Dynamics and Management of Insecticide Resistance in the Horn Fly, Haematobia Irritans Irritans (L.) (Diptera: Muscidae).

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DYNAMICS AND MANAGEMENT OF INSECTICIDE RESISTANCE IN THE HORN FLY, \textit{Haematobia irritans irritans} (L.) (Diptera: Muscidae)

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Entomology

by

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A major objective of this work was to conduct prospective and retrospective analysis of data regarding development and management of insecticide resistance. Officially, my graduate program was initiated in August 1995. Dr. Lane Foil and collaborators collected data prior to my arrival, and I greatly appreciated the opportunity to analyze these data as well as data collected from 1995 to 1998.

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Studies on resistance of the horn fly, *Haematobia irritans irritans* (L.), to insecticides were conducted in Louisiana from 1989 to 1998. These studies included monitoring resistance and resistance dynamics under different strategies of insecticide use, monitoring the efficacy of insecticide products, establishing a relationship between bioassay findings and product efficacy, and evaluation of a new insecticide class.

In four years (1989-1992), efficacy of 20% diazinon-impregnated ear tags used yearly was reduced from greater than twenty to just one week of control, and tag failure was observed with resistance ratios (RR) from 1.8 to 5.7. Diazinon resistance developed more slowly and to a lower magnitude than that reported for pyrethroids. A strong correlation was found between RR and the frequency of flies (RF) surviving a 1.72 \( \mu g/cm^2 \) discriminating concentration of diazinon. A high risk of diazinon tag failure was associated with a 5% RF in pre-season bioassays. Resistance among several organophosphate (OP) insecticides including fenthion, ethion, pirimiphos-methyl, and tetrachlorvinphos was observed following the development of resistance to diazinon. Esterase activity was significantly higher in OP-resistant flies than in susceptible flies from both laboratory colony and field population.

From 1991 to 1997, the yearly rotation of *lambda*-cyhalothrin + piperonyl butoxide and pirimiphos-methyl ear tags was evaluated for control of pyrethroid-resistant horn flies in two locations. Control efficacy was reduced to a maximum of two and seven weeks for the synergized pyrethroid and OP tags in their last year of use, respectively.
The rotation did not improve pyrethroid efficacy or prevent further development of resistance to the pyrethroid or the OP.

During 1996 and 1997, the efficacy of experimental chlorfenapyr ear tags for horn fly control was evaluated under field conditions at one location, and the susceptibility of flies to chlorfenapyr was measured at seven locations in 1997. Ear tags containing either 30 or 40% chlorfenapyr were effective for fly control, and the number of weeks with >90% fly reduction ranged from eight to eighteen. All field populations of horn flies, including those resistant to pyrethroids and OPs, were more susceptible to chlorfenapyr than were flies from a reference susceptible colony.
The horn fly, *Haematobia irritans irritans* (L.), occurs in Europe, North Africa, Asia, and the Americas (Harwood and James 1979, Palmer and Bay 1981). During high infestations, horn flies can cause intense irritation and annoyance to cattle, which can result in significant reduction in both beef and dairy production (Harwood and James 1979). In the United States, this fly is considered to be the most important economic pest of cattle, and potential annual losses to the cattle industry have been estimated to be approximately $876 million (Kunz et al. 1991). Horn fly control programs are usually based solely on chemical control and this practice can select for insecticide resistance, which results in reduced product efficacy and increased control costs.

Resistance to insecticides can be broadly defined as "the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species" (Anonymous 1957) as well as "a genetic change in response to selection by toxicants that may impair control in the field" (Sawicki 1987). Development of resistance results from the continuous selection for resistance genes that already exist in the population at very low frequencies (Roush and McKenzie 1987). Resistance genes occur due to natural mutations, which may include gene amplification, altered gene regulation, and gene structural alteration (Scott 1995). Gene amplification is a phenomenon in which copies of a normal gene are produced, resulting in specific quantitative changes in its expression and leading to
overproduction of a particular enzyme (Terriere 1983, Devonshire and Field 1991). Altered gene regulation involves a quantitative change in expression of a regulatory gene, and results either in increased or decreased product of the structural genes (Scott 1995). The third type of mutation is an altered sequence of a structural gene, which leads to a structural change in the corresponding product (Scott 1995).

Horn fly resistance was reported first in the early 1960’s to the chlorinated hydrocarbons dichlorodiphenyltrichloroethane (DDT), toxaphene (McDuffie 1960), and methoxychlor (Burns and Wilson 1963), and to the organophosphate (OP) fenchlorphos (Burns and Wilson 1963). In the late 1970’s, horn flies became resistant to the OP tetrachlorvinphos, the first insecticide used in impregnated ear tags (Sheppard 1983), and the pyrethroids fenvalerate and permethrin were used to replace tetrachlorvinphos in the ear tags (Sparks et al. 1985). Due to their effective, economic, and long-lasting control (Ahrens and Cocke 1979, Schmidt and Kunz 1980), pyrethroid impregnated ear tags had a widespread acceptance among producers. However, soon after the introduction of pyrethroid insecticidal ear tags, resistance to fenvalerate, permethrin (Quisenberry et al. 1984), flucythrinate (Sheppard 1984), cypermethrin, deltamethrin (Byford et al. 1985), and later to \textit{lambda}-cyhalothrin (Crosby et al. 1991) was reported. This widespread resistance resulted from both direct exposure of flies to the pyrethroid products and the high level of cross-resistance within this insecticide class (Byford et al. 1985).

In Louisiana, insecticide resistance has been reported in horn fly populations to the chlorinated hydrocarbons, DDT (Byford et al. 1985) and methoxychlor (Sparks et al. 1985), a carbamate (Byford et al. 1985), and the pyrethroids, fenvalerate, permethrin
(Quisenberry et al. 1984), cypermethrin, deltamethrin, and flucythrinate (Byford et al. 1985).

Mechanisms of resistance to insecticides in horn flies have been studied in pyrethroid-resistant flies and include altered penetration and increased metabolism (Sparks et al. 1990, Sheppard 1995), altered behaviors (Lockwood et al. 1985, Byford et al. 1987), and reduced target-site sensitivity (Crosby et al. 1991). Enhanced metabolism has been found to be associated with an increased activity of mixed function oxidases (Cilek et al. 1995, Sheppard 1995) and secondarily, of esterases (Bull et al. 1988, Xu and Bull 1995).

Although biological, genetic, and ecological factors can influence the resistance process, operational factors play a key role in the dynamics of resistance development (Georghiou and Taylor 1977b). The selection pressure, which depends on the compound used, time and frequency of treatment, and application technique, is the most important aspect related to the development of resistance. Although any method of insecticide application (e.g. backrubber, dustrubber, spray, spot-on, pour-on) can contribute to resistance development in horn fly populations, insecticidal ear tags are particularly effective in selecting for resistant flies. The main factors related to the efficiency of ear tags for selecting resistance are associated with their season-long selection pressure (Ahrens and Cocke 1979) along with their decreasing insecticide release rate (Miller et al. 1983, 1986). Since horn flies have a short life cycle, less than 10 days under favorable conditions (Palmer et al. 1981, Lysyk 1992), the use of ear tags for long periods provides selection of resistant flies for many generations. It has been suggested that the initial
amount of pyrethroid released by the tag is sufficient to kill all but the homozygous resistant individuals (McDonald et al. 1987); however, the rate of insecticide release declines with time (Miller et al. 1983, 1986) allowing survival of a higher proportion of heterozygous resistant flies and shifting the frequency of the resistance genes in the population (McDonald et al. 1987. Mwangala and Galloway 1993).

The widespread use of chemicals for control of horn flies has resulted in resistance to most of the commercially available insecticides (Byford et al. 1985, Sparks et al. 1985). In fact, although a relatively large number of insecticide products are currently available in the market for horn fly control, there are very few insecticide classes represented and even fewer modes of action. Because of the fast development of resistance (Quisenberry et al. 1994, Sheppard 1984) as well as widespread cross-resistance (Byford et al. 1985, Sparks et al. 1985), successful strategies for insecticide use depend ultimately on the number of available insecticide modes of action and their rational use (Georghiou and Taylor 1977a, Soderlund and Bloomquist 1990). Since WWII, a novel insecticide class for controlling adult horn flies appeared in the market roughly every decade. Around the mid-1940's, organochlorine insecticides became available, followed by organophosphates and carbamates (1950's/1960's), pyrethroids (1970's), and then ivermectins in the 1980's (Sparks et al. 1985, Drummond et al. 1988).

Organochlorine and pyrethroid insecticides act on axonal sodium channels, delaying their inactivation after action potential which leads to a continuous influx of sodium ions and consequent multiple nerve firing (Beeman 1982, Soderlund and Bloomquist 1989). Type II pyrethroids (cyanopyrethroids) secondarily target gamma-
aminobutyric acid (GABA)-gated chloride channels inhibiting the chloride ion influx and its associated inhibitory action (Soderlund and Bloomquist 1989). Organophosphates and carbamates have a similar mode of action by inhibiting acetylcholinesterase, which ultimately inhibits the hydrolysis of the neurotransmitter acetylcholine (O'Brien 1976, Eldefrawi 1985). Avermectins have been found to stimulate presynaptic GABA release and activate postsynaptic GABA-gated chloride channels resulting in an influx of chloride ions and blocking further electrical activity (Lund 1985, Lasota and Dybas 1991). More recent studies have shown that avermectins also may activate glutamate-gated chloride channels, which may lead to muscle paralysis (Bloomquist 1996).

Due to similarities in the modes of action displayed by some of these insecticide classes, cross-resistance is relatively common in horn flies (Harvey et al. 1984, Byford et al. 1985, Cilek et al. 1991). Therefore, the search for new insecticides, particularly those with novel modes of action, is of major importance to provide new tools that can be useful in more efficient pest control and resistance management strategies. Recently, a new class of insecticides, the pyrroles, has been discovered by the American Cyanamid Company (Kuhn et al. 1993). Chlorfenapyr, the lead compound of this class, interferes with cellular respiration by uncoupling oxidative phosphorylation, thus impairing ATP production in the mitochondria (Black et al. 1994, Treacy et al. 1994). Chlorfenapyr activation by oxidative metabolism (Treacy et al. 1994) has lead to an increased susceptibility to this insecticide in pyrethroid-resistant insects as reported for the tobacco budworm, *Heliothis virescens* (F.), (Pimprale et al. 1997) and the horn fly (Sheppard and
Joyce 1998). However, more extensive studies are needed before chlorfenapyr becomes available in the market for horn fly control.

In this study, we monitored horn fly susceptibility to insecticides in several populations maintained under different strategies of insecticide use. Resistance to diazinon and its dynamics under field conditions was studied and toxicological studies on possible mechanisms of resistance were conducted. The efficacy of a yearly rotation between synergized pyrethroid and OP ear tags was evaluated in two pyrethroid-resistant populations. Finally, the efficacy of experimental ear tags containing chlorfenapyr was evaluated under field conditions and susceptibility to this insecticide was studied in both OP and pyrethroid susceptible and resistant wild populations.

References


CHAPTER 2

HORN FLY (DIPTERA: MUSCIDAE) RESISTANCE TO ORGANOPHOSPHATE INSECTICIDES

Introduction

The horn fly, Haematobia irritans irritans (L.), is considered to be one of the most important economic pests of cattle in the United States (Kunz et al. 1991). Horn fly control primarily has been based on the use of insecticides, and this control strategy has lead to resistance to most commercially available products (Byford et al. 1985, Sparks et al. 1985). Despite the fact that organophosphate (OP) insecticides have been used for about forty years, there are relatively few reports on the occurrence of OP resistance in horn flies. The first report of an OP product failure in horn flies was made in the early 1960’s for fenchlorphos applied in backrubbers (Burns and Wilson 1963). Subsequently, there were reports of resistance to tetrachlorvinphos, the first insecticide used in impregnated ear tags in the late 1970’s (Sheppard 1983, Harvey et al. 1984).

In laboratory studies, pyrethroid-resistant horn flies were found to have low levels of cross-resistance to dioxathion and sulprofos (Byford et al. 1985). More recently, resistance to diazinon and pirimiphos-methyl (detected in bioassays) has been reported in field populations (Cilek et al. 1991, Steelman et al. 1994). Development of resistance to diazinon is particularly important because pyrethroid-resistant flies have been shown to have an enhanced susceptibility to this OP (Sheppard and Marchiondo 1987, Cilek and Knapp 1993, Cilek et al. 1995, Szalanski et al. 1995). Diazinon has been considered to
be a useful tool in the management of pyrethroid resistance, because it provides adequate control of pyrethroid-resistant populations under field conditions (Byford et al. 1988, Foil et al. 1990). In this study, the development of resistance to diazinon in horn fly populations was monitored under field conditions and resistance dynamics was related to bioassay findings and product efficacy. Also, studies on enzyme activity were conducted to determine possible resistance mechanisms.

**Materials and Methods**

**Efficacy Studies**

The study was conducted at the Rosepine Research Station (Rosepine, LA) a unit of the Louisiana Agricultural Experiment Station. Data collected prior to August 1995 were made available by L. Foil, LSU Department of Entomology, and collaborators from the Rosepine Research Station.

The total cattle population at the site ranged from about 300 to 350 adult mixed breed cattle and their progeny. From 1989 to 1998, the majority of the adult animals were treated annually from mid-May to mid-September with two OP tags. A control (untreated) group of 20 cows was maintained to permit comparisons for treatment efficacy as well as to provide a source of untreated flies for bioassays during the season.

Ear tags used were as follow: 20% diazinon (Terminator®), 40% diazinon (Patriot®), 36% ethion (Commando®), and 13.7% tetrachlorvinphos (Ectogard®) from Boehringer Ingelheim Animal Health (St. Joseph, MO); 21.4% diazinon (Optimizer®) and 30% diazinon + 10% chlorpyrifos (Warrior®) from YTex Corp. (Cody, WY); 20% fenthion + 15% piperonyl butoxide (PBO) (Cutter Blue®, Bayer Corp., Shawnee...
Mission, KS); 20% pirimiphos-methyl (Rotator® and Tomahawk®, Schering-Plough Animal Health Corp., Union, NJ). Pour-on insecticides, which were applied after tags were removed, were 0.5% ivermectin (Ivomec®, MSD AGVET, Rahway, NJ) used in July of 1993, and 1% permethrin + 1% PBO (Synergized DeLice®, Schering-Plough) used in September of 1995 and 1996.

In addition to the above, there was a minor use of pyrethroid ear tags during the study. In 1991, 6.5% bifenthrin + 6.5% PBO tags (provided by YTex) were used on 32 cows during the first half of the fly season (about 12 weeks); in 1992 and 1993, 31 and 24 cows were treated with 10% lambda-cyhalothrin + 13% PBO tags (Saber Extra®, Schering-Plough), respectively; in 1996, 20 heifers were treated with 10% lambda-cyhalothrin + 13% PBO tags (Saber Extra®); in 1997 and 1998, 20 heifers were treated with 10% zetacypermethrin + 20% PBO tags (Python®, YTex).

Weekly fly counts were conducted from at least one week before ear tags were applied until the end of the treatment period or until there were greater than 50 flies per side for two consecutive weeks. Counts were made with the aid of binoculars; the total number of flies on one side of ten randomly selected adult cows was estimated before 0930 hours c.d.s.t. Control provided by the OP tags was considered adequate when fly counts averaged less than 50 flies/side/animal, and control efficacy was determined by the number of weeks post-treatment below this level.

**Insecticide Susceptibility Studies**

Susceptibility of fly populations was determined by the impregnated filter paper method (Sheppard and Hinkle 1987). Insecticides used were the OPs diazinon (87.5%
purity) and ethion (92.3% purity) from Boehringer Ingelheim Animal Health, fenthion (99% purity), and pirimiphos-methyl (90.2% purity) from Chem Service (West Chester, PA), and the pyrethroids lambda-cyhalothrin (93% purity, Schering-Plough) and permethrin (99.5% purity, FMC, Philadelphia, PA). From 1989 to 1998, bioassays using diazinon, lambda-cyhalothrin and permethrin were conducted twice per year. Pre-season bioassays were conducted in the spring (May), before any insecticide treatment was applied, and post-season bioassays were conducted in the fall (October), at least two weeks after the ear tags were removed. Exceptions for the time when the post-season bioassays were conducted were 1991 (August), 1993 (September, after ivermectin pour-on in July), and 1998 (August). In 1992 and 1995, at least four bioassays were conducted. In 1997, bioassays were expanded with the use of ethion, fenthion, and pirimiphos-methyl, and were conducted monthly from April to November. In 1998, bioassays were conducted monthly from May to August using diazinon, ethion, lambda-cyhalothrin, and permethrin. Bioassays were conducted immediately after flies were collected with hand nets from untreated animals; accidental mortality due to handling was checked immediately after the flies were released into the Petri dishes. Fly mortality was determined after a 4 hr-exposure period and flies unable to walk were considered dead. Three replicates of approximately 25 flies each were used for each insecticide concentration; 10-11 concentrations were used per insecticide. Flies obtained from the colony maintained at the Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS (Kerrville, TX) were used as the reference susceptible strain.
Mortality data were corrected using Abbott’s formula (Abbott 1925) when necessary. Data were analyzed by probit analysis using POLO-PC (LeOra Software 1987). Differences between LC$_{50}$s were considered significant when their 95% fiducial limits did not overlap. Resistance ratios (RR) were calculated by dividing the LC$_{50}$ from field populations by the LC$_{50}$ from the reference strain. Although only RRs are used for comparisons and discussion, LC$_{50}$'s and their 95% fiducial limits are provided. The highest concentration of diazinon (1.72 µg/cm$^2$) which resulted in 100% mortality of flies from the reference strain was used as a discriminating concentration (DC) and the percentage of flies surviving this DC was considered as the resistance frequency (RF) of individuals in the population. Correlation analysis (PROC CORR) (SAS Institute 1989) was performed among monthly RRs for the six insecticides tested in 1997 as well as between yearly insecticide efficacy (number of weeks of control) and the respective pre-season RRs and RFs. Correlation analysis also was performed between RR and RF using all data obtained from 1992 to 1998.

Enzyme Assays

At the time of each bioassay, flies were collected, transported to the LSU Dept. of Entomology (Baton Rouge, LA), and stored at -70°C. Enzyme activity studies were conducted with samples of flies collected during 1997 at Rosepine (OP-resistant population) and susceptible flies from both the reference colony and a field population from the St. Gabriel Research Station (St. Gabriel, LA). Three collections from Rosepine (April, May, and October) were used; flies were collected in April and May, before any
insecticidal treatment was applied that year, and in October, two weeks after the OP tags were removed.

The two major groups of enzymes tested were glutathione S-transferases (GSTs) and esterases (ESTs). Preliminary assays were conducted to establish optimal protein concentration and buffer type and pH. Fly homogenates used for both GST and EST assays were prepared from pools of five abdomens dissected from frozen flies for each tested sample. Abdomens were homogenized in 500 μl of cold 1.15% potassium chloride (KCl) plus a few crystals of phenylthiourea (PTU). Homogenates were centrifuged at 4 °C for 15 minutes at 12,000 g and supernatants were tested immediately.

The GST assays included the measurement of enzyme activity toward two model substrates, 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB). The CDNB assay followed the method described by Jakoby (1978) with modifications described by Kirby et al. (1994); the GST activity toward DCNB followed the method of Booth et al. (1960) as modified by Grant et al. (1989). Both substrates, as well as the KCl and PTU, were purchased from Aldrich Chemical Company, Milwaukee, WI. Substrate solutions (0.75 mM) were prepared freshly by mixing a 0.1 M sodium phosphate buffer (pH 8) plus 15% glycerol (USP grade; EM Science, Gibbstown, NJ) solution with the respective CDNB or DCNB stock solutions (50 mM in DMSO). Reaction mixtures in the microtiter plate contained 40 μl sodium phosphate buffer (0.1 M, pH 8), 30 μl reduced glutathione (GSH, 8 mM final conc.; Sigma Chemical Co., St. Louis, MO), 30 μl homogenate, and 200 μl of either CDNB or DCNB substrate solutions per individual well. Immediately after substrate addition, the mixture was incubated at
30 °C and the rate of change in the optical density (OD) was measured at 340 nm for the initial 10 minutes using a Thermomax microplate reader (Molecular Devices, Palo Alto, CA). Each measurement was replicated two or three times and corrected for non-enzymatic activity using reactions without proteins (70 μl buffer + 30 μl GSH + 200 μl substrate) as controls. The GST activities were corrected for protein concentration, which was measured by the Bradford method (Bradford 1976) with bovine serum albumin (fraction V) as the standard. The changes in OD for CDNB and DCNB were converted to nmol conjugate/minute/mg protein using extinction coefficients of 8.5 and 10.09 mM⁻¹ 300 μl⁻¹, respectively (Grant et al. 1989, 1991).

The EST activity was measured toward α-naphthyl acetate (α-NA; Sigma Chem.) by using the method of Gomori (1953) with modifications described by Ibrahim and Ottea (1995). The substrate solution was prepared by adding 200 μl of α-NA (0.113 M dissolved in 50% acetone + 50% 0.1 M sodium phosphate buffer pH 7.4) to a fresh solution of Fast Blue B salt (6 mg in 10 ml of 0.1 M sodium phosphate buffer pH 7.4; Aldrich Chem.) and then filtered (Whatman #3 filter paper). The solution was protected from light until use. Reaction mixtures in each replicate (individual microtiter plate well) contained homogenate (10 μl) and substrate solution (240 μl). Reaction mixtures were incubated at 37 °C and measurement of the rate of change in OD was made at 450 nm as described for the GST assays. Two or three replicates were used per measurement and data were corrected by nonenzymatic hydrolysis using blanks containing 10 μl sodium phosphate buffer (pH 7.4) and 240 μl of the substrate solution. The EST data were corrected for protein concentration as described for the GST data, and converted to nmol...
α-naphthol/minute/mg protein using a 9.25 mM⁻¹ 250 μl⁻¹ extinction coefficient (Grant et al. 1989).

To check for a potential reduction of enzyme activities due to the freezing/thawing process, GST assays using both CDNB and DCNB were conducted with flies collected from untreated animals at the St. Gabriel Research Station. Assays with fresh flies were conducted immediately after flies were held at about -20 °C for five minutes (for fly immobilization) and with flies from the same collection held for 72 hr at -70 °C. Four replications per assay were used following the previously described protocol.

Statistical analysis of enzyme activity data was performed (SAS Institute 1989) by analysis of variance and mean comparison using Duncan’s Multiple Range Test (PROC GLM) at a significance level of 5%.

Results

Fly Counts and Insecticidal Ear Tag Efficacy

Mean number of flies from untreated animals during the control season, as well as the yearly efficacy of the OP ear tags, are summarized in Table 2.1. From 1989 to 1992, the efficacy of 20% diazinon tags decreased from greater than twenty to one week of control. In 1992, fenthion + PBO tags provided no fly control (zero weeks). In 1993, no control above one week was achieved with either fenthion + PBO, pirimiphos-methyl, or tetrachlorvinphos tags, but 40% diazinon tags provided at least seven weeks of control before all OP tags were removed and ivermectin pour-on was applied. In 1994, except for the tetrachlorvinphos and fenthion + PBO tags, a substantial increase in control efficacy ranging from 10 to 14 weeks was observed for diazinon tags, as well as for the
Table 2.1. History of major insecticide use and efficacy* of horn fly control by organophosphate ear tags (two tags/animal) at Rosepine, LA

<table>
<thead>
<tr>
<th>Year</th>
<th>Fly counts^b</th>
<th>Diazinon 20%</th>
<th>Diazinon 21%</th>
<th>Diazinon 40%</th>
<th>Fenthion +PBO</th>
<th>Pirimiphos-methyl</th>
<th>Tetrachlorvinphos</th>
<th>Diazinon+ chlorpyrifos</th>
<th>Ethionine</th>
<th>Other insecticides^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>121</td>
<td>≥20</td>
<td>≥20</td>
<td>ND^e</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1990</td>
<td>87</td>
<td>11</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1991</td>
<td>229</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1992</td>
<td>77</td>
<td>1</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1993</td>
<td>235</td>
<td>1^f</td>
<td>ND</td>
<td>≥7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ivermectin</td>
</tr>
<tr>
<td>1994</td>
<td>96</td>
<td>12</td>
<td>10</td>
<td>14</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>permethrin+PBO</td>
</tr>
<tr>
<td>1995</td>
<td>150</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>ND</td>
<td>permethrin+PBO</td>
</tr>
<tr>
<td>1996</td>
<td>237</td>
<td>ND</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>8</td>
<td>permethrin+PBO</td>
</tr>
<tr>
<td>1997</td>
<td>237</td>
<td>ND</td>
<td>ND</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>183</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>ND</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Number of weeks with horn fly numbers below 50 flies/side/animal

^b Mean number of flies/side on the untreated group during the control period (until all tags had failed)

c Pour-on insecticides applied in July (ivermectin) or September (permethrin+PBO), after OP tags were removed

^d OP tags were applied but fly counts were not available

^e Not done

Data from 40% diazinon tags (one tag/animal)
pirimiphos-methyl tag. In 1995, six types of OP tags provided just three weeks of control or less. In 1996, efficacy of several OP tags increased to 7-8 weeks of control, after a permethrin (+PBO) pour-on treatment in the previous fall. In 1997, despite another pyrethroid pour-on application at the end of 1996, fly control by OP tags was reduced to 5-6 weeks. In 1998, none of the five different OP tags tested provided more than three weeks of control.

**Insecticide Susceptibility**

The RRs for diazinon ranged from 0.6 (1997 pre) to 7.7 (1994 post) during the study (Table 2.2). Generally, the RRs to diazinon increased during the season (from pre to post bioassays) and decreased by the next spring. This pattern was observed for all years except 1993, when tags were removed early and the post-season bioassay was conducted in September.

When bioassays were conducted monthly, RRs for diazinon measured in 1995 increased from 3.7 (May) to 6.9 (October) after tags were applied; RRs measured in August and September were lower than those determined in July and October (Fig. 2.1). In 1997, monthly changes in RRs were similar for all OPs tested (Table 2.3). Correlation coefficients from monthly RRs between diazinon and other OPs in 1997 were 0.60, 0.68, and 0.87 to fenthion, ethion, and pirimiphos-methyl, respectively. Also, strong correlations were found among ethion, fenthion, and pirimiphos-methyl, with coefficients ranging from 0.92 to 0.99. In general, RRs dropped from April to May (before tagging) and increased after the OP tag application until October. The RRs for all insecticides...
Table 2.2. Horn fly susceptibility (LC$_{50}$ expressed as µg/cm$^2$) and resistance ratios to different insecticides at Rosepine, LA

<table>
<thead>
<tr>
<th>Year</th>
<th>Bioassay time</th>
<th>Diazinon LC$_{50}$ (95% F.L.)</th>
<th>RR</th>
<th>λ- CYHALOTHIN LC$_{50}$ (95% F.L.)</th>
<th>RR</th>
<th>Permethrin LC$_{50}$ (95% F.L.)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.10 (-)$^f$</td>
<td></td>
<td>0.44 (0.21-0.72)</td>
<td></td>
<td>2.28 (1.74-2.99)</td>
<td></td>
</tr>
<tr>
<td>1989</td>
<td>Pre</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>Pre</td>
<td>0.37 (0.26-0.46)$^e$</td>
<td></td>
<td>0.44 (0.21-0.72)</td>
<td></td>
<td>4.44 (3.19-5.67)$^d$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>Pre</td>
<td>0.54 (0.28-1.00)</td>
<td>1.2</td>
<td>0.28 (0.18-0.38)$^d$</td>
<td></td>
<td>2.25 (1.17-3.50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.05 (0.90-1.19)</td>
<td>6.1</td>
<td>0.76 (-)$^e$</td>
<td>40.5</td>
<td>4.92 (1.33-11.29)</td>
<td>8.9</td>
</tr>
<tr>
<td>1992</td>
<td>Pre</td>
<td>0.53 (0.26-0.83)</td>
<td>1.2</td>
<td>0.16 (0.06-0.31)</td>
<td>8.5</td>
<td>1.16 (0.48-1.90)</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>2.59 (-)$^e$</td>
<td></td>
<td>3.3</td>
<td>3.3</td>
<td>1.23 (0.42-3.21)</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>Pre</td>
<td>1.59 (-)$^e$</td>
<td>3.4</td>
<td>0.39 (0.17-0.75)</td>
<td>8.5</td>
<td>6.16 (3.82-9.72)</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0.92 (0.69-1.21)</td>
<td>2.0</td>
<td>0.31 (0.19-0.48)</td>
<td>6.8</td>
<td>3.46 (1.29-6.20)$^d$</td>
<td>8.7</td>
</tr>
<tr>
<td>1994</td>
<td>Pre</td>
<td>0.54 (0.39-0.74)</td>
<td>1.3</td>
<td>0.26 (0.10-0.51)</td>
<td>3.3</td>
<td>2.23 (1.42-3.21)</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>3.24 (-)$^e$</td>
<td>7.7</td>
<td>0.21 (0.09-0.40)</td>
<td>2.6</td>
<td>2.41 (1.57-3.47)</td>
<td>3.1</td>
</tr>
<tr>
<td>1995</td>
<td>Pre</td>
<td>1.47 (1.19-1.81)</td>
<td>3.7</td>
<td>0.26 (0.17-0.39)</td>
<td>11.4</td>
<td>2.99 (1.54-4.42)</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.71 (1.15-3.32)</td>
<td>4.3</td>
<td>0.27 (0.06-0.74)</td>
<td>11.9</td>
<td>1.01 (0.68-1.41)</td>
<td>1.8</td>
</tr>
<tr>
<td>1996</td>
<td>Pre</td>
<td>0.55 (0.46-0.63)</td>
<td>1.5</td>
<td>-</td>
<td>2.8</td>
<td>-</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.60 (1.29-1.96)</td>
<td>4.4</td>
<td>-</td>
<td>4.2</td>
<td>-</td>
<td>1.4</td>
</tr>
<tr>
<td>1997</td>
<td>Pre</td>
<td>0.27 (0.20-0.35)</td>
<td>0.6</td>
<td>0.11 (0.08-0.15)</td>
<td>4.5</td>
<td>2.18 (1.80-2.59)</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>2.83 (-)$^e$</td>
<td>6.6</td>
<td>0.41 (0.33-0.50)</td>
<td>16.7</td>
<td>8.51 (4.56-13.95)</td>
<td>7.8</td>
</tr>
<tr>
<td>1998</td>
<td>Pre</td>
<td>0.83 (0.57-1.05)$^d$</td>
<td>3.0</td>
<td>0.89 (0.56-1.21)$^d$</td>
<td>33.4</td>
<td>5.32 (2.45-6.81)$^d$</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.64 (1.39-1.91)</td>
<td>5.9</td>
<td>0.71 (0.52-0.92)</td>
<td>26.7</td>
<td>4.30 (3.56-5.07)</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*Pre= pre-season bioassays (April/May) conducted before any insecticide treatment; Post= post-season bioassays conducted at least one week after tags were removed, post-season bioassays were conducted in October except for 1991 (August), 1993 (September), and 1998 (August)

* Resistance ratio (LC$_{50}$ from field population / LC$_{50}$ from Kerrville reference susceptible strain)

* Data too heterogeneous to calculate 95% fiducial limits (LeOra Software 1987)

* Control mortality between 10% and 20% (control mortality <10% in all other bioassays)

* Actual LC$_{50}$s were not determined
dropped in August, then increased in September and peaked in October (just after tags were removed). The RRs for both pyrethroids peaked in September at 58.2 and 18.3 for lambda-cyhalothrin and permethrin, respectively. No significant correlation was found

![Graph showing dynamics of diazinon resistance in horn flies for three years at Rosepine, LA.](image)

Figure 2.1. Dynamics of diazinon resistance in horn flies for three years at Rosepine, LA. Resistance frequency is the percentage of flies surviving a diazinon discriminating concentration of 1.72 μg/cm².

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Table 2.3. Susceptibility (LC₅₀ expressed as μg/cm²) and resistance ratios of horn flies from field populations (Rosepine, LA) and from a reference colony (Kerrville, TX) in 1997

<table>
<thead>
<tr>
<th>Date</th>
<th>Diazinon</th>
<th>Ethion</th>
<th>Fenthion</th>
<th>Pirimiphos-methyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀(95% F.L.)</td>
<td>RR</td>
<td>LC₅₀(95% F.L.)</td>
<td>RR</td>
</tr>
<tr>
<td>April 23</td>
<td>2.95 (-)³</td>
<td>6.9</td>
<td>22.60 (-)²</td>
<td>2.4</td>
</tr>
<tr>
<td>May 20</td>
<td>0.27 (0.20-0.35)</td>
<td>0.6</td>
<td>5.00 (4.08-5.87)</td>
<td>0.5</td>
</tr>
<tr>
<td>June 23</td>
<td>0.78 (0.66-0.91)</td>
<td>1.8</td>
<td>5.44 (4.44-6.39)</td>
<td>0.6</td>
</tr>
<tr>
<td>July 25</td>
<td>1.54 (-)³</td>
<td>3.6</td>
<td>10.40 (8.24-12.75)</td>
<td>1.1</td>
</tr>
<tr>
<td>August 20</td>
<td>0.65 (0.41-1.05)</td>
<td>1.5</td>
<td>6.50 (4.51-9.18)</td>
<td>0.7</td>
</tr>
<tr>
<td>September 19</td>
<td>1.01 (0.79-1.25)</td>
<td>2.4</td>
<td>8.28 (7.00-9.67)</td>
<td>0.9</td>
</tr>
<tr>
<td>October 24</td>
<td>2.83 (-)³</td>
<td>6.6</td>
<td>123.33 (79.96-227.07)</td>
<td>13.0</td>
</tr>
<tr>
<td>November 26</td>
<td>2.91 (2.18-3.69)</td>
<td>6.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kerrville strain</td>
<td>0.43 (0.39-0.47)</td>
<td>-</td>
<td>9.48 (-)³</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>λ-cyhalothrin</th>
<th>Permethrin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀(95% F.L.)</td>
<td>RR</td>
</tr>
<tr>
<td>April 23</td>
<td>0.41 (0.23-0.70)</td>
<td>16.5</td>
</tr>
<tr>
<td>May 20</td>
<td>0.11 (0.08-0.15)</td>
<td>4.5</td>
</tr>
<tr>
<td>June 23</td>
<td>0.21 (0.15-0.29)²</td>
<td>8.4</td>
</tr>
<tr>
<td>July 25</td>
<td>1.19 (0.81-1.57)</td>
<td>48.5</td>
</tr>
<tr>
<td>August 20</td>
<td>0.63 (0.49-0.81)</td>
<td>25.8</td>
</tr>
<tr>
<td>September 19</td>
<td>1.43 (1.03-1.97)</td>
<td>58.2</td>
</tr>
<tr>
<td>October 24</td>
<td>0.41 (0.33-0.50)</td>
<td>16.7</td>
</tr>
<tr>
<td>November 26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kerrville strain</td>
<td>0.02 (-)³</td>
<td>-</td>
</tr>
</tbody>
</table>

³ Resistance ratio (LC₅₀ from field population / LC₅₀ from Kerrville susceptible strain) calculated from results before rounding to one decimal
² Mortality in the control group between 15% and 25% (control mortality <15% in all other bioassays)
⁴ Data too heterogeneous to calculate 95% fiducial limits (LeOra Software 1987)
between OPs and either *lambda*-cyhalothrin or permethrin (range -0.01 to 0.18), but a strong correlation (*r*=0.84) was observed between these pyrethroids. The pre- and post-season RRs for pyrethroids ranged from 2.6 (1994 post) to 40.5 (1992 post) and from 1.4 (1996 pre and post) to 15.2 (1993 pre) for *lambda*-cyhalothrin and permethrin, respectively (Table 2.2). No general pattern of change in susceptibility to pyrethroids was observed.

The percentage of flies surviving the diazinon 1.72 μg/cm² DC did not exceed 82% even in the post-season bioassays (Table 2.4). Generally, the RFs increased during the season, but were lower by the next spring. Pre-season RFs dropped substantially in the years after animals were treated in the summer with ivermectin (1993) and in the fall

<table>
<thead>
<tr>
<th>Year</th>
<th>Survival frequency (%)</th>
<th>Pre-season*</th>
<th>Post-season*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>0.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>0.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>6.4</td>
<td>22.1</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>9.4</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>34.0</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>1.1</td>
<td>79.3</td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>40.7</td>
<td>76.9</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>10.4</td>
<td>54.7</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>2.0</td>
<td>75.5</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>16.4</td>
<td>53.1</td>
<td></td>
</tr>
</tbody>
</table>

* Bioassays conducted in the spring (May), before any insecticide treatment
b Bioassays conducted at least two weeks after tags were removed in the fall; bioassays were conducted in October, except in 1991 and 1998 (August), and in 1993 (September, two months after animals were treated with ivermectin pour-on)

Data not available
with permethrin + PBO (1995 and 1996). During 1995, monthly RFs ranged from 40.7% in May to 76.9% in October (Fig. 2.1). In 1997, monthly RFs ranged from 2.0% in May to 76.3% in November. In 1998, RFs ranged from 16.4% (May) to 53.1% (August).

A strong positive correlation was found between RR and RF \((r=0.94)\) when data from all bioassays \((n=28)\) were analysed (Fig. 2.2); also, a high correlation \((r=0.92)\) was obtained when only pre-season yearly results \((n=7)\) were analysed. Negative correlations were found between the number of weeks of control for 20% diazinon tags and pre-season RR \((r=-0.53)\) and RF \((r=-0.71)\). Lower negative correlations were found between the efficacy of 40% diazinon tags and pre-season RR \((r=-0.41)\) and RF \((r=-0.34)\). Ear tags containing 20-21% diazinon provided greater than 10 weeks control in the years when

![Figure 2.2. Correlation between resistance ratio (RR) and frequency of horn fly survival (RF) to a 1.72 µg/cm² discriminating concentration of diazinon. Data from bioassays \((n=28)\) conducted from 1992 to 1998 at Rosepine, LA.](image-url)
RF was equal to 0% (n=3), but no OP tag provided fly control for more than eight weeks when RF was greater than 5% (n=26).

**Enzyme Assays**

Lower GST activity was measured when assays were conducted with frozen flies rather than fresh flies from the same sample (data not shown). Mean reductions in GST activity were 21.1% and 12.7% toward CDNB and DCNB, respectively. Consequently, only frozen flies were used.

Enzyme activity levels in the Rosepine flies were higher in May than in April and lowest in October for all enzymes tested (Table 2.5). GST activities for both CDNB and DCNB were significantly higher in susceptible flies from St. Gabriel than for flies from the reference colony, but EST activity did not differ statistically between these two fly populations.

Table 2.5. Glutathione S-transferase (GST) and esterase (EST) activities in adult horn flies from field populations collected in 1997 and from a laboratory strain

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>GST-CDNB (± SD)</th>
<th>GST-DCNB (± SD)</th>
<th>Esterase (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerrville</td>
<td>11.6 (± 3.4)a</td>
<td>0.4 (± 0.1)a</td>
<td>12.6 (± 3.3)a</td>
</tr>
<tr>
<td>St. Gabriel</td>
<td>63.1 (± 29.9)b</td>
<td>2.0 (± 0.6)b</td>
<td>14.8 (± 2.3)a</td>
</tr>
<tr>
<td>Rosepine Apr</td>
<td>129.5 (± 41.9)c</td>
<td>2.7 (± 1.0)b</td>
<td>33.2 (± 13.2)bc</td>
</tr>
<tr>
<td>Rosepine May</td>
<td>148.0 (± 63.7)c</td>
<td>4.2 (± 1.3)c</td>
<td>37.4 (± 9.5)c</td>
</tr>
<tr>
<td>Rosepine Oct</td>
<td>53.7 (± 29.5)b</td>
<td>2.3 (± 0.7)b</td>
<td>27.7 (± 8.1)b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within columns are not significantly different by Duncan’s test (α=0.05)

*a* Enzyme assays conducted with pools of 5 abdomens (n ≥ 10)

*b* Kerrville (susceptible reference strain), St. Gabriel (field susceptible), Rosepine (organophosphate-resistant); April and May (sampled before insecticidal treatment), October (sampled two weeks after tags removed)

*c* Mean enzyme activity (± SD) toward CDNB (1-chloro-2,4-dinitrobenzene) and DCNB (1,2-dichloro-4-nitrobenzene), expressed as nmol conjugate minute⁻¹ mg protein⁻¹

*d* Mean enzyme activity (± SD) toward α-naphthyl acetate, expressed as nmol α-naphthol minute⁻¹ mg protein⁻¹

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The GST activity toward CDNB did not exceed 20 and 115 nmol minute\(^{-1}\) mg protein\(^{-1}\) for susceptible flies from the laboratory colony and the field population, respectively. The GST activity toward DCNB did not exceed 0.6 and 2.7 nmol minute\(^{-1}\) mg protein\(^{-1}\) for susceptible flies from colony and field populations, respectively. The EST activity did not exceed 18.5 nmol minute\(^{-1}\) mg protein\(^{-1}\) for both susceptible populations.

**Discussion**

Development of resistance to diazinon was observed during four years when efficacy of 20% diazinon ear tags decreased from greater than twenty weeks to just one week of control. Diazinon tags have been reported to provide control under 50 flies per side for about 20 weeks (Crosby et al. 1991) and for 15 weeks at \(\geq 90\%\) fly reduction (Cocke et al. 1990), but lower efficacy for diazinon tags also has been reported (Lancaster et al. 1991, Williams and Towell 1992, Derouen et al. 1995). Some selection for resistance to diazinon may have occurred in 1987-1988, when diazinon was first used (records of the amount of use were not available).

Both increased (Sheppard and Marchiondo 1987, Cilek and Knapp 1993, Cilek et al. 1995) and decreased (Harvey et al. 1984, Cilek et al. 1991) susceptibility to OPs have been reported in pyrethroid-resistant populations. Although horn fly populations at Rosepine have had a history of pyrethroid resistance, which reached 32-fold to fenvalerate and 27-fold to permethrin (Quisenberry et al. 1984), a high efficacy of the OP tags was observed initially.
Although a relatively high pre-season RR was observed in 1993, the efficacy of 40% diazinon tags suggested that even after six years of diazinon use, only a moderate level of resistance and/or frequency of highly resistant flies was present in the population. In Australia, resistance to diazinon was reported in the cattle tick, *Boophilus microplus* about 7 years after this compound became available (Wharton and Roulston 1970) and took 10 years to develop in the Australian sheep blowfly, *Lucilia cuprina* (McKenzie and Whitten 1984).

Resistance to other OP products also was observed. In 1993, the fenthion (+PBO), pirimiphos-methyl, and tetrachlorvinphos tags provided one week of control or less. Foil et al. (1990) reported that the efficacy of 20% pirimiphos-methyl ear tags against horn flies was 11 weeks at two locations in Louisiana. Also, 20% pirimiphos-methyl tags initially provided 10 and 15 weeks of control at two other locations in Louisiana (Chapter 3). In this study, control provided by ethion tags in 1996 was eight weeks, but only one week of control was obtained in 1998. Similarly, limited control was obtained with tags containing the diazinon + chlorpyrifos mixture. The ethion, fenthion (+PBO), and diazinon + chlorpyrifos ear tags have been reported to provide greater than 10 weeks of horn fly control in Louisiana (Foil et al. 1997).

The application of pour-on ivermectin and permethrin (+PBO) products showed initial but somewhat limited efficacy to reverse the OP resistance. Following treatment with ivermectin, a marked increase in efficacy of all diazinon tags was observed the next year. This change also may have been influenced by the shorter OP pressure in 1993, since tags were removed in July rather than September. After the first pyrethroid
treatment in September 1995, there was an increase in the efficacy of OP products the next year, but the change was less than that observed after ivermectin use. However, OP efficacy was reduced the year following the second pyrethroid application.

The RRs to diazinon decreased between fall and spring for every year. This phenomenon has been reported previously in pyrethroid-resistant horn fly populations (Sheppard 1987, Krafsur et al. 1993, Mwangala and Galloway 1993), and possible causes include reduced biological fitness (Kunz 1991, Scott et al. 1997) as well as the entry of susceptible individuals into the resistant population (Byford et al. 1987, Guillot et al. 1988).

Despite the relatively low RRs measured from bioassays, control failure of all OP tags occurred in the field. In 1995, 20% diazinon tags failed four weeks after a 3.7 RR and a 40.7% RF were measured, and 40% diazinon tags failed one week before a 5.7 RR and a 69.1% RF were measured. In 1997, 40% diazinon tag failure was observed one week after a 1.8 RR and a 12.3% RF were measured. In 1998, a 4.8 RR and a 43.9% RF were measured at the exact time when product failure (53 flies per side) was recorded in the 40% diazinon tag group. These findings indicate that diazinon tag failure can occur at RRs of 1.8-5.7.

A strong correlation was found between RR and RF for diazinon, but these measures may not be equivalent indicators of product efficacy. The use of RRs to diazinon as predictors of product efficacy was found to be difficult, but the percentage of flies surviving a DC appeared to be a more reliable and useful indicator of diazinon resistance. The RRs are calculated from midpoint values (LC$_{50}$s), which reflect the
different levels of susceptibility present in populations, and ultimately are influenced by the frequency of both susceptible and resistant individuals. Also, the calculation of RRs depends on the susceptibility of a reference population, which may vary. On the other hand, the RF is a measure of the more resistant portion of the sampled populations, and is not influenced by fluctuations in the susceptibility of a reference strain. Furthermore, much larger samples are needed to detect resistance at any given frequency by using standard measurements (LDs, LCs) when compared to the use of diagnostic tests using discriminating techniques (Roush and Miller 1986). Additional advantages of the DC include the use of smaller test kits, as well as a reduction in time and cost of the bioassay.

During 10 years at Rosepine, when pre-season RFs were 0%, 20-21% diazinon tags provided control for more than 10 weeks. Studies at four other sites in Louisiana where OP tags have been used and diazinon pre-season bioassays were conducted support these observations (Foil and Barros, unpublished data). With pre-season RF=0%, efficacy of diazinon tags were 10 weeks at Hill Farm Research Station (Homer) in 1989, 12 and \( \geq 15 \) weeks at Red River (Bossier City) in 1989 and 1991, respectively, and \( \geq 13 \) and \( \geq 20 \) weeks at St. Gabriel in 1997 and 1996, respectively; tags provided \( \geq 16 \) weeks of control at St. Gabriel in 1995 (the RF was 2.1%). With the exception of 1994, fly control did not exceed eight weeks when pre-season RFs were \( > 0 \% \), and when RF was \( > 5 \% \) none of the OP tags provided greater than eight weeks of control. Therefore, RFs are a measure of diazinon resistance that can be used to predict expected efficacy for diazinon tags. Risks of control failure can be predicted using a 5% RF; lower frequencies represent lower risks and higher frequencies represent higher risks of failure. According to May and Dobson
(1986), a population should be considered as effectively susceptible when resistance has dropped to a frequency of about 1% after insecticide pressure is withdrawn.

The development of resistance to all OPs tested in the field was supported by data from the monthly bioassays in 1997 (Table 2.3). Correlation coefficients indicated that there was a similar pattern of changes in susceptibility observed for all OPs tested. In a recent study, Campbell et al. (1998) reported a pattern of cross-resistance between OPs in *L. cuprina*, with higher levels of cross-resistance to diethyl OPs observed in a diazinon-resistant strain, and higher levels of cross-resistance to dimethyl OPs found in malathion-resistant strains. At Rosepine, flies were shown to be resistant to the diethyl OPs, diazinon and ethion (bioassay and field results) as well as the dimethyl OPs, pirimiphos-methyl (bioassay and field results), tetrachlorvinphos (field results), and fenthion (field results). Although bioassays indicated a high susceptibility to fenthion, the failure of fenthion + PBO tags was observed in the field. The low RRs to fenthion were related mainly to the high LC₅₀ from the reference colony, which was much higher than any field population tested other than the flies from Rosepine in October (Barros and Foil, unpublished data). Higher insecticide susceptibility of wild flies compared to flies from a susceptible colony has been reported (Schmidt et al. 1985, Sheppard 1987, Szalanski et al. 1995). Furthermore, the tags contained fenthion plus the mixed-function oxidases (MFO) inhibitor PBO (Hodgson 1983, Wilkinson 1983), which may have reduced the fenthion activation.

Despite the fact that fly populations were selected with OP tags, RRs to the pyrethroids increased 12.9-fold (from 4.5 to 58.2) for *lambda*-cyhalothrin and 9.2-fold
(from 2.0 to 18.3) for permethrin during the season in 1997, which suggested that possible
cross-resistance had developed. However, correlation coefficients indicated that changes
in resistance to OP and pyrethroids were not directly associated. The occurrence of cross-
resistance between OP and pyrethroid insecticides has been reported in horn flies (Harvey

In this study, diazinon resistance developed to a lower level of magnitude and less
rapidly than has been reported for pyrethroid resistance. A low magnitude of RR for
other diazinon-resistant fly populations also has been observed for a laboratory colony of
horn flies (McKenzie and Byford 1993) as well as in L. cuprina collected from the field
(Roxburg and Shanahan 1973). Factors that could contribute to the slower development
of diazinon resistance when compared to pyrethroid resistance may include genetic
factors (initial frequency of resistance genes in the population, number of alleles
determining resistance, and the dominance/recessiveness of the resistant allele(s)) and
selection factors such as previous selection by DDT/DDT residues (in the case of
pyrethroid resistance) and more frequent and widespread exposure to pyrethroids.

Pyrethroid resistance (kdr) in horn flies is determined by a single incompletely
recessive gene (Roush et al. 1986, McDonald and Schmidt 1987), which may favor quick
selection of resistance (Georghiou and Taylor 1977, McDonald and Schmidt 1987) and
may ultimately lead to a high level of resistance in a relatively short time (Quisenberry
et al. 1984, Sheppard and Joyce 1992, McKenzie and Byford 1993). In house flies,
oxidative resistance to diazinon has been found to be polygenic and semidominant (Plapp
et al. 1976). If diazinon resistance in horn flies is driven by similar genetic characters,
this may help to explain the dynamics of resistance to diazinon observed in the field. For pyrethroids, RFs of 100% often were observed after insecticide pressure (Chapter 3). However, the plateau observed for the frequency of diazinon-resistant flies (about 75-80% in most cases) indicated that part of the population remained susceptible despite the OP pressure during the season. These data support the suggestion that resistance to diazinon is probably not recessive. McKenzie and Byford (1993) found that a 2.54-fold resistance to diazinon developed in a closed horn fly population after 31 generations under a continuous selection with diazinon, while under permethrin selection, a 7.38-fold increase to permethrin occurred at generation 21. Furthermore, the RR to diazinon decreased in their studies even under further diazinon pressure up to generation 45, which suggests that there may be some genetic constraint associated with resistance to diazinon.

Previously, resistance to diazinon has been reported (from bioassays) in wild horn fly populations only in Arkansas (Cilek et al. 1991, Steelman et al. 1994). In those studies, RRs to diazinon ranged from 8.7 to 23.3, which are higher than those observed in our study. One explanation may be that RRs in our study were based on bioassays using reference colony flies; whereas the Arkansas studies used a wild population (which was exposed to pyrethroids for several years) as the OP-susceptible population. Another factor that could be responsible for the differences observed in susceptibility is the bioassay method; in the Arkansas studies, flies were exposed to diazinon residues on glass while we used the impregnated filter paper method. Burg et al. (1995) found a higher variability in mortality of horn flies exposed to insecticides (including diazinon) on glass than on filter paper. Furthermore, there is significant variation when RRs are calculated

The EST activity was consistently higher in all three samples from Rosepine than in susceptible flies from field and the reference strain. The lowest mean level of EST activity found in OP-resistant flies was 1.9- and 2.2-fold higher than that measured in susceptible flies from St. Gabriel and the reference strain, respectively. A higher EST activity (1.53-fold) in horn flies has been previously associated with pyrethroid resistance (Xu and Bull 1995). However, Szalanski et al. (1995) found that ESTs were not a major mechanism behind pyrethroid resistance in horn flies.

Activity of GST (toward both CDNB and DCNB) was significantly higher in the OP-resistant flies than in the laboratory colony flies, suggesting that these enzymes may play a role in the OP-resistance. However, a negative relationship was observed between enzyme activity and resistance (RR) to diazinon. Therefore, despite the higher GST activity (toward model substrates) in the resistant flies, the absence of a positive correlation with bioassay results suggests that other mechanism(s) may be involved. In house flies, besides enhanced GST and EST activity, diazinon resistance also has been associated with a reduced penetration (Forgash et al. 1962, Gwiazda and Lord 1967) and a higher level of MFO activity (Folsom et al. 1970, Yang et al. 1971). In horn flies, an enhanced MFO activity has been found in pyrethroid-resistant populations (Cilek et al. 1995, Sheppard 1995).
In summary, development of resistance to diazinon associated with the yearly use of diazinon ear tags led to a decrease in product efficacy from greater than twenty to just one week of control in four years, and resistance to other OPs (both diethyl and dimethyl) was observed. These observations were based upon product failure and supported by bioassay results. Esterase activity in resistant flies was higher than in susceptible flies and this may be one of the mechanisms of the observed OP resistance. Treatment of cattle with insecticides other than OPs either in late-summer or fall appeared to partially reverse the level of OP resistance. The results of this study support the concept that basing a horn fly control program upon the use of a single class of insecticides is inappropriate. Due to their low magnitude, the RRs to diazinon were hard to interpret as measures of resistance and were not reliable indicators of product efficacy in the field. However, use of bioassay with a DC of diazinon (1.72 μg/cm²), may be a simple and reliable indicator of potential product efficacy. When no flies survived the DC in pre-season bioassays, the efficacy of OP tags was subsequently found to be greater than 10 weeks, but when more than 5% of the flies survived, OP tags provided less than eight weeks of control.

References


LeOra Software. 1987. POLO-PC a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.


CHAPTER 3

EVALUATION OF THE ALTERNATED YEARLY USE OF ORGANOPHOSPHATE AND SYNERGIZED PYRETHROID EAR TAGS FOR CONTROL OF PYRETHROID-RESISTANT HORN FLY (DIPTERA: MUSCIDAe) POPULATIONS IN LOUISIANA

Introduction

Selection by the continued use of a single type of insecticide can result in resistant populations of the horn fly, *Haematobia irritans irritans* (L.) (Sparks et al. 1985, Kunz and Schmidt 1985). Resistance has been reported in horn flies to several insecticide classes, including chlorinated hydrocarbons, carbamates, pyrethroids, and organophosphates (Sparks et al. 1985, Byford et al. 1985, Cilek et al. 1991). Resistance to pyrethroids, primarily associated with use of insecticidal ear tags, has been reported frequently (Sparks et al. 1985, Quisenberry et al. 1984, Sheppard 1984, Sheppard and Joyce 1992). Strategies that have been proposed for the use of multiple insecticides to manage resistance of horn flies include use of mosaics, rotations, and mixtures of insecticides (Byford et al. 1987b, c; Sparks and Byford 1988). The effects of the use of mixtures containing two insecticides or insecticide(s) plus synergist(s) on development of resistance has been studied under field conditions (Byford et al. 1987b, Cilek and Knapp 1993, Sparks and Byford 1988), but the effects of alternated use of insecticides over an extended period has not been studied under such conditions. The purpose of this study was to test the effects of yearly alternated use of pirimiphos-methyl and *lambda*-cyhalothrin + piperonyl butoxide (PBO) ear tags on product efficacy as a measure of
resistance for two pyrethroid-resistant horn fly populations. The use of bioassay data obtained before horn fly treatments in the spring as a potential tool for predicting product efficacy also was evaluated.

**Materials and Methods**

The study was conducted at two locations (Saint Joseph and Winnsboro, Louisiana) of the Northeast Research Station of the Louisiana Agricultural Experiment Station. Data collected prior to August 1995 were made available by L. Foil and collaborators from the Research Stations. Prior to 1991, insecticidal ear tags had not been used at either location. From 1991 to 1997, yearly rotation between organophosphate (OP) tags and pyrethroid plus synergist (PS) tags was conducted. The pyrethroid ear tags (Saber Extra®) contained 10% lambda-cyhalothrin + 13% PBO and were used every other year from 1991 to 1997. The OP tags (Tomahawk®, Dominator®, and Rotator®) contained 20% pirimiphos-methyl and were used in 1992, 1994, and 1996. Except for 1997, animals also were treated with 1% permethrin + 1% PBO pour-on (Synergized DeLice®) at the time of tagging. All insecticide products were provided by Schering-Plough Animal Health Corp. (formerly Mallinckrodt Veterinary), Union, NJ. Two ear tags were used per animal and the number of cattle ranged from 50 to 65 at both sites. The cows were crossbred Angus (37.5%-50%) x Hereford (25%-50%), *Bos taurus*, x Brahman (12.5%-25%), *Bos indicus*, at both sites. No control (untreated) groups were used at these sites in order to simulate normal farm practices and to avoid maintaining a susceptible fly source. In an effort to reduce migration of susceptible flies into the studied
populations, the same type of insecticide tag that was used at each station was provided to all cattle producers within approximately a two kilometer radius of each station.

Weekly fly counts were conducted from at least one week before ear tags were used until the end of the treatment period, or until there were greater than 50 flies per side for two consecutive weeks. The total number of flies on one side of ten randomly-selected, adult cows per site was estimated before 0830 hours c.d.s.t. with the aid of binoculars at each site. Control was considered adequate when fly counts averaged less than 50 flies/side/animal.

Susceptibility of fly populations was determined by using the impregnated filter paper method (Sheppard and Hinkle 1987). The insecticides used in the tests were diazinon (87.5% purity, Fermenta Animal Health, Kansas City, MO) and lambda-cyhalothrin (93% purity, Mallinckrodt Veterinary, Mundelein, IL). Insecticide bioassays were conducted twice per year (before treatment and at least one week after the tags were removed) using flies collected with hand nets. Fly mortality was determined after a 4 hr-exposure period; flies that could not walk were considered dead. Three replicates (approximately 25 flies each) were used for each insecticide concentration. In 1991, flies collected and tested at the St. Gabriel Research Station (St. Gabriel, LA) of the Louisiana Agricultural Experiment Station, were used as a susceptible strain.

From 1992 to 1997, the lowest concentration of each insecticide which resulted in 100% mortality of flies tested from a reference susceptible colony (Knippling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS, Kerrville, TX) was used yearly as a discriminating concentration (DC). The resistance frequency (RF) was determined
as the percentage of flies surviving the established DC. The DCs were established yearly based on the bioassays for the susceptible flies rather than using a single standard concentration for all years. When necessary, results were corrected for control mortality by Abbott’s formula (Abbott 1925). In most bioassays, control mortality did not exceed 15%, but results with control mortality between 15 and 30% are provided in Tables 3.1 and 3.2. Data for the 1991, 1992, and 1994 post-season bioassays were not included because flies were collected from tagged cattle; other missing data were excluded due to control mortality above 30%. Bioassay data were analyzed by probit analysis using POLO-PC (LeOra Software 1987). Differences between LC_{50}s were considered significant when their 95% fiducial limits did not overlap. Resistance ratios (RR) were calculated by dividing the LC_{50} from field populations by the LC_{50} from the reference susceptible strain. No RR data are presented in the tables for 1991 because the laboratory strain was not tested that year. The RR for 1991 was calculated based on the LC_{50}s from a more susceptible fly population collected at St. Gabriel. Correlation analysis (PROC CORR) (SAS Institute 1989) was performed between insecticide efficacy (number of weeks of control pooled for the years when each insecticide was used) and the respective pre-season RRs and RFs.

Results

The efficacy of OP ear tags was highest in the first year of use (1992) at both sites (Figure 3.1). There was a steady decline in the efficacy of synergized pyrethroid tags from 1993 to 1997, despite the use of OP tags in alternate years. At St. Joseph, the efficacy of pyrethroid ear tags declined continuously from seven weeks in 1991 to two
weeks in 1997, while the efficacy of OP tags dropped from fifteen weeks in 1992 to just three weeks in 1996. At Winnsboro, the efficacy of pyrethroid tags increased from four weeks in 1991 to seven weeks in 1993 but declined in 1997 to zero despite the alternated use of OP tags. The efficacy of OP ear tags decreased from ten weeks in 1992 to five weeks in 1994, but there was a slight increase in efficacy in 1996 (7 weeks).

![Graph showing the control of horn fly populations using a yearly pyrethroid (lambda-cyhalothrin+PBO)/organophosphate (pirimiphos-methyl) ear tag rotation schedule at two locations in Louisiana.]

Figure 3.1. Control of horn fly populations using a yearly pyrethroid (lambda-cyhalothrin+PBO)/organophosphate (pirimiphos-methyl) ear tag rotation schedule at two locations in Louisiana.
Table 3.1. Susceptibility (LC$_{50}$ expressed as µg/cm$^2$) and resistance ratios of horn flies from cattle under a yearly insecticide ear tag rotation schedule at Winnsboro, LA

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment (ear tag)</th>
<th>Bioassay time</th>
<th>Diazinon LC$_{50}$ (95% F.L.)</th>
<th>RR$^c$</th>
<th>λ-cyhalothrin LC$_{50}$ (95% F.L.)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>PS</td>
<td>Pre</td>
<td>0.46 (0.32-0.67)</td>
<td>-</td>
<td>3.41 (1.65-9.79)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>OP</td>
<td>Pre</td>
<td>0.49 (0.39-0.60)</td>
<td>1.2</td>
<td>7.51 (5.25-10.19)</td>
<td>399.5</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>PS</td>
<td>Pre</td>
<td>0.80 (0.38-2.20)</td>
<td>1.7</td>
<td>6.79 (4.49-8.97)</td>
<td>148.1</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>0.57 (-)$^d$</td>
<td>1.2</td>
<td>3.78 (-)</td>
<td>82.4</td>
</tr>
<tr>
<td>1994</td>
<td>OP</td>
<td>Pre</td>
<td>1.57 (1.04-2.03)</td>
<td>3.7</td>
<td>8.07 (3.41-16.16)</td>
<td>101.6</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>PS</td>
<td>Pre</td>
<td>1.01 (0.86-1.16)</td>
<td>2.5</td>
<td>9.39 (7.14-11.85)$^e$</td>
<td>412.6</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>1.34 (1.05-1.59)</td>
<td>3.4</td>
<td>22.62 (16.70-30.80)$^g$</td>
<td>993.6</td>
</tr>
<tr>
<td>1996</td>
<td>OP</td>
<td>Pre</td>
<td>0.52 (0.48-0.56)</td>
<td>1.4</td>
<td>-</td>
<td>116.6</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>0.95 (0.65-1.50)</td>
<td>2.6</td>
<td>-</td>
<td>112.9</td>
</tr>
<tr>
<td>1997</td>
<td>PS</td>
<td>Pre</td>
<td>2.04 (1.57-2.51)$^a$</td>
<td>4.8</td>
<td>8.83 (5.91-13.91)</td>
<td>359.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>1.23 (0.82-1.54)</td>
<td>2.9</td>
<td>64.52 (38.46-222.89)</td>
<td>2625.0</td>
</tr>
</tbody>
</table>

$^a$ PS = pyrethroid (λ-cyhalothrin) + synergist (piperonyl butoxide), OP = organophosphate (pirimiphos-methyl)

$^b$ Pre = bioassay conducted before any insecticide treatment, Post = bioassay conducted at least one week after tags were removed

$^c$ Resistance ratio (LC$_{50}$ from field population / LC$_{50}$ from Kerrville reference susceptible strain)

$^d$ Data too heterogeneous to calculate 95% fiducial limits (LeOra Software 1987)

$^e$ Data from bioassays with control mortality between 15% and 30% (control mortality <15% in all other bioassays)

$^f$ Actual LC$_{50}$s were not determined
Table 3.2. Susceptibility (LC$_{50}$ expressed as $\mu$g/cm$^2$) and resistance ratios of horn flies from cattle under a yearly insecticide ear tag rotation schedule at St. Joseph, LA

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment (ear tag)*</th>
<th>Bioassay time$^b$</th>
<th>Diazinon LC$_{50}$ (95% F.L.)</th>
<th>RR$^c$</th>
<th>$\lambda$-cyhalothrin LC$_{50}$ (95% F.L.)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>PS</td>
<td>Pre</td>
<td>1.07 (0.74-1.39)</td>
<td>-</td>
<td>1.94 (1.56-2.43)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>OP</td>
<td>Pre</td>
<td>0.43 (0.36-0.49)$^a$</td>
<td>1.0</td>
<td>5.76 (4.85-6.74)</td>
<td>306.4</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>92.6</td>
</tr>
<tr>
<td>1993</td>
<td>PS</td>
<td>Pre</td>
<td>0.05 (0.03-0.08)</td>
<td>0.1</td>
<td>0.95 (0.67-1.29)</td>
<td>20.8</td>
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<tr>
<td></td>
<td>Post</td>
<td></td>
<td>0.55 (0.48-0.65)</td>
<td>1.2</td>
<td>7.48 (5.46-10.97)</td>
<td>163.2</td>
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<td>1994</td>
<td>OP</td>
<td>Pre</td>
<td>0.45 (0.37-0.54)</td>
<td>1.1</td>
<td>5.72 (3.98-7.81)</td>
<td>72.0</td>
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<tr>
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<td>Post</td>
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<td></td>
</tr>
<tr>
<td>1995</td>
<td>PS</td>
<td>Pre</td>
<td>0.39 (-)$^e$</td>
<td>1.0</td>
<td>3.36 (1.21-14.70)</td>
<td>147.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>0.64 (-)</td>
<td>1.6</td>
<td>9.91 (-)</td>
<td>435.3</td>
</tr>
<tr>
<td>1996</td>
<td>OP</td>
<td>Pre</td>
<td>0.13 (0.11-0.15)</td>
<td>0.4</td>
<td>$^f$</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>0.47 (0.34-0.63)</td>
<td>1.3</td>
<td>$^f$</td>
<td>29.8</td>
</tr>
<tr>
<td>1997</td>
<td>PS</td>
<td>Pre</td>
<td>1.20 (-)</td>
<td>2.8</td>
<td>0.54 (0.16-1.25)</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td>42.74 (24.53-239.53)</td>
<td>1738.7</td>
</tr>
</tbody>
</table>

* PS= pyrethroid ($\lambda$-cyhalothrin) + synergist (piperonyl butoxide), OP= organophosphate (pirimiphos-methyl)

$^b$ Pre= bioassay conducted before any insecticide treatment, Post= bioassay conducted at least one week after tags removed

$^c$ Resistance ratio (LC$_{50}$ from field population / LC$_{50}$ from Kerrville reference susceptible strain)

$^d$ Data from bioassay with control mortality of 26% (control mortality <15% in all other bioassays)

$^e$ Data too heterogeneous to calculate 95% fiducial limits (LeOra Software 1987)

$^f$ Actual LC$_{50}$'s were not determined
At the beginning of the study, RRs to *lambda*-cyhalothrin were 7.2 and 12.7 at St. Joseph and Winnsboro, respectively, based on an LC$_{50}$ of 0.27 µg/cm$^2$ for flies from St. Gabriel. During the study, RRs for *lambda*-cyhalothrin ranged from 20.8 (1993 pre) to 1,738.7 (1997 post) and from 82.4 (1993 post) to 2,625.0 (1997 post) at St. Joseph and Winnsboro, respectively (Tables 3.1 and 3.2).

Except for the 1993 bioassays at Winnsboro, RRs for *lambda*-cyhalothrin increased from pre- to post-season tests at both sites when pyrethroid ear tags were used. After OP ear tags were used in 1992, RRs for *lambda*-cyhalothrin dropped substantially before the 1993 treatment season. However, this reduction did not occur in the second and third year of OP ear tag use. In the third year of OP tag use (1996), RRs for the pyrethroid did not change between pre- and post-season bioassays at Winnsboro and increased approximately 30% at St. Joseph. The RRs for diazinon increased every year at St. Joseph during the season, independent of the type of ear tag used. At Winnsboro, a reduction in the RR for diazinon from pre- to post-season was observed when pyrethroid tags were used in 1993 and 1997 and an increase occurred under OP pressure (1996). In contrast, the RR for diazinon decreased in 1992 despite the use of OP tags and increased in 1995 despite the use of pyrethroid tags.

The RFs for *lambda*-cyhalothrin ranged from 70.2% to 100% at Winnsboro and 49.2% to 100% at St. Joseph (Table 3.3). At Winnsboro, RFs for *lambda*-cyhalothrin were generally higher than those at St. Joseph and remained close to 100% in most tests after 1994. A change in one concentration for the DC (0.86 µg/cm$^2$ in 1993 vs. 1.72 µg/cm$^2$ in all other years) had a large effect on the calculated RF for diazinon.

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No consistent correlation was found between insecticide efficacy (expressed as the number of weeks of control) and pre-season RFs and RRs (Table 3.4). The correlation between control efficacy and bioassay parameters for diazinon varied from strongly negative ($r = -0.86$) to moderately positive ($r = 0.52$) for RR, and from -0.43 and -0.78 for RF. Correlation coefficients for control efficacy and the RR for lambda-cyhalothrin ranged from -0.01 to -0.89. The correlation coefficient for the RF and control for lambda-cyhalothrin was 0.73 at St. Joseph; analysis could not be conducted for Winnsboro because the RFs were 100% at this site for all years when the PS tags were used.

Table 3.3. Frequency of horn flies surviving discriminating concentrations\(^a\) of insecticides using impregnated filter paper bioassays

<table>
<thead>
<tr>
<th>Year</th>
<th>Bioassay time</th>
<th>Winnsboro</th>
<th>St. Joseph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diazinon</td>
<td>λ-cyhalothrin</td>
</tr>
<tr>
<td>1992</td>
<td>Pre</td>
<td>3.4</td>
<td>100.0</td>
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<tr>
<td></td>
<td>Post</td>
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<tr>
<td>1993</td>
<td>Pre</td>
<td>59.7</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>6.4</td>
<td>85.2</td>
</tr>
<tr>
<td>1994</td>
<td>Pre</td>
<td>80.2</td>
<td>70.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1995</td>
<td>Pre</td>
<td>68.3</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>82.8</td>
<td>100.0</td>
</tr>
<tr>
<td>1996</td>
<td>Pre</td>
<td>0</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>8.9</td>
<td>100.0</td>
</tr>
<tr>
<td>1997</td>
<td>Pre</td>
<td>59.7</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>20.9</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\(^a\) Lowest concentrations from each year which resulted in 100% mortality in flies from the susceptible strain

\(^b\) Pre = pre-season bioassay, conducted before any insecticidal treatment was applied; Post = post-season bioassay, conducted at least one week after tags were removed
Table 3.4. Correlation coefficients of control efficacy* on pre-season bioassay data

<table>
<thead>
<tr>
<th>Site</th>
<th>n</th>
<th>Resistance ratio**</th>
<th>Resistance frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diazinon</td>
<td>λ-cyhalothrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Joseph</td>
<td>3</td>
<td>0.52</td>
<td>-0.01</td>
</tr>
<tr>
<td>Winnsboro</td>
<td>3</td>
<td>-0.86</td>
<td>-0.89</td>
</tr>
<tr>
<td>Both sites</td>
<td>6</td>
<td>-0.13</td>
<td>-0.57</td>
</tr>
</tbody>
</table>

* Number of weeks of control pooled for the years when each insecticide ear tag was used
** LC<sub>50</sub> field population / LC<sub>50</sub> reference susceptible colony
* Percentage of wild flies surviving treatment with the lowest concentration that resulted in 100% mortality in susceptible flies
* 100% resistance frequency at this site each year

Discussion

Except when OP ear tags were used for the first time, both pyrethroid and OP ear tag treatments provided limited control during the study. Ear tags containing 10% lambda-cyhalothrin, 20% cyhalothrin + 5% PBO, and 20% pirimiphos-methyl have been reported to provide a high degree of control in pyrethroid-resistant horn fly populations in Louisiana (Sparks and Byford 1988). In the current study, the lambda-cyhalothrin + PBO ear tags provided limited control even in the first year of use. Although insecticidal ear tags had not been used at either location prior to 1991, it is possible that pesticides applied to cotton or to cattle at these sites may have influenced the susceptibility of the flies. In Georgia, lambda-cyhalothrin ear tags were effective (infestation <50 flies per side) for 19 weeks in the first year of use against a pyrethroid-resistant population and remained effective for at least 14 weeks during the next two years, but the efficacy subsequently dropped to four weeks or less (Sheppard and Joyce 1992).

Cross-resistance among pyrethroid insecticides has been described frequently and is considered to be associated with the knockdown resistance (kdr) caused by altered
target-site sensitivity (Byford et al. 1985, Sheppard and Joyce 1992, Bull et al. 1988). Although kdr could have been responsible for the initial resistance observed for PS tags, the later development of resistance to the OP tags suggests that at least another resistance mechanism (which was not effectively inhibited by PBO) was present in flies at both sites. Enzymatic metabolism has been shown to be an important secondary factor contributing to development of resistance to pyrethroids and potentially contributing to resistance to other classes of insecticides (Byford et al. 1985, Bull et al. 1988).

The rotation of OP ear tags failed to improve pyrethroid ear tag efficacy against pyrethroid-resistant fly populations, and resistance to the OP developed in a relatively short time. McKenzie and Byford (1993) reported that susceptible horn flies developed resistance to both permethrin and diazinon insecticides when they were sprayed in a 4-month rotation schedule on indoor animals, but selection was faster and resistance levels were higher when single insecticides were applied continuously.

A tag and pour-on spring treatment was recommended by the manufacturer when these studies were initiated and was used during the first six years of this study. The pour-on treatment was not used in 1997 in order to determine if the efficacy of the tags was less than the number of weeks of control expected with the permethrin pour-on. Permethrin pour-on treatments provide fly control for approximately 3-4 weeks in Louisiana (Foil, unpublished data), while ear tags have been shown to be effective for at least 20 weeks under certain conditions (Ahrens and Cocke 1979). When the study was initiated, it was considered that pyrethroid-resistant flies were highly susceptible to OPs (Byford et al. 1988). Therefore, the pyrethroid pour-on treatment potentially could have
selected flies more susceptible to the OP tags, but this did not appear to occur. In fact, the combination of the poured permethrin+PBO and the OP ear tag could have contributed to selection of individuals resistant to both insecticides. Since there was season-long selection from both PS and OP ear tag treatments, we considered the yearly rotation between the OP and the pyrethroid-synergist mixture ear tags as the basic strategy used in this study.

Although bioassay results showed that pyrethroid resistance dropped substantially at both sites after the first use of OP ear tags, this tendency did not continue in subsequent years. There was little difference in susceptibility of flies to diazinon regardless of obvious resistance to pirimiphos-methyl. Thus, data from these bioassays may not effectively measure resistance to pirimiphos-methyl when compared to product efficacy. In this study, diazinon was used rather than pirimiphos-methyl in the OP bioassays because the former has been used as our standard OP in previous studies. However, similar patterns of susceptibility have been observed when bioassays using either diazinon or pirimiphos-methyl were used for monitoring horn fly susceptibility to OPs (Barros and Foil, unpublished information). McKenzie and Byford (1993) found a maximum RR of 7.24 in horn flies selected with diazinon after 36 generations. Relatively small RRs (<6.5) also were observed in adult flies from different OP-resistant strains of the Australian sheep blowfly (*Lucilia cuprina*) after diazinon selection (Roxburgh and Shanahan 1973, Shanahan and Roxburgh 1974). The low magnitude of RR for diazinon may explain why there have been few cases of diazinon resistance reported (Cilek et al.)
1991, Steelman et al. 1994) and why there have been no studies on horn flies that relate bioassay results to OP product control failure.

Cilek and Knapp (1993) reported a 100% RF to permethrin in a pyrethroid-resistant horn fly population after 14 weeks of using either cyhalothrin+PBO or cypermethrin+PBO+chlorpyrifos ear tags. In our study, post-season bioassays showed very high RFs (>85%) for \textit{lambda}-cyhalothrin at both sites when fly populations were exposed to pyrethroid ear tags.

No clear correlation was obtained between pre-season bioassay results (RR or RF) and control efficacy. This is not surprising considering that there are multiple mechanisms for resistance to pyrethroids, some of which are not detected in the bioassay used in this study, such as the behavioral resistance mechanism (Lockwood et al. 1985, Byford et al. 1987a). Further, there is a lack of correlation between the effect of insecticides in the bioassay and on the animal. Environmental and temporal factors as well as physical-chemical differences between the treated surfaces (filter paper vs. skin) are important factors. Furthermore, there is a mosaic of insecticide concentrations on cattle (Taylor et al. 1985, Leprince et al. 1991) and a temporal variation in insecticide release from the ear tags (Miller et al. 1983). There have been limited studies on horn flies relating bioassay results and insecticide efficacy in the field (Sheppard and Joyce 1992, Weinzierl et al. 1990). The meaning of resistance levels is of limited value if not related to control efficacy under field conditions (Farnham et al. 1984). Bioassay results (RR and RF) have been used successfully to forecast insecticide efficacy for house flies; permethrin failure was observed when the frequency of insects homozygous for kdr was
at least 10% and/or the RR was at least 15 (Farnham et al. 1984). Although it was not possible to predict accurately a time for product failure based solely on RR or RF from our bioassays, in practice, such variables can be used to empirically forecast general product performance with the aid of a knowledge of prior insecticide use.

In 1997, at St. Joseph, one of the lowest RR’s (21.9) for lambda-cyhalothrin was measured before tags were applied, and the treatment failed two weeks after the animals were tagged. Thus, although much higher RRs have been reported by others and in our study, only a 22-fold RR was needed to permit a significant proportion of the fly population to survive the lambda-cyhalothrin ear tag treatment. Regardless of resistance level and/or frequency, control failure only would be observed when fly numbers on untreated animals (population size) were at least equal to the control threshold (50 flies per side). In Louisiana, fly counts on untreated cattle from four sites conducted during the seven years of this study (Foil and Barros, unpublished data) had an average peak of 245 flies per side. Considering this population size, at least 20% of flies present in the population would have to be resistant to the treatment for the number of flies to reach 50 per side. This minimum frequency of resistant flies would vary with the population size, which would vary spatially and temporally.

The alternated use of OP and PS tags for the control of pyrethroid-resistant horn flies did not prevent development of resistance to both insecticides when used separately. Cross-resistance between insecticide classes has been reported for horn flies. Harvey et al. (1984) reported that tetrachlorvinphos ear tags were unsuccessful in controlling
pyrethroid-resistant flies in Kansas. Similarly, in Arkansas, Cilek et al. (1991) reported that pyrethroid-resistant flies exposed for 15 weeks to cyhalothrin ear tags showed a decreased susceptibility to both cyhalothrin and pirimiphos-methyl, and higher resistance ratios to both cyhalothrin and pirimiphos-methyl also were found when cattle were treated with pirimiphos-methyl ear tags for 15 weeks.

Successful fly control (number of flies below a certain threshold) during a season does not necessarily prevent the development of resistance even when insecticides are rotated. The average number of flies per side following an insecticide application reflects an interaction between resistance level (RR), frequency of effectively resistant flies in the population, and the population size (which naturally fluctuates). The yearly rotation of insecticides (particularly pyrethroids and OPs) has been recommended as a strategy to manage insecticide resistance in horn flies. This study showed that the use of this strategy in pyrethroid-resistant populations was unsuccessful in improving pyrethroid efficacy or preventing further development of resistance to pyrethroid or OP compounds.

References


LeOra Software. 1987. POLO-PC a user’s guide to probit or logit analysis. LeOra Software, Berkeley, CA.


CHAPTER 4

EVALUATION OF CHLORFENAPYR EAR TAG EFFICACY AND SUSCEPTIBILITY OF HORN FLIES (DIPTERA: MUSCIDAЕ) TO CHLORFENAPYR

Introduction

Since WWII, a novel insecticide class for controlling horn flies, *Haematobia irritans irritans* (L.), has been introduced into commerce approximately every decade (Sparks et al. 1985, Drummond et al. 1988). Except for the avermectins, resistance in horn flies has been described for all of these commercially available insecticides (Byford et al. 1985, Sparks et al. 1985). Although a relatively large number of insecticide products currently are available commercially for horn fly control, the active ingredient for the majority of these products is either a pyrethroid or an organophosphate (OP) insecticide.

A new insecticide class known as the pyrroles was discovered by the American Cyanamid Company in the 1980's as a result of the synthetic improvement of a natural fermentation product from a *Streptomyces fumanus* culture (Kuhn et al. 1993, Treacy et al. 1994). The lead compound of this new class, a cyanopyrrole known as chlorfenapyr (AC303630), is a pro-insecticide, which is activated in insects by oxidative metabolism (Treacy et al. 1994). Chlorfenapyr interferes with cellular respiration by uncoupling oxidative phosphorylation, thus impairing ATP production in the mitochondria (Black et al. 1994, Treacy et al. 1994). Laboratory studies have shown that chlorfenapyr has both contact and oral activity against the tobacco budworm, *Heliothis virescens* (F.) (Treacy
et al. 1991). In field studies, chlorfenapyr has been found to provide acceptable control of several major insect and mite pests of cotton (Farlow et al. 1991). Bioassays have shown that an increased susceptibility to chlorfenapyr can occur in pyrethroid-resistant insects such as the tobacco budworm (Pimprale et al. 1997) and the horn fly (Sheppard and Joyce 1998). In this study, we evaluated the efficacy of experimental ear tags containing chlorfenapyr for control of horn flies under field conditions and measured the susceptibility of field populations of both susceptible and resistant (OP- and pyrethroid-resistant) horn flies to chlorfenapyr.

**Materials and Methods**

During 1996 and 1997, experimental ear tags containing chlorfenapyr (4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl) pyrrole-3-carbonitrile) were evaluated under field conditions at the St. Gabriel Research Station (St. Gabriel, LA), a unit of the Louisiana Agricultural Experiment Station (LAES). In 1996, three chlorfenapyr ear tags, containing 30% A.I., 40% A.I., or 40% A.I. in thicker tags (TT), and 40% diazinon tags (Patriot®, Boehringer Ingelheim Animal Health, St. Joseph, MO) were tested. Forty mixed breed adult cows were randomly assigned to the four tag groups and an untreated control group (8 animals each). In 1997, three chlorfenapyr tags, containing 20% A.I., 30% A.I., or 30% A.I. TT, and 40% diazinon tags were tested. Fifty mixed breed adult cows were randomly assigned to the four tag groups and an untreated control group (10 animals each). In both trials, tags were applied at two per animal (one per ear); the treatment periods were June 10-November 27, 1996 and May 22-October 30, 1997. All tags were provided by Fort Dodge Animal Health (FDAH), Princeton, NJ.
Cattle were maintained on pastures ranging from 15 to 40 acres. All treatment groups were separated by at least one pasture (about 30-40 m), and were separated from the untreated control group by greater than 1 km.

Fly counts were conducted weekly from about one week prior to tagging until tags were removed in both years. In 1996, the pre-treatment count was conducted before animals were assigned to treatment groups. In 1997, the pre-treatment count was taken after treatment groups were assigned to their pastures. The number of flies on one side of all animals in each group was estimated before 0900 hr c.d.s.t. by one observer at a close range. Efficacy of control provided by the tags is discussed in terms of percentage of reduction of fly numbers, which was calculated as follows:

\[
\text{Percentage Efficacy} = \frac{\text{control group mean} - \text{treated group mean}}{\text{control group mean}} \times 100
\]

Fly count data were transformed by logarithmic transformation \((y=\log_{10}(1+\text{count}))\) before analyses were performed. First, analysis of combined post treatment data was conducted using a two-way analysis of variance (PROC GLM) (SAS Institute 1989) using the model \(\text{Flycount} = \text{Week} \times \text{Treatment} \times \text{Week} \times \text{Treatment}\). Then, a one-way analysis of variance was performed with treatment groups for each week using the model \(\text{Flycount} = \text{Treatment}\). Differences in fly counts were considered statistically significant at the 5% significance level.

Susceptibility of fly populations to chlorfenapyr (99% purity, FDAH) was assessed using the impregnated filter paper method (Sheppard and Hinkle 1987). Chlorfenapyr impregnated filter papers (from 0.1953 to 50 \(\mu g/cm^2\)) were provided by FDAH. Flies were collected from untreated animals using hand nets, and the bioassays were performed.
immediately after collection. The 1996 pre-season bioassay at St. Gabriel was conducted before animals were tagged. Post-season bioassays were conducted at least two weeks after tags were removed. Three replicates (approximately 30 flies each) per concentration were used, and flies unable to walk after a 4 hr exposure period were considered dead.

Susceptibility of flies to chlorfenapyr was measured in the fall of 1997 at seven LAES locations: Ben Hur (Baton Rouge), Hill Farm (Homer), Red River (Bossier City), Rosepine (Rosepine), St. Gabriel (St. Gabriel), and two locations of the Northeast Research Station, i.e. Northeast (St. Joseph) and Macon Ridge (Winnsboro). Also, susceptibility of flies from these locations to *lambda*-cyhalothrin (93% purity, Mallinckrodt Veterinary, Mundelein, IL) and diazinon (87.5% purity, Fermenta Animal Health, Kansas City, MO) was assessed using the impregnated filter paper method. St. Gabriel was the only location where chlorfenapyr tags were used.

Flies from the Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS (Kerrville, TX) were used as the reference susceptible strain. Resistance ratios (RRs) were calculated as LC$_{50}$ from field populations/LC$_{50}$ from the reference strain. Mortality data were corrected using Abbott’s formula (Abbott 1925) when necessary, and bioassay results were analyzed by probit analysis using POLO-PC (LeOra Software 1987). The LC$_{50}$'s were considered significantly different when the 95% fiducial limits did not overlap. RRs to chlorfenapyr were not calculated for 1996 because bioassays were not conducted with the reference strain.
Results

In 1996, average number of flies per side on untreated cattle ranged from 44 to 275 (Table 4.1). Number of horn flies were significantly reduced after treatment in all treated groups. Average control efficacies for the entire study period (24 weeks) were 89, 77, 91, and 95% for chlorfenapyr 30%, 40%, 40% TT, and diazinon tags, respectively. The number of flies was reduced more than 90% for at least 10 weeks for all treatments. The 40%TT chlorfenapyr tags provided >90% control until week 16 (except for weeks 12 and 13) and provided >80% control until week 18. Ear tags containing 30% chlorfenapyr reduced fly numbers >80% until week 16, except for week 13. Greater than 90% control was achieved for 23 weeks with diazinon tags, except for week 19.

In 1997, the average number of flies per side before treatment was 210, 253, 190, and 269 for the chlorfenapyr 20%, 30%, 30% TT, and diazinon groups, respectively. The average number of flies/side on untreated cattle during the study ranged from 35 to 305 (Table 4.2). Average control efficacies for the entire study period were 63, 72, 82, and 78% for chlorfenapyr 20%, 30%, 30% TT, and diazinon tags, respectively (Table 4.2). Reduction in fly numbers above 90% did not occur until after the second week post-treatment with the chlorfenapyr tag treatments. After the second week (except for weeks 5 and 7), efficacy of 20% chlorfenapyr tags remained >90% until week 8. Efficacy of 30% chlorfenapyr tags above 90% was observed until week 8 and above 80% until week 15, except for weeks 9 and 10. The 30% TT chlorfenapyr tags reduced fly numbers >90% until week 18, except for weeks 7 and 15. Control using the diazinon tags was >90% from the first week after treatment until week 16 (except week 10).
Results from pyrethroid and OP bioassays at St. Gabriel indicated that flies were susceptible to both \textit{lambda}-cyhalothrin and diazinon at the beginning of both trials (Table 4.3). Initial RRs were 0.6 for \textit{lambda}-cyhalothrin for both years and 0.5 and 0.1 for diazinon.

Table 4.1. Mean number of horn flies per side on control cattle and percentage reduction on treated animals by insecticidal ear tags\textsuperscript{a} at St. Gabriel, LA, in 1996

<table>
<thead>
<tr>
<th>Date</th>
<th>Week post-treatment\textsuperscript{b}</th>
<th>Control flies/side</th>
<th>Chlorfenapyr 30%</th>
<th>Chlorfenapyr 40%</th>
<th>Chlorfenapyr 40% TT\textsuperscript{c}</th>
<th>Diazinon 40%</th>
</tr>
</thead>
<tbody>
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<td>05 Jun</td>
<td>-</td>
<td>209</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>219</td>
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<td>96</td>
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<td>100</td>
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<tr>
<td>27 Jun</td>
<td>2</td>
<td>275</td>
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<td>100</td>
</tr>
<tr>
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<td>244</td>
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<td>17 Jul</td>
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<tr>
<td>24 Jul</td>
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<td>165</td>
<td>95</td>
<td>98</td>
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<td>172</td>
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<td>08 Aug</td>
<td>8</td>
<td>238</td>
<td>97</td>
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<td>97</td>
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<td>228</td>
<td>98</td>
<td>95</td>
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<td>20 Aug</td>
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<td>94</td>
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<tr>
<td>10 Oct</td>
<td>17</td>
<td>100</td>
<td>78</td>
<td>76</td>
<td>89</td>
<td>93</td>
</tr>
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<td>19 Oct</td>
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<td>197</td>
<td>81</td>
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<td>92</td>
</tr>
<tr>
<td>24 Oct</td>
<td>19</td>
<td>88</td>
<td>64</td>
<td>34\textsuperscript{d}</td>
<td>74</td>
<td>72</td>
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<td>31 Oct</td>
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<td>188</td>
<td>86</td>
<td>75</td>
<td>83</td>
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<tr>
<td>06 Nov</td>
<td>21</td>
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<td>89</td>
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<td>96</td>
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<tr>
<td>14 Nov</td>
<td>22</td>
<td>109</td>
<td>96</td>
<td>39\textsuperscript{d}</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>21 Nov</td>
<td>23</td>
<td>44</td>
<td>46\textsuperscript{d}</td>
<td>16\textsuperscript{d}</td>
<td>63\textsuperscript{d}</td>
<td>90</td>
</tr>
<tr>
<td>27 Nov</td>
<td>24</td>
<td>46</td>
<td>72</td>
<td>34\textsuperscript{d}</td>
<td>85</td>
<td>88</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Fly number and percentage reduction based on arithmetic means; all animals were tagged on 10 June and tags were removed on 27 November.

\textsuperscript{b} Number of weeks were calculated as close as possible from the real dates.

\textsuperscript{c} TT = thicker tags.

\textsuperscript{d} Mean number of flies on treated animals did not differ significantly from control group (\(\alpha=0.05\)).
diazinon in 1996 and 1997, respectively. Results from pyrethroid and OP bioassays conducted at all locations in 1997 are presented in Table 4.3. Flies from all sites except St. Gabriel were resistant to pyrethroids; RRs to lambda-cyhalothrin ranged from 16.7 (Rosepine) to 2,625.0 (Winnsboro). Susceptibility to diazinon was less variable than that

Table 4.2. Mean number of horn flies per side on control cattle and percentage reduction on treated animals by insecticidal ear tags at St. Gabriel, LA, in 1997

<table>
<thead>
<tr>
<th>Date</th>
<th>Week post-treatment</th>
<th>Control flies/side</th>
<th>Chlorfenapyr</th>
<th>Diazinon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20%</td>
<td>30%</td>
</tr>
<tr>
<td>22 May</td>
<td>0</td>
<td>263</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29 May</td>
<td>1</td>
<td>131</td>
<td>78</td>
<td>49</td>
</tr>
<tr>
<td>05 Jun</td>
<td>2</td>
<td>138</td>
<td>62</td>
<td>53d</td>
</tr>
<tr>
<td>12 Jun</td>
<td>3</td>
<td>145</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>20 Jun</td>
<td>4</td>
<td>178</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>27 Jun</td>
<td>5</td>
<td>91</td>
<td>89</td>
<td>94</td>
</tr>
<tr>
<td>03 Jul</td>
<td>6</td>
<td>65</td>
<td>96</td>
<td>99</td>
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<td>10 Jul</td>
<td>7</td>
<td>35</td>
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<td>96</td>
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<td>17 Jul</td>
<td>8</td>
<td>173</td>
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<td>99</td>
</tr>
<tr>
<td>25 Jul</td>
<td>9</td>
<td>118</td>
<td>33</td>
<td>75</td>
</tr>
<tr>
<td>31 Jul</td>
<td>10</td>
<td>84</td>
<td>44</td>
<td>37d</td>
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<tr>
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<td>14</td>
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<td>90</td>
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<td>11 Sep</td>
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<td>97</td>
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<td>193</td>
<td>49c</td>
<td>79</td>
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<tr>
<td>10 Oct</td>
<td>20</td>
<td>228</td>
<td>41d</td>
<td>59d</td>
</tr>
<tr>
<td>16 Oct</td>
<td>21</td>
<td>103</td>
<td>-61d</td>
<td>2d</td>
</tr>
<tr>
<td>23 Oct</td>
<td>22</td>
<td>106</td>
<td>72</td>
<td>50d</td>
</tr>
<tr>
<td>30 Oct</td>
<td>23</td>
<td>61</td>
<td>54d</td>
<td>50d</td>
</tr>
</tbody>
</table>

* Fly number and percentage reduction based on arithmetic means; all animals were tagged on 22 May and tags were removed on 30 October
* Number of weeks were considered as close as possible from the real dates
* TT = thicker tags
* Mean number of flies on treated animals did not differ significantly from control group (α=0.05)
Table 4.3. Susceptibility (LC50 expressed as μg/cm²) and resistance ratios for horn fly populations in Louisiana

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>Chlorfenapyr</th>
<th>λ-Cyhalothrin</th>
<th>Diazinon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LC50 (95% F.L.)</td>
<td>RR</td>
<td>LC50 (95% F.L.)</td>
</tr>
<tr>
<td>1996</td>
<td>St. Gabriel&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.30 (-)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>0.10 (0.06-0.16)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>St. Gabriel&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.00 (-)&lt;sup&gt;gs&lt;/sup&gt;</td>
<td>-</td>
<td>0.40 (0.15-0.70)</td>
</tr>
<tr>
<td>1997</td>
<td>St. Gabriel&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.85 (2.08-3.39)</td>
<td>0.08</td>
<td>0.04 (0.01-0.08)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rosepine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.98 (2.69-5.14)</td>
<td>0.11</td>
<td>0.41 (0.33-0.50)</td>
</tr>
<tr>
<td></td>
<td>Ben Hur&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.58 (1.87-3.32)</td>
<td>0.07</td>
<td>2.47 (2.05-2.92)</td>
</tr>
<tr>
<td></td>
<td>Red River&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.86 (4.16-5.53)</td>
<td>0.13</td>
<td>2.47 (1.47-4.15)</td>
</tr>
<tr>
<td></td>
<td>Hill Farm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.12 (2.43-3.81)</td>
<td>0.09</td>
<td>10.17 (8.59-12.01)</td>
</tr>
<tr>
<td></td>
<td>St. Joseph&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.16 (-)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.17</td>
<td>42.74 (24.53-239.53)</td>
</tr>
<tr>
<td></td>
<td>Winnsboro&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.46 (6.51-8.69)</td>
<td>0.21</td>
<td>64.52 (38.46-222.89)</td>
</tr>
<tr>
<td></td>
<td>Kerrville&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.39 (-)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>0.02 (-)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pre-season (May/June) bioassays  
<sup>b</sup> Post-season (September/October) bioassays  
<sup>c</sup> Reference susceptible colony (Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS, Kerrville, TX)  
<sup>d</sup> Resistance ratio (LC50 from field population / LC50 from reference susceptible strain)  
<sup>e</sup> Data too heterogeneous to calculate 95% fiducial limits (LeOra Software 1987)  
<sup>f</sup> Control mortality in the bioassay between 15% and 22% (<sup>·</sup>), and 30.9% (<sup>·</sup>); control mortality <15% in all other bioassays
observed for pyrethroids, and RRs ranged from 0.1 (St. Gabriel) to 6.6 (Rosepine). Lower LC₅₀'s to chlorfenapyr were measured for field populations than for the reference susceptible strain (Table 4.3). The LC₅₀ (36.39 µg/cm²) observed for the reference strain was 4.9-fold higher than the highest LC₅₀ (7.46 µg/cm²) measured among field populations.

Discussion

Ear tags containing 30% or 40% chlorfenapyr were effective for horn fly control. The diazinon tag treatment was more effective than the chlorfenapyr treatments during the first year of study. However, in the second year, efficacy of 30% chlorfenapyr tags was equivalent to that observed using the diazinon tags, and efficacy of 30% TT chlorfenapyr tags was higher than that recorded for diazinon tags. Similar levels of horn fly control for diazinon tags have been reported elsewhere (Cocke et al. 1990, Crosby et al. 1991, Byford et al. 1988).

In 1997, pyrethroid resistance was detected at all sites except St. Gabriel, which is consistent with reports of its widespread occurrence (Sparks et al. 1985, Kunz and Schmidt 1985). If a RR of >2 for diazinon is considered as an indicator of an OP-resistant population (Chapter 2), then four of the seven populations tested were considered OP-resistant. This is consistent with control failure of OP tags observed at Rosepine, St. Joseph, and Winnsboro prior to this study (Chapters 2 and 3).

All field populations tested were more susceptible to chlorfenapyr than the reference strain, independent of their level of susceptibility to pyrethroid or OP insecticides. The highest LC₅₀ to chlorfenapyr observed among field strains was about

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5-fold lower than that found for the reference flies. Similar results were reported by Sheppard and Joyce (1998), who found that pyrethroid-resistant horn flies in Georgia were five times more susceptible to chlorfenapyr when compared to susceptible flies. Sheppard and Joyce (1998) suggested the enhanced chlorfenapyr toxicity found in pyrethroid-resistant horn flies was a consequence of a higher activation of this insecticide by mixed-function oxidases (MFO). This assumption is supported by laboratory studies, which have showed that oxidative metabolism is the major route of activation of chlorfenapyr (a pro-insecticide) to its N-dealkylated analog, which is actually the toxic uncoupling agent (Black et al. 1994, Treacy et al. 1994). Metabolic activation of insecticides by MFO activity in pyrethroid-resistant horn flies also has been proposed to occur with diazinon (Cilek et al. 1995). Pimprale et al. (1997) reported a negative correlation between pyrethroid and chlorfenapyr toxicity for the tobacco budworm. In our studies, field populations with varying levels of pyrethroid resistance were uniformly more susceptible to chlorfenapyr than the reference strain. The apparent lack of correlation between pyrethroid and chlorfenapyr toxicity found in our studies indicate that there may be differing mechanisms conferring resistance in the different populations.

Pyrethroid resistance in horn flies has been widespread in the southern United States since the mid-1980's (Kunz and Schmidt 1985, Sparks et al. 1985). Although pyrethroid-resistant horn flies have been reported to be more susceptible to diazinon than pyrethroid-susceptible flies (Sheppard and Marchiondo 1987, Cilek and Knapp 1993, Cilek et al. 1995), reports of resistance to diazinon are increasing (Cilek et al 1991, Steelman et al. 1994, Chapter 2). Furthermore, Barros et al. (Chapter 3) demonstrated
that seven years of annual rotation between OP and pyrethroid tags failed to improve pyrethroid efficacy or prevent further development of resistance to the pyrethroid. Also, development of resistance to both insecticides has been observed in flies under a pyrethroid-OP rotation in laboratory studies (McKenzie and Byford 1993). Therefore, a need exists for alternate chemicals to control horn flies. The results of our study indicate that chlorfenapyr will be useful in controlling horn flies, regardless of their susceptibility to pyrethroids or OPs. Furthermore, availability of an alternate insecticide class with toxicity against resistant populations provides a potentially valuable tool to improve resistance management.

References


LeOra Software. 1987. POLO-PC a user’s guide to probit or logit analysis. LeOra Software, Berkeley, CA.


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CHAPTER 5

SUMMARY AND CONCLUSIONS

This study reports results from three major studies conducted on horn fly resistance to insecticides in several locations throughout Louisiana. Except for one location (St. Gabriel), resistance to insecticides was present at levels high enough to impair chemical control in the field. Resistance to both organophosphates (OP) and pyrethroids, the insecticide classes most extensively used and available in the market for horn fly control, was found to be widespread.

Horn fly resistance to diazinon was reported for the first time in terms of susceptibility to bioassays and product (insecticidal ear tags) efficacy. Resistance to diazinon developed in four years and led to resistance to other OP insecticides. Our findings support the concept that basing a horn fly control program upon the use of a single class of insecticides is inappropriate. Due to their low magnitude, resistance ratios were difficult to interpret and to use as indicators of product efficacy. A measure of frequency of resistant flies based on 5% survival to a discriminating concentration of diazinon in pre-season bioassays was proposed for prediction of diazinon ear tag efficacy.

The use of rotation between insecticides belonging to different classes (with distinct modes of action) has been recommended as a resistance management strategy for horn flies. In two different locations where pyrethroid resistance was previously established, we studied a yearly rotation between synergized pyrethroid (lambda-cyhalothrin + PBO) and OP (pirimiphos-methyl) ear tags. This strategy did not provide
acceptable results in a long-term control program, and after seven years of rotation, the flies had become resistant to both OP and pyrethroid insecticides. The rotation with the OP compound did not improve pyrethroid ear tag efficacy or prevent further development of resistance to the pyrethroid. Thus, the inclusion of pyrethroids, even combined with synergists, should be avoided in management strategies against pyrethroid-resistant horn fly populations.

Since horn fly resistance to most available insecticides is widespread, there is a need for novel chemicals that could improve horn fly control and resistance management of this livestock pest. The efficacy of experimental chlorfenapyr ear tags suggested that this insecticide can be effective in controlling horn fly populations. Furthermore, both OP- and pyrethroid-resistant flies showed a high susceptibility to chlorfenapyr. These results indicated that this novel insecticide may become an important and useful tool for management of horn fly resistance.
VITA

Antonio Thadeu Medeiros de Barros was born on September 27, 1962 in Rio de Janeiro, Rio de Janeiro State, Brazil. He received a bachelor of science degree in Veterinary Medicine in 1984 from the Universidade Rural Federal do Rio de Janeiro (UFRRJ), and in 1985 he began his master's program in the same university. In 1987, he was hired by the EMBRAPA (Brazilian Enterprise for Agricultural Research) as a researcher in the Area of Animal Health of the Centro de Pesquisa Agropecuária do Pantanal (Center of Agricultural Research for the Pantanal). In 1989, he concluded his master's in Veterinary Parasitology at UFRRJ. Since 1987, he has conducted studies on parasites of livestock in the Pantanal region, with particular interest on haematophagous flies. In 1995, he was accepted into the Department of Entomology at the Louisiana State University, where he is a candidate for the degree of Doctor of Philosophy.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate:  Antonio Thadeu Medeiros de Barros

Major Field:  Entomology

Title of Dissertation:  Dynamics and Management of Insecticide Resistance in the Horn Fly, Haematobia irritans irritans (L.) (Diptera: Muscidae)

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

October 23, 1998