

EFFECTS OF MID-SEASON AVERMECTIN TREATMENTS OF CATTLE ON  
PYRETHROID RESISTANCE IN THREE POPULATIONS OF HORN FLIES,  
*HAEMATOBIA IRRITANS IRRITANS* (L.) (DIPTERA: MUSCIDAE)

A Thesis

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  
Master of Science

in

The Department of Entomology

by

Glenn R. O'Remus

B.S. The University of Massachusetts at Amherst, 1999

December 2003

## **ACKNOWLEDGEMENTS**

First and foremost, I would like to thank my family for their love and support. In particular, my mother and father for without them I would never have made it this far.

I would like to thank my committee members, Felix Guerrero and Jim Ottea, for their advice and understanding. Also, I would like to thank all of my technical help, especially Richard Streb, who helped me keep my head above water and make it across the finish line on time.

I greatly thank Dr. Lane Foil for his guidance, support, and friendship throughout my entire time at Louisiana State University. It was a pleasure and an honor.

Lastly, to Kimberly, whose patience, love, and compassion were immeasurable in the completion of this thesis.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS .....	ii
LIST OF TABLES .....	iv
LIST OF FIGURES .....	v
ABSTRACT.....	vi
CHAPTER	
1    INTRODUCTION .....	1
2    EFFECTS OF MID-SEASON AVERMECTIN TREATMENTS ON PYRETHROID RESISTANCE IN HORN FLY POPULATIONS AT THREE LOCATIONS IN LOUISIANA.....	8
3    CHANGES IN THE FREQUENCY OF <i>KDR</i> AND <i>SKDR</i> MUTATIONS ASSOCIATED WITH PYRETHROID RESISTANCE IN HORN FLIES UNDER DIFFERENT ENVIRONMENTAL PRESSURES.....	21
4    SUMMARY AND CONCLUSIONS.....	36
REFERENCES .....	37
VITA.....	42

## LIST OF TABLES

2.1. Summary of bioassay data and weeks of control at Macon Ridge Research Station.....	13
2.2. Summary of bioassay data and weeks of control at Red River Research Station.....	13
2.3. Summary of bioassay data and weeks of control at St. Joseph Research Station.....	15
2.4. Summary of bioassay data and weeks of control at St. Gabriel Research Station.....	16
3.1. Primer sequences used for resistant and susceptible alleles.....	25
3.2. 1999-2002 LC <sub>50</sub> (95% FL) for $\lambda$ -cyhalothrin, diazinon and permethrin for horn flies collected at St. Joseph Research Station.....	27
3.3. Distribution of genotypes for individual horn flies tested from St. Joseph Research Station.....	29
3.4. Frequency of RR- <i>kdr</i> resistant horn flies collected at St. Joseph Research Station....	29
3.5. Frequency of RR- <i>kdr</i> resistant horn flies collected at St. Joseph Research Station....	30
3.6. Frequency of R- <i>kdr</i> alleles, detected in horn flies collected at St. Joseph Research Station.....	30
3.7. Frequency of R- <i>kdr</i> alleles, detected in horn flies collected at St. Joseph Research Station.....	30
3.8. Frequency of R- <i>skdr</i> alleles detected in horn flies collected at St. Joseph Research Station.....	31
3.9. Frequency of R- <i>skdr</i> alleles detected in horn flies collected at St. Joseph Research Station.....	31

## LIST OF FIGURES

3.1. Description of gel electrophoresis.....	28
3.2. The frequency of R- <i>skdr</i> and R- <i>kdr</i> alleles in horn fly populations at St. Joseph Research Station.....	35

## ABSTRACT

The effects of mid-season avermectin treatments on pyrethroid-resistant horn fly populations were examined at three separate farms. The study was conducted at three Louisiana State University Agricultural Center research stations: Red River Research Station (Bossier City, LA), Macon Ridge Research Station (Winnsboro, LA) and St. Joseph Research Station (St. Joseph, LA) between 1999-2002. The cattle were treated with pyrethroid ear tags in all years at all farms, and each farm received a mid-season avermectin treatment in one year. With consecutive yearly use of pyrethroids the number of weeks of control decreased for all farms. The number of weeks of control rebounded at Red River from 2 to 13 weeks in the year following the mid-season treatment of avermectin. At Macon Ridge there was a minimal reversal of weeks of control from 0 to 2 weeks in the year following the mid-season treatment. No change was observed at St. Joseph. The concentration required to kill 50% of the flies tested ( $LC_{50}$ 's) for fly populations at Macon Ridge and St. Joseph were found to increase for pyrethroids from the spring populations to the fall populations between 2000 and 2002. The  $LC_{50}$ 's for fly populations at Red River followed the same trends except in 2000, the year when the avermectin treatment was administered, when a decrease was seen from spring to fall.

Flies from St. Joseph were assayed for two alleles (*kdr* and *skdr*) associated with target site resistance to pyrethroids. There was a general decline in the frequency of homozygous susceptible *skdr* (SS-*skdr*) and homozygous susceptible *kdr* (SS-*kdr*) individuals, as well as a general increase in homozygous resistant *skdr* (RR-*skdr*) and homozygous resistant *kdr* (RR-*kdr*) individuals, during the four year study. In every year, the frequency of RR-*kdr* increased significantly in flies collected in the fall compared to flies collected in the spring. In all comparisons, the frequency of RR-*kdr* was significantly higher in flies collected in the fall

compared to flies collected in the spring in the following year. The frequency of R-*skdr* alleles was significantly higher in fly populations tested in the fall compared to fly populations tested in the spring for 1999, 2000 and 2002. The frequency of R-*skdr* alleles was significantly higher in fly populations tested in the fall in 2001 compared to in the spring the following year. The frequency of RR-*kdr* resistant horn flies collected in the fall at St. Joseph increased each year. By fall of 2002, the frequency of RR-*kdr* at St. Joseph had reached 100%.

# CHAPTER 1

## INTRODUCTION

Since its introduction into the United States around 1887, the horn fly, *Haematobia irritans* (L.), has been considered to be a major pest of cattle (Bruce 1964). The horn fly was introduced from southern Europe and eventually spread rapidly throughout the U.S. The horn fly is a small gray-colored biting fly measuring approximately 3.5-4mm in length; both male and female flies are hematophagous. Adult horn flies spend most of their lives on cattle. In the U.S., peak populations of horn flies occur in the summer months, and populations of 500-1000 flies per animal are common on adult cattle (Kunz and Schmidt 1985). Horn fly infestation of cattle is associated with reduction in rate of weight gain, feeding efficiency, and milk production due to blood loss, stress, and annoyance (Campbell, 1976 and Kinzer, et al., 1984). For pastured cattle production in the U.S., economic losses due to horn fly damage have been estimated at \$876 million annually (Kunz et al. 1991).

Economic threshold is defined as “the population level where the incremental value of production loss due to pests is just equal to the incremental cost of reducing the pest problem” (Headley 1971). Gordon et al (1984) indicated that under most conditions the economic threshold for horn fly control is at a low level of infestation. Economic damage from horn flies has been associated with weaning weights of calves and lower weight gains for yearling cattle. Campbell (1976) showed a 5.86 kg increase in weaning weights for calves of treated cows compared to calves of untreated cows. Quisenberry and Strohhahn (1984) reported an average increased weaning weight of 5.6 kg for calves of treated cows compared to calves of untreated cows.

The stable loss of 17-20% potential growth rate of yearling cattle represents the minimum loss in production of immature cattle on pasture due to horn fly infestation (Haufe 1982). The lowest mean threshold defined in laboratory studies was 12 flies per animal for yearling cattle (Haufe 1981). Howell (1952) and Butler (1975) both reported weight gain advantages from horn fly control on yearling animals. Other studies also have shown that effective control methods for horn flies were associated with increased weight gains in yearling stocker beef cattle (Harvey and Brethour, 1979; Haufe, 1982). In Louisiana, Derouen et al. (1995) reported that yearlings treated for horn flies achieved a total weight gain advantage of 12kg or 17% more than untreated cattle. Currently, it is considered economically important to control horn fly infestations when population levels exceed 200 flies per animal (Foil and Hogsette 1994).

Horn fly control is based upon treatment of cattle with insecticides, and insecticide resistance has reduced the ability of certain cattle producers to control horn flies for economic benefit. Topical treatment of cattle with DDT was used as an effective means of horn fly control (Knipling 1954), but reports of resistance of horn flies to DDT were confirmed by Harris (1964). Similarly, resistance of horn flies to organophosphates (OPs) was reported by Burns and Wilson (1963), when resistance to fenchlorphos was demonstrated following intensive use of backrubbers.

Insecticide impregnated ear tags (containing dichlorvos) were developed by Harvey and Brethour (1970), and the use of the tags was proposed to reduce the amount of environmental contamination with insecticides compared to other applications (Kunz and Schmidt 1985). Ahrens (1979) reported up to 20 weeks of control using stirofos impregnated ear tags. However, stirifos resistance in horn fly populations was soon observed (Sheppard 1983). Ear

tags impregnated with pyrethroids (fenvalerate and permethrin) were introduced in 1977; but within 5 years, the first report of horn fly resistance to pyrethroids in the U.S. was made (Schmidt et al. 1982). Resistance to both fenvalerate and permethrin in horn flies in Louisiana was reported by both Quisenberry et al. (1984) and Byford et al. (1985). Byford et al. (1985) also demonstrated pyrethroid cross-resistance in the horn fly by testing pyrethroid resistant flies with a broad spectrum of pyrethroids.

There are multiple mechanisms of insecticide resistance. Penetration resistance affects the ability for the insecticide to penetrate the insect integument. As described by Brooks (1976), the distribution of insecticides depends on their ability to cross semi-permeable membranes. Penetration resistance was shown by Ottea et al (1995); decreased pyrethroid penetration was detected following selection in both larval and adult tobacco budworms, *Heliothis virescens* (F.).

Metabolic resistance involves enzymatic modifications of insecticides into less toxic products. Examples of enzymes associated with metabolic resistance are mixed function oxidases, glutathione S-transferases and esterases. Pyrethroid resistance has been shown to be affected by metabolic processes, such as oxidative enzyme systems (Guerrero et al. 1997). The authors used the mixed function oxidase inhibitor piperonyl butoxide (PBO) in combination with pyrethroids and showed that PBO increased susceptibility of pyrethroid resistant flies to pyrethroids.

Target site resistance is the alteration of a target site that prevents binding and action of an insecticide. The target site for pyrethroids is the voltage-dependent sodium channel, which in mammals is made up of three subunits;  $\alpha$ ,  $\beta_1$  and  $\beta_2$  (Catterall 1995). In a normal resting neuron, the sodium channel is triggered to open by depolarization of the adjacent membrane, and sodium cations flow into the cell causing an increase of the membrane potential (Shankland 1976).

When the membrane potential surpasses the threshold for nerve firing, an action potential is generated and is transmitted through the entire membrane. Efflux of potassium ( $K^+$ ) is triggered by the resulting depolarization and the equilibrium potential of the membrane potential is restored (Shankland 1976).

Pyrethroids have been shown to prolong the inactivation (closing) of the sodium channels allowing an increased flow of sodium into the cell (Bloomquist and Miller 1985). This mode of action is described for both Type I (allethrin, tetramethrin) and Type II (deltamethrin) pyrethroids, although neurological symptoms of poisoning differ between types. Pyrethroid neurotoxicity ranges from repetitive firing to conduction block (Wouters & van den Berken 1978). Type I pyrethroids produce repetitive discharges while Type II pyrethroids produce stimulus-dependent nerve depolarization and block (Clements et al. 1977, Lund and Narahashi 1983).

Reduced sensitivity to pyrethroids was first recognized in the house fly and termed knockdown resistance (Busvine 1951). Target site resistance for pyrethroids has been shown to involve at least two alleles. In the house fly (*Musca domestica*), a *para*-type sodium channel gene has been implicated as the site of the knockdown resistant gene, or *kdr* gene (Williamson et al. 1996). Along with the identification of *kdr* alleles, a more potent component, the super-*kdr* (*skdr*) allele, was found to produce up to a 500-fold resistance to type II pyrethroids (Sawicki 1978).

Analysis of house fly populations containing *kdr* alleles revealed two amino acid mutations that correlate with resistance (Williamson et al. 1996). The mutations were recognized as a substitution of phenylalanine for leucine at position L1014F in the house fly sodium channel sequence with an additional methionine to threonine replacement (Williamson et

al. 1996). This mutation was shown to decrease the sensitivity of the house fly sodium channel to cismethrin and decrease the duration of the open state of the cismethrin modified channel (Smith et al. 1997).

In the horn fly, the knockdown (*kdr*) mechanism was correlated with a sodium channel locus in the house fly (Williamson et al., 1993). Linkage studies were done for the house fly (Williamson et al. 1993), cockroaches (Dong and Scott 1994), and tobacco budworm (Taylor et al. 1994) showing that resistant fragment length polymorphisms (RFLP's) within a region homologous to the *para* sodium channel gene are tightly associated with *kdr* phenotypes. Guerrero et al. (1997) subsequently reported the mutation to be present in two separate resistant strains of horn flies.

Guerrero et al. (1998) identified point mutations of *kdr* and *skdr* in the horn fly, and correlated the point mutations to pyrethroid resistance. Resistance ratios for cyhalothrin and frequency of resistant *kdr* and *skdr* alleles were higher in a "super resistant" colony of horn flies compared to a "resistant" colony of horn flies. Jamroz et al (1998) compared susceptible lab flies, resistant lab flies and wild populations and concluded that the *kdr* mechanism conferred significant levels of pyrethroid resistance. The frequency of homozygous resistant (RR) *kdr* individuals in the resistant colony was twice that of pyrethroid pressured wild populations and much higher than in susceptible populations.

*Skdr* is directly correlated to the *kdr* mechanism in that it confers resistance to DDT and pyrethroids and is unaffected by piperonyl butoxide and FDMC, an analogue of DDT, (Sawicki 1978); *skdr* is associated with higher levels of resistance towards DDT-like compounds and pyrethroids than *kdr* (Sawicki 1978).

Pyrethroid resistance has been associated with decreased biotic fitness in horn flies. Scott et al. (1997) showed that susceptible horn flies from susceptible colonies emerged sooner than resistant horn flies obtained from a pyrethroid resistant colony with a resistance factor of 17. Susceptible flies pupated approximately twice as successfully as resistant flies and resistant flies produced approximately 50% less eggs than susceptible flies

Several resistance management strategies for horn fly control have been proposed. Both field and laboratory studies have demonstrated that continuous use of a single chemical class of insecticides leads to resistance in horn flies (Byford et al. 1998). Continuous selection of a colony of horn flies with permethrin resulted in a significant increase in  $LC_{50}$ 's for permethrin in 31 generations. In field studies, horn fly control dropped from 13 to 3 weeks when pyrethroids were used in three consecutive years, and from 16 to 6 weeks when OP's were used in four consecutive years (Byford et al. 1998). One control strategy that showed promise was the use of mosaics of insecticides (Byford et al. 1998). When a mosaic (cattle herds with different treatments and maintained apart) of OP and pyrethroid ear tags was used at two sites, there was no change in the level of horn fly control at either site over a three year period.

Alternation between the use of OP's and pyrethroids each year has been recommended as a management strategy to delay development of resistance of horn fly populations. Barros et al. (1999) reported that a yearly alternation of pyrethroid and OP tag treatments did not increase susceptibility to pyrethroids. During the seven year study, there was a decrease in number of weeks of control from seven to two weeks in the pyrethroid treated years and from 15 to 2 weeks in the OP treated years. Subsequently, Guerrero et al. (2002) genotyped these populations and showed that the frequency of susceptible wild type *kdr* alleles decreased in fall horn fly populations following treatment in the pyrethroid treatment years.

The effects of alternating between chemical classes within one season also has been studied. Barros et al. (2001) examined the effects of a mid-season treatment of avermectins on an OP-resistant horn fly population. Cattle were treated with OP ear tags each year and the number of weeks of control declined from greater than 20 weeks in 1989 to 1 week in 1993. In 1993, the cattle were treated in mid-season with an avermectin (Ivomec pour-on®, Rahway, NJ), and in the following year the number of weeks of control rebounded to 12 weeks.

## **CHAPTER 2**

### **EFFECTS OF MID-SEASON AVERMECTIN TREATMENTS ON PYRETHROID RESISTANCE IN HORN FLY POPULATIONS AT THREE LOCATIONS IN LOUISIANA**

#### **Introduction**

The horn fly is considered to be the primary insect pest of cattle in the United States. For pastured cattle production, economic losses due to horn fly damage have been estimated at \$876 million (Kunz et al. 1991). Horn fly control is based upon the treatment of cattle with insecticides, and insecticide resistance has reduced the ability of certain cattle producers to control horn flies and obtain the associated economic benefits. Resistance in horn flies has been reported for chlorinated hydrocarbons (Harris 1964), organophosphates (Sheppard 1983), and pyrethroids (Quisenberry et. al. 1984 and Byford et. al. 1985).

Several resistance management strategies for horn fly control have been proposed. Both field and laboratory studies have demonstrated that continuous use of a single chemical class of insecticides leads to resistance in horn flies (Byford et al. 1998). Continuous selection of a colony of horn flies with permethrin resulted in a significant increase in  $LC_{50}$ 's for permethrin in 31 generations. In field studies, horn fly control dropped from 13 to 3 weeks when pyrethroids were used for three consecutive years, and from 16 to 1 week when organophosphates (OP's) were used for four consecutive years. One control strategy that showed promise was the use of mosaics of insecticides (Byford et al. 1998). When a mosaic (cattle herds with different treatments and maintained apart) of OP and pyrethroid ear tags was used at two sites, there was no change in the level of horn fly control at either site over a three year period.

Alternation between the use of OP's and pyrethroids each year has been recommended as a management strategy to delay development of resistance of horn fly populations. Barros et al.

(1999) reported that a yearly alternation of pyrethroid tag treatments and OP tag treatments did not increase susceptibility to pyrethroids. During the seven year study, there was a decrease in number of weeks of control from seven to two weeks in the pyrethroid treated years and from 15 to 2 weeks in the OP treated years.

The effects of alternating between chemical classes within one season also has been studied. Barros et al. (2001) examined the effects of a mid-season treatment of avermectins on an OP resistant horn fly population. Cattle were treated with OP ear tags each year and the number of weeks of control declined from greater than 20 weeks in 1989 to 1 week in 1993. In 1993, the cattle were treated in mid-season with an avermectin (Ivomec pour-on®, Rahway, NJ), and in the following year the number of weeks of control rebounded to 12 weeks. The purpose of this study was to determine the effects of mid-season avermectin treatments on populations of pyrethroid-resistant horn flies at three research stations in Louisiana.

### **Materials and Methods**

The study was conducted between 1999-2002 at three Louisiana State University Agriculture Center research stations: Red River Research Station (Bossier City, LA), Macon Ridge Research Station (Winnsboro, LA) and St. Joseph Research Station (St. Joseph, LA). All the cattle at each station were tagged with two Saber Extra® ear tags (10% lambda-cyhalothrin + 13% piperonyl butoxide; Schering-Plough, Kenilworth, NJ) in the spring. The average number of cows per station was 64, 83 and 98 at Macon Ridge, St. Joseph (St. Jo), and Red River, respectively. At Red River, the cattle were tagged on May 19, 1999, May 25, 2000, May 18, 2001, and June 6, 2002, at Macon Ridge the cattle were tagged on May 28, 1999, May 26, 2000, May 15, 2001 and June 4, 2002, and at St. Jo the cattle were tagged on May 20, 1999, May 18, 2000, May 15, 2001 and June 4, 2002. In 1999, 2000, and 2001, each farm was assigned one of

three mid-season treatments: tags not removed, tags removed, tags removed and cattle treated with doramectin (Dectomax® pour-on, .5% doramectin solution; Pfizer Animal Health, NY, NY). Each farm received all three treatments during the three year study in a continued Latin Square design.

The cattle were treated at Macon Ridge, St. Jo and Red River with Dectomax ® pour-on August 6, 1999, August 9, 2001, and August 10, 2000, respectively. Tags were removed at Macon Ridge on August 6, 1999, September 14, 2000, August 10, 2001, and September 4, 2002. Tags were removed at St. Jo August 6, 1999, September 12, 2000, August 9, 2001, and September 4, 2002. Tags were removed at Red River on September 21, 1999, August 10, 2000, September 13, 2001, and September 2, 2002, respectively.

### **Bioassay**

Horn flies were collected before the spring ear tag treatment (PRE) and at least two weeks after tags were removed (POST) from cattle by the use of aerial nets and tested for susceptibility using the impregnated filter paper method (Sheppard and Hinkle 1987). Stock solutions of insecticides were prepared using technical grade materials; diazinon (Boehringer Ingleheim Inc., Ridgefield, CT), permethrin (Y-TeX Corporation, Cody, WY) and  $\lambda$ -cyhalothrin (Syngenta, Wilmington, DE) were prepared in acetone at 0.875 mg/ml, 25.44 mg/ml, and 10.176 mg/ml, respectively. Then, two-fold dilutions were made; and 1ml of each dilution (the control was 1ml of acetone) was applied to separate Whatman ® 1 filter papers [90mm in diameter, surface area of  $63.62 \text{ cm}^2$  ( $A=\pi r^2$ )]. The concentration for each dose was expressed as  $\mu\text{g}/\text{cm}^2$ . The ranges of dilutions used for diazinon,  $\lambda$ -cyhalothrin, and permethrin were from 13.76-0.03  $\mu\text{g}/\text{cm}^2$ , 160-0.02  $\mu\text{g}/\text{cm}^2$ , and 400-0.2  $\mu\text{g}/\text{cm}^2$ , respectively. Filter papers were allowed to dry overnight and then three filter papers for each dilution were wrapped in aluminum foil. Prior to

the assays, the papers were individually placed into 100 x 15mm polysterene petri dishes, each with a hole burned in the top to allow the addition of horn flies. Approximately 25 horn flies were placed into each of the petri dishes and mortality (the inability for the fly to translocate) was assessed at four hours. The total number of flies in each petri dish was then counted. Probit analysis (LeOra Software 1987) was used to obtain the concentration required to kill 50% of the flies tested ( $LC_{50}$ 's), and 95% fiducial limits (FL). The  $LC_{50}$ 's were considered significantly different when the FL did not overlap. Resistance ratios, subsequently referred to as resistance factor (RF's), were calculated by dividing the  $LC_{50}$ 's for the wild flies by those obtained for the susceptible lab colony from the Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS, Kerrville, TX. Flies also were collected and tested at St. Gabriel Research Station (St. Gabriel, LA), where there was no major use of pyrethroids during the study.

The number of horn flies per side on 10 randomly selected cattle was estimated once per week at each station. Control was considered successful when the average number of horn flies was less than 50 flies per side. Fly counts began at least 2 weeks before tags were administered, and were continued until the average number of flies was above control. The counts were made by one individual at each station with the aid of binoculars before 8:00 am.

## **Results**

### **Fly Control**

#### Macon Ridge

The number of weeks of control increased from zero in 1999 to two in 2000 after the mid-summer treatment with doramectin in 1999 (Table 2.1). The number of weeks of control declined each year between 2000 and 2002.

### Red River

The number of weeks of control decreased from eight weeks in 1999 to two weeks in 2000 (Table 2.2). Following the mid-summer treatment with doramectin in 2000, the number of weeks of control increased from two weeks in 2000 to thirteen weeks in 2001, then decreased in 2001 to seven weeks in 2002.

### St. Joseph

The number of weeks of control increased from three weeks in 1999 to four weeks in 2000 (Table 2.3). The number of weeks of control then decreased from four weeks in 2000 to one week in 2001. Following the mid-summer treatment with doramectin in 2001, the number of weeks of control in 2002 was the same as 2001.

### **Bioassay**

#### Macon Ridge

The  $LC_{50}$ 's for permethrin were significantly lower for fly populations in PRE compared to POST in 2000, 2001, and 2002; no difference was detected in 1999. The RF's were higher for flies tested POST than for flies tested PRE in the same year except in 1999 when the RF's were lower.

The  $LC_{50}$ 's for  $\lambda$ -cyhalothrin were significantly lower for fly populations tested PRE compared to POST in 2000, 2001, and 2002; no significance was detected in 1999. The RF's were higher for flies tested POST than for flies tested PRE in the same year except in 1999 where the RF's were lower for flies tested POST than for flies tested PRE in the same year.

The  $LC_{50}$ 's for diazinon were significantly lower for fly populations tested PRE compared to POST fly population in 1999, 2000, and 2002. RF's for diazinon were higher for flies tested

POST than for flies tested PRE in all years except in 2001 when the RF's were lower for flies tested POST than for flies tested PRE in the same year.

Table 2.1. Summary of bioassay data and weeks of control at Macon Ridge Research Station.

Year	Time	Permethrin		$\lambda$ -cyhalothrin		Diazinon		Weeks Control <sup>b</sup>
		LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF <sup>a</sup>	LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF	LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF	
1999 <sup>c</sup>	Pre <sup>d</sup>	27.0(20.3-35.6)	122.6	126.9(87.4-256.2)	555.5	1.2(1.1-1.4)	3.1	0 (5) <sup>e</sup>
	Post <sup>f</sup>	17.9(13.7-23.6)	81.2	76.4(63.7-93.7)	334.1	2.9(2.7-3.1)	7.5	
2000	Pre	17.4 (5.9-63.6)	79.0	10.9(7.5-16.3)	47.9	0.3 (0.3-0.5)	0.9	2
	Post	43.2(21.4-71.7)	195.7	51.3(24.8-148.1)	224.3	1.2(1.0-1.3)	3.1	
2001	Pre	23.2(14.4-35.0)	105.0	5.3(4.0-6.8)	23.1	0.9 (0.6-1.1)	2.2	1
	Post	107.7(55.9-260.6)	488.3	122.6(66.0-317.6)	536.4	0.6(0.4-0.8)	1.5	
2002	Pre	12.1(8.9-16.1)	54.9	3.7(3.1-4.4)	16.1	0.39 ( <sup>g</sup> )	0.9	0
	Post	366.5(215.2-919.1)	1662.5	ND <sup>g</sup>	ND <sup>g</sup>	1.8(1.6-2.1)	4.8	

<sup>a</sup>Resistance Factor (LC<sub>50</sub> from field population/ LC<sub>50</sub> from Kerrville reference colony strain)

<sup>b</sup>Control was considered significant only when the number of horn flies was less than 50 flies/side.

<sup>c</sup>Designates treatment of Dectomax ® pour-on

<sup>d</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>e</sup>Number in parentheses represent control after Dectomax® treatment

<sup>f</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>g</sup>Not determined

### Red River

The LC<sub>50</sub>'s for permethrin were significantly lower for fly populations tested PRE compared to POST in 1999, 2001, and 2002; no significant difference was detected in 2000, when the doramectin treatment occurred. The RF's were higher for flies tested POST than for flies tested PRE in the same year for each year, except for 2000 where the RF's were lower for flies tested POST than for flies tested PRE in the same year.

Table 2.2. Summary of bioassay data and weeks of control at Red River Research Station.

Year	Time	Permethrin		$\lambda$ -cyhalothrin		Diazinon		Weeks Control <sup>b</sup>
		LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF <sup>a</sup>	LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF	LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF	
(Table Continued)								

1999	Pre <sup>c</sup>	3.8(1.9-6.9)	17.4	5.8(3.5-9.8)	25.6	1.9(1.0-3.9)	5.1	8
	Post <sup>d</sup>	62.1(36.9-116.2)	281.8	222.4(54.9-7310.4)	972.9	0.96(0.8-1.2)	2.5	
2000 <sup>e</sup>	Pre	3.1(2.4-4.2)	14.2	0.9 <sup>(f)</sup>	3.9	0.8(0.6-1.0)	2.0	2 (5) <sup>g</sup>
	Post	1.9(1.3-2.6)	8.6	0.1(0.03-0.31)	0.6	0.8 <sup>(f)</sup>	1.9	
2001	Pre	3.4 (2.1-5.1)	15.3	0.6(0.5-0.7)	2.6	0.7(0.6-0.8)	1.7	13
	Post	289.7(197.3-500.7)	1313.9	93.7(35.9-1006.5)	409.7	0.8(0.7-0.9)	2.0	
2002	Pre	4.02(1.9-7.8)	18.2	0.4(0.2-0.63)	1.6	0.7(0.5-0.8)	1.7	7
	Post	245.7 <sup>(f)</sup>	1114.2	ND <sup>f</sup>	ND <sup>f</sup>	1.6 <sup>(f)</sup>	4.1	

<sup>a</sup>Resistance Factor (LC<sub>50</sub> from field population/ LC<sub>50</sub> from Kerrville reference colony strain)

<sup>b</sup>Control was considered significant only when the number of horn flies was less than 50 flies/side.

<sup>c</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>d</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>e</sup>Designates treatment of Dectomax ® pour-on

<sup>f</sup>Not determined

<sup>g</sup>Number in parentheses represent control after Dectomax® treatment

The LC<sub>50</sub>'s for λ-cyhalothrin were significantly lower for fly populations PRE compared to POST in 1999 and 2001; no significant difference was detected in 2000. In 2000 the RF's were lower for flies tested POST than for flies tested PRE in the same year. In 1999 and 2001, the RF's were higher for flies tested POST than for flies tested PRE in the same year.

There were no significant differences in the LC<sub>50</sub>'s for diazinon for fly populations tested PRE compared to POST. RF's for diazinon were lower for fly populations tested PRE compared to POST in 2001 and 2002, and higher for fly populations tested PRE compared to POST in 1999 and 2000.

### St. Joseph

The LC<sub>50</sub>'s for permethrin were significantly lower for fly populations tested PRE compared to POST in 2000, 2001, and 2002; no significant difference was detected in 1999. The RF's were higher for flies tested POST than for flies tested PRE in the same year for each year,

except for 1999 when the RF's decreased slightly for fly populations tested POST compared to PRE.

Table 2.3. Summary of bioassay data and weeks of control at St. Joseph Research Station.

Year	Time	Permethrin		$\lambda$ -cyhalothrin		Diazinon		Weeks Control <sup>b</sup>
		LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF <sup>a</sup>	LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF	LC <sub>50</sub> (95% FL) μg/cm <sup>2</sup>	RF	
1999	Pre <sup>c</sup>	10.6 (8.6-13.1)	48.1	34.4 (21.1-65.1)	150.5	0.8 (0.6-0.9)	2.1	3
	Post <sup>d</sup>	9.4 (7.5-11.7)	42.6	31.8 (27.5-36.5)	139.1	1.3 (0.9-1.8)	3.4	
2000	Pre	24.8 (7.8-66.1)	112.5	8.3 (5.2-12.1)	36.3	0.3 (0.2-0.3)	0.7	4
	Post	122.4 (58.7-301.8)	555.2	79.3 (52-152.8)	346.9	0.84 (0.8-0.9)	2.2	
2001 <sup>e</sup>	Pre	29.9 (21.9-41.9)	135.6	8.5 (5.9-12.2)	37.2	0.3 (0.3-0.4)	0.9	1 (5) <sup>f</sup>
	Post	74.9 (54.4-104.6)	339.7	29.5 (17.7-58.8)	129.1	0.5 (0.4-0.5)	1.3	
2002	Pre	38.2 (27.6-54.7)	173.3	10.2 (7.4-14.3)	44.6	0.7(0.4-1.3)	1.9	1
	Post	85.8 (73.7-100.1)	389.2	ND <sup>g</sup>	ND <sup>g</sup>	1.2 (0.9-1.4)	3.1	

<sup>a</sup>Resistance Factor (LC<sub>50</sub> from field population/ LC<sub>50</sub> from Kerrville reference colony strain)

<sup>b</sup>Control was considered significant only when the number of horn flies was less than 50 flies/side.

<sup>c</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>d</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>e</sup>Designates treatment of Dectomax<sup>®</sup> pour-on

<sup>f</sup>Number in parentheses represent control after Dectomax<sup>®</sup> treatment

<sup>g</sup>Not determined

The LC<sub>50</sub>'s for  $\lambda$ -cyhalothrin were significantly lower for fly populations PRE compared to POST in 2000 and 2001; no significant difference was detected in 1999. In 1999 the RF's were lower for fly populations tested POST compared to PRE in the same year. In 2000 and 2001, the RF's were higher for fly populations tested POST compared to PRE in the same year.

The LC<sub>50</sub>'s for diazinon were significantly lower for fly populations tested PRE compared to POST in 2000 and 2001; no significance was detected in 1999 and 2002. The RF's for diazinon increased for fly populations from PRE compared to POST in all years.

## St. Gabriel

There were no significant differences in the LC<sub>50</sub>'s for permethrin for fly populations tested PRE compared to POST in all years (Table 2.4).

The RF's decreased for fly populations tested POST compared to PRE in 1999, 2000, and 2002. In 2001 the RF's increased for fly populations tested POST compared to PRE in the same year.

Table 2.4. Summary of bioassay data at St. Gabriel Research Station.

Year	Time	Permethrin		$\lambda$ -cyhalothrin		Diazinon	
		LC <sub>50</sub> (95% FL) $\mu\text{g}/\text{cm}^2$	RF <sup>a</sup>	LC <sub>50</sub> (95% FL) $\mu\text{g}/\text{cm}^2$	RF	LC <sub>50</sub> (95% FL) $\mu\text{g}/\text{cm}^2$	RF
1999	Pre <sup>b</sup>	1.9 (0.9-5.5)	8.5	2.8 (1.6-4.4)	12.3	0.7 (0.2-1.3)	1.9
	Post <sup>c</sup>	1.5 (0.8-2.5)	7.8	2.4 (0.6-4.6)	10.3	0.4(0.3-0.6)	1.1
2000	Pre	0.6(0.2-1.0)	2.5	0.09 ( <sup>d</sup> )	.04	0.2( <sup>d</sup> )	0.4
	Post	0.4(1.1-1.6)	1.6	0.03(0-0.02)	0.01	0.2(0.1-0.3)	0.5
2001	Pre	2.9(1.8-4.3)	13.2	0.3(0.2-0.5)	1.3	1.3(1.2-1.5)	3.5
	Post	5.2(3.8-7.1)	23.4	0.7(0.5-0.9)	2.9	2.0(1.7-2.4)	5.3
2002	Pre	5.9(4.8-7.1)	26.7	0.4(0.3-0.5)	1.6	2.1(0.9-6.9)	5.7
	Post	4.3(1.5-12.3)	19.5	ND <sup>d</sup>	ND <sup>d</sup>	1.5(1.1-1.9)	3.9

<sup>a</sup>Resistance Factor (LC<sub>50</sub> from field population/ LC<sub>50</sub> from Kerrville reference colony strain)

<sup>b</sup> Flies collected before treatment of the animals with ear tags (PRE)

<sup>c</sup> Flies collected at least two weeks after tags are removed (POST)

<sup>d</sup> Not determined

There were no significant differences for the LC<sub>50</sub>'s for  $\lambda$ -cyhalothrin for fly populations tested PRE compared to POST in all years. The RF's were lower for fly populations tested POST compared to PRE in the same year for 1999 and 2000, and the RF's were higher for fly populations tested POST compared to PRE for 2001 and 2002.

The LC<sub>50</sub>'s for diazinon were significantly lower for fly populations tested PRE compared to POST in 2001; no significance was detected in 1999, 2000, and 2002. The RF's for

diazinon increased for fly populations tested PRE compared to POST in 2000 and 2001. The RF's for diazinon decreased for fly populations tested POST compared to PRE in 1999 and 2002.

### **Discussion**

In the year following the mid-season avermectin treatment at Red River, the number of weeks of control increased 11 weeks over the previous year. These results are similar to those reported by Barros et al. (2001) who observed an 11 week increase in control with diazinon tags in the year following a mid-season treatment with an avermectin (ivermectin).

Barros et al. (2001) reported that RF's for OP's increased from spring to fall populations in every year of the 8 year study except for 1993, when there was a decrease after the mid-season avermectin treatment. We observed similar trends at Red River: RF's for both pyrethroids decreased from spring to fall in 2000 after the mid-season avermectin treatment. In 2001 and 2002, the  $LC_{50}$ 's for both pyrethroids were significantly higher for flies tested POST compared to PRE.

Jamroz et al. (1998) reported that RF's for cyhalothrin between 3 and 8 measured in wild horn fly populations were associated with a low frequency of resistant-*kdr* (*R-kdr*) alleles (15-31%). The level of pyrethroid resistance in flies at Red River between 2000, when the mid-season avermectin treatment was administered, and 2001 (RF's for  $\lambda$ -cyhalothrin were 2.6-3.9) were similar to that of Jamroz et al. (1998). With a low frequency of *R-kdr* found in their study and similarly low RF's we propose that Red River populations will also have a low frequency of *R-kdr* ruling out target site resistance as the dominant resistance mechanism at Red River.

Five weeks of control was obtained at each station following the doramectin treatment. Although the RF's for both pyrethroids decreased from spring to fall in the year the mid-season avermectin treatment was administered at Macon Ridge, the level of resistance was still high.

The change from 0 to 2 weeks is not considered to be significant in practical terms. Similarly, there was no change in the susceptibility to pyrethroids in the flies at St. Jo (indicated by control or bioassay data) associated with the mid-season doramectin treatment. Following topical treatment of cattle with doramectin, horn fly larvae do not develop in the manure for up to 35 days post treatment and adult control lasts for 2 to 3 weeks (Marley et al. 1993). The concept of resistance management with the mid-season treatment of all cattle on the farm is that the horn fly population that reinfests the cattle should come from surrounding areas (assuming a totally effective treatment).

There are several possible explanations for why the horn fly populations at three sites responded differently to the treatments. The different levels of pyrethroid resistance at the three locations are obviously representative of different suites of resistance mechanisms. Jamroz et al. (1998) reported that their resistant colony had an RF of 17 for cyhalothrin and a frequency of *R-kdr* of 66%. Therefore, high RF's for both permethrin and  $\lambda$ -cyhalothrin measured in flies at St. Jo and Macon Ridge throughout the study might indicate a high frequency of *R-kdr* alleles in those populations of flies. Guerrero et al. (2002) concluded that the *kdr* and *skdr* resistance trait became fixed in the population and contributed to the fact that pyrethroid resistance was unaffected by the yearly alternation of OP's and pyrethroids at St. Jo.

Since target sites for avermectins and pyrethroids are different (avermectins act at glutamate-gated chloride channels whereas pyrethroids act on sodium channels) we would not expect any negative or positive cross-resistance between the two chemistries (Wright 1986, Bloomquist and Miller 1985). That is, there should be no selective disadvantage for pyrethroid target site resistant flies associated with avermectin treatments. However, considering our results

at Red River and those of Barros et al. (2001), it may be warranted to study interactions of metabolic resistance and avermectin treatments.

The most likely explanations for the absence of change in the high frequency of pyrethroid resistant flies at St. Jo following the doramectin treatment are either that the treatment was not 100% effective or that resistant flies emigrated from neighboring farms. Of course, biotic potential also may be a factor of the success of the recolonizing flies. Scott et al. (1997) showed that susceptible horn flies from susceptible colonies emerged sooner than resistant horn flies obtained from a pyrethroid resistant colony with a RF of 17. Also, susceptible flies pupated approximately twice as successfully as resistant flies and resistant flies produced approximately 50% less eggs than susceptible flies. Guerrero et al. (2002) proposed that despite fitness costs associated with target site pyrethroid resistance, when the trait for that resistance is fixed within the populations only short or medium term control can be achieved. Our results from the studies at Macon Ridge and St. Jo would support their observations.

The changes in  $LC_{50}$ 's for pyrethroids during the year that doramectin was applied were indicators of the change in control in the following year. When doramectin was applied in 2001, the  $LC_{50}$ 's for both pyrethroids were significantly higher for flies tested at St. Jo POST compared to PRE, and the level of horn fly control was the same in the subsequent year. In contrast, the RF's for pyrethroids for flies collected at Red River and Macon Ridge decreased from PRE to POST in the year that doramectin was applied, and the number of weeks of control provided by tag treatments increased in the following year.

Preseason  $LC_{50}$ 's were not good indicators of subsequent horn fly control during this study. For example, one to two weeks of control was observed at the three locations subsequent to obtaining RF's in the range of 3.9 to 47.9, while eight weeks of control was observed

subsequent to measuring an RF for  $\lambda$ -cyhalothrin of 25.6 at Red River. Barros et al. (2001) reported that the frequency of survival of flies following exposure to a discriminating dose was a better indicator of subsequent control of horn flies with OP ear tag treatments. Therefore, further analysis of the bioassay data in this study may allow development of a discriminating dose that could be used as a general indicator of pyrethroid resistance.

## CHAPTER 3

### CHANGES IN THE FREQUENCY OF *KDR* AND *SKDR* MUTATIONS ASSOCIATED WITH PYRETHROID RESISTANCE IN HORN FLIES UNDER DIFFERENT ENVIRONMENTAL PRESSURES

#### Introduction

The horn fly is considered to be the primary insect pest of cattle in the United States. For pastured cattle production, economic losses due to horn fly damage have been estimated at \$876 million (Kunz et al. 1991). Horn fly control normally is based upon the use of insecticides and insecticide resistance has reduced the ability of certain cattle producers to control horn flies for economic benefit. Resistance in horn flies has been reported for chlorinated hydrocarbons (Harris 1964), the organochlorines (McDuffie 1969), organophosphates (Sheppard 1983), and pyrethroids (Quisenberry et al. 1984 and Byford et al. 1985).

Byford et al. (1998) reviewed potential management strategies for controlling resistance in horn fly populations. The authors indicated that the use of mosaics was preferred to sequential use of an insecticide or mixtures of two insecticides. Barros et al. (1999) reported that an alternation of yearly treatments of pyrethroids and organophosphates (OP's) did not increase susceptibility to pyrethroids. However, Barros et al. (2001) did report an increase in susceptibility of OP resistant horn flies following a mid-season avermectin treatment.

The effects of mid-season avermectin treatments on horn fly populations at St Joseph Research Station (St. Jo), Macon Ridge Research Station and Red River Research Station were described in Chapter 2. At Red River, the number of weeks of control increased from 2 to 13 weeks in the year following the mid-season treatment. However, at Macon Ridge or St. Jo there was no substantial treatment effect on pyrethroid resistance in fly populations.

Jamroz et al. (1998) reported that RF's for cyhalothrin between 3 and 8 measured in wild horn fly populations were correlated with a low frequency of resistant-*kdr* (*R-kdr*) alleles (15-31%). The level of pyrethroid resistance in flies at Red River between 2000, when the mid-season avermectin treatment was administered, and 2001 (RF's for  $\lambda$ -cyhalothrin were 2.6-3.9) were similar to that of Jamroz et al. (1998). Therefore, we proposed that target site resistance was not the dominant resistance mechanism at Red River (Chapter 2).

Jamroz et al. (1998) also reported that their resistant colony had an RF for cyhalothrin of 17 and a frequency *R-kdr* of 66%. Guerrero et al. (2002) reported that pyrethroid resistance in horn flies at St. Jo between 1991 and 1997 was attributed to contributions of the *kdr* and *skdr* mutations. The purpose of this study was to determine whether a high frequency of *kdr* and *skdr* mutations could be associated with the level of pyrethroid resistance at St. Jo during the study described in Chapter 2.

### **Materials and Methods**

The study was conducted at the Louisiana State University Agricultural Center St. Joseph Research Station (St. Jo), St. Joseph, LA, between May, 1999, and Oct, 2002. Each year, all of the cattle (approximately 85) were tagged in the spring with two Saber Extra® ear tags (10% lambda-cyhalothrin + 13% piperonyl butoxide, Schering-Plough, Kenilworth, NJ). The cattle were tagged on May 20, 1999, May 18, 2000, May 15, 2001 and June 4, 2002. Subsequent to tagging, three different treatments (following the protocol described in Chapter 2) were administered: (1) the tags were removed from the cattle on August 6, 1999, (2) the tags were not removed until September 12, 2000, and (3) tags were removed on August 9, 2001, all cattle were treated with Dectomax® pour-on (Pfizer Animal Health, NY, NY) at a rate of 1ml (5 mg doramectin) per 10 kg body weight. In 2002, tags were removed on September 4, 2002.

The number of horn flies per side on 10 randomly selected cattle was estimated once per week. Fly counts began at least 2 weeks before tags were administered, and were continued until the average number of flies was above 50 flies per side. The counts were made by one individual with the aid of binoculars before 8:00 am. Before the spring ear tag treatment (PRE) and at least two weeks after tags were removed (POST), flies were collected for bioassay and subsequent genomic assays.

### **Bioassay**

Horn flies were collected from cattle by the use of aerial nets and tested for susceptibility using the impregnated filter paper method (Sheppard and Hinkle 1987). Stock solutions of insecticides were prepared using technical grade materials; diazinon (Boehringer Ingelheim Inc., Ridgefield, CT), permethrin (Y-Tex Corporation, Cody, WY) and  $\lambda$ -cyhalothrin (Syngenta, Wilmington, DE) were prepared in acetone at 0.875 mg/ml, 25.44 mg/ml, and 10.176 mg/ml, respectively. Then, two-fold dilutions were made; and 1ml of each dilution (the control was 1ml of acetone) was applied to separate Whatman® 1 filter papers [90mm in diameter, surface area of 63.62 cm<sup>2</sup> ( $A=\pi r^2$ )]. The concentration for each dose was expressed as number of  $\mu\text{g}/\text{cm}^2$ . The ranges of dilutions used for diazinon,  $\lambda$ -cyhalothrin, and permethrin were from 13.76-0.03  $\mu\text{g}/\text{cm}^2$ , 160-0.02  $\mu\text{g}/\text{cm}^2$ , and 400-0.2  $\mu\text{g}/\text{cm}^2$ , respectively. Filter papers were allowed to dry overnight and then three for each dilution were wrapped in aluminum foil. Prior to the assays, the papers were individually placed into 100 x 15mm polystyrene petri dishes, each with a hole burned in the top to allow the addition of horn flies. Approximately 25 horn flies were placed into each of the petri dishes and mortality (the inability for the fly to translocate) was assessed at four hours. The total number of flies in each petri dish was then counted. Probit analysis (LeOra Software 1987) was used to obtain the concentration required to kill 50% of the flies tested

( $LC_{50}$ 's), and 95% fiducial limits (FL). The  $LC_{50}$ 's were considered significantly different when the FL did not overlap. Resistance ratios, subsequently referred to as resistance factors (RF's), were calculated by dividing the  $LC_{50}$ 's for the wild flies, by those obtained for the susceptible lab colony from the Knippling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS, Kerrville, TX.

### **Genomic Assay**

The polymerase chain reaction (PCR) was used to determine the genotypes of flies for two described sodium channel mutations (Jamroz et al. 1998). Flies were collected at St. Jo PRE and POST from 1999 through 2002, and stored at  $-80^{\circ}\text{C}$  until processed. The assay was developed to detect resistant (R) and susceptible (S) *kdr* and *skdr* sodium channel alleles and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a metabolic enzyme expressed constitutively in many tissues (Jamroz et al. 1998). The protocol was also designed to locate the E7 allele, but this study did not collect or utilize the information.

For each collection, at least 20 males and 20 females were prepared for the PCR assay. The PCR was done at Louisiana State University and the USDA-ARS Livestock Insect laboratory in Kerrville, TX. First, DNA was isolated from individual frozen ( $-80^{\circ}\text{C}$ ) flies using a disposable pellet pestle to crush the flies in 1.5ml centrifuge tubes. The pestles and tubes were prechilled on dry ice. Next, 25  $\mu\text{l}$  of buffer (Perkin Elmer PCR buffer II) were added and samples were ground for at least 30 seconds. Grinding was also done on dry ice to prevent thawing and damage to the DNA. The tubes were then placed on a rack and set in boiling water for 3 minutes to inactivate DNA-degrading enzymatic activity. After boiling, the samples were set on liquid ice for 2 minutes and then centrifuged at 14,000g for 5 minutes at  $4^{\circ}\text{C}$ . A dilution of 1:10 was made using 9 $\mu\text{l}$  of PCR grade water and 1 $\mu\text{l}$  of homogenate.

The PCR was performed in 500µl thin-walled microcentrifuge tubes (BioRad). Two separate 20µl mixtures are prepared for each individual to assay for both the S and R *kdr* and *skdr* alleles. The mixtures were prepared for each reaction by using the following measurements: Using a microliter pipettor, 13.3µl of PCR grade water, 1.6µl of 25mM magnesium chloride (MgCl<sub>2</sub>), 2.0µl of Perkin Elmer 10x PCR buffer II (500mM KCl, 100mM Tris, pH 8.3) and 0.4µl of a 10mM dNTP mix (2.5mM of each base pair) were used for both the susceptible and resistant reactions. As for the primers (Table 3.1), in both reactions, 0.1µl FG 236, 0.2µl FG 138, 0.2µl FG 235, 0.2µl FG 243 and 0.2µl FG 234 were used. To assay for susceptible alleles, 0.1µl FG 238, 0.1µl H<sub>2</sub>O, 0.2µl FG130 and 0.2µl FG154 were also added. To assay for resistant alleles, 0.1µl FG 239 0.1µl FG 240, 0.1µl FG 241, 0.2µl FG 134 and 0.2µl FG 155 were added.

Table 3.1. Primer sequences used for resistant and susceptible alleles

Primer	Sequence
FG130	5'-TACTGTTGTCATCGGCAATC
FG134	5'-TACTGTTGTCATCGGCAATT
FG138	5'-CAATATTACGTTTCACCCAG
FG234	5'-CTTCTTCATCGGTGTAGC
FG235	5'-CTTCGTGTATTCAAATTGGCA
FG236	5'-TTGTTGTCATGCTGCCTCC
FG238	5'-CGCCACAAATGAAACC
FG239	5'-GCCACAAATGAAACG
FG240	5'-GCCACAAATGAAACA
FG241	5'-GCCACAAATGAAACT
FG243	5'-GGCATGGCTTTCCGTGTCC
FG154	5'-ACCCATTGTCCGGCCCA
FG155	5'-ACCCATTGTCCGGCCCG

A 1:1 vol:vol mix of AmpliTaq DNA Polymerase (5 units/µl stock; Perkin-Elmer) and TaqStart Antibody (1.1µg/µl stock; Clontech) was prepared according to Clontech instructions, and 0.1µl was added to the mixtures. For each reaction, 19µl of the mixture and 1µl of the 1:10 genomic DNA solution were mixed gently and set in the thermocycler for amplification. The

cycle ran 96°C for 2 minutes followed by 36 cycles, each consisting of denaturation at 94°C for 50s, annealing at 62°C for 1 min, and extension at 68°C for 1 min. Next, a final extension step at 68°C for 7 min was done and then samples were cooled to 10°C for an unlimited time period. The reaction products were electrophoresed in a 3.5% agarose TBE (tris, boric acid, EDTA) gel and DNA was stained for 30 minutes in an ethidium bromide solution of 12.5µl ethidium bromide and 250ml TBE and a photograph taken using UV lighting.

Comparisons of genetic data were made by using Chi-square analysis and Fishers exact test (Graphpad Software, Inc. ©1992-1998); differences were considered significant at  $P \leq 0.05$ .

## **Results**

### **Fly Control**

In 1999 and 2000, there were three to four weeks of control (less than 50 flies per side) and there was just one week of control in 2001. Following the treatment of cattle with doramectin in 2001, five weeks of horn fly control was recorded. In 2002, the tag treatment again provided just one week of control.

### **Bioassay**

The LC50's for permethrin were significantly lower for fly populations PRE compared to POST in 2000, 2001, and 2002; and the LC50's for permethrin were significantly higher for flies collected POST in 2000 and 2001, when compared to flies tested PRE in the following year. Except for 1999, the RF's for permethrin were higher for flies tested POST than for the flies tested PRE in the same year. In 2000 and 2001, the RF's for permethrin for flies tested POST were higher than flies tested PRE in the following year (Table 3.2).

The LC50's for  $\lambda$ -cyhalothrin were significantly lower for fly populations PRE compared to POST in 2000 and 2001; and the LC50's for  $\lambda$ -cyhalothrin were significantly higher for flies

collected POST in 2000 and 2001, when compared to flies tested PRE in the following year. Except for 1999, the RF's for  $\lambda$ -cyhalothrin were lower for fly populations tested PRE compared to POST. In 2000 and 2001 the RF's for  $\lambda$ -cyhalothrin for fly populations tested POST compared to PRE in the following year. In every comparison of PRE to POST treatment or POST to PRE, the change in RF's for the two pyrethroids followed the same trend.

Table 3.2. LC<sub>50</sub>'s (95% FL) for  $\lambda$ -cyhalothrin, diazinon and permethrin for horn flies collected at St. Joseph Research Station

Year	Time	Permethrin		$\lambda$ -cyhalothrin		Diazinon		Control <sup>b</sup> (weeks)
		LC <sub>50</sub> (95% FL) $\mu\text{g}/\text{cm}^2$	RF <sup>a</sup>	LC <sub>50</sub> (95% FL) $\mu\text{g}/\text{cm}^2$	RF	LC <sub>50</sub> (95% FL) $\mu\text{g}/\text{cm}^2$	RF	
1999	Pre <sup>c</sup>	10.6 (8.6-13.1)	48.1	34.4 (21.1-65.1)	150.5	0.8 (0.6-0.9)	2.1	3
	Post <sup>d</sup>	9.4 (7.5-11.7)	42.6	31.8 (27.5-36.5)	139.1	1.3 (0.9-1.8)	3.4	
2000	Pre	14.1(5.1-28.8)	63.8	8.3 (5.2-12.1)	36.3	0.3 (0.2-0.3)	0.7	4
	Post	122.4 (58.9-301.8)	555.2	79.3 (52-152.8)	346.9	0.8 (0.8-0.9)	2.2	
2001 <sup>e</sup>	Pre	29.9 (21.9-41.9)	135.6	8.5 (5.9-12.2)	37.2	0.3 (0.3-0.4)	0.9	1 (5 <sup>f</sup> )
	Post	74.9 (54.4-104.6)	339.7	29.5 (17.7-58.8)	129.1	0.5 (0.4-0.5)	1.3	
2002	Pre	38.2 (27.6-54.7)	173.26	10.2 (7.4-14.3)	44.6	0.74(0.4-1.3)	1.9	1
	Post	85.8 (73.7-100.1)	389.2	ND <sup>g</sup>	ND <sup>g</sup>	1.17 (0.9-1.4)	3.1	

<sup>a</sup>Resistance Factor (LC<sub>50</sub> from field population/ LC<sub>50</sub> from Kerrville reference colony strain)

<sup>b</sup>Control was considered significant only when the number of horn flies was less than 50 flies/side.

<sup>c</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>d</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>e</sup>Designates treatment of Dectomax<sup>®</sup> pour-on

<sup>f</sup>Number in parentheses represents control after Dectomax<sup>®</sup> treatment

<sup>g</sup>Not determined

The LC50's for diazinon were significantly lower for fly populations in PRE compared to POST in 2000 and 2001, but not different in 1999 and 2002. The RF's for diazinon were lower for flies treated PRE compared to POST each year. The RF's were lower for fly populations tested POST in one year compared to PRE in the following year for all years except between 2001 and 2002.

### Genomic Assay

The genotypes were examined for the presence or absence of the appropriate bands in the gel. The diagnostic product for *kdr* should appear at 285bp, the diagnostic product for GAPDH allele should appear at 145bp and the diagnostic product for *skdr* should appear at 72bp. Homozygous resistant (RR) flies have the R mutation in the R column at the 285bp marker with no product shown in the susceptible column, as seen in individual #1 (Fig 3.1). Individual #1 was heterozygous for *skdr*, having a diagnostic product visible at 72bp in both the S and R columns. The same is shown for individual #2.

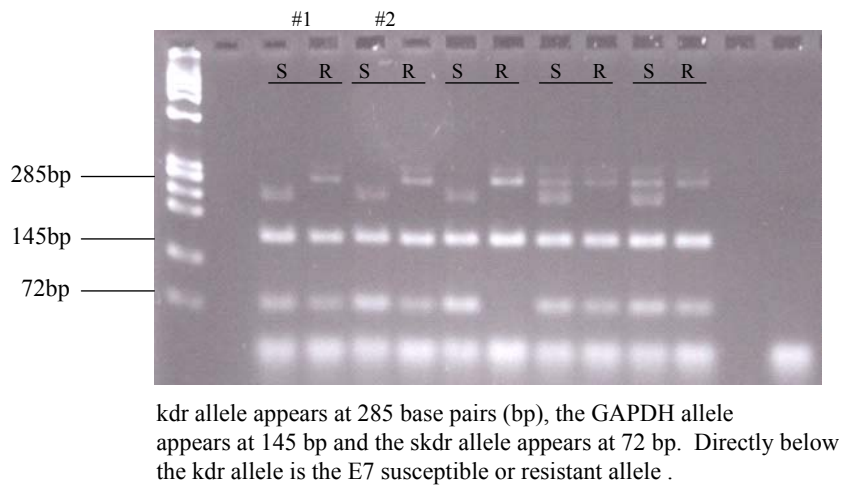


Figure 3.1. Description of gel electrophoresis

There was a general decline in the SS-*skdr* and SS-*kdr* as well as a general increase in RR-*skdr* and RR-*kdr* during the four year study (Table 3.3). The sample size for PRE 1999 was larger than those for the other collections due to a protocol of another project (Foil, unpublished).

Table 3.3. The percent of different genotypes of individual horn flies tested from St. Joseph Research Station

Year	Season	<i>skdr</i>				<i>kdr</i>			
		n <sup>a</sup>	SS	SR	RR	n <sup>a</sup>	SS	SR	RR
1999	PRE <sup>b</sup>	224	56.7	39.3	4.0	224	6.3	41.5	52.2
	POST <sup>c</sup>	39	43.6	41.0	15.4	39	0.0	15.4	84.6
2000	PRE	70	37.1	61.4	1.4	39	7.7	15.4	76.9
	POST	74	5.4	81.1	13.5	30	0.0	3.3	96.7
2001	PRE	77	18.2	77.9	3.9	36	2.7	22.2	75.0
	POST	74	14.9	75.7	9.5	29	0.0	3.4	96.6
2002	PRE	39	51.3	41.0	7.7	39	5.1	28.2	66.7
	POST	41	7.3	29.3	63.4	41	0.0	0.0	100.0

<sup>a</sup>Sample size

<sup>b</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>c</sup>Flies collected at least two weeks after tags are removed (POST)

There were at least 40 flies processed for the other collection periods, but data were not obtained for all flies processed. There were individual flies for which only the *kdr* or *skdr* alleles were detected and these data were included when the bands for GAPDH were visible.

In every year, the frequency of RR-*kdr* was significantly lower in the flies from PRE collections compared to POST collections (Table 3.4). In all comparisons, the frequency of RR-*kdr* was significantly higher in flies collected POST than flies collected PRE in the following year (Table 3.5).

Table 3.4. Frequency of RR-*kdr* resistant horn flies collected at St. Joseph Research Station.

Year	PRE <sup>a</sup>	POST <sup>b</sup>	p-value <sup>c</sup>
1999	52	84.6	0.0001
2000	76.9	96.67	0.0067
2001	75	96.55	0.0225
2002	66.67	100	0.0001

<sup>a</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>b</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>c</sup>p-value determined by chi-square analysis

Table 3.5. Frequency of RR-*kdr* resistant horn flies collected at St. Joseph Research Station.

Year	POST <sup>a</sup>	PRE <sup>b</sup>	P-value <sup>c</sup>
1999-2000	84.6	76.9	0.0026
2000-2001	96.67	75	0.0118
2001-2002	96.55	66.67	0.0021

<sup>a</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>b</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>c</sup>P-value determined by chi-square analysis

In all comparisons, the frequency of R-*kdr* alleles was significantly lower in flies collected PRE than flies collected POST (Table 3.6). In all comparisons, the frequency of R-*kdr* was significantly higher in flies collected POST than flies collected PRE in the following year (Table 3.7).

Table 3.6. Frequency of R-*kdr* alleles, detected in horn flies collected at St. Joseph Research Station.

Year	PRE <sup>a</sup>	POST <sup>b</sup>	P-value <sup>c</sup>
1999	72.99	96.36	0.0001
2000	84.61	98.33	0.0067
2001	86.11	98.27	0.0225
2002	80.76	100	0.0001

<sup>a</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>b</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>c</sup>P-value determined by chi-square analysis

Table 3.7. Frequency of R-*kdr* alleles, detected in horn flies collected at St. Joseph Research Station.

Year	POST <sup>a</sup>	PRE <sup>b</sup>	P-value <sup>c</sup>
1999-2000	96.36	84.61	0.0026
2000-2001	98.33	86.11	0.0118
2001-2002	98.27	80.76	0.0021

<sup>a</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>b</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>c</sup>P-value determined by chi-square analysis

The frequency of R-*skdr* alleles was significantly lower in fly populations tested PRE compared to POST in 1999, 2000 and 2002 (Table 3.8). The frequency of R-*skdr* alleles was significantly higher in fly populations tested POST in 2001 compared to PRE in the following year (Table 3.9).

Table 3.8. Frequency of R-*skdr* alleles detected in horn flies collected at St. Joseph Research Station.

Year	PRE <sup>a</sup>	POST <sup>b</sup>	P-value <sup>c</sup>
1999	23.66	35.89	0.0249
2000	32.14	54.05	0.0002
2001	42.85	47.29	0.4879
2002	28.2	78.04	0.0001

<sup>a</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>b</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>c</sup>P-value determined by chi-square analysis

Table 3.9. Frequency of R-*skdr* alleles detected in horn flies collected at St. Joseph Research Station.

Year	POST <sup>a</sup>	PRE <sup>b</sup>	P-value <sup>c</sup>
1999-2000	35.89	32.14	0.6536
2000-2001	54.05	42.85	0.0652
2001-2002	47.29	28.2	0.0067

<sup>a</sup>Flies collected at least two weeks after tags are removed (POST)

<sup>b</sup>Flies collected before treatment of the animals with ear tags (PRE)

<sup>c</sup>p-value determined by chi-square analysis

## Discussion

Barros et al. (1999), reported that an annual rotation between pyrethroid and organophosphate (OP) ear tag treatments at St. Jo between 1991 and 1997 had no effect on development of pyrethroid resistance in the horn fly populations. The number of weeks of control for pyrethroid tags declined from eight weeks in 1991 to two weeks in 1997 in spite of an alternation with organophosphates. Bioassay data confirmed these observations. Therefore, the decline in the number of weeks of control with sequential use of pyrethroid tags (the same tags as used in the study above) between 1999 and 2001 observed in this study was not unexpected. Our bioassay data also supported these observations. For example, when the tags were left in at St. Jo for the entire season in 2000 and 2002, the RF's for pyrethroids increased. When the tags were removed midseason in 1999, there was no change.

Barros et al. (2001) also observed a decline in the number of weeks of control provided by the OP tags in the alternate years; control was reduced from seven to two weeks. In this

study, the RF's for diazinon increased in every year for flies tested POST as compared to PRE (Table 3.2) and the LC<sub>50</sub>'s were significantly lower for flies tested PRE compared to POST in 2 of the 4 years. These data may provide support for development of partial cross-resistance for OP's and pyrethroids in horn flies via either increased metabolism or decreased penetration.

After the mid-season treatment with doramectin in 2001, there was no increase in fly control in the following year. In 2001, the LC<sub>50</sub>'s for both pyrethroids were significantly higher for flies tested POST compared to PRE. This was the pattern for 2000 and 2002 when the tags were removed in the fall. These results are in contrast to the observations at Red River and Macon Ridge. The RF's for pyrethroids for flies collected at both stations decreased from PRE to POST in the year that doramectin was applied, and the number of weeks of control provided by tag treatments increased in the following year (Chapter 2). The results at St. Jo also are in contrast with the study of Barros et al. (2001), who reported a significant increase in susceptibility to OP's following a mid-season treatment of cattle with avermectins.

Since the study sites were distant from each other, different suites of mechanisms of pyrethroid resistance in the flies at the different farms may have existed. There are several explanations for why the fly populations at St. Jo, Macon Ridge, and Red River responded differently to the different treatments and those were discussed in detail in Chapter 2.

Regardless of the mid-season treatment, we observed an increase in the percentage of RR-*kdr* individuals and frequency of R-*kdr* alleles from the PRE populations to the POST populations in every year. Bioassay data for flies tested for permethrin in 2001 (the year of mid-season treatment with doramectin) and 2002 were equivalent, and the number of weeks of control was the same in both years. These data indicate that the genetic makeup of the horn fly

population at St. Jo did not change in spite of the five weeks of control following the treatment with doramectin (and absence of pyrethroid pressure).

Following topical treatment of cattle with doramectin, horn fly larvae do not develop in the manure for up to 35 days post treatment, and adult control lasts for 2 to 3 weeks (Marley 1993). The concept of resistance management with the mid-season treatment of all cattle on the farm is that the horn fly population that reinfests the cattle should come from surrounding areas (assuming a totally effective treatment).

As discussed in Chapter 2, the sources and genotypes of flies that reinfest the cattle following effective treatment are the most important factors for changes in pyrethroid resistance in horn fly populations. Biotic fitness differences associated with pyrethroid resistance may further increase susceptibility. For example, Scott et al. (1997) showed that horn flies from susceptible colonies emerged sooner than horn flies obtained from a pyrethroid resistant colony. Susceptible flies pupated approximately twice as successfully. We did not test for fitness, but from 2000-2002, RF's for both pyrethroids decreased from POST compared to PRE of the following year which may be partially indicative of a fitness deficit. Similarly, there was a significant decrease in the percentage of RR-*kdr* individuals and frequency of R-*kdr* alleles from the POST populations to the PRE populations in each year. Guerrero et al. (2002) also found an increase in the frequency of S-*kdr* alleles between removal of the ear tags in POST and treatment again with ear tags in PRE of the following year at St. Jo. However, the changes in the frequency of R-*kdr* alleles were not large enough in any of the years to allow susceptibility to pyrethroids to be established in the fly populations before the reapplication of pyrethroids in the following year.

Guerrero et al. (2002) showed that in years when the cattle were treated with pyrethroids the frequency of R-*skdr* alleles significantly increased from PRE compared to POST for every year and we observed the same trend. Guerrero et al. (2002) also reported that the frequency of R-*skdr* decreased from POST compared to PRE in the following year for all years. The authors speculated that higher fitness costs were associated with R-*skdr* alleles than the R-*kdr* alleles. In our study, the frequency of R-*skdr* alleles significantly decreased from POST of one year to PRE of the next year only once. The patterns for changes for the frequency of R-*skdr* and R-*kdr* were similar throughout our study, and we did not find evidence to support the concept of a higher fitness deficit of *skdr* compared to *kdr*.

Guerrero et al. (2002) concluded that the *kdr* and *skdr* resistance trait became fixed in the population and contributed to the fact that pyrethroid resistance was unaffected by the yearly alternation of OP's and pyrethroids at St. Jo. The results of this study indicate that a mid-season treatment of doramectin also had no effect on the frequency of the resistant traits. Since currently recommended management strategies are ineffective in control of the pyrethroid resistant populations at St. Jo, future studies on the mechanisms of fitness deficits associated with the *kdr* and *skdr* mutations are warranted.

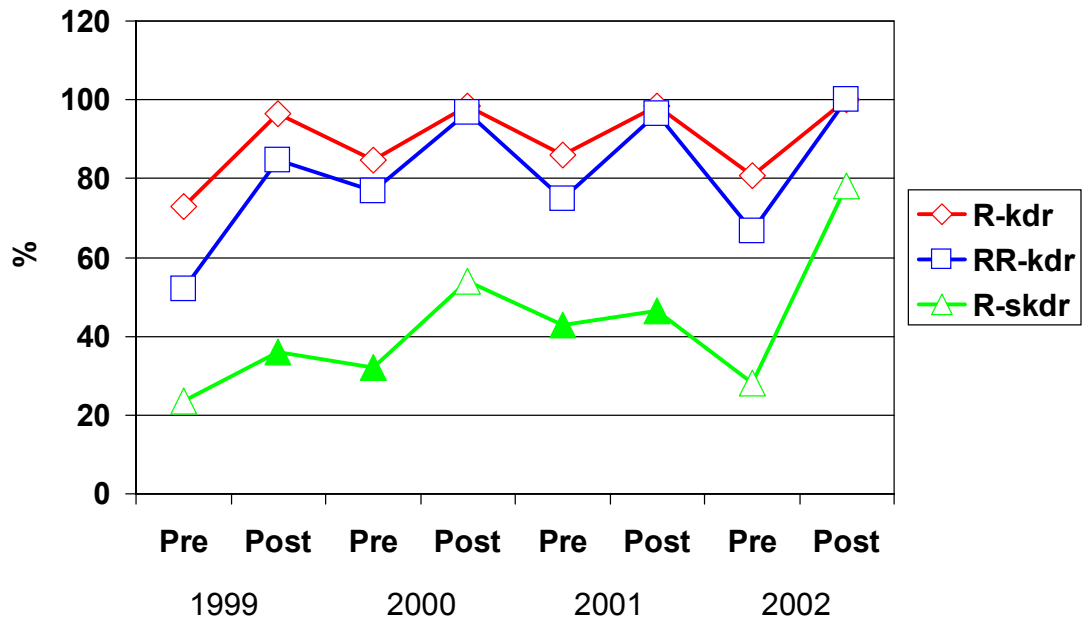


Figure 3.2. The frequency of *R-skdr* and *R-kdr* alleles and *RR-kdr* individuals in horn fly populations at St. Joseph Research Station. The frequency for open symbols was significantly different from the frequency measured in the previous and subsequent samples.

## CHAPTER 4

### SUMMARY AND CONCLUSIONS

It is possible that a mid-season treatment with avermectin pour-ons can be used for management of pyrethroid resistance in certain horn fly populations. We found that when a mid-season treatment of doramectin pour-on was applied to cattle, pyrethroid resistance was reversed, in the following year, in two out of the three cases. At Red River the number of weeks of horn fly control increased by 11 weeks in the year following the mid-season treatment. We propose that target site resistance may be the dominant mechanism for pyrethroid resistance at Macon Ridge and St. Joseph, and consider this to be the reason there were no significant changes in pyrethroid resistance following the mid-season treatment of avermectin, at those two locations.

At St. Joseph, a mid-season treatment of doramectin had no effect on the frequency of the resistant traits. Regardless of the mid-season treatment, we observed an increase in the percentage of RR-*kdr* individuals and frequency of R-*kdr* alleles for the fly populations sampled pre-treatment compared to populations sampled post-treatment in every year. Guerrero et al. (2002) speculated that higher fitness costs were associated with R-*skdr* alleles than the R-*kdr* alleles. During this study, the frequency of R-*skdr* alleles significantly decreased from post-treatment in one year to pre-treatment in the next year only once. The patterns for changes in the frequency of R-*skdr* and R-*kdr* were similar throughout our study, and we did not find evidence to support the concept of a higher fitness deficit of *skdr* compared to *kdr*. Since currently recommended management strategies are ineffective in control of the pyrethroid resistant populations at St. Jo, future studies on the mechanisms of fitness deficits associated with the *kdr* and *skdr* mutations are warranted.

## REFERENCES

1992. Graphpad Software, Inc. Instat.

**Ahrens, E. H. and J. Cocke. 1979.** Season long horn fly control with an insecticide-impregnated ear tag. *J. Econ. Entomol.* 72:215.

**Barros, A. T. M., M. W. Alison, Jr., and L. D. Foil. 1999.** Evaluation of a yearly insecticidal ear tag rotation for control of pyrethroid-resistant horn flies (Diptera: Muscidae). *Veterinary Parasitology.* 82:317-325.

**Barros, A. T. M., J. Ottea, D. Sanson, and L. D. Foil. 2001.** Horn fly (Diptera: Muscidae) resistance to organophosphate insecticides. *Veterinary Parasitology* 96:243-256.

**Barth, D. and E. S. Brokken. 1980.** The activity of 22,23-dihydroavermectin B<sub>1</sub> against the pig louse, *Haematopinus suis*. *Vet. Rec.* 106:388.

**Bloomquist, J.R. and T.A. Miller. 1985.** Carbofuran triggers flight motor output in pyrethroid-blocked reflex pathways of the house fly. *Pesticide Biochemistry and Physiology* 23 (2):247-255.

**Brooks, G.T. 1976.** Penetration and distribution of insecticides. IN *Insecticide Biochemistry and Physiology*, Ed. C.F. Wilkinson. Plenum Press, New York. pp. 3-58.

**Bruce, W. G. 1964.** The history and biology of the horn fly, *Haematobia irritans* (Linnaeus); with comments on control. North Carolina, North Carolina Agricultural Experimental Station and Entomology Research Division, Agricultural Research Service. 157.

**Burns E.C. and B. H. Wilson. 1963.** Field resistance of horn flies to the organic phosphate insecticide ronnel. *Journal of Economic Entomology* 56:718.

**Busvine, J. R. 1951.** Mechanisms of resistance to insecticide in house flies. *Nature* 168:193-195.

**Butler, J. F. 1975.** Economics and control of livestock insects. Proc. Short course FAO-IAEA training manual-radio isotopes and radiation in entomology.143pp.

**Byford, R. L., S. S. Quisenberry, T. C. Sparks, and J. A. Lockwood. 1985.** Spectrum of insecticide cross-resistance in pyrethroid-resistance populations of *Haematobia irritans* (Diptera: Muscidae). *J. Econ. Entomol.* 78:768-773.

**Byford, R. L., M. E. Craig, S. M. DeRouen, M. D. Kimball, D. G. Morrison, W. E. Wyatt, and L. D. Foil. 1999.** Influence of permethrin, diazinon and ivermectin treatments on insecticide resistance in the horn fly (Diptera: Muscidae). *International Journal for Parasitology* 29:125-135.

**Campbell, J. B. 1976.** Effect of horn fly control on cows as expressed by increased weaning weights of calves. *J. Econ. Entomol.* 69:711-712.

**Catterall, W. A. 1995.** Structure and function of voltage-gated ion channels. *Annu. Rev. Biochem.* 64:493-531.

- Chamberlain, W.F. 1981.** Dispersal of horn flies: Effect of host proximity. *Southwest. Entomol.* 6(4): 316-325.
- Chamberlain, W.F. 1984.** Dispersal of horn flies: III. Effect of environmental factors. *Southwest. Entomol.* 9(1): 73-78.
- Clements, A. N. and T. E. May. 1977.** The actions of pyrethroids upon the peripheral nervous system and associated organs in the locust. *Pestic. Sci.* 8:661-680.
- DeRouen, S. M., L. D. Foil, J. W. Knox, and J. M. Turpin. 1995.** Horn fly (Diptera:Muscidae) control and weight gains of yearling beef cattle. *Veterinary Entomology* 88:666-668.
- Dong, K. and J. G. Scott. 1993.** Linkage of *kdr*-type resistance and the *para*-homologous sodium channel gene in German cockroaches (*Blattella germanica*). *Insect Biochemistry and Molecular Biology* 24:647-654.
- Duce, I. R. and R. H. Scott. 1985.** Actions of dihydroavermectin B<sub>1a</sub> on insect muscle. *Br. J. Pharmac.* 85:395-401.
- Duce, I. R. and R. H. Scott. 1985.** Interactions of avermectin, GABA and ibotenic acid on input conductance of locust (*Schistocerca gregaria*) muscle. Neurotox '85.2nd International Symposium on Insect Neurobiology and Pesticide Action, Bath, 31 March - 4 April 1985, abstract.
- Foil, L.D. and J.A. Hogsette. 1994.** Biology and control of tabanids, stable flies and horn flies. *Rev. Sci. Tech. Int. Epiz.* 13(4):1125-1158.
- Gordon, D.V., W.O. Haufe, and K.K. Klein. 1984.** Determination of economic thresholds for horn fly control in Western Canada: A farm level simulation approach. *Cdn. J. of Agric. Economics.* 32: 390-421.
- Guerrero, F. D., R. C. Jamroz, D. Kammlah, and S. E. Kunz. 1997.** Toxicological and molecular characterization of pyrethroid-resistant horn flies, *Haematobia irritans*: identification of *kdr* and *super-kdr* point mutations. *Insect Biochemistry and Molecular Biology* 27:747-755.
- Guerrero, F. D., S. E. Kunz, and D. Kammlah. 1998.** Screening of *Haematobia irritans irritans* (Diptera: Muscidae) populations for pyrethroid resistance-associated sodium channel gene mutations by using a polymerase chain reaction assay. *J. Med. Entomol.* 35:710-715.
- Guerrero, F.D., M.W. Alison, Jr., D.M. Kammlah, and L.D. Foil. 2002.** Use of the polymerase chain reaction to investigate the dynamics of pyrethroid resistance in *Haematobia irritans irritans* (Diptera: Muscidae). *J. Med. Entomol.* 39(5): 747-754.
- Guillot, F.S., J.A. Miller, and S.E. Kunz. 1988.** Physiological age of female horn flies (Diptera: Muscidae) emigrating from a natural population. *J. Econ. Entomol.* 81(2): 555-561.
- Harvey, T. L. and J. R. Brethour. 1970.** Horn fly control with Dichlorvos-impregnated strips. 63:1689.

- Harvey, T. L. and J. R. Brethour. 1979.** Effect of horn flies on weight gains of beef cattle. *J. Econ. Entomol.* 72:516-518.
- Haufe, W. O. 1982.** Growth of range cattle protected from horn flies (*Haematobia irritans*) by ear tags impregnated with fenvalerate. *Can. J. Anim. Sci.* 62:567-573.
- Headley, J. C. 1972.** Defining the Economic Threshold. National Academy of Science, Pest Control Strategies for the Future 1972:100-108.
- Howell, D. E. 1952.** Fly Control in Oklahoma. *Okla. Agric. Exp. Stn. Bull.* B-385:18.
- James, P. S., J. Picton, and R. F. Riek. 1980.** Insecticidal activity of the avermectins. *Vet. Rec.* 106, 59, abstract.
- Jamroz, R. C., F. D. Guerrero, D. Kammlah, and S. E. Kunz. 1998.** Role of the *kdr* and *super-kdr* sodium channel; mutations in pyrethroid resistance: correlation of allelic frequency to resistance level in wild and laboratory populations of horn flies (*Haematobia irritans*). *Insect Biochemistry and Molecular Biology* 28:1031-1037.
- Kinzer, H. G., W. E. Houghton, J. M. Reeves, S. E. Kunz, J. D. Wallace, and N. S. Urquhart. 1984.** Influence of horn flies (*Haematobia irritans*) on weight loss in cattle with notes on prevention of loss by insecticide treatment. *Southwest Entomol.* 9:212-217.
- Knipling, E. F. 1954.** On the insecticide resistance problem. *Agric. Chem.* 9:46-155.
- Kunz, S. E. and C. D. Schmidt. 1985.** The pyrethroid resistance problem in the horn fly. *Agric. Entomol* 2:358-363.
- Kunz, S. E. 1991.** Dynamics of permethrin resistance in a colony of horn flies (Diptera: Muscidae). *Med. Entomol* 28:63-65.
- LeOra Software. 1987.** POLO-PC A user's guide to probit or logit analysis. LeOra Software, Berkeley CA.
- Lund, A. E. and T. Narahashi. 1983.** Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs. *Pestic. Biochem. Physiol.* 20:203-216.
- Marley, S.E., R.D. Hall, and R.M CCorwin. 1993.** Ivermectin cattle pour-on: duration of a single late spring treatment against horn flies, *Haematobia irritans* (L.) (Diptera: Muscidae) in Missouri, USA. *Vet Parasitol.* 51, 167-172.
- Ostlind, D. A., S. Cifelli, and R. Lang.** Insecticidal activity of the antiparasitic avermectins. *Vet. Rec.* 105[168]. 1979.
- Ottea, J.A., S.A. Ibrahim, A.M. Younis, R.J. Young, B.R. Leonard, and A.R. McCaffery. 1995.** Biochemical and physiological mechanisms of pyrethroid resistance in *Heliothis virescens* (F.). *Pesticide Biochemistry and Physiology* 51:117-128.

- Quisenberry, S. S., J. A. Lockwood, H. K. Wilson, and T. C. Sparks. 1984.** Pyrethroid resistance in the horn fly *Haematobia irritans* (L.) (Diptera: Muscidae). *Journal of Economic Entomology* 77:1095-1098.
- Quisenberry, S.S. and D.R. Strohbehn. 1984.** Horn fly (Diptera: Muscidae) control on beef cows with permethrin-impregnated ear tags and effect on subsequent calf weight gains. *J. Econ. Entomol.* 77:422-424.
- Sawicki, R. M. 1978.** Unusual response of DDT-resistant houseflies to carbene analogues of DDT. *Nature* 275:443-444.
- Schmidt, C. D., C.T. Ward, and J.L. Eschle. 1973.** Rearing and biology of the horn fly in the laboratory: Effects of density on survival and fecundity of adults. *Environ. Ecol.* 2:223-224.
- Schmidt, C. D., S. E. Kunz, H. D. Petersen, and J. L. Robertson. 1985.** Resistance of horn flies (Diptera: Muscidae) to permethrin and fenvalerate. *J. Econ. Entomol.* 78:402-406.
- Scott, J.A., F.W. Plapp, Jr., D.E. Bay. 1997.** Pyrethroid resistance associated with decreased biotic fitness in horn flies (Diptera: Muscidae). *Southwest. Entomol.* 22(4): 405-410.
- Shankland, D.L. 1976.** The nervous system: Comparative physiology and pharmacology. in insecticide biochemistry and physiology, Ed. C.F. Wilkinson. Plenum Press, New York. pp. 229-270.
- Sheppard, D.C., and N.C. Hinkle. 1985.** Pyrethroid resistance in horn flies: the problem, causes, and possible solutions. *J. Agric. Entomol.* 2(4):317-324.
- Sheppard, D.C., and N.C. Hinkle. 1987.** Differential pyrethroid resistance selection in horn fly populations treated with pyrethroid cattle ear tags and pyrethroid sprays. *J. Agric. Entomol.* 4(2):167-178.
- Sheppard, D.C. 1987.** A field procedure using disposable materials to evaluate horn fly insecticide resistance. *J. Agric. Entomol.* 4(1):87-89
- Sheppard, C. 1983.** Stirofos resistance in a population of horn flies. *Georgia Entomol.* 18:370-376.
- Smith, T. J., S. H. Lee, P. J. Ingles, D. C. Knipple, and D. M. Soderlund. 1997.** The L1014F point mutation in the house fly *Vssc1* sodium channel confers knockdown resistance to pyrethroids. *Insect Biochemistry and Molecular Biology.* 807-812.
- Taylor, M. F. J., D. G. Heckel, T. M. Brown, M. E. Kreitman, and B. Black. 1993.** Linkage of pyrethroid insecticide resistance to a sodium channel locus in the tobacco budworm. *Insect Biochemistry and Molecular Biology* 23:763-775.
- Usherwood, P. N. R. and H. Grundfest. 1965.** Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* 28:497-518.

**Williamson, M. S., I. Denholm, C. A. Bell, and A. L. Devonshire. 1993.** Knockdown resistance (*kdr*) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). *Mol. Gen. Genet.* 240:17-22.

**Williamson, M. S., D. Martinez-Torres, C. A. Hick, and A. L. Devonshire. 1996.** Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. *Mol. Gen. Genet.* 252:51-60.

**Wouters, W. and J. van den Bercken. 1978.** Action of pyrethroids. *Gen. Pharmac.* 9:387-398.

**Wright, D.J. 1986.** Biological Activity and Mode of Avermectins. IN Neuropharmacology and Pesticide Action, Ed. M.G. Ford. Weinheim, Federal Republic of Germany: VCH, 174-202.

## VITA

Glenn Robert O'Remus was born on September 21, 1975 in Livingston, New Jersey. He received a Bachelor of Science degree in animal science from the University of Massachusetts at Amherst. In 1999, he accepted the position of research associate, under the direction of Lane Foil, in the Department of Entomology at Louisiana State University, where he researched insects of veterinary importance. In 2001, he was accepted into the Department of Entomology at Louisiana State University, where he is a candidate for the degree of Master of Science.