Mechanisms of Insecticide Resistance

Ronnie L. Byford, *Ph.D.*
Department of Entomology, Plant Pathology & Weed Science,
New Mexico State University,
Las Cruces, New Mexico 88003

Introduction

Resistance to insecticides is a problem of critical importance since it limits our ability to control pests of agriculture.[1,2] Resistance to one or more insecticides has evolved throughout the world and has been documented in more than 450 species of insects and mites, with costs of resistance estimated at $1 billion per year.[3]

Resistance has been traditionally defined as "the developed ability in a strain of insects to survive doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species". This developed ability is the result of selecting individuals with a heritable capacity to withstand the toxicant; and is not due to the action of the insecticide on the individual insect. Thus, the development of resistance is dependent on genetic variability already present in a population or arising during the period of selection. As a result of the practical and economic implications of these genetic changes, Sawicki[4] proposed the following: "Resistance marks a genetic change in response to selection by toxicants that may impair control in the field."

Brief History of Insecticide Resistance

The first scientific documentation of insecticide resistance was that of Melander in 1914 who reported resistance of the San Jose' scale, *Quadraspidiotus perniciosus* (Comstock), to lime-sulphur.[5] The number of documented cases of insecticide resistance gradually increased over the next 30 years and by 1946 insecticide resistance was reported in a total of 11 species of Arthropods including the cattle tick, *Boophilus microplus* Cannestrini, and the blue tick, *B. decoloratus* (Koch).[6]

Following the introduction of DDT after World War II, insecticide resistance began to receive increasing attention. Resistant strains of the house fly, *Musca domestica* L., appeared almost simultaneously in Sweden and Denmark in 1946. The mosquitoes *Culex pipiens* L. and *Aedes sollicitans* (Walker) were documented as resistant in Italy and Florida in 1947. The bed bug, *Cimex lectularius* Linneaus was reported to be resistant in Hawaii in 1947 and the human body louse, *Pediculus humanus humanus* L., was identified as resistant in Korea and Japan in 1951.[6,7]

As a result of the introduction of new insecticides, the number of documented cases of insecticide resistance has increased at an exponential rate.[3] Resistance was reported in 224 species in 1970, 364 in 1976, and 447 in 1984 (Table 1). Available records, updated through 1978, are summarized in Table 2 according to the taxonomic order of the species, whether of medical or agricultural importance, and the chemical class of the insecti-

Table 1 - Development of insecticide resistance

Year	Resistant Species	Pesticide group*				
		DDT	Cyclod	OP	Carb	Pyr
1938	7	----	----	----	----	----
1948	14	1	----	----	----	----
1956	69	36	24	17	----	----
1970	224	98	140	54	4	3
1976	364	203	225	147	36	7
1980	428	229	269	200	51	22
1984	447	223	276	212	64	32

Adapted from Metcalf 1989
*Cyclod = Cyclodienes, OP = Organophosphates, Carb = Carbamates, Pyr = Ppyrethroids.

Table 2 - Number of species of Arthropods resistant to insecticides

Order	Pesticide group*					Importance+		
	DDT	Cyclod	OP	Carb	Other	Med	Agr	Total
Acarina	17	15	41	6	28	15	38	53
Anoplura	4	3	2	1	----	5	----	5
Coleoptera	24	56	28	10	21	----	64	64
Dermaptera	1	----	----	----	----	----	1	1
Diptera	91	100	50	7	5	115	27	142
Ephemeroptera	2	----	----	----	----	----	2	2
Hemiptera	20	27	34	7	5	5	56	61
Hymenoptera	1	2	----	----	----	----	3	3
Lepidoptera	41	40	31	15	4	----	64	64
Mallophaga	----	3	----	----	----	3	----	3
Orthoptera	2	3	1	1	1	3	----	3
Siphonaptera	6	5	1	----	----	6	----	6
Thysanoptera	3	5	1	----	2	----	7	7
Totals	212	259	189	47	66	152	262	414

Adapted from Georghiou 1980
*Cyclod = Cyclodiene, OP = Organophosphate, Carb = Carbamate.
+Med = Medical, Agr = Agricultural.

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cide. It is now evident that resistance involves at least 447 species of insects and mites and that the most significant increases during the past few years have occurred in species of agricultural importance, especially in the Orders Lepidoptera and Acarina.

Early examples of insecticide resistance were identified primarily in insect vectors of human diseases due to the widespread use of DDT and the cyclodienes in vector control programs. However, by 1970 insecticide resistance was documented in 118 pests of crop, forest, and stored products as compared with 166 pests of humans or animals.[8] By 1980, resistance was established in 260 agricultural pests compared with 168 pests of humans and animals.[9,10]

As each new class of insecticide was introduced, the rate of development of resistant species has followed a similar pattern of exponential growth. This is best characterized by the average doubling time in the number of resistant pests (Table 3). The doubling time for the numbers of resistant species has steadily declined with each new class of insecticide being introduced;[10] DDT/methoxychlor 6.3 years, lindane/cyclodienes 5.0 years, organophosphates 4.0 years, carbamates 2.5 years, and pyrethroids 2.0 years.

Table 3 - Rates of development of insecticide resistant species*

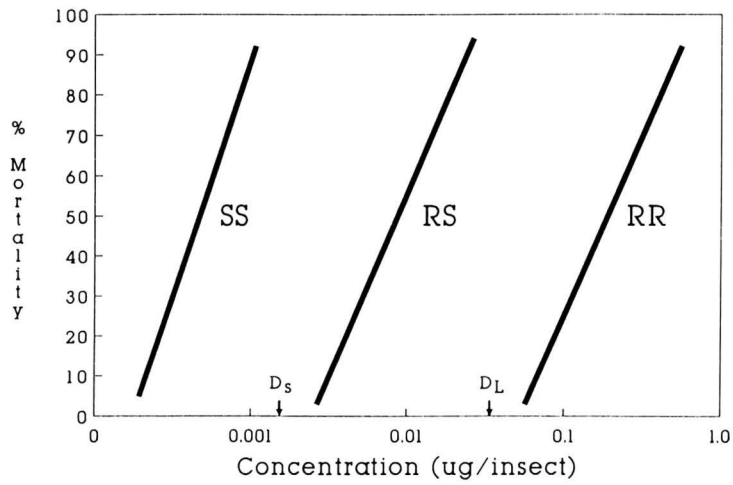
Number of pests	Year resistance attained				
	DDT/ Methoxy	Cyclod	OP	Carb	Pyr
5	1951	1954	1959	1971	1976
10	1952	1955	1962	1972	1979
20	1955	1956	1964	1974	1980
40	1960	1959	1968	1977	----
80	1968	1965	1972	----	1985
160	1974	1971	1976	----	----
Years to double	6.3	5.0	4.0	2.5	2.0

Adapted from Metcalf 1989
*Methoxy = Methoxychlor, Cyclod = Cyclodienes, OP = Organophosphate, Carb = Carbamate, Pyr = Pyrethroid.

Genetic Factors Influencing the Evolution of Resistance

The development of resistance is a product of the persistence of resistant (R) genes in the species genome and the interaction of these genes through a variety of resistance mechanisms that affect both the detoxification of and the target site sensitivity to various classes of insecticides. Insecticide resistance is the result of random mutation that establishes an R-allele in the natural population of the species.[11] Widespread application of the insecticide propagates the R-allele and it becomes dispersed throughout the population. As the R-allele becomes sufficiently common, the effectiveness of the insecticide is reduced. Resistant populations evolve rapidly if resistance is dominant, slower if it is recessive. However, the expression of dominance is dependent upon the dose applied.[12,13] Regression lines presented in Fig. 1 illustrate the three genotypes (SS, RS and RR) one might encounter when resistance is monogenic. Application of a small dose of an insecticide (D_S) results in the survival of heterozygotes and the R-allele is functionally dominant. If a large dose is applied (D_L), heterozygotes are subjected to a lethal concentration and the R-allele is functionally recessive.

Figure 1. Theoretical regression lines illustrating discrimination between three genotypes (SS, RS, RR) and dominance of resistance. Resistance is functionally dominant with a small dose (D_S), resistance is functionally recessive with a large dose (D_L).



Mechanisms of Insecticide Resistance

We currently recognize that a combination of biochemical, physiological, and genetic adaptations influence the development of resistance to insecticides.[6,7,14] A listing of insecticide resistance mechanisms to various insecticides, and their relative importance are shown in Table 4. Simplistically, insects have three basic resistance mechanisms: reduced cuticular penetration, increased metabolic detoxification, and target site insensitivity. The selectivity of an insecticide for its target site is not absolute and these mechanisms may interact to confer a high degree of resistance; for example, reduced penetration may allow greater opportunity for detoxification of the insecticide.

Decreased penetration was first described as a resistance mechanism in the 1960's.[15-17] This mechanism usually confers only low levels (<3-fold) of resistance;[18] yet it does provide protection to a wide variety of insecticides.[19]

Metabolic resistance involves detoxification of the

Table 4 - Influence of different resistance inechanisms on various insecticides*

Mechanism	DDT	Cyclod	OP	Carb	Pyr
Insensitive AChe			+++	+++	
Kdr	+++				+++
Metabolism					
Hydrolases			+++	+	+
MFO	++		++	+++	+
GSH S			++		
DDT ase	+++				
Penetration	+	+	+		+
Cyclodiene R		+++			

Adapted from Oppenoorth 1985
*Cyclod = Cyclodienes, OP = Organophosphates, Carb = Carbamates, Pyr = Pyrethroids.

insecticide by enzymatic process including esterases, microsomal oxidases, glutathione transferases, and epoxide hydrolyses. Enhanced metabolism has been implicated as a major mechanism of resistance for all classes of insecticides except the chlorinated cyclodienes. The specific detoxification enzymes can be inhibited by synergists (compounds that enhance toxicity of an insecticide and are thought to inhibit certain types of metabolism) that may act to restore some of the effectiveness of the insecticide;[20-23] examples include: a) the DDT ase inhibitor for DDT, b) a carboxyesterase inhibitor for malathion, c) DEF for esterases hydrolyzing organophosphates, and d) PBO, a microsomal oxidase inhibitor, for carbamates and pyrethroids.

A totally different mechanism, involving alteration of the site of action of an insecticide, is common for organophosphates and carbamates. These insecticides exert their toxic effects by inhibiting acetylcholinesterase (AChE), thereby prolonging the residence time of acetylcholine at cholinergic synapses and producing hyperexcitation of cholinergic pathways. AChE that is less sensitive to inhibition by organophosphates and carbamates has been documented in resistant strains of insects, ticks, and mites.

The mechanism involving reduced neuronal sensitivity of DDT and the pyrethroid insecticides, is known as target site insensitivity or *kdr*. DDT and pyrethroids are capable of disrupting the normal function of many enzymes, neuroreceptors, and ion channels, but the toxic actions of these insecticides in insects are best explained by their action on the voltage-sensitive sodium channel of nerve membranes.[24]

Pyrethroid Resistance in *Haematobia Irritans* (L.)

The development of resistance of insects to insecticides has been intensively studied. The primary aim of these studies has been to find solutions to the problem of controlling resistant insects, but at the same time they attempt to obtain a better knowledge of the biochemical and physiological changes that underlie this phenomenon.

I now want to concentrate on a particular insect pest, the horn fly, *Haematobia irritans* (L.), because it represents a worst-case scenario for resistance development due to its biology and insecticide treatment regimen. The horn fly is an economically important haematophagous parasite of cattle throughout the United States. Control of this pest has depended exclusively on the use of insecticides. Difficulty controlling the horn fly was first recognized in the early 1960's (Table 5), when field efficacy of a 0.5% toxaphene spray was found to be reduced.[25] Similarly, resistance to fenchlorphos was reported in 1962 after intensive use of the insecticide in backrubbers for three consecutive years.[26] Horn fly resistance received very little attention during the next 15 years, although reports of control failure continued.

Table 5 - Insecticide resistance in the horn fly, *Haematobia irritans* (L.)

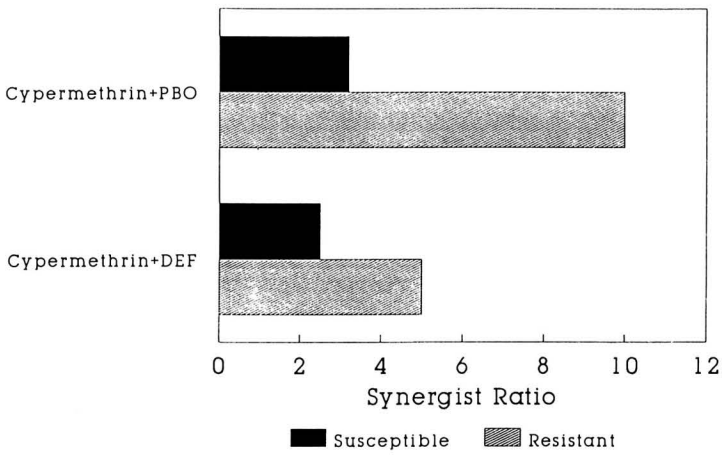
Class of Insecticide	Insecticide	Year	State
Chlorinated hydrocarbons	Toxaphene	1960	TX
	DDT	1961	TX
		1984	LA
	Methoxychlor	1965	LA
Organophosphates	Fenchlorphos	1962	LA
	Stirofos	1978	GA
Pyrethroids	Fenvalerate	1982	GA
		1983	LA
	Permethrin	1983	FLA
		1983	LA
	Flucythrinate	1983	GA
		1983	LA
	Cypermethrin	1984	LA
	Deltamethrin	1984	LA
	Cyhalothrin	1992	AL
		1992	LA

Sheppard[27] demonstrated that orally feeding stirofos over a period of 1.5 years resulted in the development of resistance in a population of horn flies in Georgia. The introduction of insecticide-impregnated cattle ear tags to control horn flies provided effective and season-long control.[28-34] Unfortunately, resistance soon

developed to the insecticides used in these ear tags. Control problems with the pyrethroid insecticide ear tags were reported when the tags had only been in use for 2-4 years. Pyrethroid resistance in the horn fly now represents one of the more established cases of insecticide resistance in the field. This resistance occurs throughout the southeastern and mid-western U.S., as well as in California and Hawaii.[35-38]

Although pyrethroid resistance in the horn fly has been widely documented, the mechanisms of resistance have received only limited study. One of the first studies of the mechanisms of horn fly resistance involved the use of the synergists PBO, an oxidase inhibitor, and DEF, an esterase inhibitor, to determine the possible role of metabolism in pyrethroid resistance.[39] The low level of synergism observed (< 8-fold; Fig. 2) suggested involvement of increased metabolism, but did not account for the relatively high degree (35-fold) of resistance displayed by this population.

Figure 2. Effects of synergists on cypermethrin toxicity to horn flies.



In subsequent studies by Bull *et al.*[40] and Sparks *et al.*,[41] the rate of metabolism in pyrethroid resistance and susceptible horn flies was examined along with the penetration and excretion of pyrethroids. In a laboratory-selected population of horn flies with low levels of resistance, Bull *et al.*[40] did not detect any differences in rate of penetration, metabolism or excretion of permethrin. However, in a field-selected population, Sparks *et al.*[41] detected a higher rate of penetration and a faster metabolism (Table 6 and 7) of permethrin in pyrethroid resistant versus susceptible horn flies. Although the resistant horn flies were able to metabolize permethrin to a greater extent, the observed difference in metabolic capability was insufficient to account for the levels of resistance observed.

These synergism and metabolism studies along with the insecticide resistance pattern observed for the horn fly (i.e. cross-resistance to all pyrethroids, DDT, and methoxychlor; with little cross-resistance to the organophosphates and carbamates) suggests that the primary resistance mechanism in pyrethroid resistant horn flies is target site insensitivity or *kdr*.

Table 6 - Metabolism of topically applied ^{14}C-permethrin by pyrethroid-resistant and -susceptible horn flies

Time, h	Strain*	Avg % of dose (±SD) in indicated fraction				
		Total	Permethrin	2'/4-OH-permethrin	DCVA	Origin
1	S	47.4(2.2)a	30.7(3.0)a	3.8(0.6)a	4.8(0.9)a	14.0(3.4)a
	R	51.8(9.1)b	30.3(5.5)a	7.9(1.1)b	8.5(2.3)b	10.6(2.9)b
2	S	58.3(2.4)a	38.9(3.9)a	2.8(1.5)a	7.0(2.9)a	9.6(1.5)a
	R	70.7(5.3)b	41.5(5.7)a	6.8(1.4)b	5.6(1.9)a	15.3(3.2)b
6	S	71.3(4.4)a	32.2(3.3)a	4.1(0.7)a	6.5(3.5)a	27.3(2.9)a
	R	68.6(4.6)a	28.7(5.4)a	4.9(0.9)a	6.0(0.7)a	27.3(4.5)a

Sparks et al. 1990
*S= Susceptible, R= Resistant.
For each time point, pairs of data (susceptible and resistant) followed by a different letter are significantly different ($P < 0.05$; t test).

Table 7 - Penertration and internal accumulation of topically applied ^{14}C-permethrin in pyrethroid-resistant and -susceptible horn flies

Time, h	Strain*	Avg % of dose (±SD) in indicated fraction				
		Internal extract	External rinse	Flask extract	Flask cover	Unextractable radioactivity
1	S	47.4(2.2)a	44.7(2.9)a	6.9(1.5)a	0.1(0.1)a	0.8(0.2)a
	R	58.8(6.6)b	35.3(6.6)b	3.7(0.6)b	1.4(0.7)b	1.0(0.6)a
2	S	58.3(2.4)a	32.1(2.4)a	8.3(1.8)a	0.4(0.2)a	0.9(0.3)a
	R	70.7(5.3)b	18.1(5.1)b	4.9(1.6)b	2.6(3.2)a	1.7(0.6)a
6	S	71.3(4.4)a	15.8(4.6)a	11.3(1.2)a	0.3(0.2)a	1.2(0.2)a
	R	68.6(4.6)a	12.6(1.4)a	10.1(1.2)a	7.5(4.1)a	1.2(0.3)a

Sparks et al. 1990
*S = Susceptible, R = Resistant.
For each time point, pairs of data (susceptible and resistant) followed by a different letter are significantly different ($P < 0.05$; t test).

Because pyrethroids are known to affect the peripheral nerves, particularly sensory cell bodies and motor nerve terminals, Crosby *et al.*[42] conducted a larval bioassay to detect *kdr*-type resistance (Table 8). Their results detected high levels (42-fold) of *kdr*-resistance to the pyrethroids and DDT; but not to organophosphates; further documenting target site insensitivity as the major mechanism of pyrethroid resistance in the horn fly.

Whenever an entire pest population is intensively selected with an insecticide for several generations, resistance is expected to develop quickly. A variety of factors can influence the rate at which this resistance develops.[11,22,43-46] These authors described a number of operational conditions that, if present along with other genetic and biological factors, would ensure the rapid development of insecticide resistance (Table 9). In the case of horn fly control with pyrethroid-impregnated ear

Table 8 - Knockdown toxicities of insecticides to pyrethroid resistant and susceptible horn fly larvae

Compound	Population[a]	Total no. treated	Slope ± SE	KD_{50}(95% FL)[b] (μg/cm²)	Resistance ratio
Premethrin	S	260	1.71 ± 0.38	0.0052 (0.0035-0.0073)	----
	R	237	1.48 ± 0.19	0.2186 (0.1586-0.2922)	42.0
Fenvalerate	S	218	3.34 ± 0.34	0.0046 (0.0038-0.0056)	----
	R	277	1.76 ± 0.22	0.1292 (0.0946-0.0023)	28.1
Cyhalothrin	S	247	1.15 ± 0.20	0.0015 (0.0009-0.0023)	----
	R	195	2.22 ± 0.40	0.0438 (0.0337-0.0579)	29.2
DDT	S	293	1.77 ± 0.19	0.3707 (0.1450-0.6230)	----
	R	280	----	>4.00	10.8

Crosby et al. 1991
[a]S = Susceptible horn fly larvae, R = Resistant horn fly larvae.
[b]Based on 15 minute paralysis data.
[c]resistance ratio = KD_{50} of pyrethroid resistant population/KD_{50} of the pyrethroid susceptible population.

Table 9 - Conditions necessary for the rapid development of insecticide resistance*

Genetic
1- Number of R alleles
2- Frequency of R alleles
3- Dominance of R alleles

Operational
1- Prolonged exposure to a single insecticide
2- Relationship to earlier used insecticides
3- Insecticide selection pressure is high
4- Every generation is selected
5- No functional refugia
6- A large geographical area is covered
7- Selection occurs prior to mating

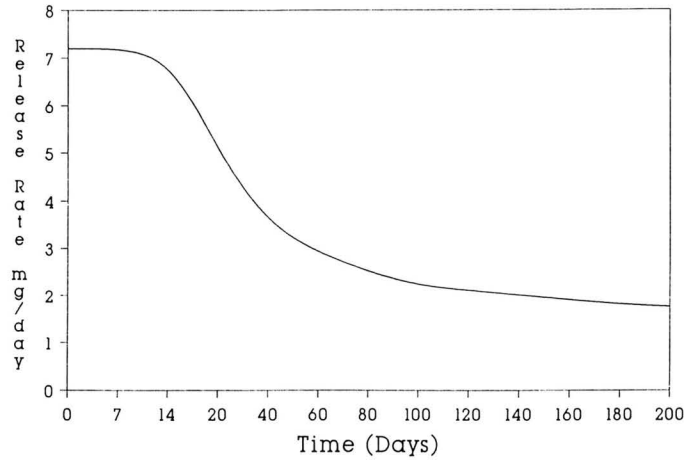
Biological
1- Monophagous insect
2- Short generation time
3- Numerous offspring per generation
4- Highly mobile insect

*Adapted from Georghiou and Taylor (1976) and Forgash (1984)

tags, essentially all of these conditions are present.

Foremost, ear tags release the insecticides slowly (Fig. 3) to provide relatively complete control of susceptible flies for 120-140 days.[47] This long term presence of the insecticide combines with several aspects of horn fly biology to accelerate the rate of resistance development. For example, all horn flies infesting a herd of cattle undergo selection since all cattle in a herd are often tagged. The slow release of insecticide from the ear tags, combined with the tendency of horn flies to spend essentially all of their time on cattle (host specificity), ensures that any susceptible flies that manage to immigrate are likely to be killed before they mate and reproduce in treated herds. In susceptible populations the selection pressure resulting from the ear tags is very high (>95%) which also contributes to the rapid development of resistance. Finally, the generation time of horn flies is only 9-14 days during the summer months which translates to at least 15-20 generations per season in most regions of the U.S. that are exposed to insecticide selection.

Figure 3. Theoretical release rate of insecticide impregnated ear tags.



Given the conditions associated with the use of ear tags for horn fly control, the accelerated evolution of resistance to any insecticide used would be expected and the rapid development of pyrethroid resistance supports this conclusion. Either component, the horn fly or the ear tag, by itself, does not necessarily present problems in terms of the development of resistance. For example, resistance to ear tags has not occurred in ticks or stable flies, *Stomoxys calcitrans* (Linneaus), which spend less time on the host and are less effectively controlled by the ear tags. However, the interaction of ear tags with horn flies almost ensures the rapid development of resistance and presents a very difficult situation relative to resistance management.

Summary

Resistance to insecticides has been documented in approximately 450 insect and mite species, with costs of resistance estimated at $1 billion annually. Studies suggests that there is a combination of biochemical, physiological, and behavioral adaptations responsible for the development of insecticide resistance. Organophosphate and carbamate insecticides usually exhibit a type of resistance involving biochemical processes that detoxify the insecticide by enzymatic activities of esterases, hydrolases, microsomal oxidases, and glutathione transferases. Insects resistant to pyrethroid and organochlorine insecticides may exhibit some metabolic resistance, however, the dominant resistance mechanism is active site insensitivity.

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