis of branched chain amino acids leucin, valine and isoleucine (Stidham, 1991)

The enzyme catalyzes 2 parallel reactions

1. Conjugation of ketobutyrate with pyruvate to form acetohydroxybutyrate (hence called AHAS).
2. Conjugation of 2 molecules of pyruvate to form acetolactate (hence called ALS).

AHAS /ALS inhibitors

Chemical family	Herbicide
Sulfonylureas	Chlorosulfuron, Sulfosulfuron
Imidazolinones	Imazapyr
Triazolopyrimidines	Diclosulam, flumetsulam, metosulam
Pyrimidinyl(thio)benzoate	Pyriminobac-methyl, bispyribac, pyriftalid

These herbicides molecules when present in the system will bind with AHAS/ ALS and make the enzyme inactive. So the synthesis of valine, isoleucine and leucine will not take place and plant suffers (Stidham, 1991). Due to this phloem transport in the plant is hampered (Hall and Devine, 1993).

Resistance to AHAS/ ALS

First resistance was spotted in *Lactuca serriola*. Resistance was reported from fields where herbicide was used continuously for 5 years (Eberlein *et al.*, 1999). Till recently 70 weed species have reported resistance against this group of herbicides (Heap, 2002)

Species in which resistance has been spotted are :

<i>Amaranthus</i> sp.	(Pigweed)
<i>Avena fatua</i>	(Wild oat)
<i>Conyza</i> sp.	
<i>Eleusine indica</i>	(Goose grass)
<i>Lolium</i> sp.	(Rye grass)

Mechanism of resistance to ALS inhibitor

a) Due to less sulfonylurea sensitive ALS enzyme

This was observed in *Kochia scoparia*. Resistant biotypes of Kochia were observed in fields that have received 5 application of chlorosulfuron for a 5 year period. The resistant species needed more than 350 fold post emergent rate than susceptible type (dry and fresh weight were criteria).

Metabolism study revealed that the detoxification of the herbicide as observed in wheat was not the factor that conferred the R biotypes of Kochia resistance of sulfonylureas.

The inhibition of ALS activity from susceptible and resistant Kochia by chlorosulfuron was studied. At the highest concentration of 2.8 μM chlorosulfuron, the ALS activity from the susceptible biotype was completely inhibited where as ALS from resistant Kochia still retained 30% activity (Fig.11).

The I_{50} value for chlorosulfuron with susceptible and resistant ALS enzyme was 22 and 400 μM respectively (Saari *et al.*, 1990).

In common chickweed (*Stellaria media*), perennial ryegrass (*Lolium peresine*) and Russian thistle (*Salsola iberica*) the resistance against ALS inhibitors was due to a less sensitive ALS enzyme (Saari *et al.*, 1992).

Biochemical and physiological effects of target site resistance to herbicides inhibiting ALS were evaluated using sulfonylurea resistant and susceptible lines for *Lactuca sativa*. Sequence data suggest that resistance in *L. sativa* is conferred by a single point mutation that encodes a proline₁₉₇ to histidine substitution in Domain A of ALS protein (Eberlein, 1999).

Similarly several point mutations within the gene encoding ALS can result in herbicide resistant biotypes. Till recently 5 conserved amino acids have been identified in ALS that on substitution can confer resistance to ALS inhibitors (Table-4; Tranel and Wright, 2002).

(b) Due to rapid metabolic inactivation of herbicide

It was observed in *Lolium rigidum* (Rigid ryegrass). Based on experimental evidence, a proposed pathway for degradation of chlorosulfuron was given by Cotterman (1992) (Fig.12).

This was further corroborated by studies which showed that during the first 6 hr, radioactivity in the glucose conjugate increased as percentage of total radioactivity extracted from both root and shoot of both resistant and susceptible *Lolium rigidum*. However, the percentage glucose conjugate increased more rapidly and reached a higher level in resistant than in susceptible biotype (Fig. 13)

Barnyard grass is tolerant to primisulfuron because it can rapidly metabolize the herbicide. Studies showed that the pyrimidine side of compound is the site of metabolic activity. Hydroxylation followed by glycosylation is considered as the mechanism of metabolism (Neighbors and Privalle, 1990).

6. Isoproturon resistance in Phalaris minor

Isoproturon was used in India since early 1980's for the control of *Phalaris minor* in wheat fields. Isoproturon is a 'tailor-made' herbicide for Indian farmers for its flexibility in application as pre-emergence or post emergence, apply through sand urea soil etc. Also it controls wide variety of weeds. Resistance in *Phalaris minor* was reported by Malik and Singh (1995). Resistance was observed in field where isoproturon was used for over 10 years. Due to resistance the control of *P. minor* dropped from 78% to 21% in a time span of 3 years (1990-1993). This is the most serious case of herbicide resistance in the world, which may cause 30-90 per cent reduction in wheat yield and a total crop failure under heavy infestation. (Malik and Singh, 1995).

Resistance mechanism

It is thought that this resistant *P. minor* biotype is degrading the isoproturon through the same metabolic pathway as that in wheat (degradation via N-dealkylation and ring alkyl oxidation by NADPH-cytochrome p-450 monooxygenase) (Singh, 1999).

Long term approaches to manage resistant Phalaris minor

a) Exhaustion of soil seed bank

It has been reported that 150 plants of *P. minor*/m² can cause 30 % yield loss in wheat. Weed emergence ranging from 2000-3000 seedlings/m² is a common feature in problem areas. So any approach for successful control of weed must aim at reducing the seed load in the soil. The most effective method is to go for stale-seedbed technique.

b) Alternate crop and cropping system

Toria, barley, fodder oats and berseem reduces *P. minor* population due to their faster canopy cover and change in their dates of planting (Yaduraju, 1999). The incidence of isoproturon resistance in *P. minor* was lower in rice wheat sequence when it was rotated to incorporate other crops in cropping pattern (Malik and Singh, 1995) (Fig.14).

c) Changes in planting of wheat and scheduling of first irrigation

Germination of *P. minor* is greater under decreased temperature and higher moisture conditions. Delay in sowing of wheat from November to late December may favour *P. minor* more than wheat (Singh, 1999). So early planting of wheat (October end to first week of November) is effective in reducing *P. minor* emergence. This can be more effective with first irrigation being delayed by a week or two.

d) Herbicide mixtures and rotation

Alternate herbicides proposed to control the isoproturon resistant *P. minor* include clodinafop, fenoxaprop, flufenacet, sulfosulfuron and tralkoxydim (Yaduraju, 1999) (Table 5). Of these, except for sulfosulfuron none of the other herbicides can check broad leaved weeds. Thus continued use of these herbicides will shift the weed flora in favour of dicot weeds, unless mixed with other broad leaved herbicides.

e) Integrated weed management practices

Integration of chemical, cultural and mechanical methods of weed control must be adopted wherever they are feasible.

7. Cross Resistance

It is the phenomenon whereby, following exposure to a herbicide, weed population evolve resistance to herbicides from chemical classes to which it has never been exposed.

Negative cross- resistance / collateral sensitivity

It is the phenomenon whereby individual resistant to one chemical or chemical family of herbicides have a higher sensitivity to other herbicides (Table 6).

Table 6. Negative cross- resistance exerted by selected herbicides on *Echinocloa crusgalli*

Chemical family	Herbicide	GR ₅₀ (kg/ha)		
		Susceptible	Resistant	RI ₅₀
Triazines	Atrazine	0.60	32.00	53.33
AOPP	Fluazifop butyl	0.21	0.01	0.03
CHD	Sethoxydim	0.09	0.04	0.47

(Gadamaski *et al.*, 2000)

GR₅₀ – rate of herbicide that causes a 50% reduction of added plant growth

RI₅₀ – GR₅₀ of resistant biotype/ GR₅₀ of susceptible biotype

Here biotype of *E. crusgalli* which is resistant to triazine (53 times more resistant than susceptible) is 33 and 2 times more sensitive to fluazifop and sethoxidim respectively.

8. Strategies for managing and preventing herbicide resistant weeds

The management practices must be primarily focused on reducing the selection pressure.

a) Rotation of herbicides with different mode of action

Use of the same herbicide or different herbicides with the same mode of action will exasperate the problem of resistant weeds. So adopt rotation of herbicides with different mode of action.

b) Use of herbicide mixtures

Herbicide mixtures are presently employed to broaden the spectrum of activity. But resistant management requires both the components of mixture control the same spectrum of weeds so that the weeds resistant to vulnerable herbicides will be destroyed by the mixing partner, or at least be rendered relatively comfit compared to the wild type.

Evolution of target-site resistance to both vulnerable and partner herbicide, though possible when mixtures are used, are much delayed. The following reasoning based on compound resistance has been used to support this supposition. If frequency of individual resistant to each component of a pesticide in a mixture is independent in susceptible species, then joint probability of evolution of co-resistance to both herbicide in one individual equals the product of the probabilities of resistance for each partner. Thus if a weed has a natural mutation frequency of 10^{-5} for resistance to vulnerable herbicide and 10^{-10} to mixing partner having different target site and if genes for resistance are inherited independently of each other, then the joint probability of resistance to both the herbicide in an individual will be 10^{-15} which is very rare (Wrubel and Gressel, 1994).

Characteristics of effective mixing partner (Wrubel and Gressel, 1994)

- a) It must kill same spectrum of weeds as the vulnerable partner.
- b) It must have a mode of action different from that of vulnerable partner.
- c) Both must have some effectiveness in weed control: It may not be helpful, if at the rate used, the mixing partner kill 75% of the weeds and vulnerable kills 95% unless the 20% remaining are severely inhibited such that they have less reproductive capacity than wild type. Otherwise resistance could quickly evolve in remaining 20% of weeds.
- d) Both components must have similar persistence: Otherwise there will be period when only the vulnerable one is present and since the weeds have many flushes of germination during a cropping season the target weeds will not be exposed to mixture.
- e) The mixing partner should not be degraded in the same manner as vulnerable partner.
- f) It will be an added advantage if the mixing partner posses negative cross resistance i.e. where individuals resistant to vulnerable herbicides are more susceptible than the wild type to the mixing partner.

C. Use of herbicides when only necessary

Indiscriminate use of herbicide like pre-emergent application of herbicide must be avoided wherever there is an option for selective post-emergent herbicide. Adoption of herbicide resistant crops can also help us in this respect.

D. Control of weed escapes and sanitation of equipment to prevent spread of resistant weeds

Weed escapes must be prevented by adopting optimum dose, time and method of application of herbicides. Dissemination of resistant weed must be prevented.

E. Use of herbicides with short residual life

If we are using herbicides having long residual life then the selection pressure will be more. So use herbicides having short residual life. Also, if we are increasing the dose of herbicide the residual period will be high. So use the recommended dose.

F. Scout the fields for resistant weeds

Before and after herbicide spray take walk through the fields observing the weed flora. If, after the application of herbicide, you are running into a patch of weed escape, destroy it.

G. Adopt integrated weed control practices

H. Adopt crop rotation

Crop rotation usually means using diverse herbicide program, making it difficult for resistant weed to increase.

Assessment of risk of developing herbicide resistance based on management options followed (Valverde *et al.*, 2000)

Management options	Risk of resistance		
	Low	Moderate	High
Herbicide mix or rotation in cropping system	>2 modes of action	2 modes of action	1 mode of action
Weed control in cropping system	Cultural, mechanical and chemical	Cultural and chemical	Chemical only
Use of same mode of action per season	Once	More than once	Many times
Cropping system	Full rotation	Limited rotation	No rotation
Resistance status to mode of action	Unknown	Limited	Common
Weed infestation	Low	Moderate	High
Control in last three years	Good	Declining	Poor

9. Conclusion

Herbicide resistance is evolution in action. Through the employment of herbicides to control weeds in cultivated fields, we were moving against Nature's laws of biodiversity. The Nature retorted with herbicide resistant weeds. But our battle against the pest is not inevitably the one we are going to loose, it must be fought as a complex war with all available weapons. Commonsense and laws of nature tell us this is a game we can never entirely win. Yet there is no reason to believe that we cannot maintain a satisfactory level of crop protection. System that involves the use of herbicides should always incorporate practices to prevent and manage for eventual occurrence of resistance. We must keep available all the tool we ever had, including the hoe, while we continue searching for a new and better answer.

10. Tables

Table1. Development of resistance to different herbicides

Herbicides	Year of introduction	Year resistance first reported
2, 4-D	1945	1963
Dalapon	1953	1962
Atrazine	1958	1968
Piclorom	1963	1988
Trifluralin	1963	1973
Diclofop	1977	1982
Trillate	1962	1987
Chlorosulfuron	1982	1987

(Le Baron, 1991)

Table 2: Herbicide resistant weeds summary table

Herbicide Group	Mode of Action	HRAC Group	Example Herbicide	Total
ALS inhibitors	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)	B	Chlorsulfuron	70
Photosystem II inhibitors	Inhibition of photosynthesis at photosystem II	C1	Atrazine	63
ACCase inhibitors	Inhibition of acetyl CoA carboxylase (ACCase)	A	Diclofop-methyl	27
Bipyridiliums	Photosystem-I-electron diversion	D	Paraquat	21
Synthetic Auxins	Synthetic auxins (action like indoleacetic acid)	O	2,4-D	21
Ureas and amides	Inhibition of photosynthesis at photosystem II	C2	Chlorotoluron	20
Dinitroanilines and others	Microtubule assembly inhibition	K1	Trifluralin	10
Thiocarbamates and others	Inhibition of lipid synthesis - not ACCase inhibition	N	Triallate	6
Triazoles, ureas, isoxazolidiones	Bleaching: Inhibition of carotenoid biosynthesis (unknown target)	F3	Amitrole	4
Glycines	Inhibition of EPSP synthase	G	Glyphosate	4
Chloroacetamides and others	Inhibition of cell division (Inhibition of very long chain fatty acids)	K3	Butachlor	2
Nitriles and others	Inhibition of photosynthesis at photosystem II	C3	Bromoxynil	1
Carotenoid biosynthesis inhibitors	Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS)	F1	Flurtamone	1
Mitosis inhibitors	Inhibition of mitosis / microtubule polymerization inhibitor	K2	Propham	1
Organoarsenicals	Unknown	Z	MSMA	1
Arylamino propionic acids	Unknown	Z	Flamprop-methyl	1
Pyrazoliums	Unknown	Z	Difenzoquat	1
Total Number of Unique Herbicide Resistant Biotypes				254

(Heap, 2002)

(accessed on February 15, 2002)

Table 3. Effect of graminicides on ACCase activity isolated from leaf tissue of resistant and susceptible biotypes of *L. multiflorum*

Herbicides	I ₅₀ (μM) ^b		
	Susceptible	Resistant	R/S ^a
Aryhoyphenoxypropionic acid			
Diclofop	0.3 ± 0.1	8.3 ± 1.2	27.7
Haloxypop	1.8 ± 0.1	16.4 ± 1.2	9.1
Quizalofop	0.07 ± 0.05	0.7 ± 0.2	10.0

^a Ratio of I₅₀ values for resistant and susceptible biotypes. (Gronwald, 1992)

^b Values represent means ± SD.

Table 4. Amino-acid substitution that confer herbicide resistance^a

Amino-acid residue and number ^b	Substitution conferring resistance	Weed species	Resistance ^c	
			SU	IMI
Ala 122	Thr	<i>Xanthium strumarium</i>	S	R
	Thr	<i>Amaranthus hybridus</i>	S	R
	Thr	<i>Solanum ptycanthum</i>	S	R
Pro 197	His	<i>Lactuca serriola</i>	R	R
	Thr	<i>Kochia scoparia</i>	R	S
	Arg	<i>Kochia scoparia</i>	R	ND
	Leu	<i>Kochia scoparia</i>	R	ND
	Gln	<i>Kochia scoparia</i>	R	ND
	Ser	<i>Kochia scoparia</i>	R	ND
	Ala	<i>Kochia scoparia</i>	R	ND
	Ala	<i>Brassica tournefortii</i>	R	S
	Ile	<i>Sisymbrium orientale</i>	R	R
Ala 205	Leu	<i>Amaranthus retroflexus</i>	R	R
	Val	<i>Xanthium strumarium</i>	r	r
Trp 574	Leu	<i>Xanthium strumarium</i>	R	R
	Leu	<i>Amaranthus rudis</i>	R	R
	Leu	<i>Amaranthus hybridus</i>	R	R
	Leu	<i>Kochia scoparia</i>	R	R
	Leu	<i>Sisymbrium orientale</i>	R	R
	Leu	<i>Ambrosia artemisiifolia</i>	R	R
	Leu	<i>Ambrosia trifida</i>	R	R
Ser 653	Thr	<i>Amaranthus powelli</i>	S	R
	Thr	<i>Amaranthus retroflexus</i>	S	R
	Asn	<i>Amaranthus rudis</i>	S	R
	Thr	<i>Amaranthus rudis</i>	S	R

^a Abbreviations: ALS, acetolactate synthase; ND, not determined.

(Tranel and Wright, 2002)

^b Amino-acid number is standardized to *Arabidopsis thaliana* sequence.

^c S, r and R indicate little or no resistance (sensitive), moderate resistance (<10-fold relative to S biotype), and high resistance (>10-fold) respectively to sulfonylurea (SU) or imidazolinone (IMI).

Table 5. New herbicides for controlling isoproturon –resistant *P. minor*

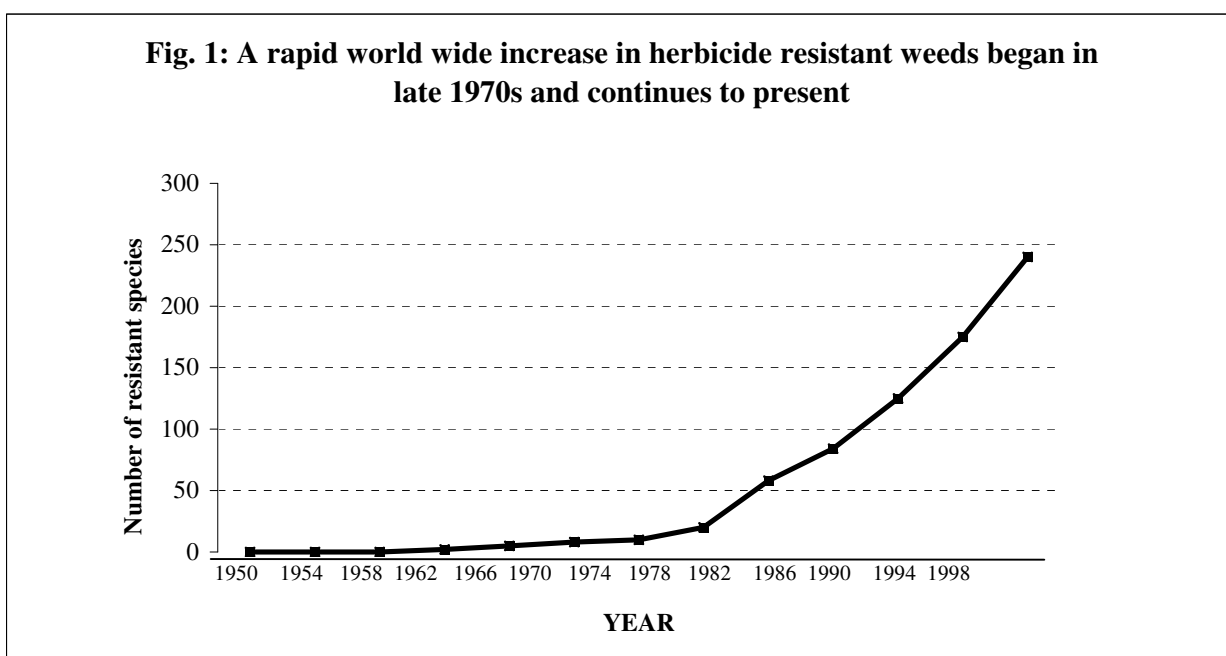
Herbicide	Dose (g/ha)	Application (WAS)
Clodinafop	40-60	4-5
Fenoxaprop	100-120	4-6
Flufenacet	180-300	0-2
Sulfosulfuron	25-30	4-5
Tralkoxydim	350-400	4-5

WAS- Weeks after sowing

(Yaduraju, 1999)

11. Figures

Some of the figures are not included due to the copyright rules. However the reader can access them from respective references cited.



Application of the same herbicide for many years

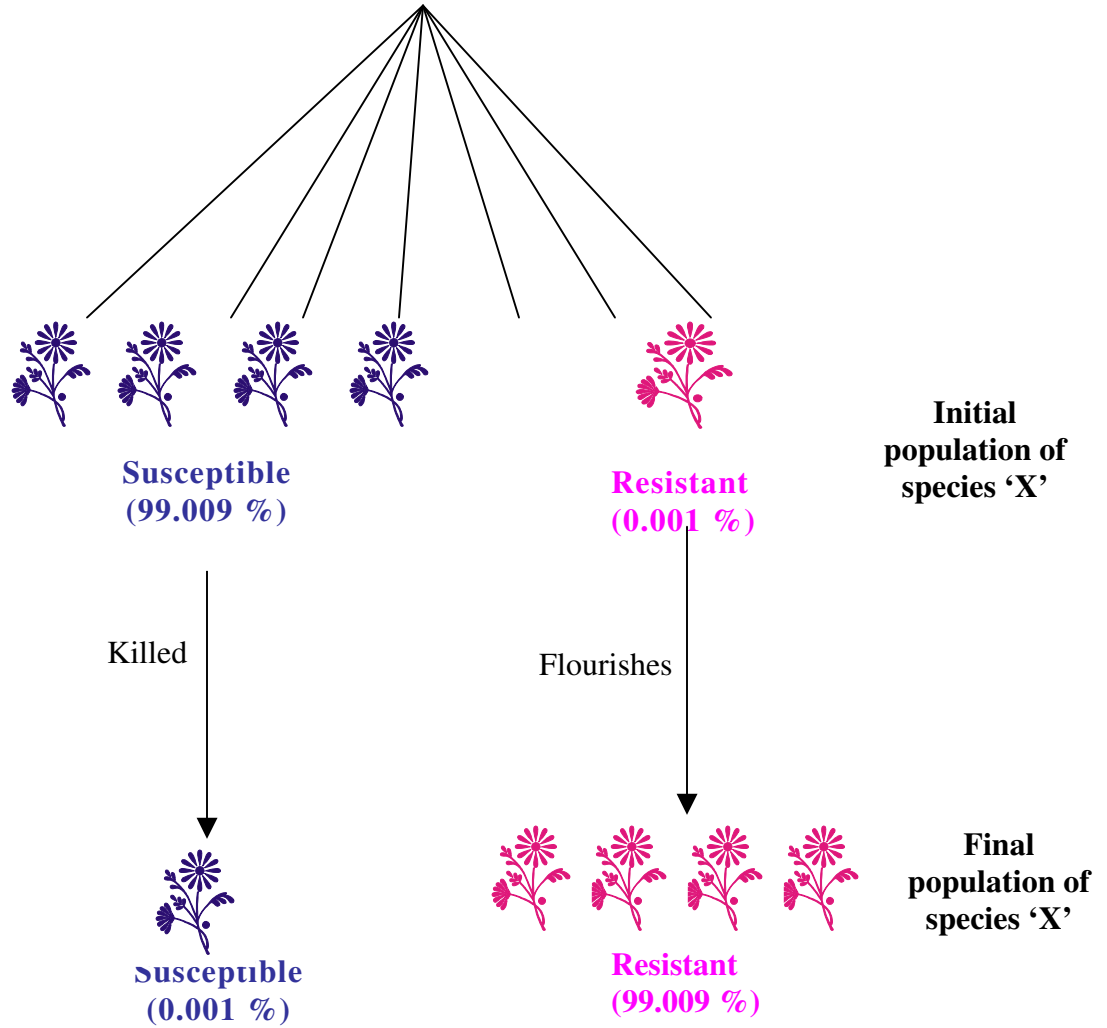


Fig. 2: The evolution of herbicide resistance (percent values are arbitrary)

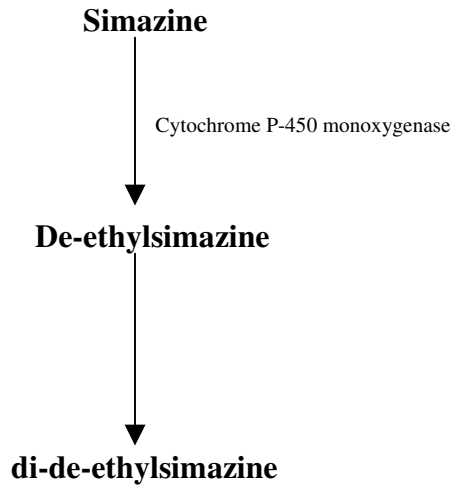
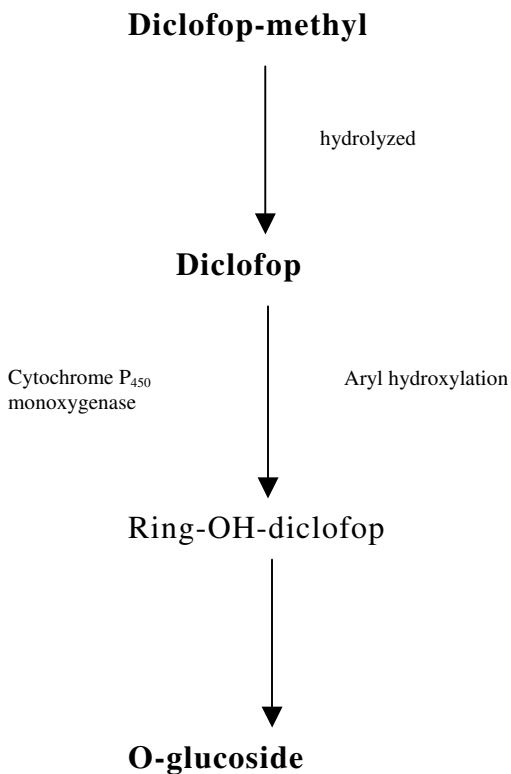


Fig. 5: Metabolic de-toxification of simazine in resistant *Lolium rigidum*.
(Burnet *et al.*, 1993)

Fig. 10: Detoxification mechanism of diclofop as in *Lolium perenne*
(Romano *et al.*, 1993)



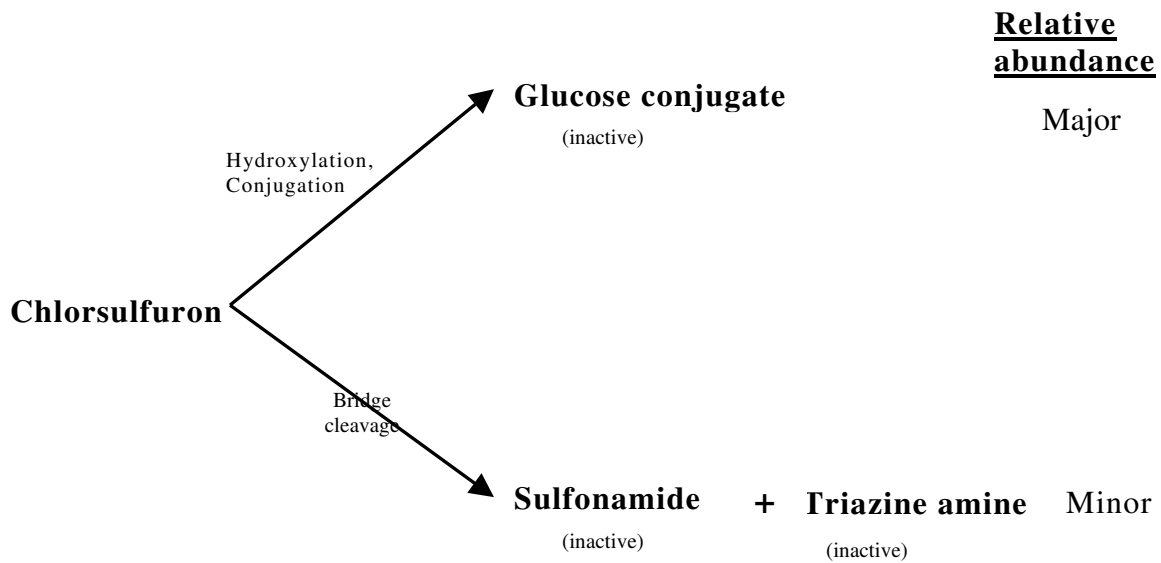
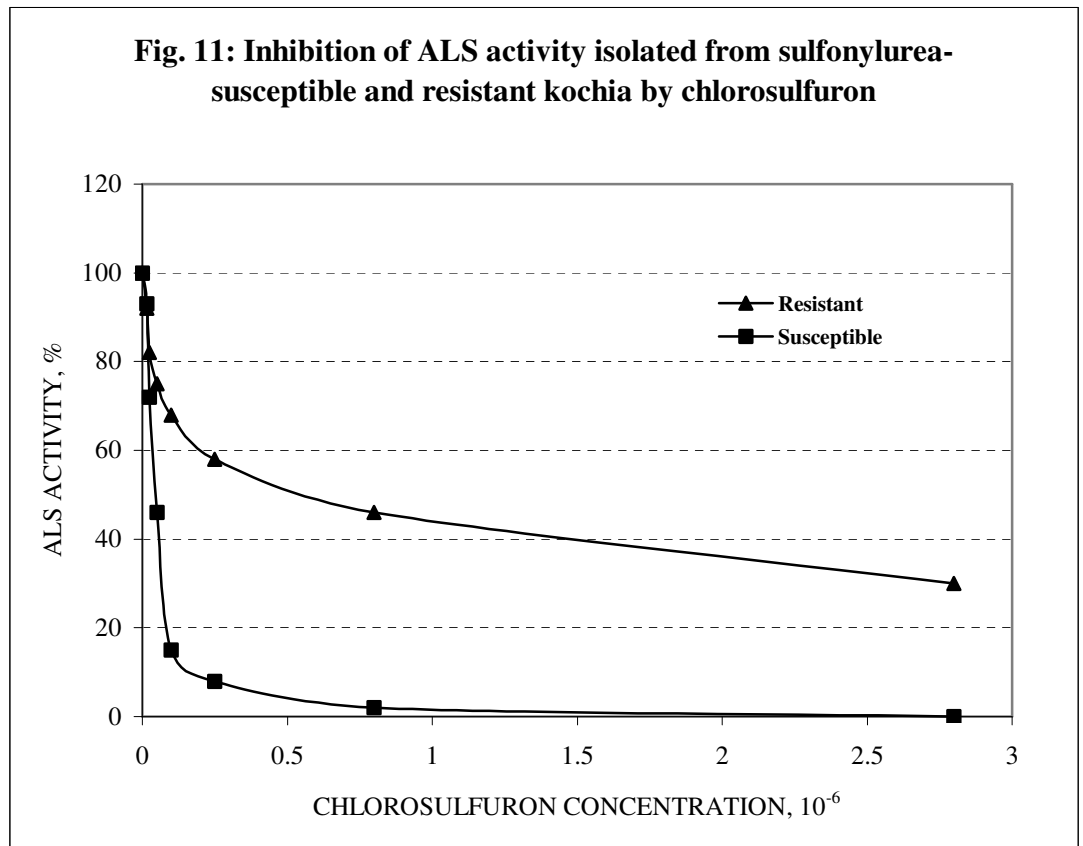


Fig.12: Metabolic pathway of chlorosulfuron inactivation by susceptible and resistant *Lolium rigidum*. (Cotterman, 1992)

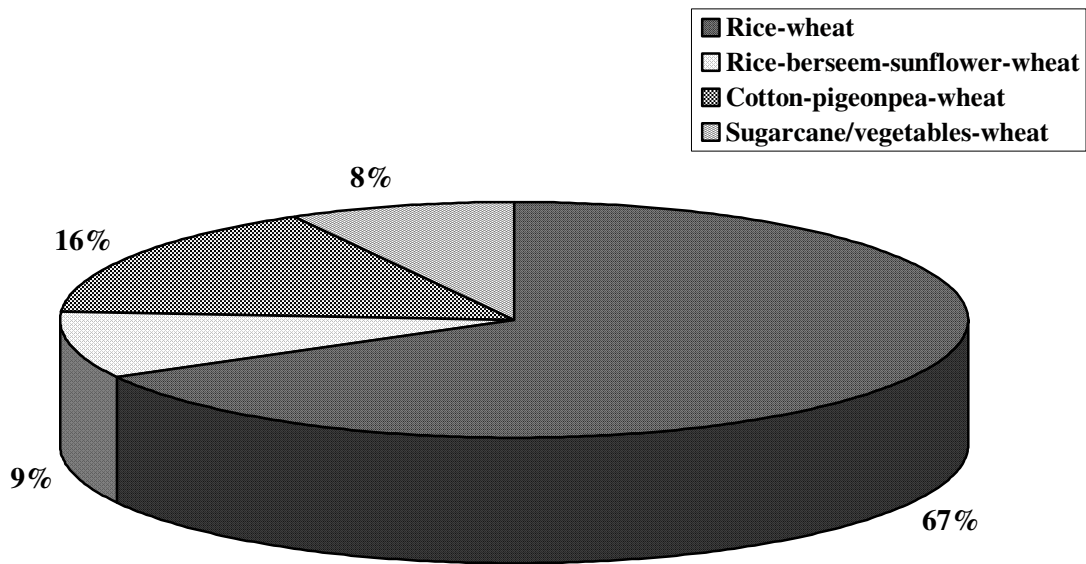


Fig.14: Incidence of *Phalaris minor* resistance in different cropping systems
(Malik and Singh, 1995)

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