THE STATUS OF RESISTANCE IN *Culex quinquefasciatus* SAY (DIPTERA: CULICIDAE) POPULATIONS IN BRAZOS AND HARRIS COUNTIES, TEXAS

A Dissertation

by

MARK MILLER JOHNSEN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Entomology

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ABSTRACT

The Status of Resistance in *Culex quinquefasciatus* Say (Diptera: Culicidae) Populations in Brazos and Harris Counties, Texas. (May 2007)

Mark Miller Johnsen, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Jimmy K. Olson

In 2002, West Nile virus was isolated for the first time in Harris County, Texas. The subsequent epidemic led the Harris County Mosquito Control Division to initiate an extensive spraying operation to suppress infected adult mosquitoes. The control program was aimed at the predominate disease-carrying mosquito for the southern United States, *Culex quinquefasciatus* Say. With the increase of insecticide pressure on the mosquito populations, the possibility of resistance was brought into question. A three year study using a vial bioassay test was conducted in Harris (2004-2005) and Brazos (2005-2006) counties to determine the resistance status of *Cx. quinquefasciatus* to the six chemicals (malathion, naled, resmethrin, permethrin, sumithrin, and pyrethrum) used most frequently in adult mosquito control programs.

The resistance ratios acquired from the vial bioassay tests were mapped onto shapefiles for Harris and Brazos counties, which revealed clustering of areas with pyrethroid resistance mosquito populations in the northeastern, southeastern, and southwestern corners of Loop 610 in Harris County. An additional six-month preliminary study, involving six operational areas in Harris County and three in Brazos

County, was conducted, demonstrating only minor fluctuations in the monthly resistance ratios occurring in both counties in 2005.

A significant correlation was documented between the two years of resistance ratios for mosquitoes to the three pyrethroids in Harris County and all the insecticides except pyrethrum in Brazos County. A significant relationship was also found between the resmethrin resistance ratios and the number of spray events performed during the previous year and the malathion resistance ratios with the insecticide treatments conducted in the same year. The correlation analyses provide data used to predict areas where resistance can develop in the mosquito population, thus providing the control agency more data to plan future control tactics.

The overall analysis indicated that Harris County has localized pockets of resistant mosquitoes; but, on a whole, it does not seem to have widespread resistance in its mosquito populations. The only resistance that was detected was in the mosquitoes tested against the three pyrethroids. Mosquitoes in Brazos County, which has no organized mosquito control, demonstrated county-wide susceptibility to all six insecticides tested.

DEDICATION

I dedicate this dissertation to my family for the support they have shown me over the past 11 years. I truly appreciate my sister Erin Johnsen, grandparents Mr. Arne "Swede" and Mary Johnsen, Mr. Ralph and Dolores Hodges, and my parents Dr. Guy and Kathy Johnsen for all their love and encouragement they have given me throughout my "college years."

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

CHAPTER I

INTRODUCTION

Since the development of synthetic chemical compounds for the control of insects, a battle has raged between scientific advancement and the capacity of insects to adapt and overcome chemical challenges. To date mosquitoes have developed resistance to every chemical class used for control of adult mosquitoes by organized control agencies around the world (Smith 1949, Bohart and Murray, 1950, Gjullin and Peter 1952, Mulla, et al. 1960, Malcolm 1988).

In recent years the development of insecticide resistance has become a great concern for vector control programs in Texas. Resistance may result in the loss of the primary control component of most mosquito control programs bases in Texas, possibly leading to failure of entire programs. The types of mosquito control programs in Texas vary in their scope, capacity, and mission and are the responsibility of the local city/county governments to manage. Specialized agencies termed Mosquito Control Districts (MCD), are mostly clustered in six counties located in the Upper Gulf Coast region of southeast Texas and are funded through local county taxes to provided vector and annoyance mosquito control for the county (Gray 1961). The primary control tactic used by Texas MCDs to combat adult disease vectoring and annoyance mosquitoes in their districts is the use of insecticides sprayed from ultra low volume (ULV) generators mounted in airplanes and on trucks (Dr. Jimmy K. Olson, personal communication). Most control districts have added a larvicide component to their control program, where conventional insecticides, bacterial agents, and monomolecular films are applied to

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This dissertation follows the style and format of the Journal of Medical Entomology.

larval breeding sites. Larvicidal tactics are still secondary to adult control operations.

To prevent an operational failure, some of the MCD's in Texas now employ some type of insecticide resistance management program to maintain the effectiveness of their vector control programs. Otherwise, the majority of the districts participate in a resistance monitoring program offered as a service of the Mosquito Research Laboratory at Texas A&M University (TAMU). This program generates insecticide resistance ratios for the mosquitoes under chemical selection pressure that are then used in certain cases to modify an agency's control tactics or choice of chemicals. The primary type of resistance management practiced by mosquito control agencies in Texas when such is done, is the alternation of insecticides from different chemical classes (Georghiou 1983). This manner of resistance management is recommended by the TAMU Mosquito Research Laboratory as the most economical and operationally-feasible method and is currently practiced in Texas by Jefferson County MCD, Orange County MCD, and Harris MCD (Dr. Jimmy K. Olson personal communication).

This study was conducted in Harris and Brazos counties to determine the status of insecticide resistance of mosquito populations sampled from a multitude of sites in each county and which receive differing amounts of chemical control over a give year. In addition to this primary objective several methodologies were tested to determine their ability to be used as means for predicting the occurrence of insecticide of resistance and their value in planning and modifying current mosquito control tactics.

This dissertation addresses the following objectives:

1. Determine the insecticide resistance ratios of the indigenous *Culex quinquefasciatus* Say populations in Harris and Brazos Counties, Texas, to resmethrin, permethrin, sumithrin, malathion, naled, and pyrethrum.
2. Monitor the month to month variation in insecticide resistance in *Cx. quinquefasciatus* populations in six operational areas in Harris County and three collection areas in Brazos County, Texas.
3. Determine if the annual variation in insecticide resistance ratios of *Cx. quinquefasciatus* populations from Brazos and Harris County, Texas can be correlated between the two years that the studies were run in each county.
4. Correlate the insecticide resistance ratios obtained for the *Cx. quinquefasciatus* populations in Harris County to the number of spray events conducted in the operational areas where each population was sampled.
5. Develop resistance maps for the six adulticides used by mosquito control agencies in Harris and Brazos counties, Texas.

CHAPTER II

LITERATURE REVIEW

Insecticide Resistance

Insecticide resistance was initially defined by Brown and Pal (1971) as any population, within a species, normally susceptible to a given insecticide that is no longer controlled by the insecticide in the area concerned. This definition is a strictly operational one and does not encompass the various aspects that interact to cause resistance. With recent research increasing the knowledge of resistance mechanisms at the molecular level, the definition of resistance needed to be modified. In 1987, Sawicki proposed that resistance was a genetic change in response to selection by toxicants that may impair control in the field. This adaptation of Crow's (1960) definition of resistance satisfied both the operational and genetic aspects that encompass the totality of resistance.

Insecticide resistance was first observed when chemicals used to control pest species began to fail operationally. In 1914, Melander noted the first case of resistance when sprays containing lime-sulfur failed to control the San Jose scale (*Aspidiotus perniciosus*) in Washington orchards. This observation was followed by a report by Quayle (1916) on resistance of the California red scale (*Aonidiedella aurantii*) to hydrogen cyanide fumigation in 1916. The initial documented cases of resistance were downplayed as aberrations and forgotten.

Insecticide resistance was first recognized in as occurring in mosquitoes from as early as 1947 when Deonier and Gilbert (1950) reported insecticide resistance in salt marsh mosquitoes located in Florida. Insecticide resistance has now been confirmed in 109 mosquito species (Georghiou and Mellon 1983, Brown 1986, WHO 1992). The majority of the documented insecticide resistance has been recorded in mosquitoes from the genus *Anopheles* (Brown 1986, WHO 1992). This is due to the malaria eradication programs that targeted *Anopheles* by using insecticides as the primary means to control the disease (Busvine 1969), with indoor spraying of residual insecticides having been a possible contributing factor here. In recent years, the number of resistant mosquito species has been increasing due to the prophylactic use of pesticides in mosquito control programs in addition to their wide-scaled use in agricultural pest control (Brown 1986, Lines 1988, Georghiou 1990, Roberts and Andre 1994). With the increase of insecticide resistant mosquito species worldwide, the mechanisms of resistance are being more intensely investigated to determine techniques that may be used to control resistant mosquito species or otherwise manage insecticide resistance in these insects.

Resistance has been divided into two broad categories termed behavioral resistance and physiological resistance. Behavioral resistance can be defined as a population-based change in a species’ genetics resulting from differential survival of individuals responding to insecticide use through insecticide avoidance behavior (Roberts and Andre 1994). Behavioral resistance has been reported in both the presence and absence of physiological resistance. Resistance is considered behavioral only if the population changes a previously observed behavior. Behavioral resistance has been documented mostly in anopheline mosquitoes as it relates to the use of residual indoor insecticide treatments and insecticide impregnated bednets (Curtis et al. 1990, Kolaczinski and Curtis 2004).

Physiological resistance occurs when the insect survives direct contact with the insecticide through one or more of a variety of biochemical mechanisms (Georghiou 1965). The physiological resistance mechanisms that have been identified to date are reduced cuticular penetrations, target site insensitivity, and increased detoxification of the insecticide through distribution, storage, and/or metabolism in internal tissues.

Reduced insecticide penetration is one physiological mechanism that can, theoretically, occur at any biological membrane but has only been demonstrated at the cuticular level (Scott 1990). Alone, reduced penetration is considered a minor mechanism; but, the effects can be magnified when it occurs in conjunction with one or more additional physiological mechanisms (Brooks 1976, Oppenoorth 1985).

Target site resistance occurs when the amino acids responsible for insecticide binding at its action site are altered causing the insecticide to be less effective or entirely ineffective (Brogdon and McAllister 1998a). The target site of organophosphorus (OPs) and carbamate insecticides is the enzyme acetylcholinesterase (AChE) in nerve synapses (Soderlund and Bloomquist 1990) and inhibition of AChE results in increased levels of neurotransmitter acetylcholine, thereby disrupting the insect’s neural and motor functions. The target of organochlorines and synthetic pyrethroids are the voltage-sensitive sodium channels of the nerve sheath and resistance to these insecticides has been associated with reduced neuronal sensitivity (Soderland and Bloomquist 1990). This mechanism confers resistance to the rapid paralytic action of these insecticides and has been termed knockdown resistance (KDR) which has been isolated in several mosquito species (Priester and Georghiou 1980, Chandre et al. 1998, Kasai et al. 1998).

Enzyme-based detoxification resistance occurs when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferase prevent the insecticide from reaching its site of action (Plapp and Wang 1983, Brogdon and McAllister 1998a). The glutathione S-transferase (GSTs) are a group of enzymes that conjugate insecticides and other xenobiotics with glutathione resulting in a more water-soluble product (Soderlund and Bloomquist 1990). GSTs exist is several molecular forms and have been shown to be an important resistance mechanism for organophosphates and DDT (Dauterman 1983, Oppenoorth 1985).

The oxidative enzymes known as cytochrome P450-dependent monooxygenases or mixed-function oxidases (MFOs) are another enzymatic group associated with insecticide resistance (Oppenoorth 1985). Like GSTs, MFOs catalyze reactions that result in products with increased water solubility thereby promoting excretion. They have been implicated in resistance for all insecticide classes with the exception of cyclodienes (Soderlund and Bloomquist 1990).

The third group of enzymes involved in physiological resistance are broadly classified as esterases. Esterases have been shown to have a significant effect on the detoxification of organophosphate, carbamate, and pyrethroids (Dauterman 1985). In mosquitoes, numerous studies have demonstrated a correlation between decreased sensitivity to organophosphates and elevated esterase activity detected (Apperson and

Georghiou 1975, Voss 1980, Hemingway et al. 1986, Raymond et al. 1987, Brogdon et al 1988, Brogdon 1989, Grant et al. 1989, Dary et al. 1990, Bisset et al. 1995).

Resistance Bioassays

There have been a variety of bioassays developed to determine the insecticide resistance existing in adult and larval mosquito populations. The first attempt to develop a standardized resistance test was undertaken by the World Health Organization (WHO), resulting in the development of the WHO adult bioassay test. This standardization was part of the world wide cooperative program on insect resistance to insecticides that promoted the study of resistance in the field and laboratory (Wright 1957, Shidrawl 1990). The WHO bioassay was based on the concept of a "diagnostic dose," where mosquitoes are exposed to papers impregnated with a lethal dose (established in the laboratory) of one of the various chemical used in mosquito control for a determined amount of time and then, mortality readings are made. This bioassay has lost favor due to lack of pesticides availability (synthetic pyrethroids) and the frequent false results occurring from deteriorated insecticide-impregnated papers.

New bioassays have been developed to replace the WHO test, including topical, bottle, and vial bioassays. Each of these tests has unique properties, but ultimately, test selection should be based on the resources of the agency performing the test. Topical assays involve the application of a small amount of pesticide directly to individual insects through the use of a hyperdermic syringe (Ludvik 1953, Busvine 1971). This assay tests the susceptibility of the population to a range of insecticide concentrations. The advantage of this test is that a known amount of insecticide is applied directly to the mosquito instead of being absorbed through the tarsi. This bioassay has been extensively abandoned by the industry due to the expensive equipment essential for conducting the test, the necessity for skilled labor, and the considerable time required to complete the test. The bottle bioassay is a time-mortality test developed by Brogdon and McAllister (1998b) to provide a simple test that records mortality over a short period of time using one chemical concentration. The disadvantages of this test include an inherit bias against organophosphates due to the lag time between exposure to death in the insect and the problems of relating the lethal time recorded from this test to lethal concentration/dosages calculated from other bioassays.

Since being established in 1976, the modified scintillation vial bioassay (Plapp 1971) has been the preferred insecticide resistance screening test of the Texas A&M University Mosquito Research Laboratory insecticide resistance monitoring program. To facilitate the comparison with pervious insecticide resistance data collected by Mosquito Research Laboratory personnel for *Cx. quinquefasciatus* populations from the Gulf Coast Mosquito Control Districts (including Harris County Mosquito Control Division) this bioassay was selected for the current study and is described in detail below.

Organized Mosquito Control in Texas

In 1949, the Texas Legislature legally recognized the fact that mosquitoes posed a threat to the health and well-being of the citizens of Texas (Micks 1965). With this declaration, the 51st Texas State Legislature provided for the creation, regulation, and financing of mosquito control districts (Micks 1965). This act initially only applied to the counties which border on the Gulf of Mexico, but the legislation was later amended to include any county in the state of Texas. The first organized mosquito control district in Texas was established in Jefferson County in 1950, and was followed by Galveston (1954), Orange (1955), Brazoria (1955), Hale (1957), Harris (1964), and Chambers (1971) counties (Micks 1965, Mr. Roy Burton personal communication). Since the inception of mosquito control in Texas, the principal reliance has been placed upon adulticiding measures which are aimed first and foremost at mosquitoes in the coastal salt marshes. The primary responsibility of the first mosquito control districts was to control the salt marsh mosquitoes, *Aedes sollicitans* (Walker) and *Ae. taeniorhynchus* (Wiedemann). Control of disease outbreaks were a secondary consideration during the initial years of organized mosquito control in Texas.

During the years immediately following the establishment of the first organized MCDs in Texas, insecticide resistance testing was performed on a sporadic basis with the majority of tests conducted either by the established MCDs or public universities. Resistance testing in Texas has historically involved various disease vectoring and annoyance mosquito species in the Gulf Coast region of Texas. The first documented occurrence of resistance in Texas involved resistance of *Cx. quinquefasciatus* to DDT and dieldrin (Micks et al. 1961). The prevalence of mosquito resistance has increased since this initial discovery, with new chemical classes (organophosphates, pyrethroids) proving to be ineffective in some cases (Micks and Rougeau 1977, Micks et al. 1980).

The Harris County Mosquito Control (HCMCD) division was founded in response to the 1964 outbreak of St. Louis encephalitis in Houston that resulted in 711 human cases and 27 deaths (Micks 1965, Henderson et al. 1970). As a result of this epidemic, the mission statement of the division was developed so as to provide disease abatement by targeting the Southern House mosquito, *Cx*. *quinquefasciatus*. Based on this mosquito's vector status, HCMCD's resistance monitoring program has historically been focused solely on this mosquito. Most of the resistance monitoring conducted by HCMCD has been a combination of topical and bottle bioassays. Pietrantonio et al. (2000), used bottle bioassays to identify *Cx*. *quinquefasciatus* resistance to malathion in eight HCMCD operational areas (42, 51, 54, 55, 66, 106, 206, and 512), and possible resistance to resmethrin in one HCMCD area (51). With the current increase in mosquito control activity due to the introduction of West Nile virus into the Houston area, the interest in monitoring *Cx*. *quinquefasciatus* for insecticide resistance has resurfaced (Mr. Kyle Flatt personal communication).

Since 1976, the Texas A&M University (TAMU) Mosquito Research Laboratory has provided a resistance monitoring service for counties or health departments across Texas. The tests are conducted for agencies that do not have the technical proficiency or the financial resources to conduct the tests themselves. The TAMU resistance monitoring program has focused primarily on disease-vectoring mosquitoes, but tests have been conducted on annoyance mosquitoes when requested by the submitting agency (Robert and Olson 1989, Sames et al. 1996, Sukontason et al. 1998).

Insecticide Resistance Management

Integrated vector control is the rational use of all appropriate means of control in a mutually compatible, safe, and cost-effective manner in order to achieve vector suppression and control of disease (WHO 1992). Most organized mosquito control programs in Texas follow an integrated pest management (IPM) philosophy which includes both chemical and non-chemical tactics. However, the primary method of control employed by these agencies are chemical agents applied from aircraft or truck-mounted ultra low volume (ULV) spray systems. The tactics target adult mosquitoes and are therefore referred to as adulticides. Due to this reliance on chemical control a insecticide resistance management (IRM) has been implemented and incorporated into their integrated mosquito management programs by several mosquito control agencies.

Insecticide resistance management is defined as the development of control strategies that prevent or delay the onset of resistance to pesticides in naïve populations, or reduce pesticide resistance in populations already tolerant to a toxicant (Croft 1990). Insecticide resistance management is primary practiced by agencies in an attempt to 1) avoid resistance development in pest populations, 2) slow the rate of resistance development, and 3) cause resistant populations to “revert” to more susceptible levels and thereafter keep resistance below some threshold (Croft 1990, WHO 1992). Specific measures which can be applied in the IRM process are 1) selection and sequence of pesticide use, 2) selective application of pesticide (spot and seasonal application), 3) rotation of pesticides, 4) mixture of pesticides, 5) use of synergists, 6) use of biological control and biopesticides, and 7) environmental management (WHO 1992). These measures were organized by Georghiou (1983) into three principal categories 1) management by moderation, 2) management by saturation, and 3) management by multiple attack.

Management by moderation is based on the principle of conservation of susceptible genes in a population through reduction of selection pressure. The current method of application of insecticides selects heterozygous and homozygous resistant portions of the population shifting the frequencies of genotypes in favor of the resistant genes. To “conserve the susceptibility,” applications of insecticide with a lower dose is prescribed to preserves a portion of the susceptible population. Since this method of management recognizes the value of conserving susceptible genes to preserve susceptibility, it calls for leaving untreated areas or “refugia” in treatment zones, through incomplete coverage of an area during treatment (Georghiou and Taylor 1977a, b). This is accomplished more readily in mosquito control in Harris County due to the control method used to treat for adult mosquitoes and the establishment of ecological sanctuaries for wildlife which refuse chemical control methods. The final plank in the platform of resistance manage through moderation is the reduction of selection pressure through a decline in insecticide applications (Georghiou 1983). The methods prescribed for resistance management by moderation may be considered too extreme in nature and impractical in an operational sense based on the objectives of the control agency.

The second approach of resistance management is through saturation of the insect’s defense mechanisms by dosages that can overcome resistance. The first option of management through saturation is recommend for untreated populations of insects only because it is based on the genetic make up of the population. This method is undertaken to make the resistance gene recessive through treatment with doses of insecticide lethal to susceptible as well as heterozygous-resistance individuals thus eliminating the resistance gene in the population (Curtis et al. 1978, Taylor and Georghiou 1979). Laboratory studies have supported this approach with insecticide, but there is limited evidence to confirm its success under field conditions (LeBaron et al 1986). This method is inadvisable in areas which already have been under select pressure from insecticidal treatments.

A method that falls under the saturation category that is currently practiced by most pest control and mosquito control agencies is the use of synergists to suppress resistance mechanisms in the insect population. Synergists act by inhibiting specific detoxification enzymes and thus are capable of reducing or eliminating the selective advantage of individuals possessing such enzymes (Georghiou 1983). The chemical mixture of Scourge® (resmethrin) purchased for use in adulticide operations in Harris County has a synergist added by the manufacturer.

The final management category is based on the use of multiple attacks independently acting on the insect selected for control so that the select pressure of any one attack is below that required to develop resistance in the population. A mixture of chemicals from different chemical classes is one method used as a multiple attack tactic. The theory behind this method is that, since the two chemical class attack different target sites in the insect, it can not develop resistance to either chemical used. Published reports on observations of mixture of chemicals show positive, negative, and no effect on the level of resistance when employed (Asquith 1961, Burden et al. 1961, Graves et al. 1967, Ozaki et al 1973, Takahashi 1979).

Mosaics use a spatial patchwork of insecticide applications so that adjacent areas are treated simultaneously with different insecticides (Tabashnik 1990). This tactic has not been tested in the field; but, modeling suggests that it will not slow the evolution of resistance (Curtis 1985) and in some cases, will increase it (Comins 1986).

The rotation of chemicals falls under the category of multiple attacks and is one of the most used resistance management technique by mosquito control organizations in Texas (Jimmy K. Olson, personal communication). The concept of rotation of chemicals as an anti-resistance measure assumes that individuals that are resistant to one chemical have lower fitness than susceptible individuals so that their frequency declines during the intervals between applications of that chemical (Georghiou 1980, Georghiou 1983, Georghiou et al. 1983). The program consists of alternating insecticides from different chemical classes that work on different target sites to suppress the development of resistance. There must be no cross resistance on the part of the target insect population to the second chemical selected with the other chemical selected for the rotation (Mellon and Georghiou 1984). This is the insecticide resistance management strategy that has been chosen by the HCMCD to implement

An IRM program may include some or all of the methods promoted in various papers published and summarized in this section. When undertaking an IRM program, the most important component to incorporate is that of an insecticide resistance monitoring program for the target mosquito population. This is the first step in determining the effectiveness of the mosquito control being practiced. Monitoring provides an early warning system to detect initial development of insecticide tolerance in the target mosquito population (Brogdon and McAllister 1998a). The baseline data gathered from resistance tests associated with these monitoring programs provide supplemental information for determining the type of mosquito control to perform and the type of pesticides to select.

Bionomics and Distribution of *Culex quinquefasciatus* Say

The systematic classification of *Cx. quinquefasciatus* has had a tumultuous existence, going through several promotions and demotions from species recognition as well as a long fought battle over nomenclature. Thomas Say first described this "exceedingly numerous and troublesome species" during an expedition to the Rocky Mountains in 1823. However, until the late 1970's the southern house mosquito was often referred to as *Culex fatigans* (Wiedemann 1828) even though the description came fives years later than Say's (Say 1823, Belkin 1977, Sirivanakarn and White 1978).

Culex quinquefasciatus is closely related to the northern house mosquito, *Cx. pipiens* Linnaeus, and has at various times been classified as a subspecies of the *Cx. pipiens* species complex (i.e., *Culex pipiens quinquefasciatus*), a separate species, and a geographic race (Mattingly et al. 1951, Sirivanakarn and White 1978, Barr 1982, Harbach et al. 1985). The advent of new molecular systematic techniques has added to the debate, with the identification of shared genetic markers supporting the subspecies nomenclature of *Cx. pipiens quinquefasciatus* (Miller et al. 1996). However, Crabtree et al (1997) identified a 600 bp DNA sequence unique to *Cx. pipiens* and developed a PCR assay that clearly separates between *Cx. pipiens* and *Cx. quinquefasciatus*. The classification of *Cx. quinquefasciatus* will likely continue to fluctuate, but this author will follow the currently accepted designation of *Cx*. *pipiens* and *Cx*. *quinquefasciatus* as separate species (Knight 1978, Bosik 1997).

Culex quinquefasciatus is globally distributed in the tropical and subtropical regions of the world and is replaced by *Cx*. *pipiens* in cooler, temperate regions (Rozeboom and Kitzmiller 1958, Barr 1982). In North America, the southern house mosquito has a distribution that stretches across the southern United States (Hill et al. 1958, Darsie and Ward 1981). The northern limit of *Cx*. *quinquefasciatus*' range varies between the 36° and 39° north latitude where it overlaps with the distribution of *Cx*. *pipiens* and hybridization is known to occur between the species (Mattingly et al. 1951, Barr 1957). The southern house mosquito has been collected throughout Texas and is found in large numbers in the major urban areas of the state (McGregor and Eads 1943, Hill et al. 1958).

Cx. *quinquefasciatus*' annoyance and vector potential is augmented by its ability to produce multiple generations a year that overlap to such an extent that there is no differentiation between broods, with the only limitation on population size being the availability of larval breeding sites (Horsfall 1955). Female *Cx*. *quinquefasciatus* preferentially oviposit in drainage ditches, septic ponds, artificial containers, and many persistent water sources with a high organic content (Laird 1988). Eggs are laid in adherent masses or “rafts” on the surface of water that is protected from wind and wave action (Howard 1900). These well-defined egg rafts contain 100 or more eggs per raft (Gerberg 1970). The length of the life cycle is temperature dependent, but embryonic development is completed and larvae emerge within 24 to 36 hours after oviposition.

The larvae go through four instars within 7 to 14 days post hatching (Kettle 1995). The fourth instar larval stage is followed by a non-feeding, motile pupal stage that lasts approximately two days (Harwood and James 1979). Adult mosquitoes can fly within 10-15 minutes of eclosion and females are sexually receptive within two days (Nasci and Miller 1996). Female *Cx. quinquefasciatus* generally mate once (Craig 1967) and both male and female adults seek out nectar sources for a carbohydrate supply (Nayar and Sauerman 1975). Gonotrophic development requires a blood meal, with adult females utilizing a variety of hosts (Horsfall 1955, Irby and Apperson 1988). Within two to seven days of blood feeding, gonotrophic development is complete and females select a suitable site for oviposition (Nasci and Miller 1996).

This species is one of the most important disease-vectoring mosquitoes in the United States and the World. It has been implicated in the transmission of parasites that cause human filariasis (*Wuchereria bancrofti*) (Edeson and Wilson 1964, Harwood and James 1979), canine heartworm (Villavaso and Steelman 1970, Loftin et al. 1995) and avian malaria (Reeves et al. 1954). *Culex quinquefasciatus* is also involved in the transmission of several arboviruses (arthropod-borne viruses) including Japanese Encephalitis (Robin et al. 1963), St. Louis encephalitis (Wiseman et al. 1959), West Nile virus (Pavri and Singh 1965, Turell et al. 2005), and Western Equine encephalitis (Reeves 1965, Kettle 1995). Prior to 2002, St. Louis encephalitis has been the most predominate threat to human health in Texas, with numerous outbreaks occurring in Hidalgo (Beadle et al. 1957), Cameron (Brody and Browning 1960), Nueces (Williams et al. 1975), Tarrant and Dallas (Hopkins et al. 1975), and Harris (Baylor University et al. 1965, Kokernot et al. 1974) counties. In 2002, West Nile virus was isolated from mosquitoes for the first time in Texas (Lillibridge et al. 2004). It has since become endemic in the state, with multiple cases reported yearly.

Culex quinquefasciatus has been incriminated as the primary vector of St. Louis encephalitis and West Nile virus in Harris County, Texas (Baylor University et al. 1965, Lillibridge et al. 2004, Turell et al. 2005). Populations of the southern house mosquito can be found at varying densities throughout the year in Harris County, peaking in the summer months (Hayes 1975, Mr. Martin Reyna, personal communication). This peak coincides with an increase in bird activity, outdoor human activity, and the natural cycling of the various encephalitic viruses. During spring and early summer, the southern house mosquito moves opportunistically from underground breeding habitats (e.g., storm sewers, catch basins) to take advantage of ground pools (i.e., roadside ditches) created by seasonal rain and fouled with sewage or human refuse (Hayes 1975). As ground pools evaporate, mosquito populations retreat to underground refugia where breeding habitats persist through for the winter months and during extended periods of drought during the warmer months of a given year (Strickman and Lang 1986).

CHAPTER III

GENERAL MATERIALS AND METHODS

Mosquito Collection

Due to its status as the primary target for insecticidal control, *Cx. quinquefasciatus* was the mosquito selected for insecticide resistance assessment in this study. To accumulate the number of adult mosquitoes needed to complete the resistance testing effort and to ensure the age of insects was consistent, the egg was chosen as the most practical life stage to collect.

Egg rafts, each consisting of 100 or more eggs glued together, were collected using black plastic food service tubs (50 x 38 x 17 cm) filled with 0.5 L of a coastal Bermuda hay infusion (Fig. 1). Use of these tubs is based on the same principle exploited by the CDC gravid traps (Reiter 1983, 1987) used in disease surveillance programs in Harris and Brazos Counties. This collection method provides a suitable artificial site for gravid *Cx. quinquefasciatus* females seeking to oviposit on an appropriate media which is provided by the putrefying infusion. The tubs were left at selected collection sites over night (approximately 12 hours) and the egg rafts were collected the following morning by skimming the water surface with 47 mm filter paper discs (Fig. 2). Approximately 25 egg rafts were collected on filter paper from each black tub trap for a total of 200 egg rafts from each area and transported back to Harris County Mosquito Control Division or Texas A&M University in Petri dishes for hatching.

Fig. 1. Black tub oviposition trap.

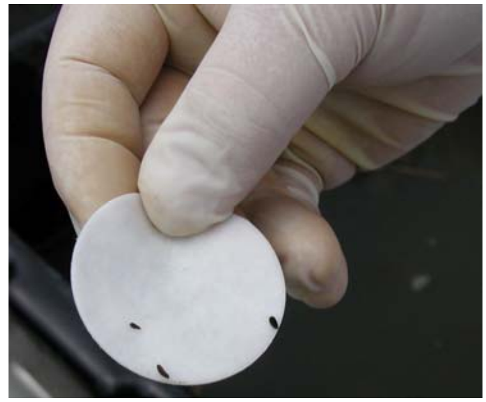

Fig. 2. *Culex quinquefasciatus* egg rafts collected on filter paper.

The oviposition media was prepared by submerging one slab (Fig. 3) of coastal Bermuda hay in 30 gallon trash cans (Rubbermaid® Incorporated, Fairlawn, Ohio) filled with tap water (Fig. 4). The trash cans were sealed and the hay was allowed to putrefy for two weeks before use. This mixture was chosen because its attractiveness for gravid *Culex* mosquitoes (Hazard et al. 1967, Murphey and Burbutis 1967) and in particular, *Cx. quinquefasciatus* (Reisen and Meyer 1990, Millar et al. 1992).

Fig. 3. A slab of coastal Bermuda hay.

Fig. 4. Thirty gallon trash can with coastal Bermuda hay infusion.

Collection Site Selection

Harris County is divided into 268 operational areas which aid in coordinating surveillance and spraying operations conducted by the Mosquito Control Division (Fig. 5). Each area is designated with a numerical value (1-940) that is used when referring to mosquito populations collected there. The 39 areas within the Interstate Highway 610 Loop are the oldest and have received the most insecticide treatments in the county (Baylor et al. 1965, Lauderdale 1969, Unpublished HCMCD data).

Brazos County was divided by this author into seven areas to facilitate collection of mosquitoes for resistance testing in a manner consistent with collection and designations in Harris County (Fig. 6). Areas 1-4 were composed mainly of Bryan and College Station, i.e. primarily urban habitats. Areas 5-7 were composed of rural habitats such as agricultural fields, ranches, and small rural communities.

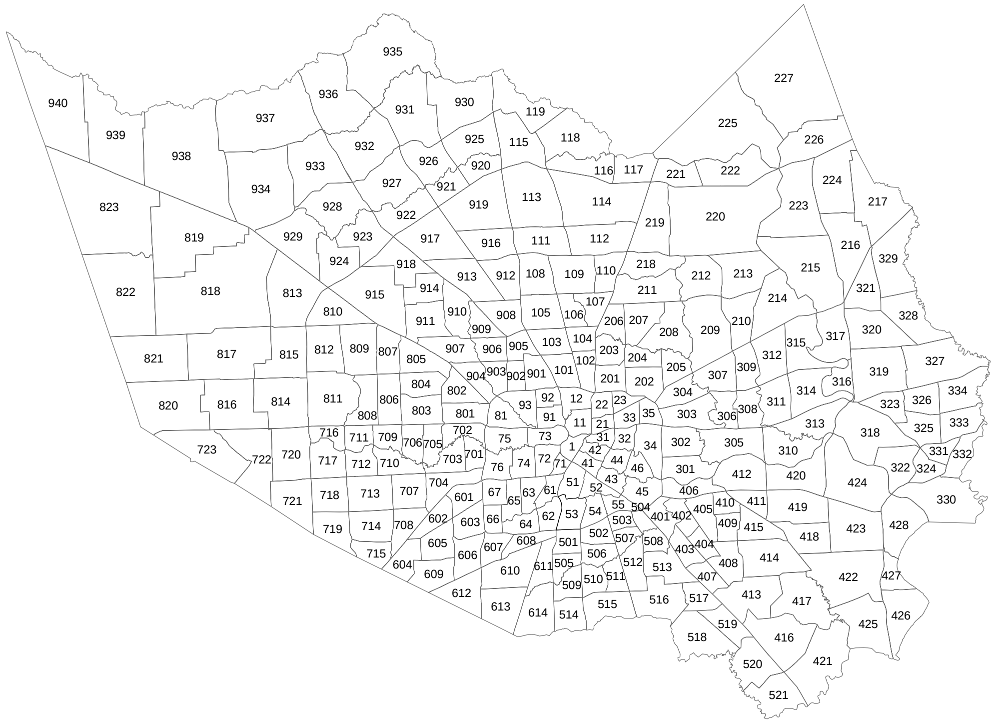

Fig. 5. Harris County Mosquito Control Division operational areas.

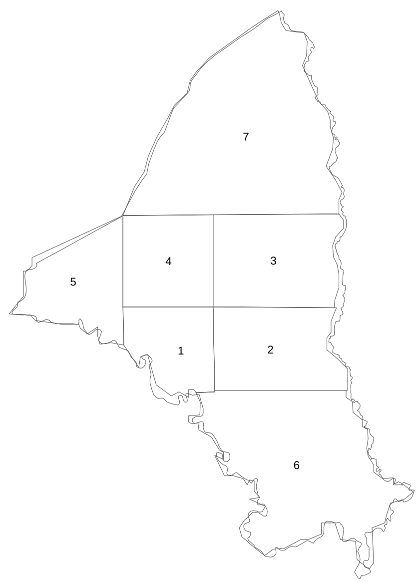

Fig. 6. Brazos County collection areas.

A minimum of four collection sites per area were established. Because some areas were larger, these areas required more collection sites, up to a maximum of six collection sites for any one area. The sites were chosen primarily on the basis of the amount of human traffic and the accessibility of the site which consisted of permission by the land owner and if it was physically possible to access the site. Two black tubs were set per collection site to maximize the number of egg rafts obtained.

Mosquito Rearing

Egg rafts collected in Brazos County were transferred to a walk-in incubator at the Mosquito Research Laboratory located on the West Campus of Texas A&M University College Station, TX (Fig. 7). Those collected in Harris County were taken to the incubators at Harris County Mosquito Control Division, Houston, Texas (Fig. 8). The incubators were maintained at 21°C, 80% relative humidity, and a photoperiod of 14:10 (L:D). Five egg rafts were placed into each aluminum larval rearing pan (32.5 x 23 x 6 cm) containing 1500 ml of purified water. A slurry of ground tropical fish food (Tetramin®, Tetrawerke, Melle, Germany) suspended in reverse-osmosis purified water was deposited on the bottom of each larval rearing pans, and then, added as needed throughout the larval growth period. The larvae were maintained in rearing pans until the majority molted to fourth instar larvae or pupae. The larvae and pupae were strained from the rearing pans using a number 80 sieve (USA Standard Sieve Series) and placed in plastic emergence cups (14 x 14 x 6 cm). The contents of two larval pans were pooled into each emergence cup and placed inside a 30.5 cm^3 adult mosquito cage for emergence. A cotton wick soaked in a 5% sucrose solution placed in the cage provided a carbohydrate source. The adult mosquitoes were kept in the incubators for one week to ten days post emergence for utilization in insecticide resistance tests.

Fig. 7. Texas A&M University Mosquito Research Laboratory walk-in incubator.

Fig. 8. Harris County Mosquito Control Division rearing room.

Bioassay Procedure

Mosquito samples were tested using a modified vial bioassay based on the methods described by Plapp (1971) for insecticide resistance testing in *Heliothis* larvae. To completely test a population of adult mosquitoes for the six adulticides (resmethrin, permethrin, sumithrin, malathion, naled, pyrethrum), three pallets consisting of 216 scintillation vials (36 per insecticide) was needed. Each insecticide test was comprised of a control and six doses of pesticide (in µg), with six replications per dose (Fig. 9). A single insecticide test required 210 female mosquitoes (30 per concentration) and 1260 females were needed to complete the total series (Fig 10).

Technical grade insecticides were used to make a 1:1 (20 mg insecticide: 20 ml acetone) stock solution in a 40 ml series 300 VOA closed cap vial (I-Chem). The stock solution was serially-diluted with acetone to develop a set of stock dilutions for testing.

The appropriate quantity of insecticide was added to scintillation vials in multiples of 0.1, 0.3, and 0.6 ml per concentration using a 1 ml pipette. A 0.2 ml aliquot of acetone was added to the control vials to ensure this reagent did not contribute to the observed mortality. A 0.2 ml aliquot of acetone was added to the vials containing 0.1 ml of pesticide to ensure the vial was completely coated. The insecticide coated vials were then placed on their side on a running hotdog roller (with the heating element disabled) and left to dry (Fig. 11). Square, 5 x 5 mm pieces of blot paper were soaked in a Petri dish containing a 5% sucrose solution and one piece was introduced into each vial to serve as a source of carbohydrate for the mosquito while they were in the vials.

Fig. 9. Insecticide-coated vials for bioassay test.

Fig. 10. Completed bioassay test.

Fig. 11. Hot dog roller used to uniformly coat the scintillation vials with insecticide dilutions.

Samples of F_0 mosquitoes were removed from the adult cages maintained in the walk-in incubators using a battery-powered, hand-held aspirator (Hausshер Machine Works, Toms River, New Jersey). These mosquitoes were anesthetized with a gentle stream of CO_2 and scattered onto a 50°F chill table. At this time, the mosquitoes were sorted by sex and the species identification checked to ensure they were *Cx. quinquefasciatus*. Five female mosquitoes were transferred into each treated vial and a cotton ball used to plug the opening (Fig. 12). After 24 hours the mosquito mortality per vial was recorded. A mosquito was considered dead if it could not walk.

Fig. 12. Insecticide coated vials with five female mosquitoes and a 5x5 mm piece of filter paper soaked in 5% sucrose.

To determine the level of resistance in the field collected *Cx. quinquefasciatus* populations, a susceptible laboratory strain of *Cx. quinquefasciatus* is needed for comparison. The susceptible strain chosen for this study was the Sebring strain that was originally colonized by USDA-ARS in Gainesville, Florida, and has been the susceptible strain used in the insecticide resistance monitoring program offered by the Texas A&M University (TAMU) Mosquito Research Laboratory since 1999. The *Cx. quinquefasciatus* Sebring strain is also the current susceptible laboratory strain used by the Centers of Disease Control and Prevention Fort Collins Infectious Disease Laboratory for their insecticide resistance testing (Dr. Jimmy K. Olson, personal communication). This colony is currently housed in incubators at TAMU and the Harris County Mosquito Control Division (HCMCD) and maintained in the same conditions (21°C, 80% RH, and 14:10 photoperiod) as the feral mosquito populations.

The *Cx. quinquefasciatus* Sebring strain was tested using the modified vial bioassay test (Plapp 1971) to develop a susceptibility baseline to the six chemicals (resmethrin, permethrin, sumithrin, malathion, naled, and pyrethrum) selected for this study. The results produced from these tests were analyzed using the Probit procedure of SAS (SAS Institute 2002) with Abbott's C correcting for mortality in the controls (Abbott 1925) to determine the concentration of insecticide necessary to kill a portion of the population. The most commonly-used concentrations for comparison between mosquito populations are lethal concentrations (LC) 50, 95, and 99 (Busvine 1971), which refer to the percentages of the population that are killed at each given concentration. The results of the field collect mosquito bioassays are analyzed in the same method described for the Sebring strain with the lethal concentrations calculated by the Probit procedure of SAS (SAS Institute 2002).

The lethal concentrations of the *Cx. quinquefasciatus* Sebring strain were used to calculate resistance ratios (RR) which demonstrate how much more resistant or susceptible field-collected *Cx. quinquefasciatus* populations are relative to the susceptible Sebring laboratory strain. The resistance ratios are calculated for any concentration by dividing the lethal concentration of the feral mosquito population by the lethal concentration of the Sebring laboratory strain and is represented as: $RR_x = LC_x$ feral mosquitoes/LC_x laboratory mosquitoes, where $_x$ = LC in question (e.g. LC_{50}).

CHAPTER IV

THE RESISTANCE STATUS OF *Culex quinquefasciatus* SAY TO SIX COMMONLY USED ADULTICIDES IN BRAZOS AND HARRIS COUNTIES, TEXAS

In 2002, West Nile virus (WNv) was isolated for the first time in Harris County, Texas (Lillibridge et al. 2004). The subsequent epidemic led the Harris County Public Health and Environmental Services Mosquito Control Division (HCPHES-MCD) to intensify its existing spraying operation in an attempt to suppress infected adult mosquito populations. The control program targeted the primary disease vectoring mosquito for the southern United States, the southern house mosquito, *Culex quinquefasciatus* Say (Baylor University et al. 1965, Sardelis et al. 2001, Goddard et al. 2002, Godsey et al. 2005, Turell et al. 2005). The increase in insecticide use for disease abatement was commensurate with the WNv outbreak caused a renewed interest in the insecticide resistance status of *Cx. quinquefasciatus* populations in Harris County. The current research project was conducted to identify the insecticide resistance status in *Cx. quinquefasciatus* and to provide baseline data to Harris County Mosquito Control (HCMCD). This data may then be used to make better choices regarding insecticides to effectively control disease-vectoring *Cx. quinquefasciatus* populations in various areas of the county.

In contrast to the HCMCD, Brazos County has no organized mosquito control program. Following the outbreak of West Nile virus in 2002, the Brazos County Health

Department instituted a program based on personal protection, human behavior modification, and source reduction. In 2003, Brazos County initiated a strategy devised by Dr. Jimmy Olson and the local city health departments of using resmethrin to thermal fog adult mosquito resting sites under bridges, in culverts, and within storm sewer systems in close proximity to positive West Nile virus cases (mosquito, bird, and human) (Dr. Jimmy K. Olson, personal communication). This is the only active mosquito control conducted that puts chemical selection pressure on the mosquito populations in Brazos County.

The current investigation was initiated when an insecticide efficacy check was conducted against *Cx. quinquefasciatus* adults in Harris County in the form of a field-cage spray test in early 2004 garnered negative results for three synthetic pyrethroids commonly used against adult mosquitoes in the United States. At that time, the lowest labeled rate of Scourge® (.003 lb ai/A) was ineffective against a population of mosquitoes collected from operational area 51 in Harris County (Fig. 5). This alerted the HCMCD and TAMU researchers that there was a possible insecticide resistance problem developing in mosquito populations in Harris County, and an intensified insecticide resistance program was initiated by this investigator to determine the level and extent of insecticide resistance that was present in the Harris County *Cx. quinquefasciatus* populations. For comparison, resistance testing was also conducted in Brazos County to determine the resistance ratios for mosquito populations that were not under pressure from chemical control tactics. This testing also provided baseline insecticide susceptibility data, if future mosquito control is conducted in Brazos County.

The vial assays performed by this investigator in Harris County became incorporated into an expanded insecticide resistance monitoring program initiated by the Harris County Mosquito Control Division's Test and Evaluation Section in response to the insecticide resistance problem they had detected in their *Cx. quinquefasciatus* populations in 2004. The resistance monitoring program in Harris County consisted of weekly (2004) or bi-weekly (2005) field cage tests conducted in conjunction with laboratory bioassays (vial, bottle, and topical) to determine the resistance status of the mosquito population in various operational areas in Harris County. The insecticide resistance status of mosquito populations tested in Harris and Brazos Counties by this investigator using the scintillation vial bioassay method to the six chemicals (malathion, naled, resmethrin, permethrin, sumithrin, and pyrethrum) most frequently used in adult mosquito control programs in the United States are presented in this chapter.

Materials and Methods

Scintillation vial bioassay tests were conducted over two year spans (2004-2005 for Harris County and 2005-2006 for Brazos County), with the results of the tests being analyzed using the Probit procedure in SAS (SAS Institute 2002) to determine the lethal concentration 50 (LC_{50}) for the six chemicals tested as described in Chapter III. Resistance ratios were calculated by dividing the LC_{50} of the feral mosquito population by the LC_{50} of the Sebring laboratory strain for each chemical tested.

The resistance ratios were compared using the RR_{50} values since the response to insecticides at this level (LC_{50}, RR_{50}) by any given insect population are less variable between different tests of the same population over time than is the case for the response at the RR_{95} (LC_{95}) level (Likitvong 1996). The LC_{95} and RR_{95} are determined in any given test so that the slopes of the probit dosage/mortality curves can be determined. This provides insight to the degree of homogeneity or heterogeneity that exists between individuals comprising insect populations as to their respective responses to different dosages of an insecticidal agent being tested against them (Likitvong 1996). A resistance ratio threshold of 10 has been established through laboratory work at Texas A&M University (Likitvong 1996, Sames et al. 1996, Sukontason 1998) as the point when a given feral mosquito population is determined to have developed a degree of resistance to a given chemical tested and operational control begins to fail. Any resistance ratio below 10 is considered to be in an acceptable range. However, if a population has a resistance ratio greater than 8, the population is considered in the process of developing resistance and is at risk of an operational failure occurring and additional monitoring is necessary to track the future resistance ratio levels. Monitoring is usually conducted on an annual basis; but, shorter time periods have been used by counties serviced by, the Texas A&M University Mosquito Research Laboratory insecticide resistance screening program, with the number of tests performed each year being contingent on the extent of the labor force and other resources made available to the screening program.

Results

Vial bioassay tests were performed on the *Cx. quinquefasciatus* Sebring strain with the results used to develop a susceptibility baseline (Table 1) to the six chemicals (resmethrin, permethrin, sumithrin, malathion, naled, and pyrethrum) that were tested during this study. The lethal concentration 50s (LC_{50}) were identified (Table 1) and used to calculate the resistance ratio 50s (RR_{50}) of the field collected *Cx. quinquefasciatus* as described in Chapter III.

Mosquito populations from twenty-six areas were tested during 2004 (Fig. 13) and forty-five areas during 2005 (Fig. 14) in Harris County. For operational purposes the highest priority was placed on resmethrin in 2004 because it was the only insecticide used for ground based chemical treatments. Due to lack of mosquitoes and availability of chemicals during certain points of the testing period certain chemicals were unable to be tested. In 2005, this situation was corrected and all areas were tested with all six chemicals. Mosquito populations from all seven collection areas in Brazos County (Fig 6.) were tested for resistance against all six insecticides in both test years (2005-2006).

Of the six chemicals tested on mosquito populations in Harris County over the two year study, only resmethrin, sumithrin, and permethrin produced resistance ratios that exceeded the threshold of 10 signifying the mosquitoes exposed to these particular chemicals to be resistant.

In 2004, the vial bioassays for resmethrin detected resistance to this chemical in mosquito populations from operational areas 33 (RR_{50} = 16.34), 43 (RR_{50} = 11.01), and 51 (RR_{50} = 10.73) (Fig. 15).

Table 1. Lethal concentration 50 (LC_{50}) and 95 (LC_{95}) for the *Culex quinquefasciatus* Sebring laboratory strain.

Chemical	**LC_{50} (95% C.I.)**	**LC_{95} (95%C.I.)**	**Slope (S.E.)**
Malathion	0.84 (0.72-1.02)	1.81 (1.38-3.20)	4.99 (± 0.98)
Naled	2.90 (N.A.)	3.43 (N.A.)	22.30 (N.A.)
Resmethrin	0.76 (0.56-0.94)	2.38 (1.68-5.29)	3.32 (± 0.73)
Permethrin	0.72 (0.51-0.91)	2.55 (1.77-5.72)	3.01 (± 0.65)
Sumithrin	0.70 (0.60-0.82)	1.38 (1.10-2.30)	5.62 (±1.21)
Pyrethrum	1.33 (1.08-1.64)	3.45 (2.58-5.63)	3.97 (± 0.60)

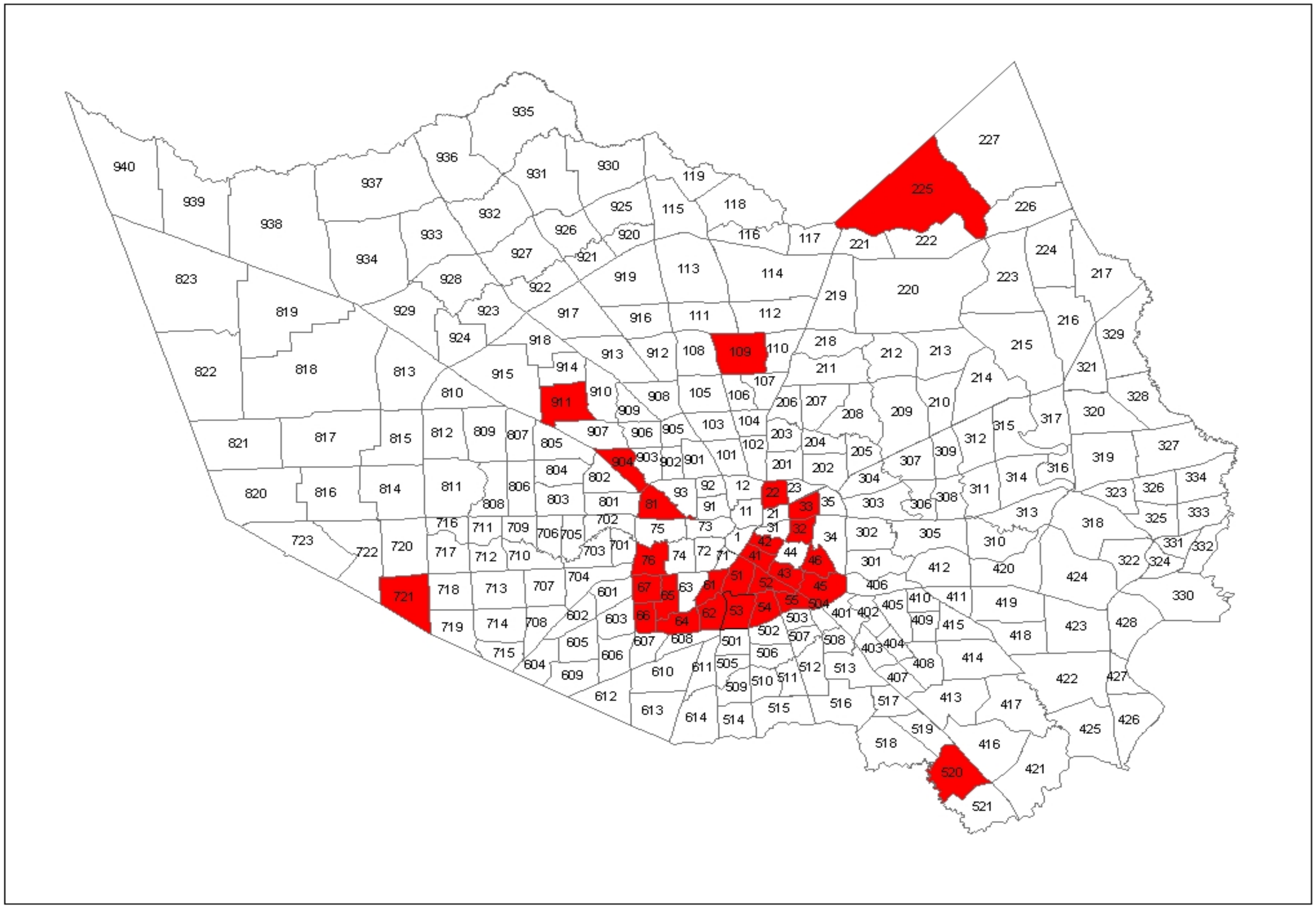

Fig. 13. Operational areas in Harris County, Texas, where resistance testing was conducted in 2004.

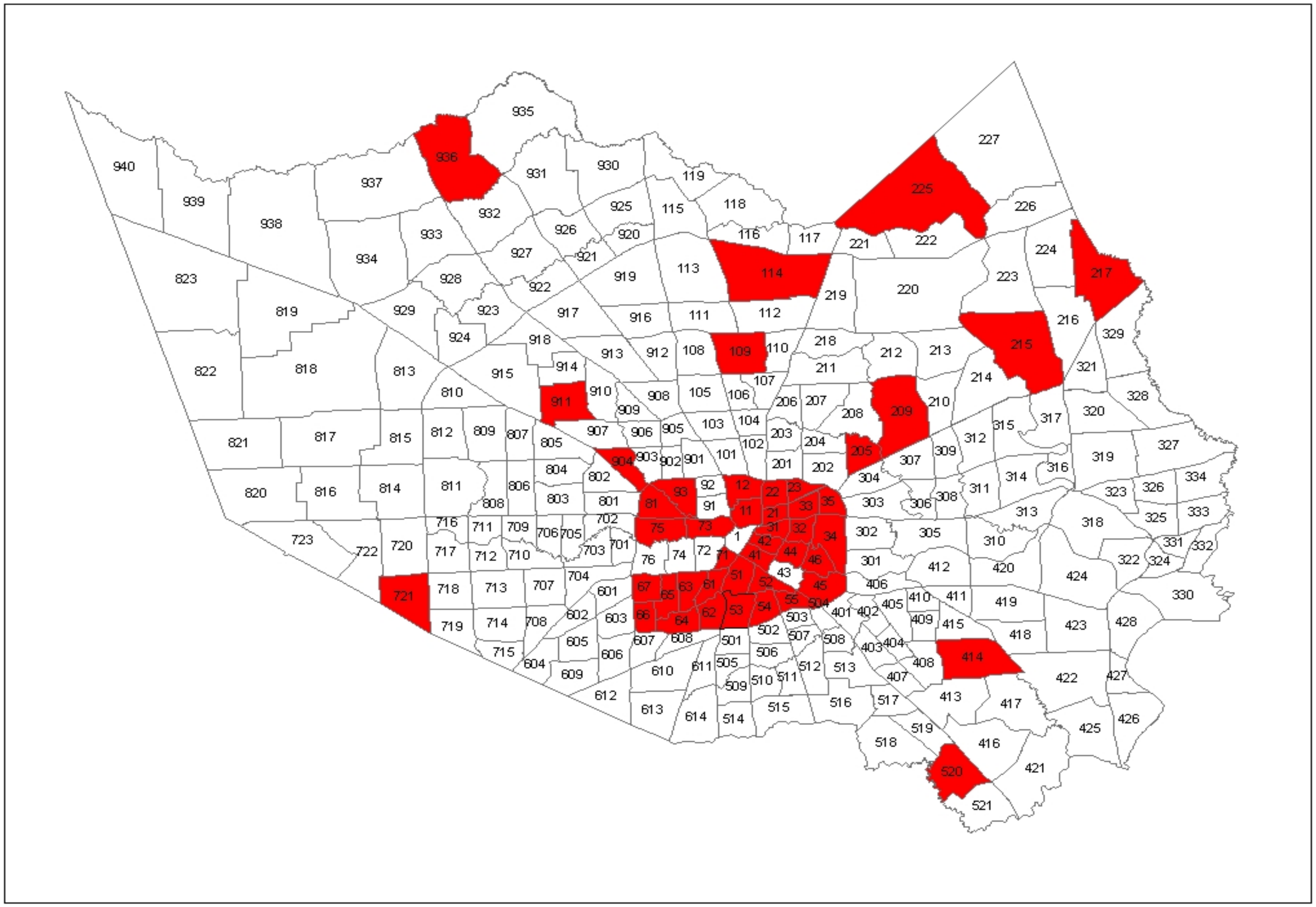

Fig. 14. Operational areas in Harris County, Texas, where resistance testing was conducted in 2005.

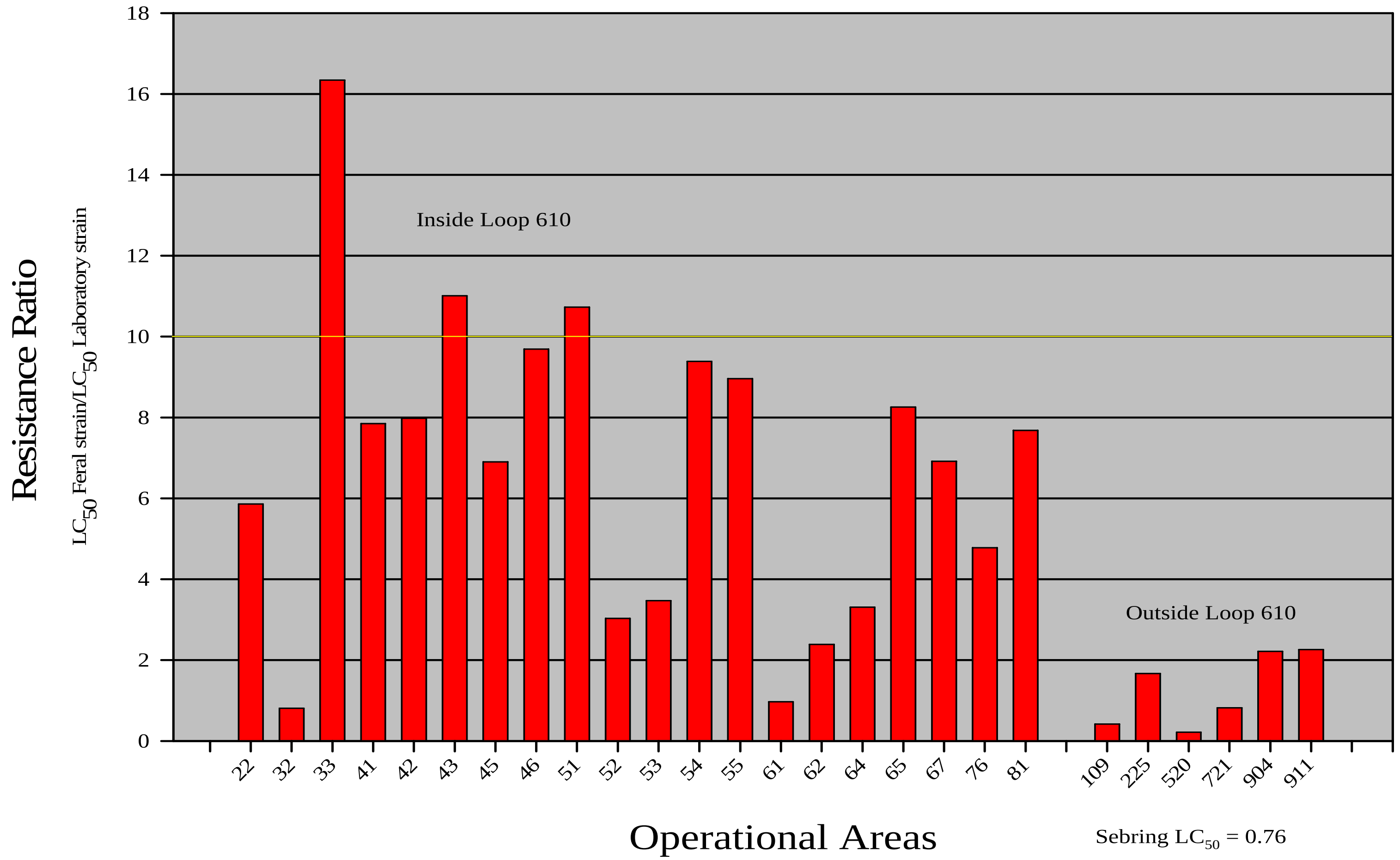

Fig. 15. Resmethrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2004.

Populations from area 33 demonstrated the highest resistance ratio detected in Harris County during the two year study for any mosquito population or chemical tested. Operational areas 46 (RR_{50} = 9.69), 54 (RR_{50} = 9.39), 55 (RR_{50} = 8.96), and 65 (RR50 = 8.26) had mosquito populations with resistance ratios that exceeded 8 for resmethrin (Fig. 15). These areas were considered to be developing resistance to resmethrin and monitoring was conducted in 2005 to determine the change in resistance status based on an IRM program that was initiated in this area by the Harris County Mosquito Control Division in 2005. Resmethrin resistance ratios for mosquitoes in the majority of areas where at risk populations were detected in 2004 dropped to acceptable levels when the populations were tested in 2005 (Area 55 RR_{50} = 4.14), except for mosquito populations collected from area 46 (RR_{50} = 9.00) and 65 (RR_{50} = 11.63) (Fig. 16-17). The resmethrin resistance ratios for the population from area 46 did decrease from the previous year; but it was still over the resistance ratio of eight which means the population still has the possible of developing resistance to resmethrin. Area 65 mosquito populations increased in resmethrin resistance and crossed the resistance ratio threshold of 10 which alerted the control personal to begin resistance management procedures in that particular operational area.

Also, in 2005, additional operational areas were included in the study and resistance to resmethrin was detected in mosquito populations from areas 21 (RR_{50} = 12.31), 22 (RR_{50} = 15.76), 23 (RR_{50} = 13.25), 33 (RR_{50} = 10.77), 61 (RR_{50} = 14.01), 65 (RR_{50} = 11.63), and 66 (RR_{50} = 10.44) (Fig 16).

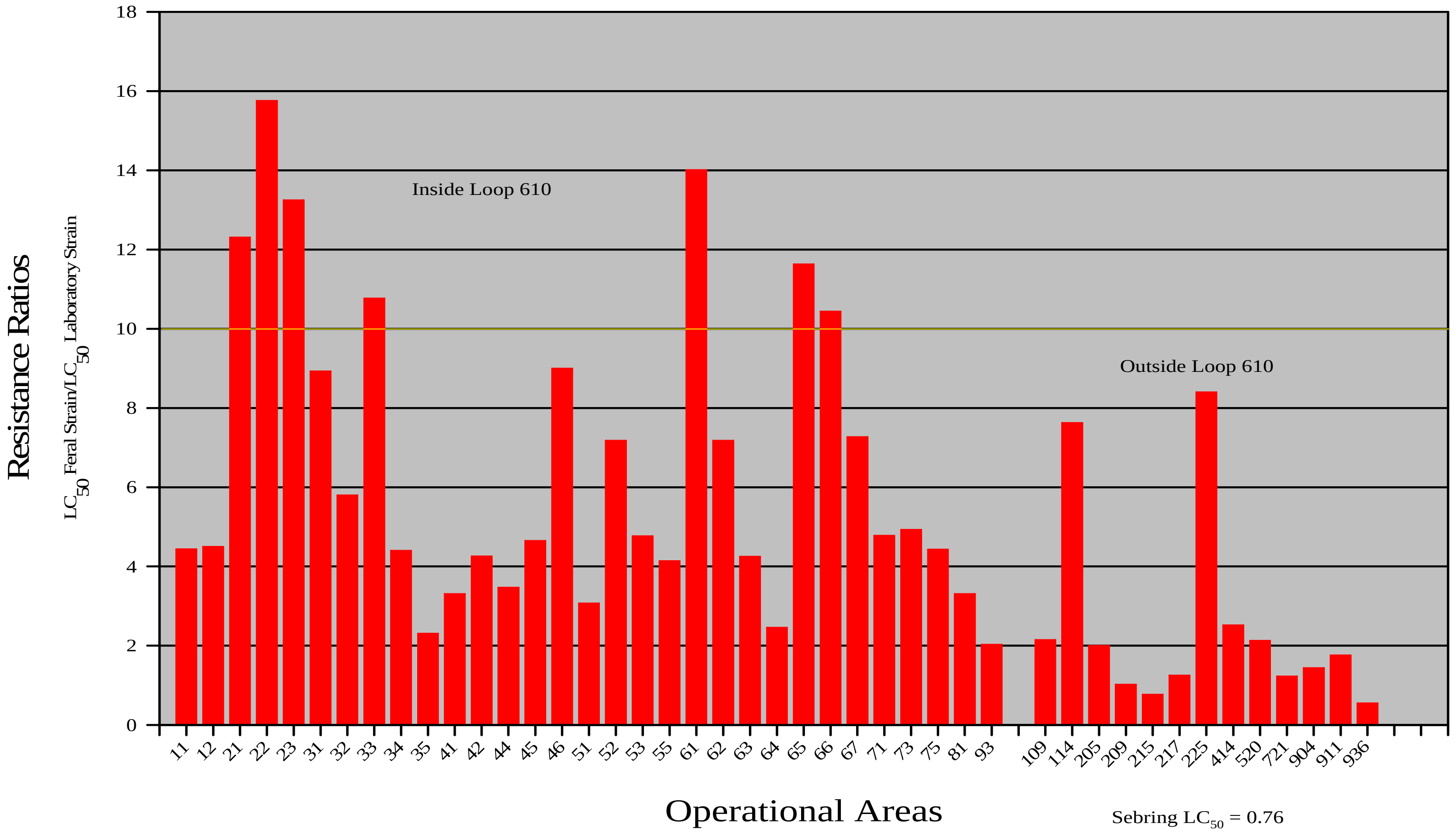

Fig. 16. Resmethrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2005.

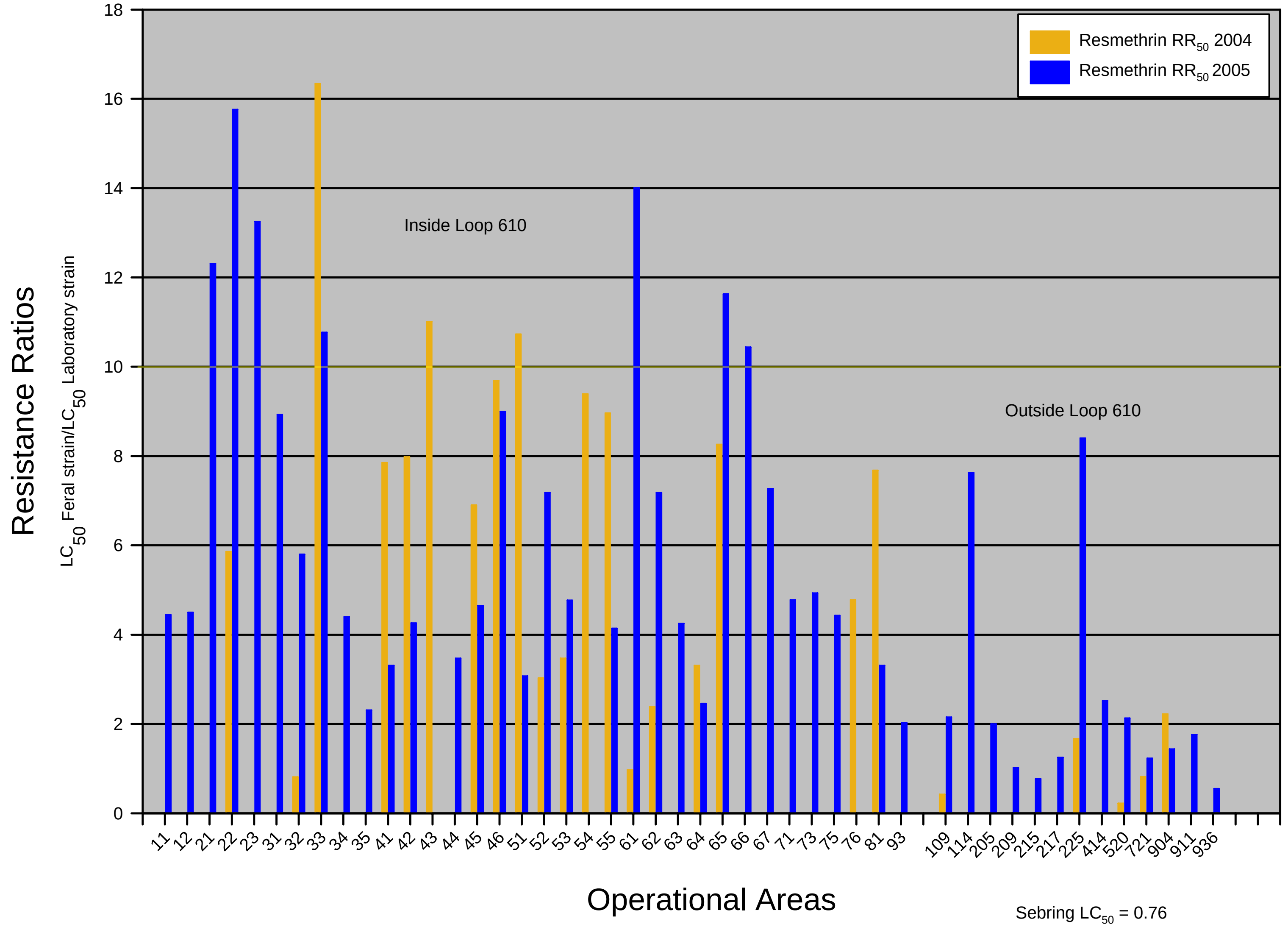

Fig. 17. A comparison of resmethrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas between 2004 and 2005.

Populations from area 33 again surpassed the threshold for resistance, but decreased considerably from the previous year. All the areas with populations that demonstrated resistance or are designated "at risk" were located inside Interstate Highway Loop 610 (Fig. 5) except for mosquitoes collected from area 225 which encompasses the Houston suburb of Kingwood. The resmethrin resistance ratio for this mosquito population increased considerably from 2004 (RR_{50} = 1.67) to 2005 (RR_{50} = 8.40) and is the only area outside the Interstate Highway Loop 610 to demonstrate the development of high resistance ratios to the chemical tested (Fig. 17).

Brazos County mosquito populations did not show any evidence of resistance to resmethrin over the two years that this study was conducted (Figs. 18-19). The highest resmethrin resistance ratios that was recorded for Brazos County mosquitoes over the two year period were from these collected from area 2 in 2004 (RR_{50} = 1.82) and 2005 (RR_{50} = 2.23) with the collection sites in this area being located in the City of College Station.

Mosquitoes from Harris County tested for resistance to permethrin had only a single population that had a resistance ratio that exceeded the resistance threshold over the two years this project was conducted (Figs. 20-21). The permethrin resistant population from area 51 (RR_{50} = 10.73) demonstrated cross resistance to resmethrin and sumithrin in 2004 (Table 2). Areas 46 (RR_{50} = 9.69) and 55 (RR_{50} = 8.96) had populations with resistance ratios higher than 8 which denoted development of resistance in the populations to permethrin (Fig. 20).

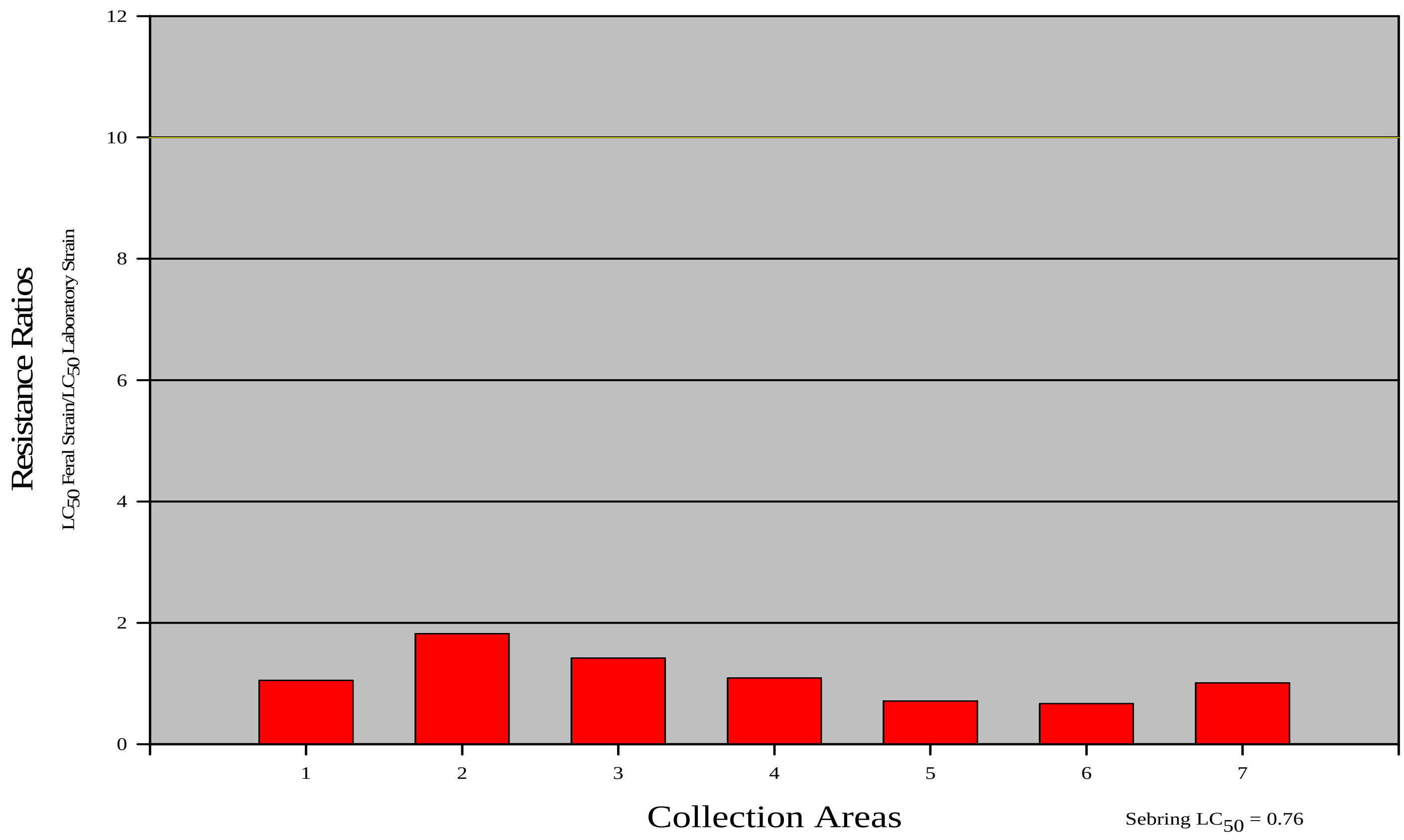

Fig. 18. Resmethrin resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2005.

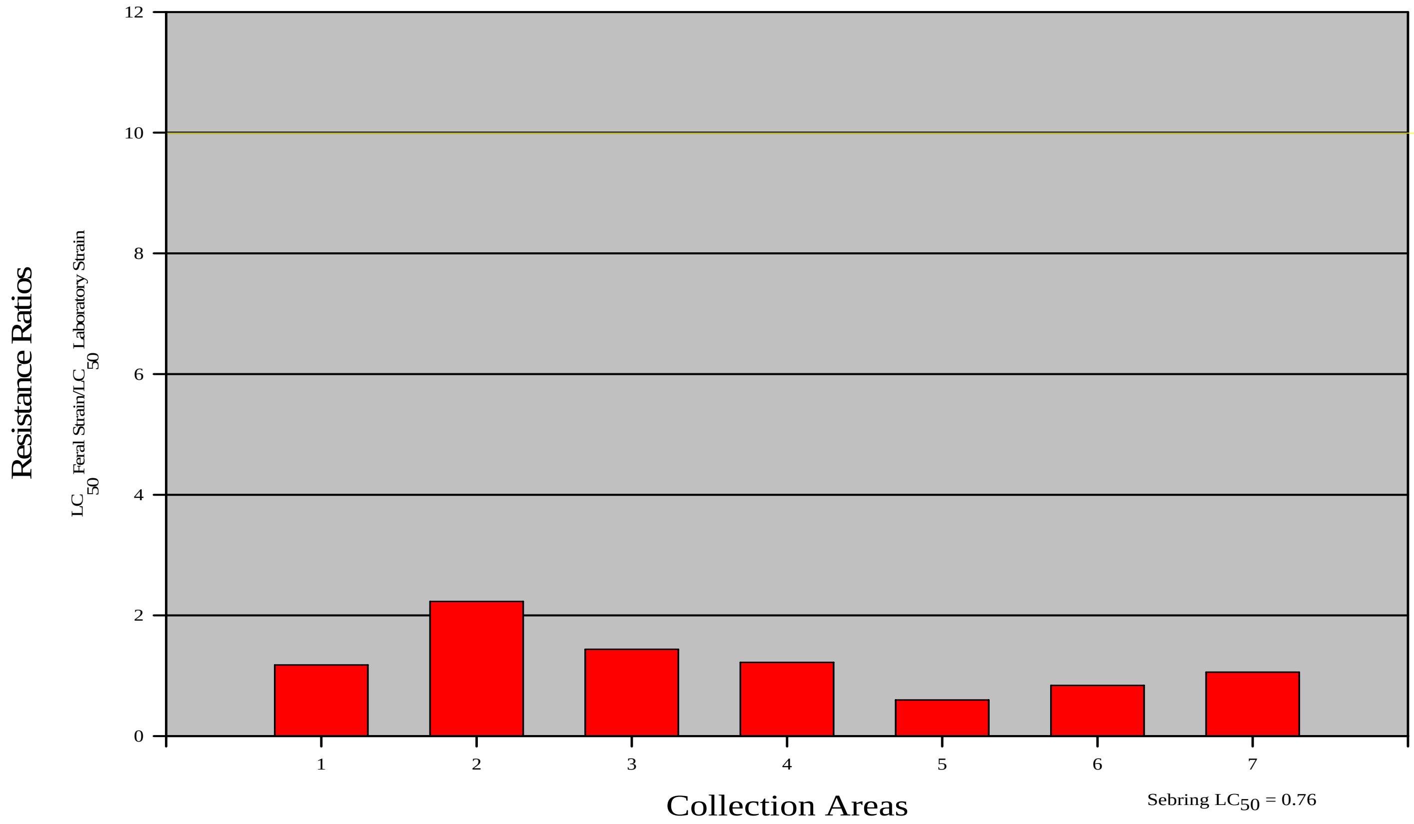

Fig. 19. Resmethrin resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2006.

Table 2. Examples of cross resistance in the *Culex quinquefasciatus* populations to the three synthetic pyrethroids tested in Harris County, Texas.

	Resmethrin (RR_{50})	Permethrin (RR_{50})	Sumithrin (RR_{50})
2004			
Area 46	9.69	9.87	3.40
Area 51	10.73	11.06	9.11
Area 54	9.39	7.88	8.70
Area 55	8.96	9.62	11.68
2005			
Area 21	12.31	8.04	8.57
Area 225	8.40	2.30	8.47

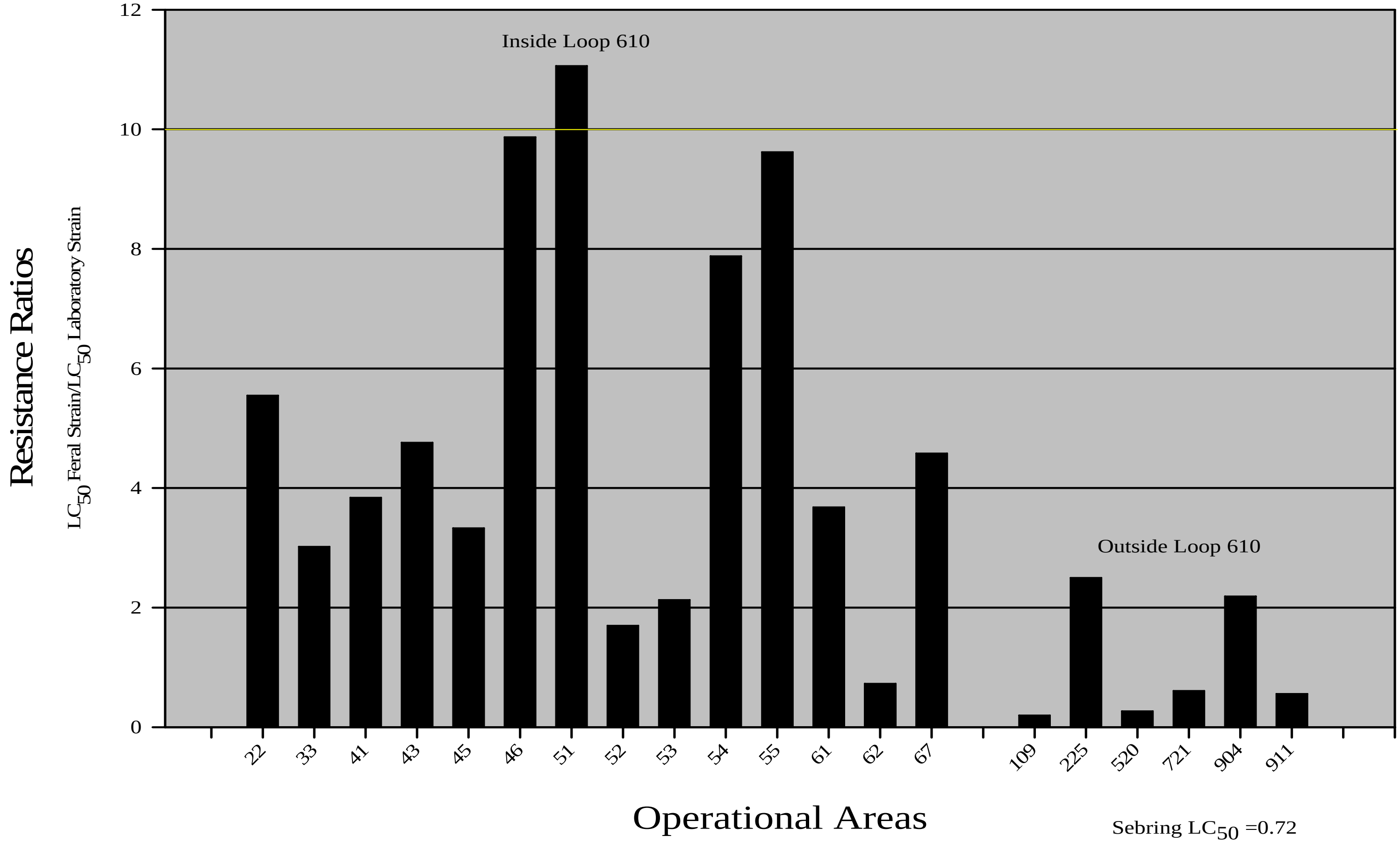

Fig. 20. Permethrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2004.

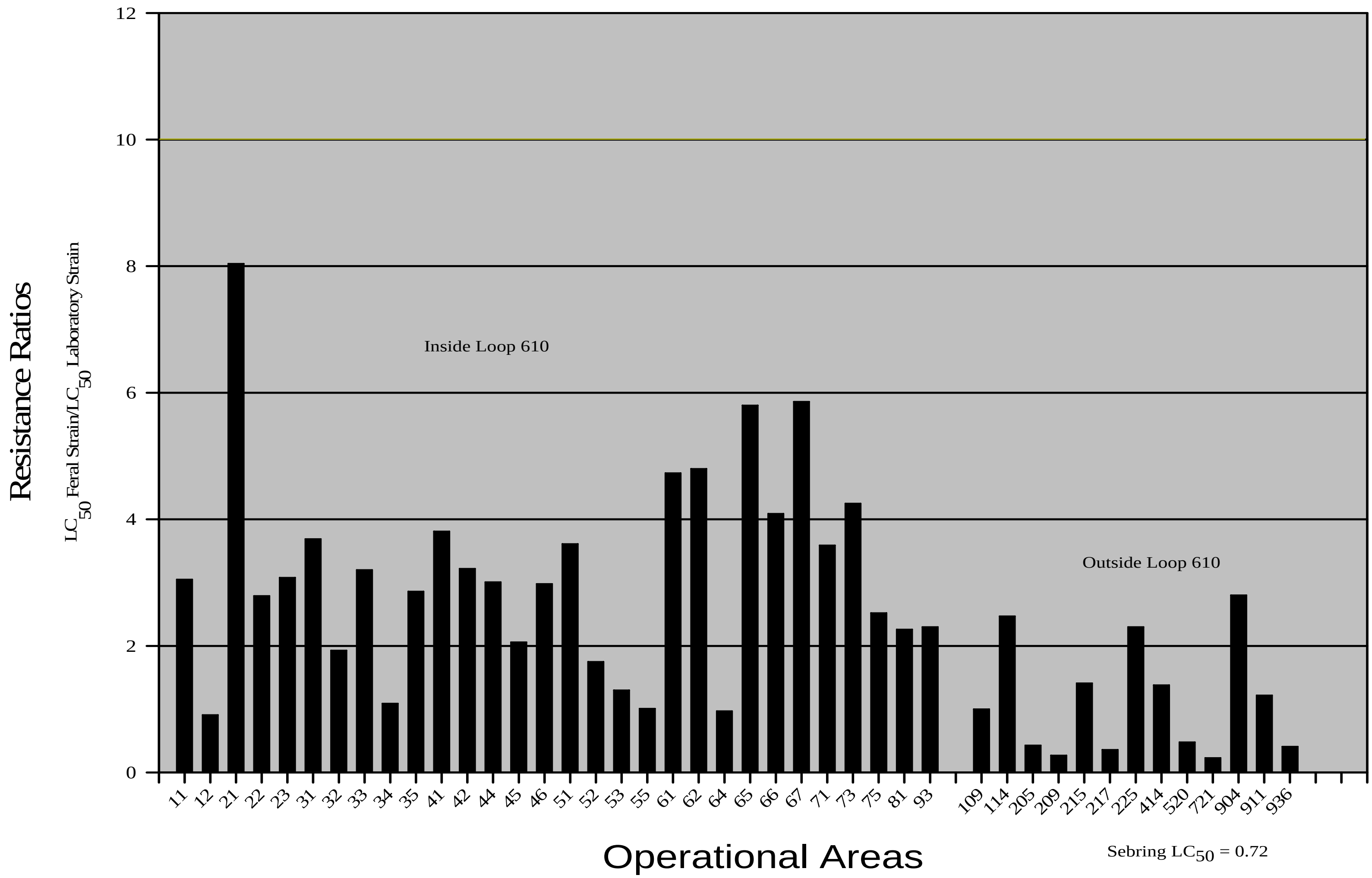

Fig. 21. Permethrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2005.

The following year the populations that demonstrated resistance characteristics in 2004 had their resistance ratios return to acceptable levels in areas 46 (RR_{50} = 2.98), 51 (RR_{50} = 3.61), and 55 (RR_{50} = 1.01). No resistance was detected in the populations test in 2005 against permethrin and only a single population from area 21 (RR_{50} = 8.04) had a resistance ratio that reached 8 (Fig. 21).

The bioassay results for the Brazos County mosquito populations tested against permethrin were similar in scope as those recorded for resmethrin. Populations from all seven collection areas exhibited susceptibility to permethrin over the two years resistance monitoring was conducted (Figs 22-23). The highest resistance ratios recorded during the study was for mosquitoes collected in area 2 in 2004 (RR_{50} = 1.50) and 2005 (RR_{50} = 1.47) with these mosquitoes again coming from collection sites located in the City of College Station.

Resistance to sumithrin, the third pyrethroid tested, was detected in a single mosquito population from Harris County operational area 55 (RR_{50} = 11.68) in 2004 (Fig. 23). The population from this area illustrated the cross resistance phenomenon, by demonstrating high resistance ratios to the other two synthetic pyrethroids tested on this population (Table 2). Mosquito populations from area 51 (RR_{50} = 9.11), 53 (RR_{50} =8.62), 54 (RR_{50} = 8.70), and 67 (RR_{50} = 8.70) all had resistance ratios for sumithrin over 8, which marked them as mosquitoes on the brink of developing resistance ratios high enough to start affecting control options. In 2005, mosquitoes in four areas (51 RR_{50} = 7.04, 53 RR_{50} = 1.37, 55 RR_{50} = 4.65, and 67 RR_{50} = 6.14) that had high resistance ratios to sumithrin the pervious year returned to satisfactory levels (Fig. 24).

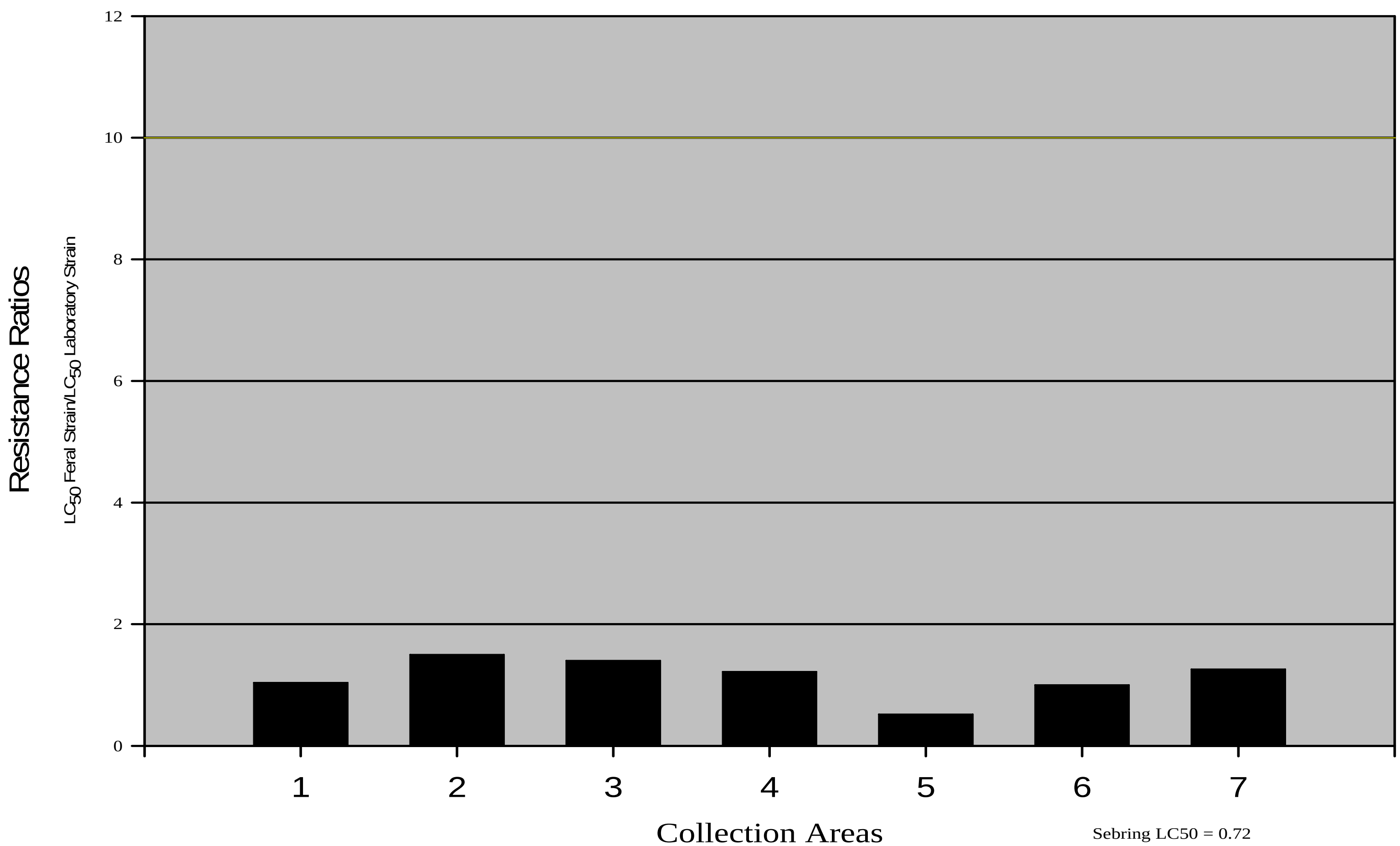

Fig. 22. Permethrin resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2005.

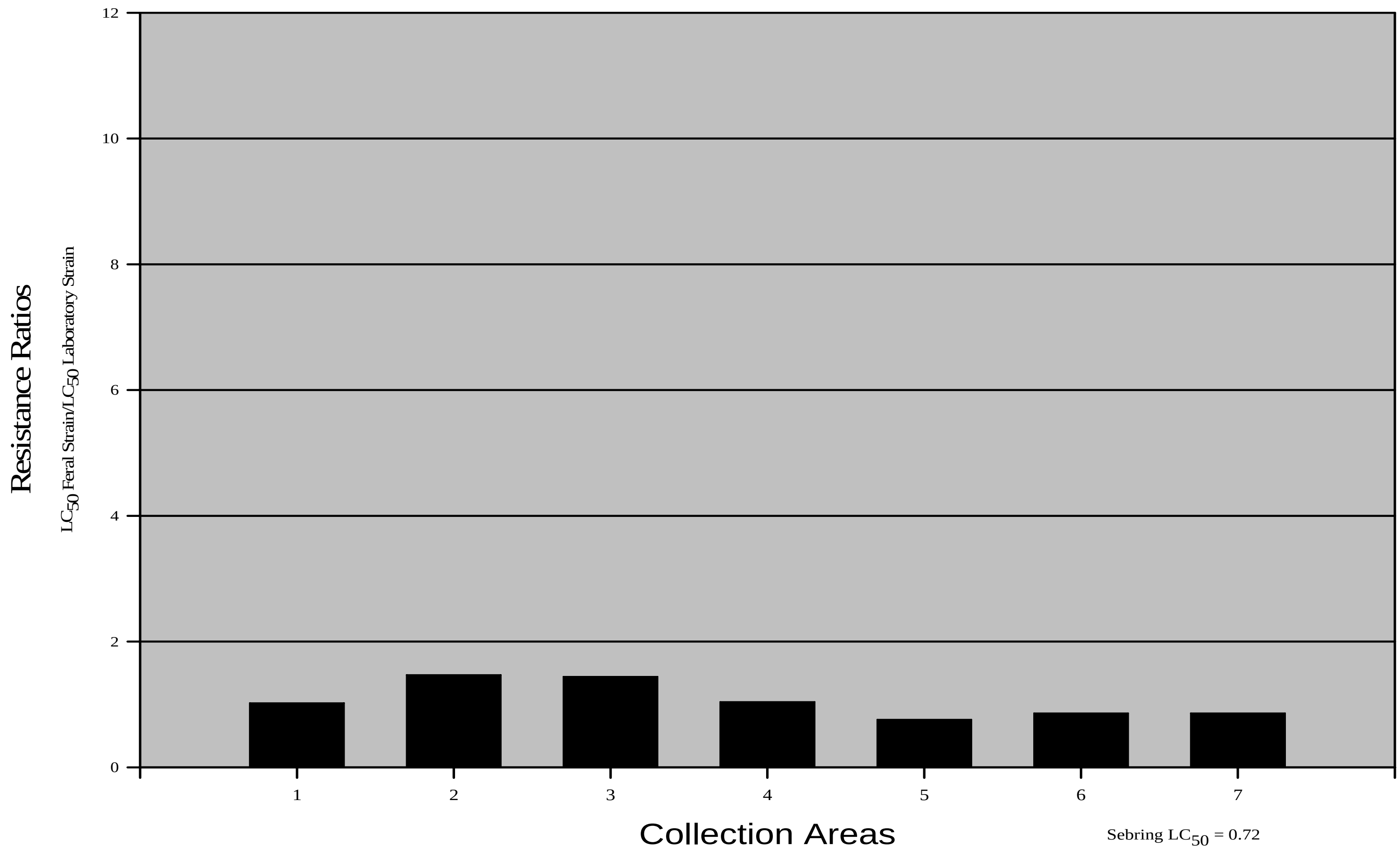

Fig. 23. Permethrin resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2006.

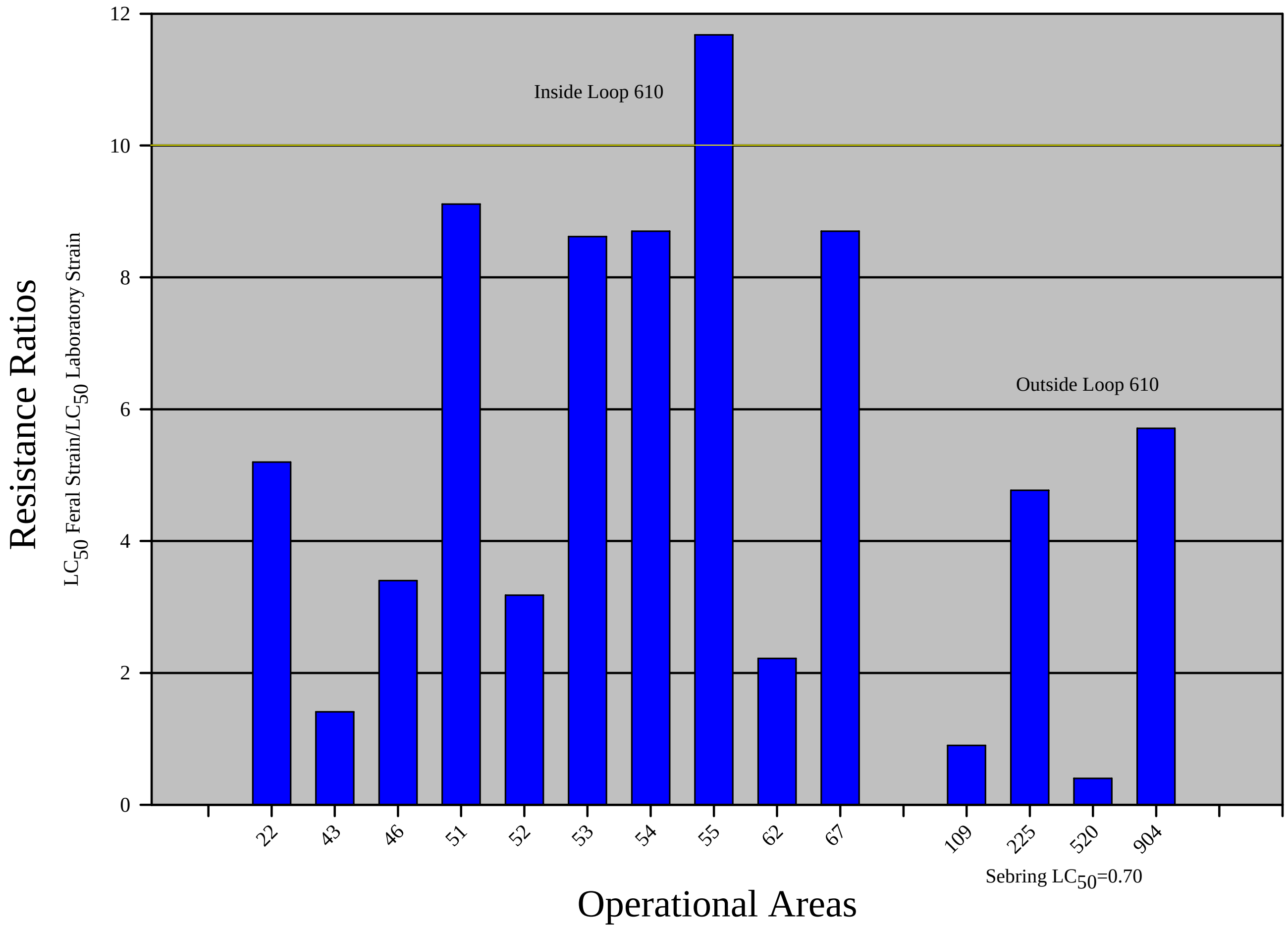

Fig. 24. Sumithrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2004.

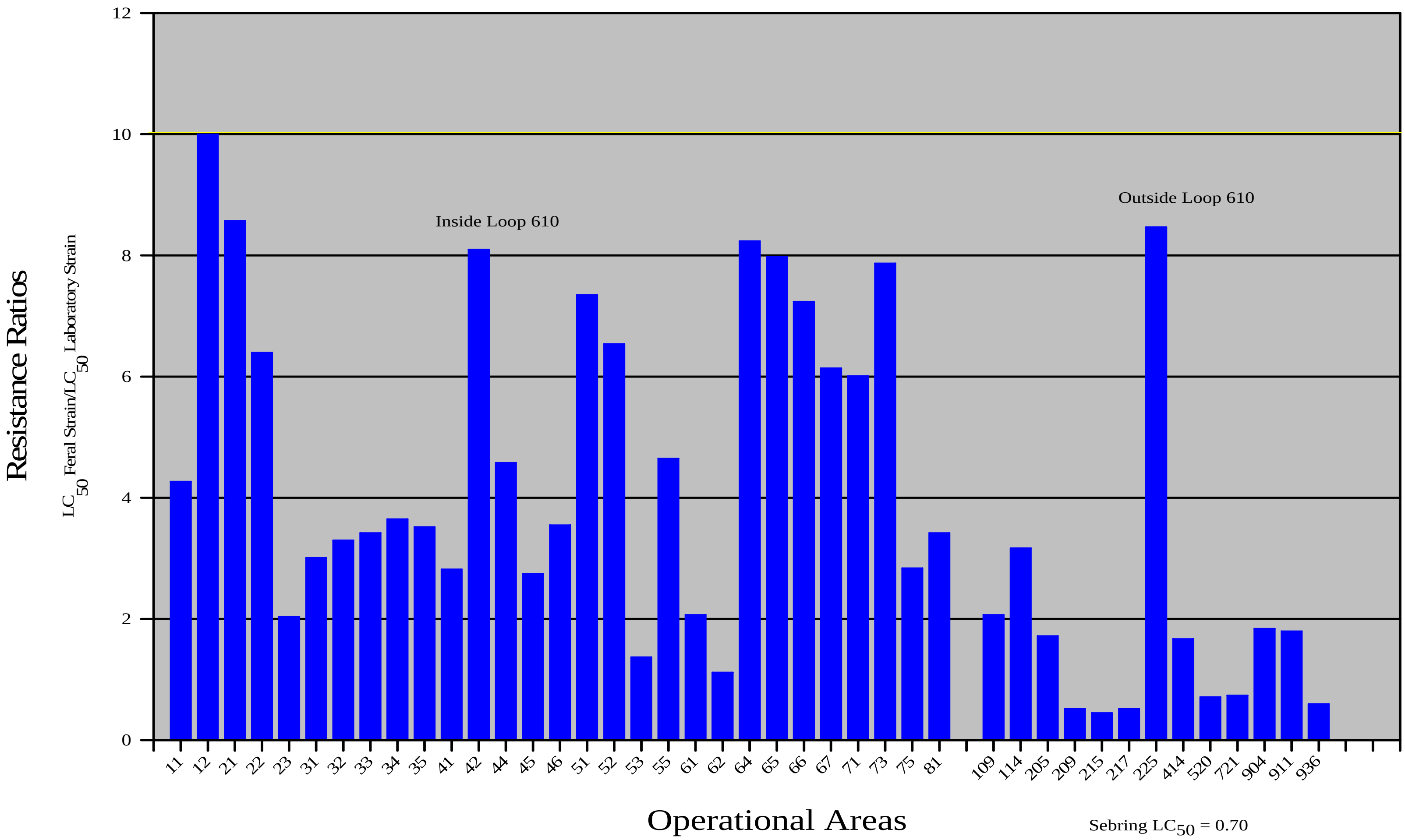

Fig. 25. Sumithrin resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2005.

However, mosquito populations from area 21 (RR_{50} = 8.57), 42 (RR_{50} = 8.10), and 64 (RR_{50} = 8.24) demonstrated resistance ratios that surpassed 8 and became areas of concern needing future monitoring. Mosquitoes from area 12 (RR_{50} = 10.00) was the only population that had resistance ratio that met the resistance threshold for sumithrin (Fig. 25). Area 225 mosquito populations doubled their resistance ratio from the pervious year from RR_{50} = 4.77 (2004) to RR_{50} = 8.47 (2005). As previously noted this increase was also seen in resistance ratios recorded for this mosquito population to resmethrin (Fig. 16).

The mosquito populations from all Brazos County's seven collection areas demonstrated good susceptibility to sumithrin over the two year resistance monitoring project (Figs 26-27). The highest resistance ratio recorded for 2004 was for mosquitoes collected from area 7 (RR_{50} = 1.42) in rural northern Brazos County. The highest resistance ratios recorded for 2005 was for mosquitoes from area 3 (RR_{50} = 1.87), with collection sites of this area being in the cities of Bryan and College Station (Fig 27).

Amongst the mosquito populations in Harris and Bryan Counties tested against the two organophosphates (malathion and naled), none had resistance ratios that exceeded the threshold and only a few populations had resistance ratios fall within the range that would cause them to be considered "at risk" of developing resistance to the organophosphate agents (Figs. 28-36). In the case of mosquitoes tested against malathion in Harris County in 2004, only a single population from area 42 (RR_{50} = 8.77) was considered in jeopardy of continued development of resistance and thereby, required close monitoring the following year.

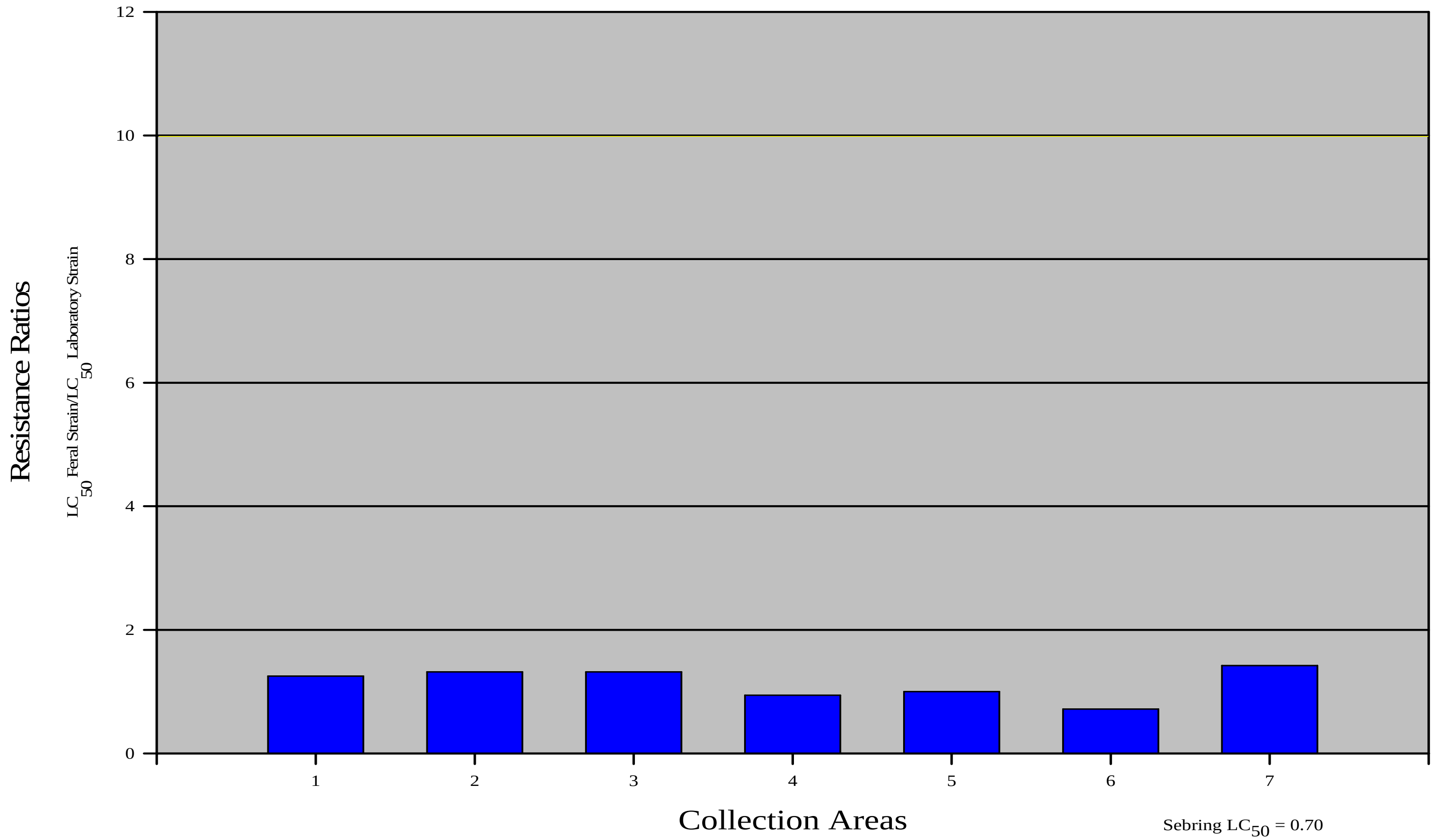

Fig. 26. Sumithrin resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2005.

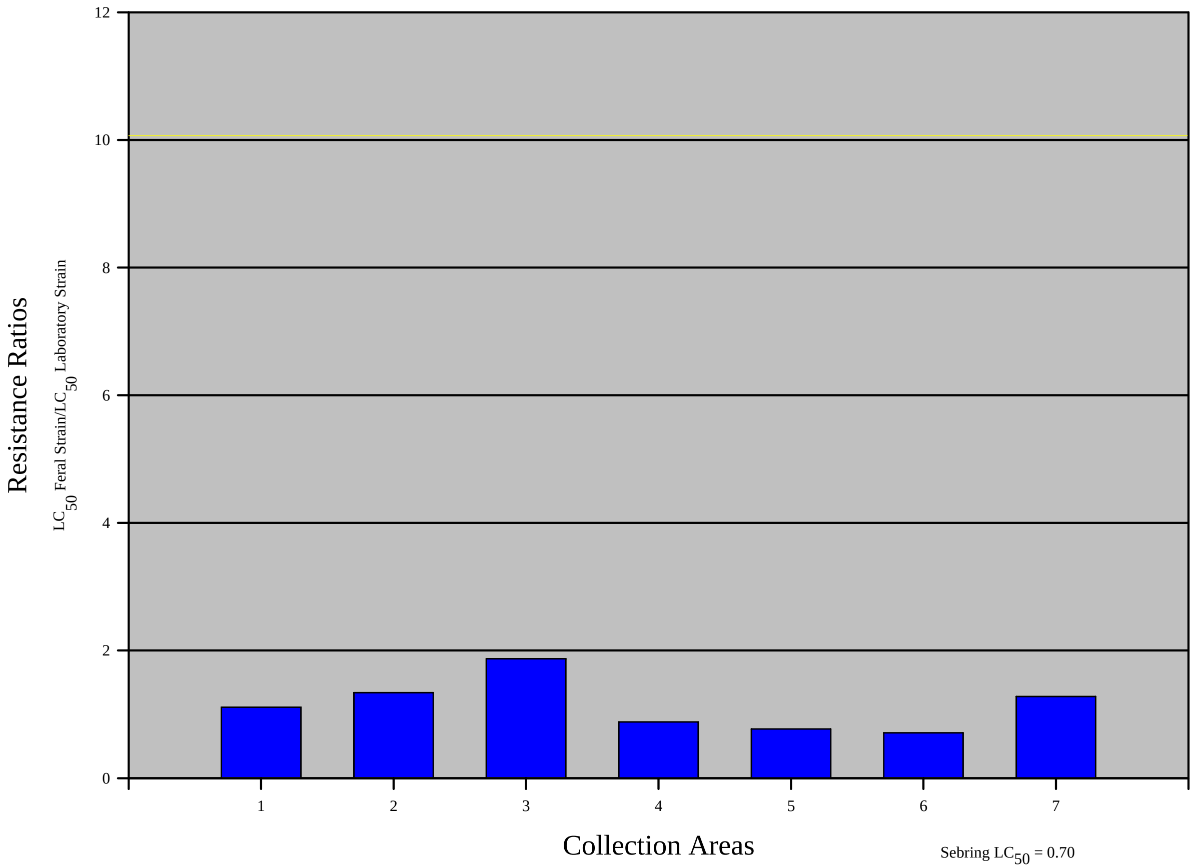

Fig. 27. Sumithrin resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2006.

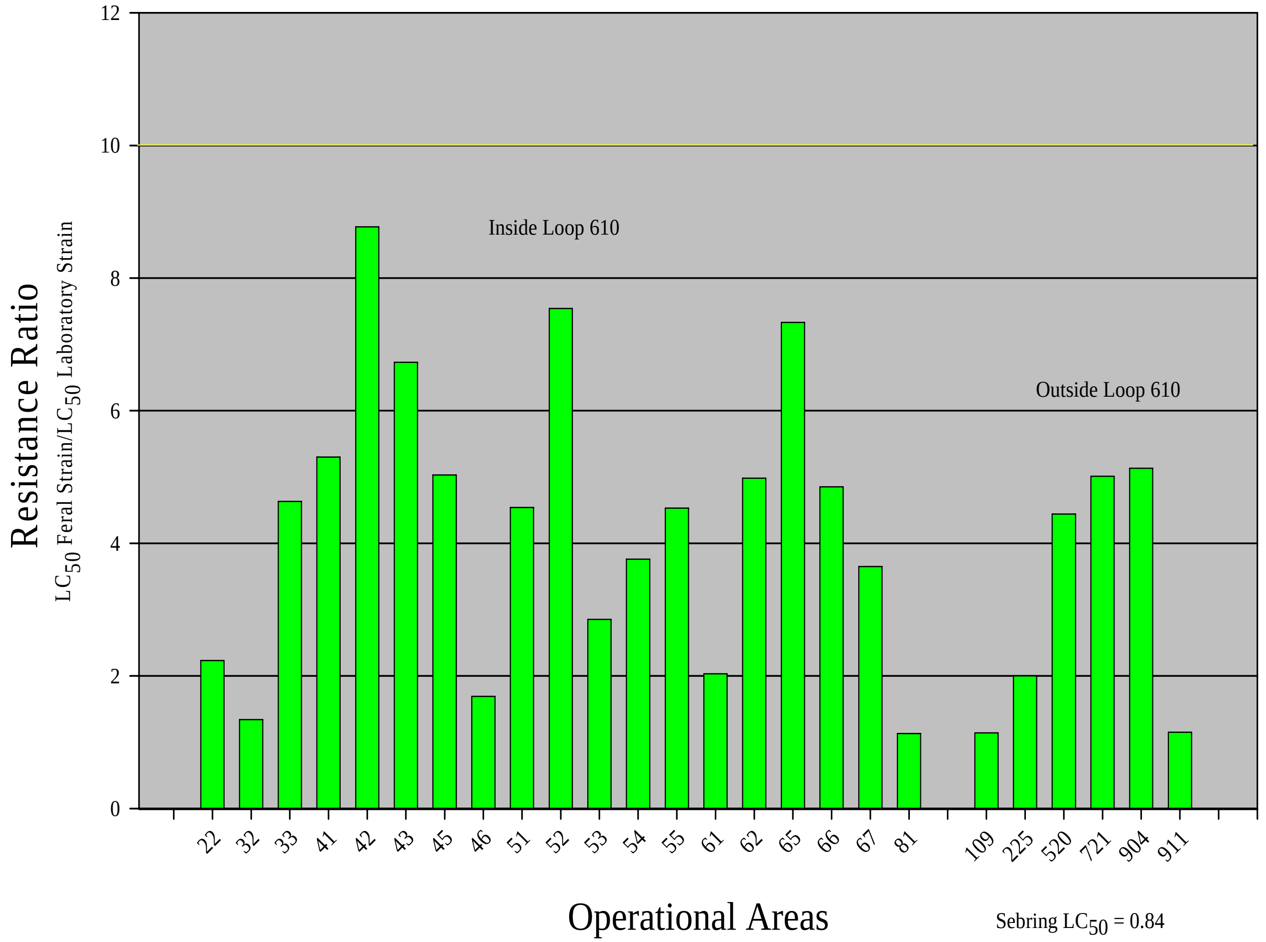

Fig. 28. Malathion resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2004.

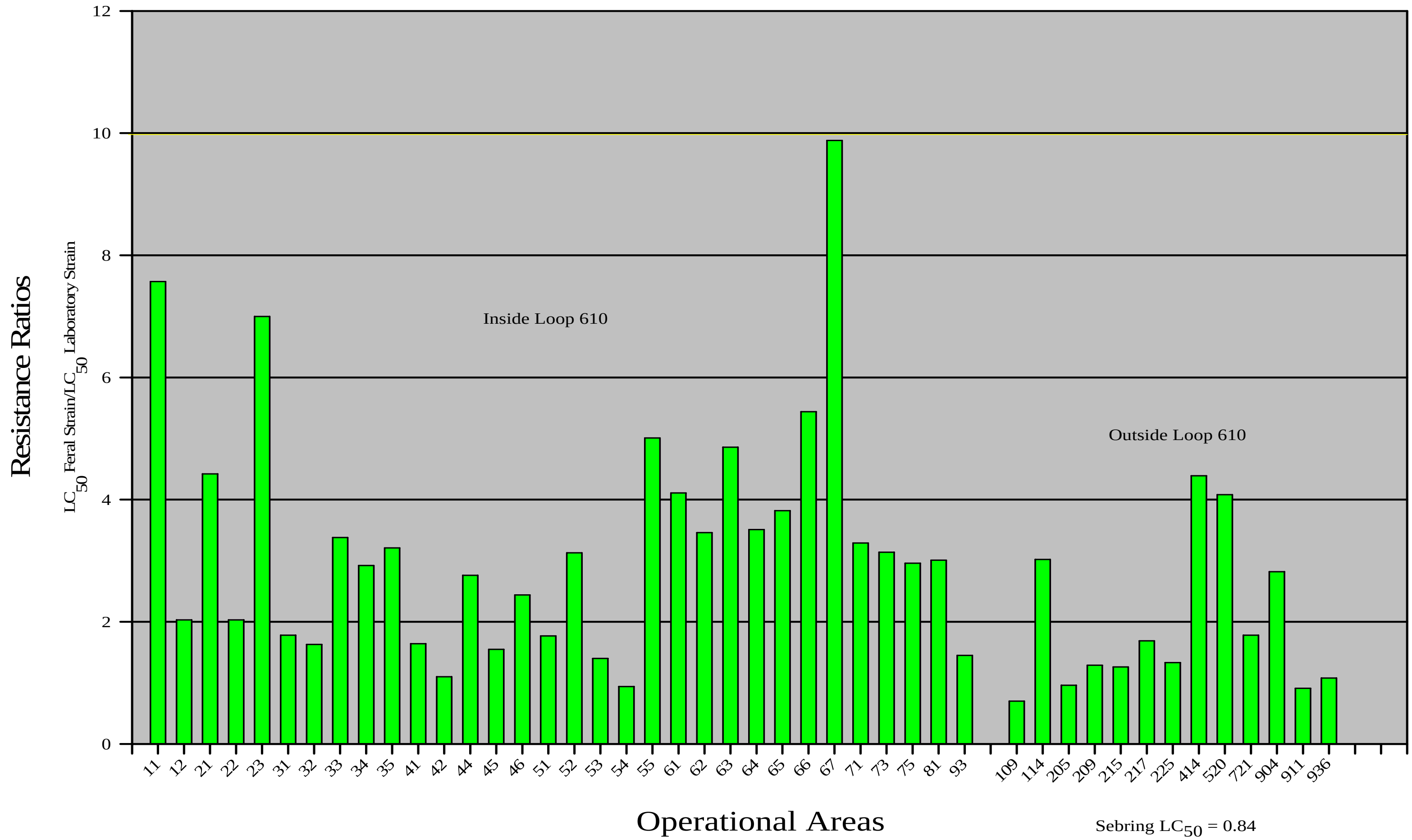

Fig 29. Malathion resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2005.

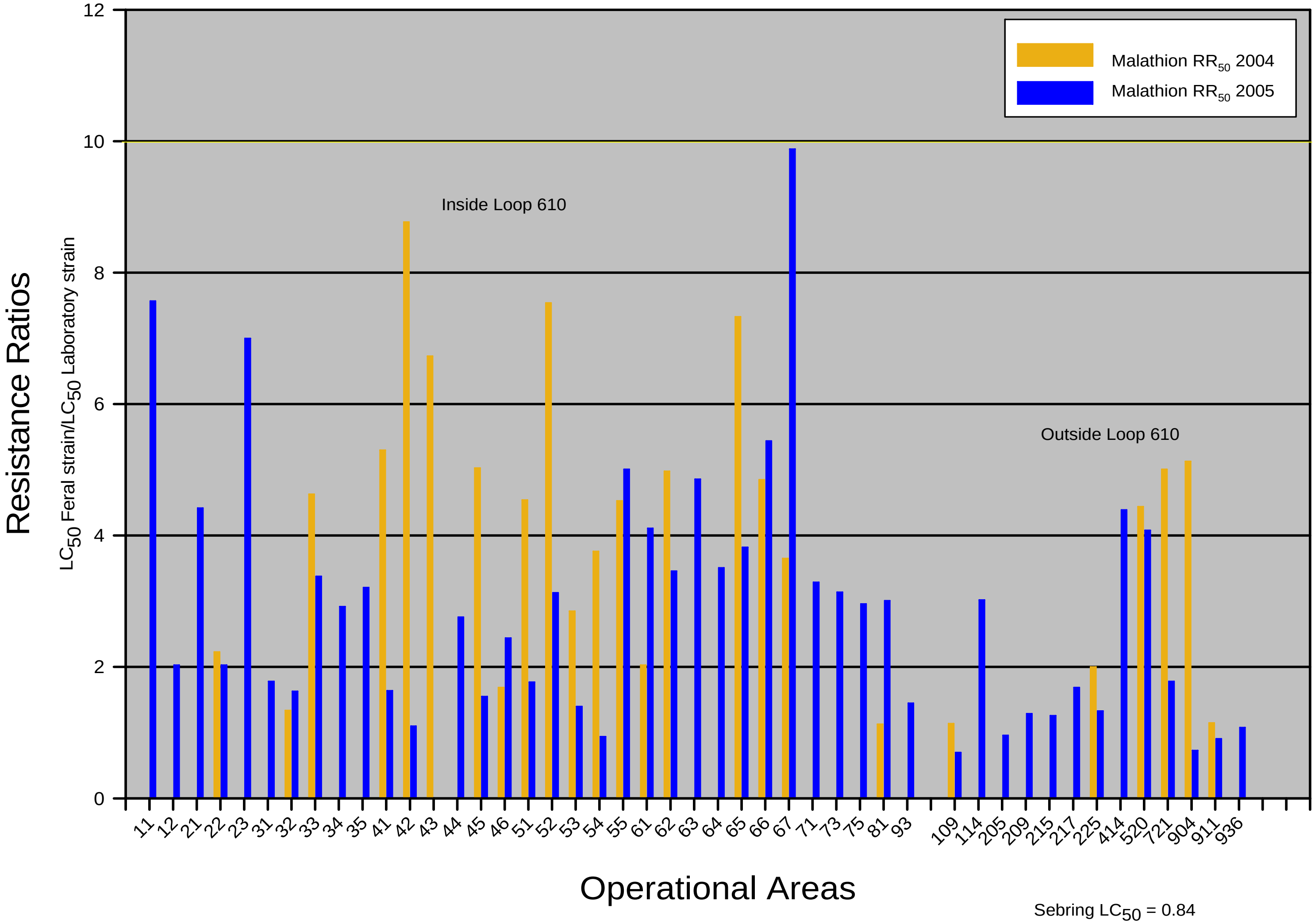

Fig. 30. A comparison of malathion resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas in 2004 and 2005.

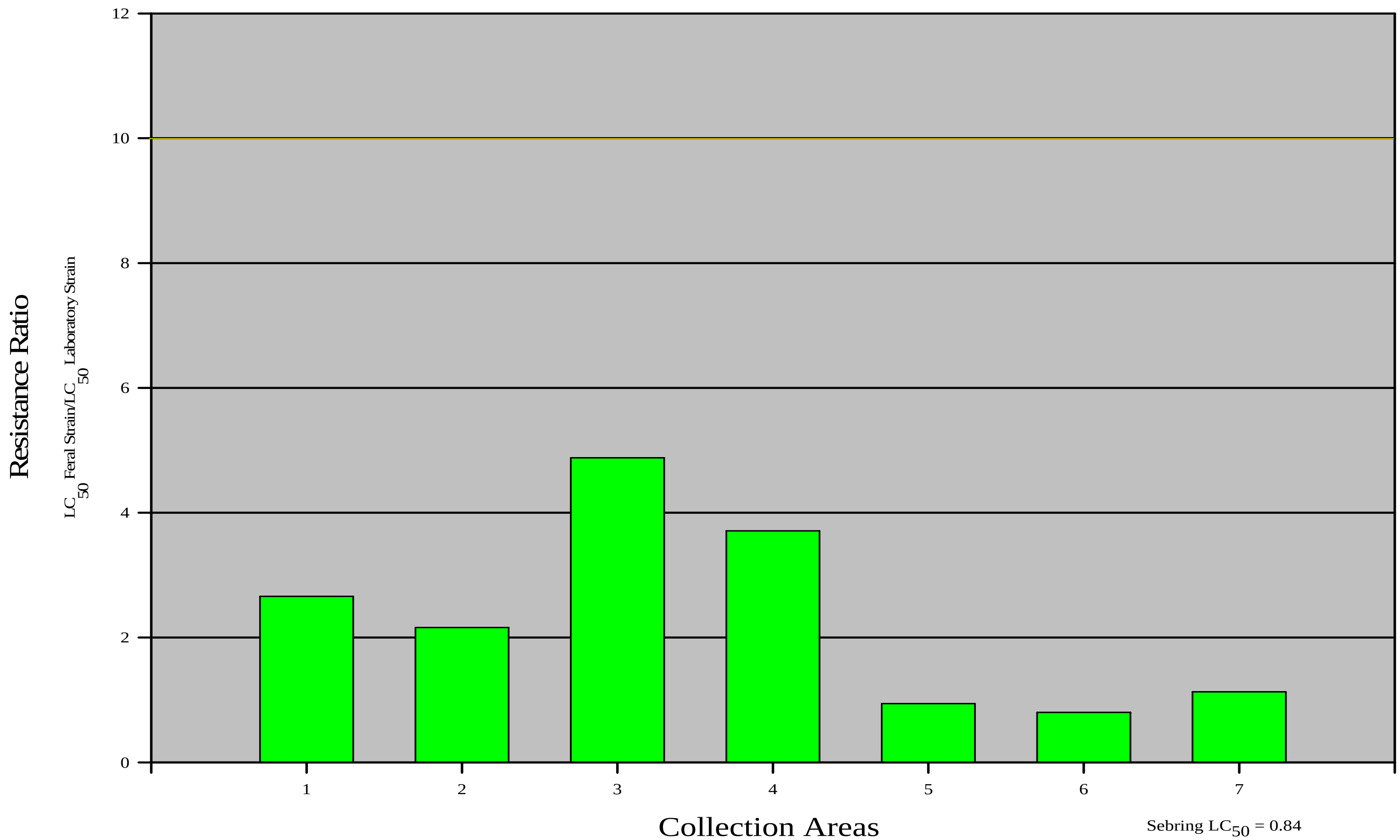

Fig. 31. Malathion resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2005.

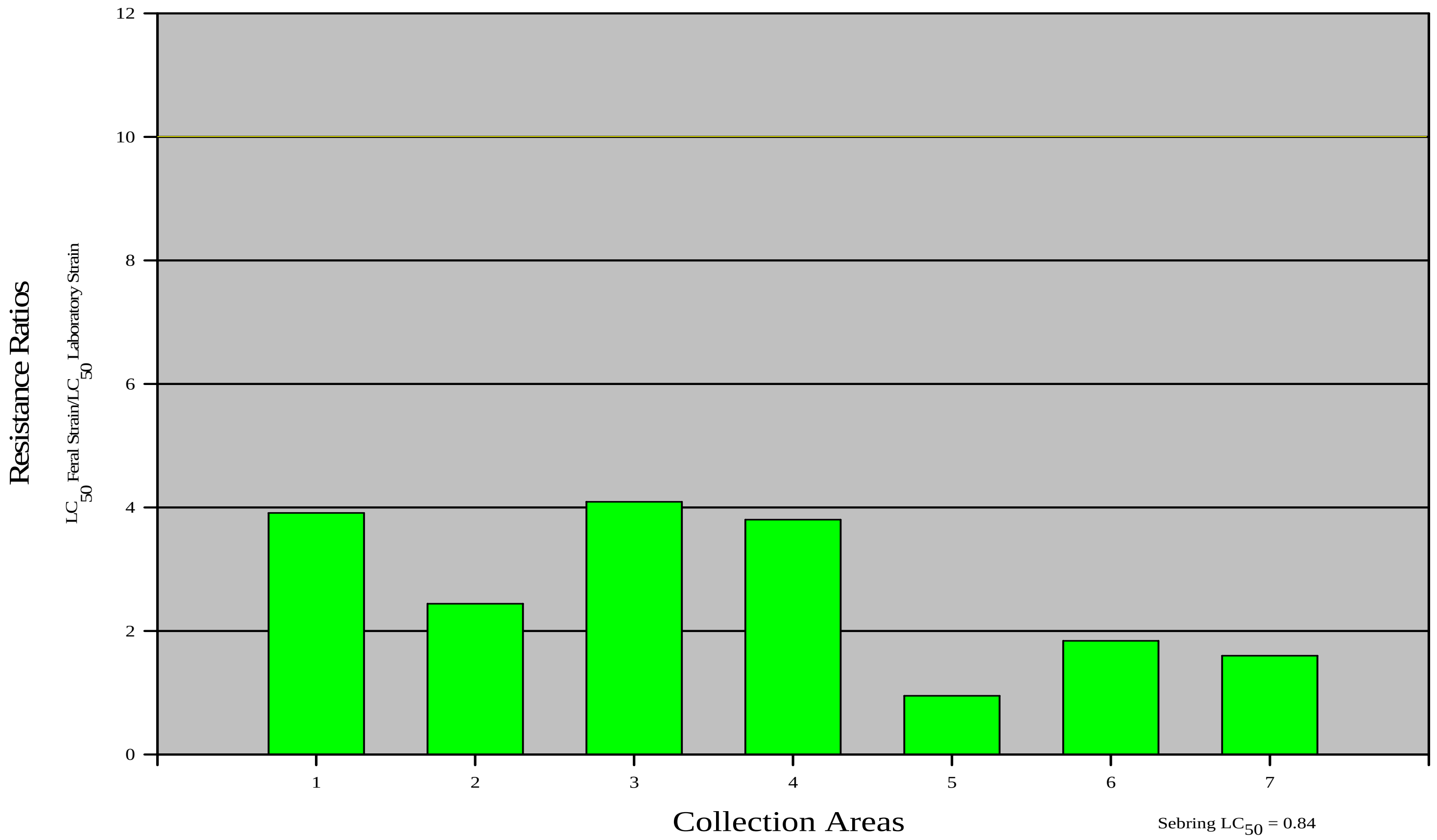

Fig. 32. Malathion resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2006.

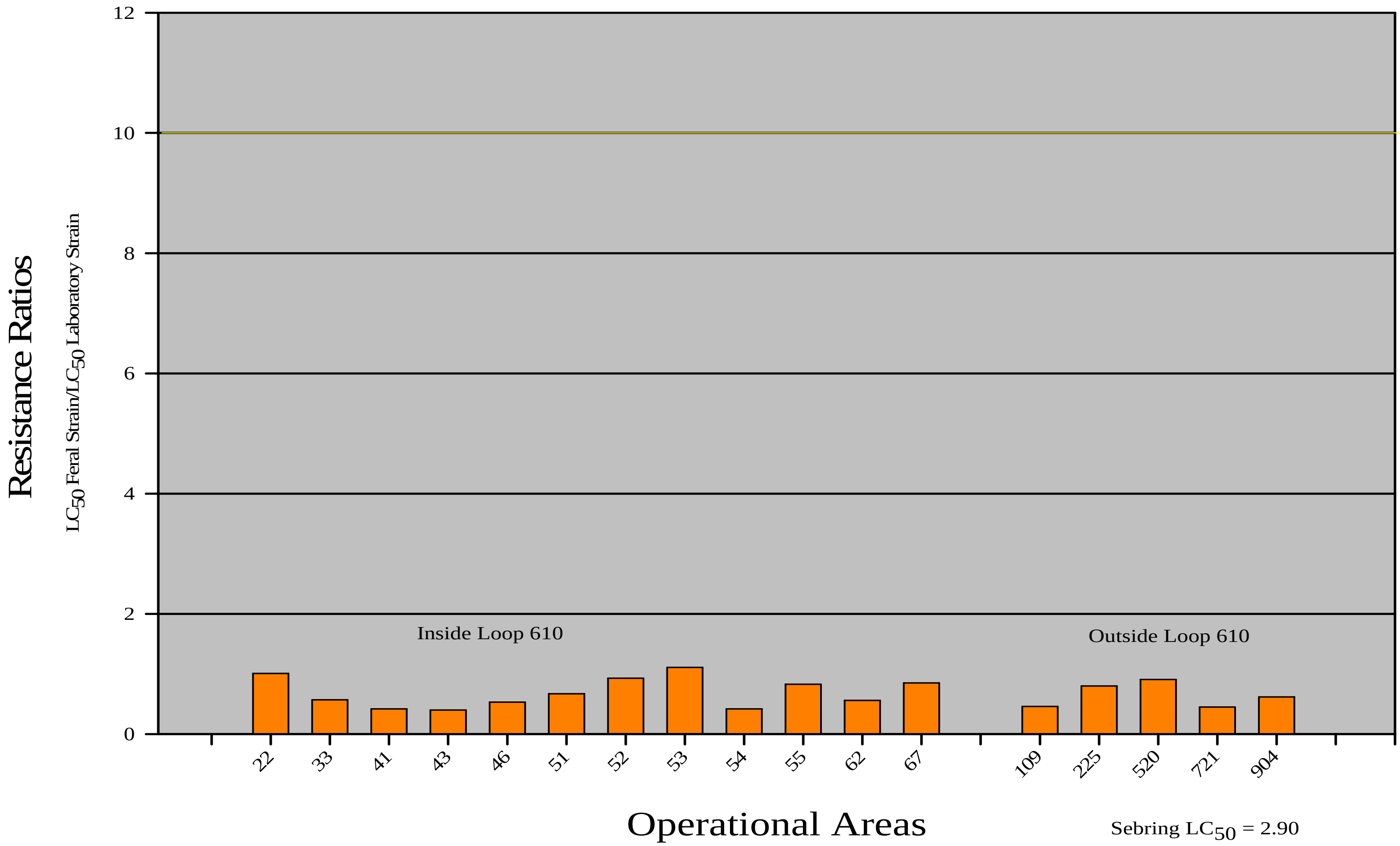

Fig. 33. Naled resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2004.

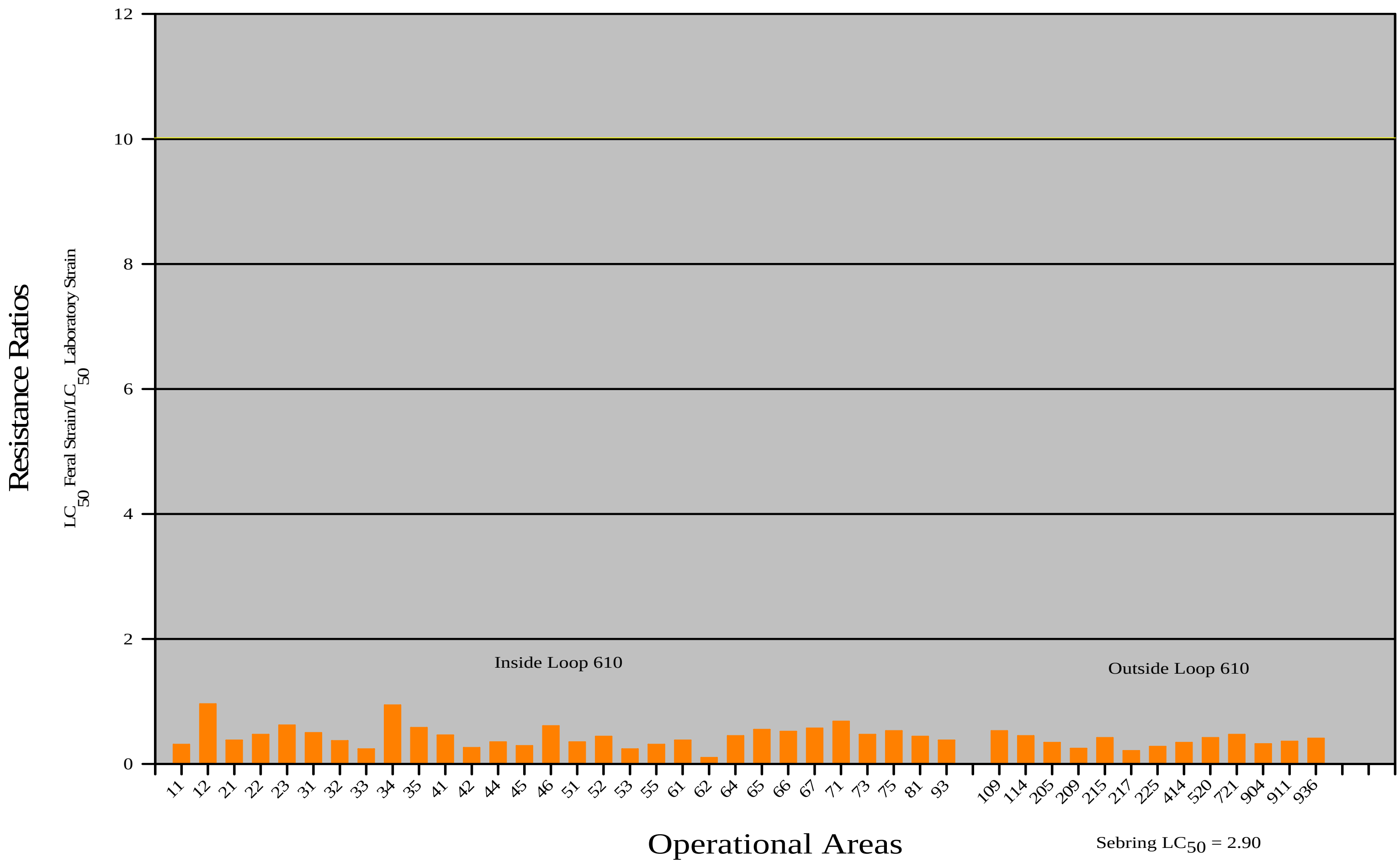

Fig. 34. Naled resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2005.

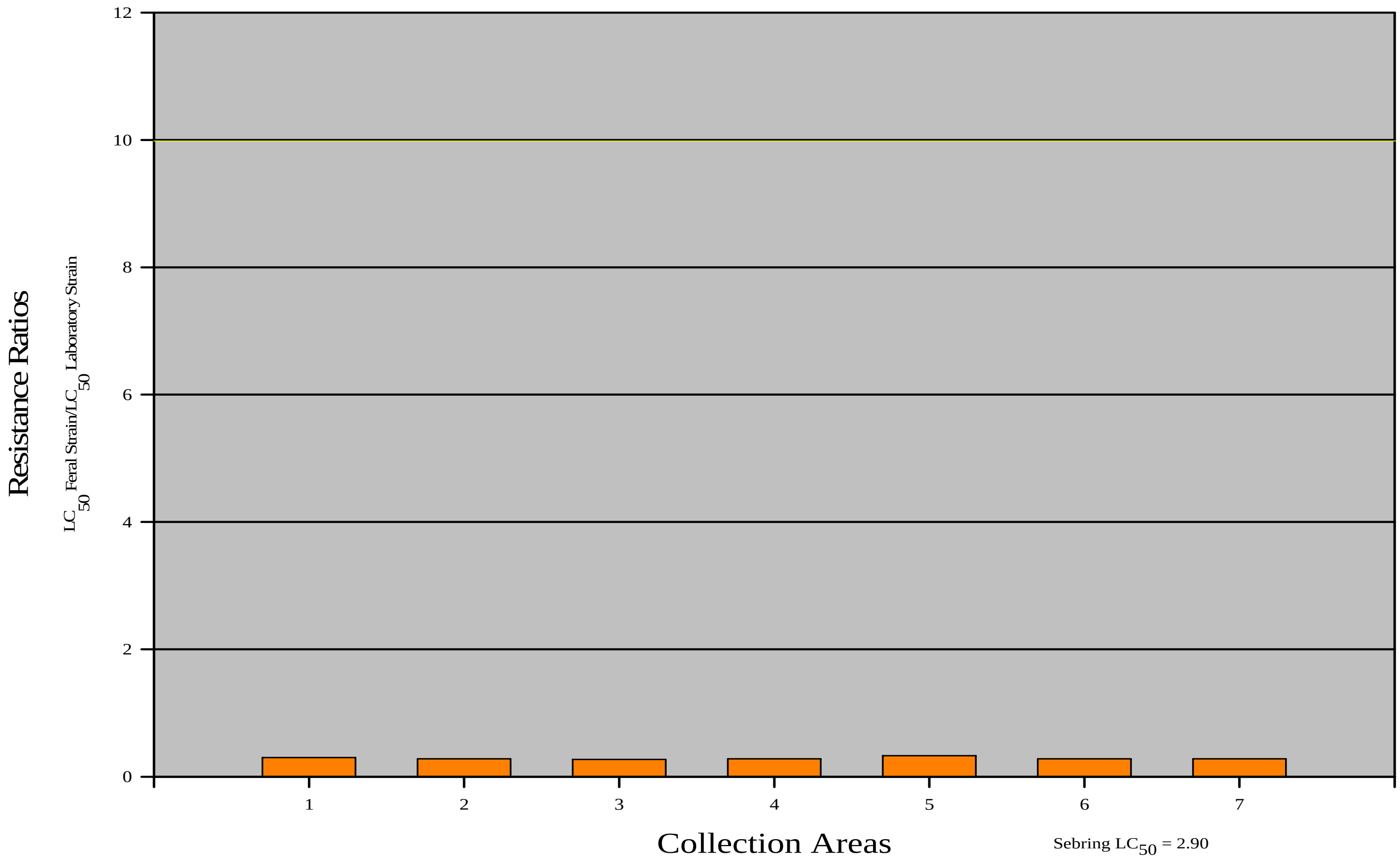

Fig. 35. Naled resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2005.

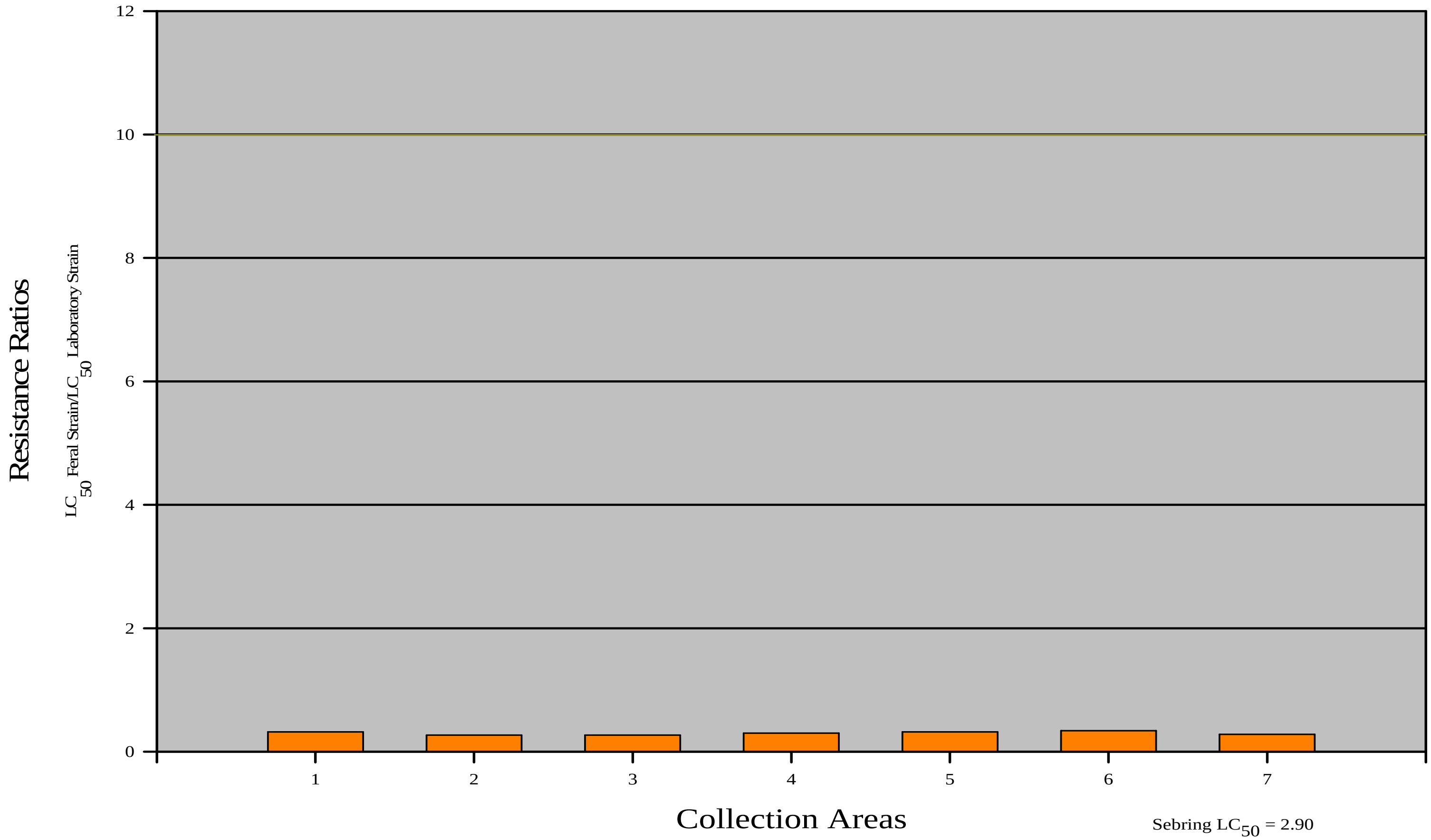

Fig. 36. Naled resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2006.

All other populations tested from inside and outside Interstate Highway Loop 610 had resistance ratios that fell within the acceptable range (Fig. 28). During the 2005 testing period, the resistance ratio of mosquitoes from area 42 returned to acceptable levels (RR_{50} = 1.10); but, the population collected from area 67 increased considerably from 2004 (RR_{50} =3.65) to a level just below the resistance threshold in 2005 (RR_{50} = 9.88) (Fig. 29-30). All other areas had resistance ratios that fell within the acceptable range designated by the test procedure used in this study.

Brazos County mosquito populations in all collection areas displayed susceptibility to malathion there were no mosquito populations that exceeded a resistance ratio of five (Figs. 31-32). The highest resistance ratios recorded over the two year project was for mosquito populations from area 3 (RR_{50} = 4.88) in 2004 and (RR_{50} = 4.09) in 2005, with collection sites in this area being in College Station and Bryan, Texas.

Naled is an insecticide that is used sparingly in Harris County and is only applied aerially. This chemical is not used at all in Brazos County. The resistance ratios calculated from the mosquito populations tested over the two year research project proved naled to be an extremely effective chemical in Harris and Brazos Counties. Every mosquito population tested against naled showed remarkable susceptibility compared to the other chemicals tested with resistance ratios that did not approach a value of 2. The highest resistance ratios recorded in Harris County to naled was from populations collected from area 53 (RR_{50} = 1.11) in 2004 and area 34 (RR_{50} = 1.11) in 2005 (Figs. 33-34).

Brazos County mosquitoes showed the same level of susceptibility to naled as those collected in Harris Co. Populations collected from sites in the cities of Bryan and College Station that made up area 1 (RR_{50} = 0.33) in 2004 and sites from rural southern Brazos Co. that made up area 6 (RR_{50} = 0.34) in 2005 had the highest resistance ratios recorded during the two year resistance monitoring program to naled (Figs. 35-36).

The bioassay tests of mosquito populations for resistance against natural pyrethrum showed results similar to those for naled, with all areas in Brazos and Harris Counties having mosquito populations showing susceptibility to the insecticide over the time period this research was conducted. Pyrethrum is used on a limited basis in Harris County and only applied only by thermal fogging of the sewer systems. This agent is not used at all for mosquito control in Brazos County. The highest resistance ratios recorded in Harris County over the two year project were for mosquito populations from area 81 (RR_{50} = 4.22) in 2004 and area 61 (RR_{50} = 4.23) in 2005 (Figs. 37-38).

Brazos County mosquito populations did not have one resistance ratio that approached a ratio of 2 over the two year testing period. The highest resistance ratios recorded were from populations from area 1 in 2004 (RR50 = 0.75) and area 3 in 2005 (RR_{50} = 0.78) and consisted of collection sites in the cities of Bryan and College Station (Figs 39-40).

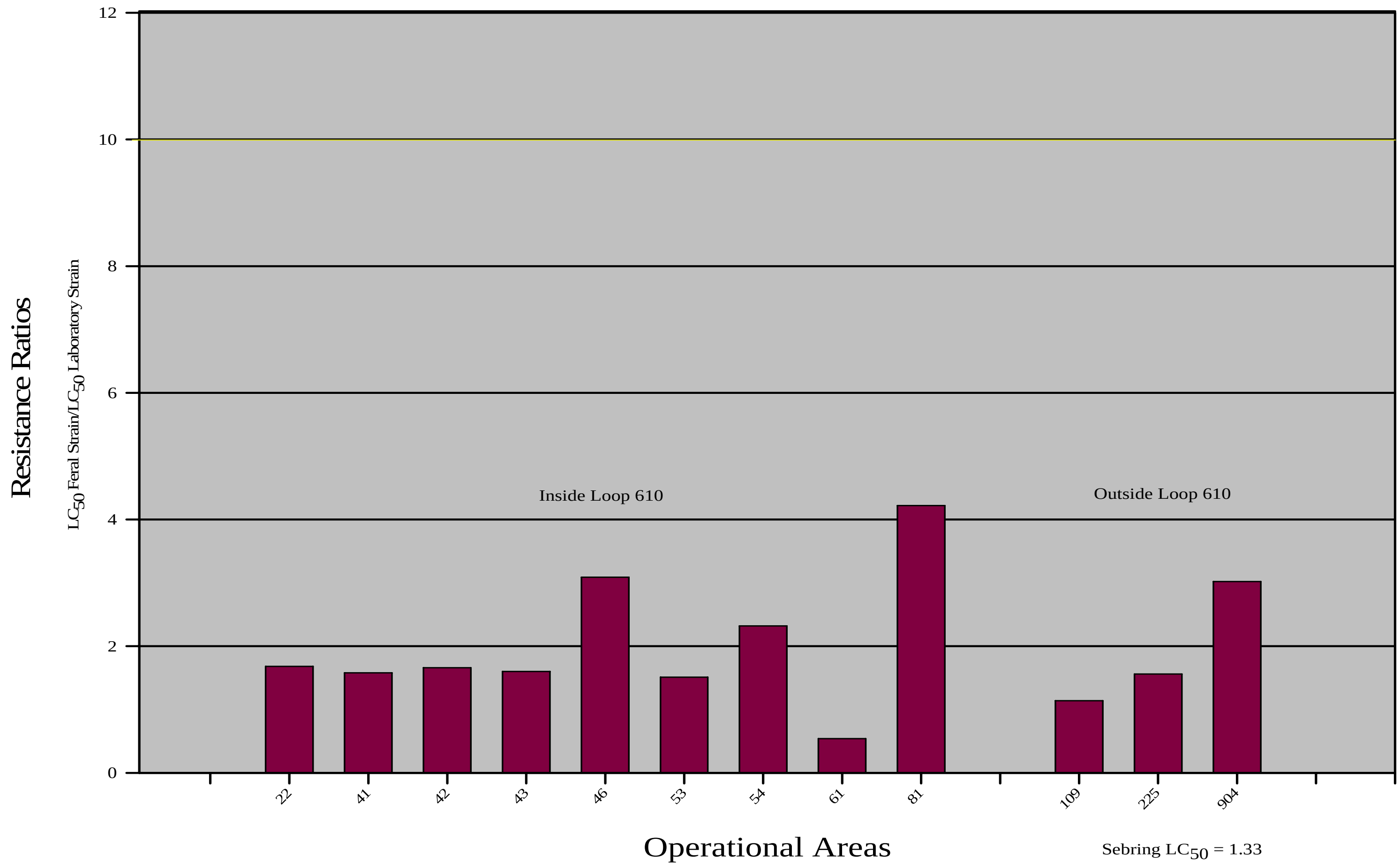

Fig. 37. Pyrethrum resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2004.

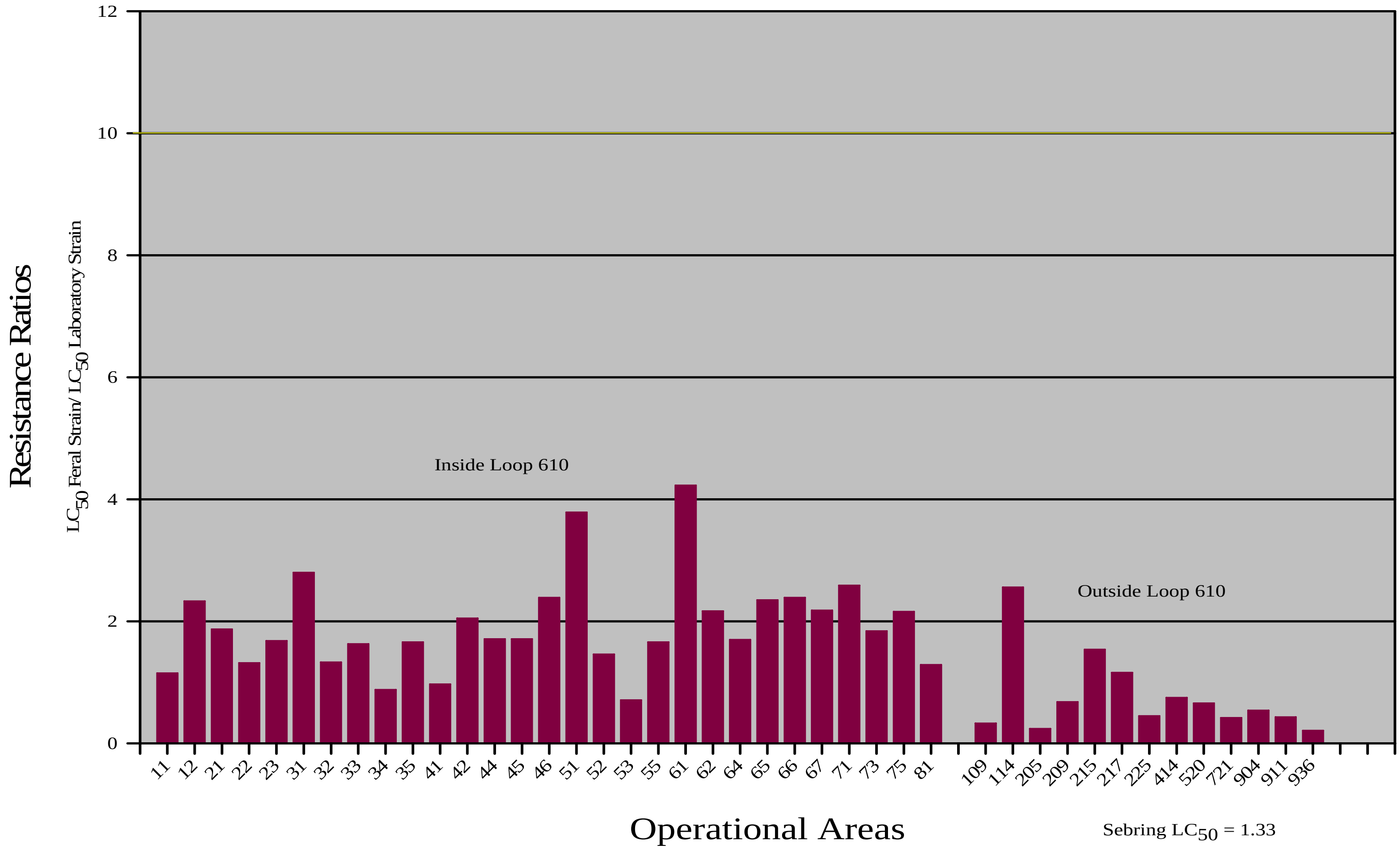

Fig. 38. Pyrethrum resistance ratios for female *Culex quinquefasciatus* collected from operational areas in Harris County, Texas 2005.

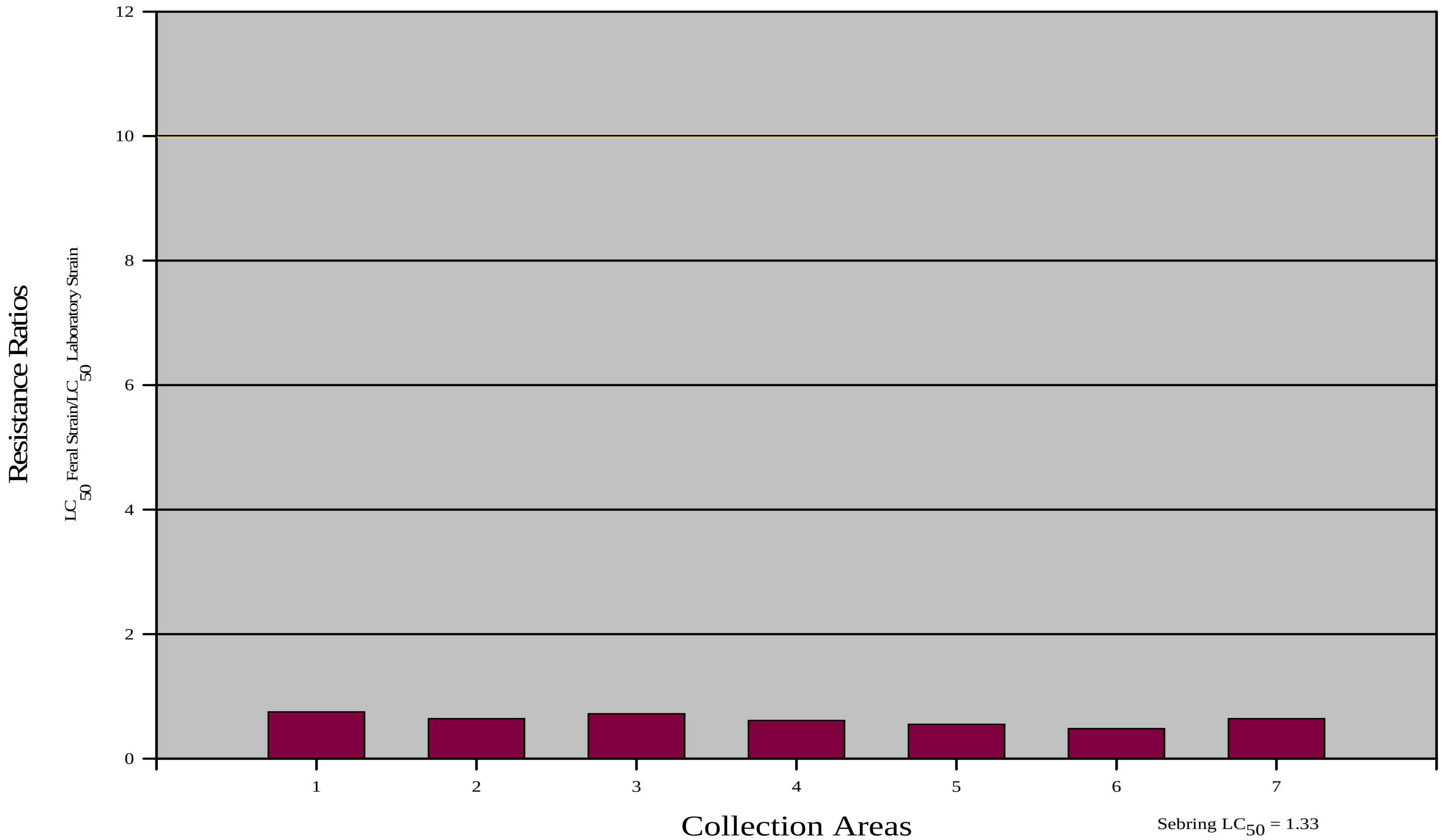

Fig. 39. Pyrethrum resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2005.

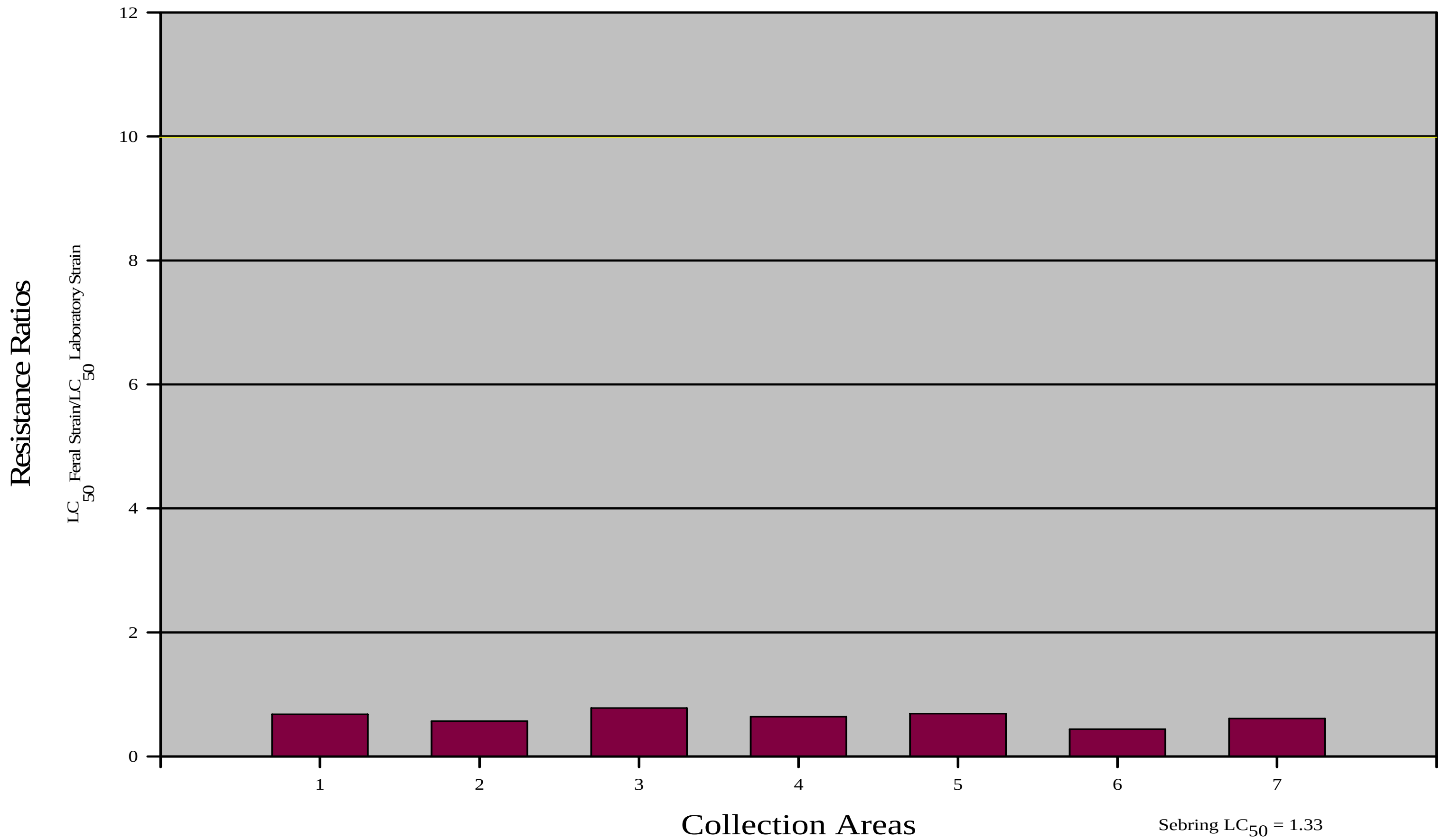

Fig. 40. Pyrethrum resistance ratios for female *Culex quinquefasciatus* collected from areas in Brazos County, Texas 2006.

Discussion

The results from the resistance-coated vial bioassay assessment conducted on adult *Cx. quinquefasciatus* populations in Harris County, Texas, are that mosquito populations in this county do not have county-wide resistance to any particular chemical but there are a few localized populations that exhibited resistance characteristics. Populations from Harris County operational areas 12, 21, 22, 23, 33, 43, 51, 55, 61, 65, and 66 all exhibited resistance ratios that exceeded 10 to at least one chemical, which designates each of them as a resistant population. All of the populations were resistant to one or more of the pyrethroids tested. No resistance was detected to pyrethrum or the two organophosphates in the populations tested.

Prior to this study, mosquito populations in Harris County areas 42, 51, 54, 66, 106, 206, and 512 were determined to be highly resistant to malathion by bottle bioassays (Pietrantonio et al. 2000). This study also detected the first recorded resistance to resmethrin in Harris County for populations sampled in area 51 (Pietrantonio et al. 2000). The data generated from the Pietrantonio et al. (2000) resistance screening study in conjunction with operational preferences led to the suspension of the use of malathion in all control operations in 2001 by the Harris County Mosquito Control Division. Malathion had previously been restricted to use on annoyance mosquitoes and was almost completely abandoned for a lack of efficacy in disease abatement during 1994 based on unpublished spray test data from field tests conducted by the Test and Evaluation Section of the HCMCD (Mr. Kyle Flatt personal communication). In contrast, data produced from the current study indicated that the mosquito populations demonstrated susceptibility to malathion and the resistance reported in the previous studies was not fixed in the Harris County mosquito population.

Of the six chemicals tested on mosquito populations in Harris County, only four of them are used in control operations on a regular basis. Resmethrin and malathion are the primary chemicals used in truck-mounted disease control efforts. Malathion was reinstated for use in control operations when its effectiveness was established from data generated from vial bioassays and spray tests conducted during 2004. Prior to this development, only resmethrin was used in above-ground truck-mounted control activities. Naled and pyrethrum were used to a lesser extent in Harris County, with use limited by problems associated with the cities infrastructure and legal regulations. Pyrethrum is fogged into storm sewer systems by truck-mounted thermal fog units, but successful treatments is inhibited by structural deficiencies of obstructions of the storm sewer system. Although naled is an extremely effective chemical based on data from the current study; its use in ULV is not practical due to its corrosive nature. However, naled is the primary chemical used in aerial spraying program conducted in Harris County. The County does not have its own spray plane, but it does utilize specialized contractors when the need arises. State regulations require chemical applications can only be made by twin engine aircraft when spraying over metropolitan areas. Aerial applications are conducted in Harris County only when there is measured West Nile virus activity in rural parts of the county that do not contain adequate road networks for effective spraying with ground-based spray units.

In 2005, a management program for resistance was begun in Harris County that was based on the rotation of insecticides from different chemical classes as advocated by Georghiou (1980) and Georghiou and Mellon (1983). This program could only be undertaken with the discovery of the return of effectiveness of malathion. This gives the Harris County Mosquito Control Division (HCMCD) a second chemical class (organophosphates) to rotate with the pyrethroidal ones already in use. In addition to the discovery of susceptibility in the Harris County *Cx. quinquefasciatus* populations to malathion, the data also indicated a return of resmethrin susceptibility in many of the population which coincided with the implementation of the insecticide resistance management program by the HCMCD in 2005. The start of this program was a positive step in delaying the development of resistance in the county; but, this return of susceptibility to malathion in the Harris County mosquito populations may lead to some future problems. Mosquito control operations are not exclusive to the HCMCD; others are carried out by private pest control firms and local public works departments in the county. These other control agencies are not governed by the same regulatory restrictions as the HCMCD and are, thereby, they are held to different standards. Little to no coordination and communication occurs between these agencies and the HCMCD when conducting control efforts in the various cities and gated communities located in Harris County. On a monetary basis, malathion is a cheaper chemical to use in ground-based mosquito spraying operations than are the synthetic pyrethroids. This economic disparity may lead to a switch by local city and private contractors to malathion, thus increasing selection pressure immensely. With little oversight over the private contractors (i.e., pest control operators) mosquito control operations and homeowners insecticide use (i.e., backyard spray systems) the insecticide resistance management program practiced by HCMCD may prove futile in some areas. This increases the importance of continuing the resistance monitoring portion of the management program.

According to the vial bioassay, results for assessment conducted over the years 2005-2006, the Brazos County mosquito populations did not have resistance in any of the seven collection areas. As noted previously, only a minimal amount of chemical mosquito control activity occurred in Brazos County over the span of this research project and consisted primary of thermal fogging storm sewer systems, culverts, and under bridges with resmethrin in a four city block radius around the location of mosquito, bird, and/or human positive cases of West Nile virus (Dr. Jimmy K. Olson, personal communication). This lack of chemical pressure on the mosquito populations helps to explain the county-wide susceptibility to all six chemicals tested. However, the slightly higher resistance ratios from collection areas within the cities of Bryan and College Station might be traced to increased chemical usage by private citizens in recent years because of West Nile virus outbreaks in these cities.

Mosquito resistance ratios for malathion in Brazos County were slightly higher in the urban areas of the county. This may be due to use of this chemical by the general public in trying to control other insect pest species or residual resistance still present in the city-based populations from when the city of Bryan conducted a minimal annoyance mosquito control spray programs with Dursban, which is also a organophosphate. This trend was also present in the results to resmethrin and to a lesser extent for the other chemicals tested.

Vial bioassay results for this study detected some cross resistance to the three synthetic pyrethroids (resmethrin, permethrin, and sumithrin) tested on the part of certain Harris County mosquito populations. Cross-resistance is defined as the protection from more than one insecticide through the action of a single mechanism (Scott 1990). The three chemicals for which cross-resistance was detected in the Harris County mosquito populations belong to the same chemical class (pyrethroid) and work on the same target site (sodium ion channel); so, some amount cross resistance was expected between these insecticides. Variation in the chemistry of the insecticides and the disparity in efficiency of the resistance mechanism to protect the insect from any one member of the chemical group over another member may account for differences in resistance ratios recorded in the current study (Busvine 1968). The cross-resistance occurred in Harris County mosquito populations which had low resistance ratios as well as populations that had high resistance ratios. However, the cross-resistance is especially evident in the populations with high resistance ratios for pyrethroids; so, they are the examples used in this dissertation.

Cross-resistance was best illustrated in a Harris County mosquito population collected in 2004 from operational area 51. This population had resistance to both resmethrin and permethrin and had a sumithrin resistance ratio that was above 8 (Table 2). Mosquito populations from areas 46, 54, and 55 also had this pattern in 2004 collections (Table 2). The populations from these areas were either resistant to a given pyrethroid or had resistance ratios that exceed 8. This was evidence for the development of resistance in two or more of the pyrethroids tested. The same phenomenon was again recognizable during the 2005 test period. Resistance to resmethrin was detected in the Harris County mosquito population from area 21 and this same population had high resistance ratios to the other two pyrethroids tested (Table 2).

The only incident of pyrethroidal cross-resistance in a mosquito population outside U.S. Loop 610 in Harris County occurred in area 225 which encompasses the Kingwood subdivision a suburb of Houston. Prior to being annex by Houston in 1996, Kingwood was an unincorporated area of Harris County, and had contracts with private contactors to conduct mosquito control operations in addition to the control operations conducted by HCMCD. When the subdivision was annexed, these contracts were voided and control operations fell totally under HCMCD direction. In recent years, this area has had multiple cases of WNv isolated from mosquito pools (group of 50 mosquitoes for virus testing) in this area. This led to heavy spraying (Table 3) by the HCMCD in the adulticide control operations it executes in the Kingwood area of the county only Scourge® (resmethrin) was used in. This was demonstrated with 22 spray events taking place in 2003 and 28 spray events taking place in 2004 in the Kingwood subdivision to suppress the vector mosquito populations occurring in that subdivision (Unpublished HCMCD records).

In addition to the control activities conducted by HCMCD, several neighborhood associations had contracted with private pest control operators to provide annoyance mosquito control for their gated communities.

Table 3. Categories assigned to represent the amount of spray activities undertaken in control activities by Harris County Mosquito Control Division.

Number of Spray Events	
0-5	Light
5-10	Moderate
10-15	Moderate/Heavy
15-20	Heavy
20+	Extremely Heavy

This jump in control activities over the past few years lead to increased selection pressure on the population especially in area 225 (the Kingwood area), which also probably caused the dramatic increase in the resistance ratios recorded for the mosquito populations from this area from one year to the next. With the rotational resistance management program instituted in 2005 in place, this trend will hopefully be blunted and begin to decline. This is where the resistance monitoring program will be most important to monitor areas for large increases in resistance from one year to the next, as had occurred in Harris County (i.e., area 225) and to monitor what happens over the future years as treatment operations are modified. As opposed to the pyrethroids tested, no cross-resistance was seen in Harris County mosquito populations for the two organophosphates tested.

There was a trend for the mosquito populations tested in Harris County to decrease in resistance the further the operational area was located from Interstate Highway Loop 610, with the exception of populations from area 225 (Kingwood). This trend could be because of the demographics of the operational areas as one moves out from downtown Houston. Several of the operational areas are primarily rural and do not contain the infrastructure to support ground-based mosquito control operations conducted by HCMCD. Also, the lack of human habitation may lead to a reduction in breeding sites for *Cx*. *quinquefasciatus*.

This trend was not as readily apparent for the two organophosphates, naled and malathion. The resistance ratios for populations against malathion tended to be consistent in relationship to Interstate Highway Loop 610. This might be due to the heavy use of malathion for other purposes other than mosquito control which apply selection pressure when applications are directed against other key pests such as these in agriculture (Lines 1988, Diabate et al. 2002). Mosquito populations proved to be extremely susceptibility to naled from all operational areas in Harris County, whether located inside or outside Loop 610. The wide ranging susceptibility to naled could be caused by the lack of use in the county or the unique way the chemical affects the nervous system of the insect (Dr. Jimmy K. Olson, personal communication).

Brazos County mosquito populations appear to be in the initial development of the trend where resistance ratios are higher in the Bryan/College Station metroplex and decrease as one moves into the surrounding rural communities. The development of this trend is far more evident in Harris County where mosquito populations in some areas have been under selection pressure since 1964. The trend in Brazos County is apparent during both years that the research was conducted and is most obvious in the results collected for malathion (Figs. 31-32), but to a lesser extent for the three synthetic pyrethroids (Figs. 18-19, 22-23, 26-27).

The primary result of this research was the determination that malathion was again a viable chemical for use in mosquito control activities in Harris County. This led to the development of an insecticide resistance management program by HCMCD based on the rotation of chemical classes in 2005. The 739 bioassays conducted during the assessment program described herein gave the operational agencies of Harris and Brazos counties a snap shot of the resistance activity ongoing in the populations of *Cx. quinquefasciatus* occurring in both counties and the variance in resistance ratios from one year to the next. The continuation of this program, especially in areas where resistance or high resistance ratios in mosquito populations have been previously detected, is essential to the future success of the insecticide resistance management program in certain areas of Harris County.

Future research is needed to be performed in Harris County is an expansion to other operational areas to develop baseline data for mosquito populations and to detect other hot spots for resistance. More populations from outside Interstate Highway Loop 610, including areas that contain the larger cities of Baytown and Katy, need to be tested to determine the resistance status of the mosquitoes. The trend where mosquito populations resistance ratios tend to decrease as one travels out from Interstate Highway Loop 610 needs to be further assessed to determine if there is an association with the data of the first years of the assessment program. Better coordination is needed between the HCMCD and the local city mosquito control programs and private contractors to synchronize mosquito control activities and the chemicals utilized for control if the insecticide resistance management program initiated by Harris County is to succeed.

CHAPTER V

THE MONTHLY VARIATION OF RESISTANCE IN POPULATIONS OF *Culex quinquefasciatus* FROM SELECT OPERATIONAL AREAS IN BRAZOS AND HARRIS COUNTIES, TEXAS

An important factor in the development of an insecticide resistance management program is the implementation of a resistance monitoring strategy. Resistance monitoring is the attempt to measure changes in the frequency or degree of insecticide resistance in time and space (Croft et al. 1986). Monitoring should be undertaken before a resistance problem has been identified in the mosquito populations; but, monitoring can be implemented during any phase of a chemical control program. The monitoring for insecticide resistance should take place, at the very least, on an annual basis and be conducted using a classical bioassay test (Busvine 1971). Classical bioassays are characterized as precise tests that measure the potency of an insecticide by reference to standardized susceptible insect colonies and are simple to perform without the resources of a fully-equipped laboratory (Busvine 1971). These include the WHO bioassay, bottle bioassay, and vial bioassay described in the literature review section of this dissertation.

In recent years, biochemical tests have been developed and substituted for classical bioassays for identifying unique detoxification enzymes associated with resistant pests. These tests are usually conducted on the homogenates of single insects using electrophoretic analysis, filter paper tests, or microtiter plate assays (Georghiou and Pasteur 1978, Brogdon et al. 1988, Beyssat-Arnaouty et al. 1989). These biochemical tests only give the levels of detoxifying mechanisms present in a single mosquito tested and does not give the level of resistance in the population to the actually chemicals used in a control program or quantify how the levels of resistance mechanisms detected equate to resistance in the field. For this reason, the scintillation vial bioassay modified from Plapp (1971) was selected for use in the accomplishments of research described for this objective and; research on this objective was conducted in conjunction with the resistance screening assessment the first objective (See Chapter IV).

In the case of mosquito control results from periodic insecticide susceptibility bioassay tests contribute information that is essential in creation of informed operational decisions for future control strategies. Insecticide resistance monitoring is most prominently used to validate of the effectiveness of a chemical in controlling a target mosquito population. In addition to this primary objective, monitoring identifies the geographic distribution of resistance mosquito populations, determines if ineffective control is due to resistance, and provides assessment of the effectiveness of a chemical before it is widely used in a control program.

This study was undertaken to determine what variation in insecticide resistance occurred in the mosquito population over a six month period in Harris and Brazos Counties. The majority of resistance monitoring is conducted on an annual basis with little information collected in the intervening months. Little is know about the fluctuations within the populations on a month to month basis. Populations of *Cx. quinquefasciatus* from six areas in Harris County having a variety of historical backgrounds of chemical selection pressure from mosquito control operations were selected as were three populations from areas in Brazos County, where little to no current or historical insecticidal control has been or is now conducted. These selected mosquito populations were monitored over a six month period (June-November) in 2005 to determine if or how much the resistance ratios of the various populations varied during the year.

Materials and Methods

The selection of areas in Harris County for this aspect of the research project was based on a high number of spray events having been conducted in a given area (Unpublished HCMCD data) and/or if resistance was previously identified in the operational area by bioassay testing. The six areas chosen consisted of two areas located within Interstate Highway Loop 610, two areas between Interstate Highway Loop 610 and the Sam Houston Tollway (Beltway 8), and the final two areas were located outside Beltway 8 (See Fig. 5 for the HCMCD operational area map). Areas 11 and 51 were the operational areas chosen inside the Interstate Highway 610 Loop. Area 11 had received the most spray events since spraying for West Nile virus began in 2002 (Harris County unpublished data). Resistance was detected from area 51 for malathion and resmethrin by bottle bioassays (Pietrantonio et al. 2000). Area 109 and 904 were the operational areas chosen between Interstate Highway 610 Loop and Beltway 8. These areas were chosen based on the spray data provided by HCMCD and the baseline data obtain from 2004 bioassay testing. The final areas selected for this study were areas 520 and 225 located outside of Beltway 8. These areas were chosen on the basis of unpublished spray data HCMCD, vial bioassay and field cage test results conducted during the pervious year. The methods for mosquito collection were the same procedures as described in Chapter III of this dissertation with the same collection sites used in each area during each month throughout the experimental period as were used in the study described in Chapter III.

The three areas where mosquito populations were collected in Brazos County consisted of an urban site (Bryan), a rural area comprised of ranching, farming, and small communities (Kurten, Wixon Valley), and an area that ranges from suburban (Wellborn) to rural farms and ranches. These sites were chosen on the basis of their differences in human and animal populations and diversity of environment. These areas were thought to give an adequate insight as to how mosquito populations in three distinct areas of Brazos County might vary monthly in their resistance to six chemicals (malathion, naled, resmethrin, permethrin, sumithrin, and pyrethrum) used by mosquito control agencies for adult mosquito control that were included in this study. The mosquitoes were tested using the scintillation vial bioassay test described in Chapter III of this dissertation.

The resistance ratio 50s (RR_{50}) were used for this objective since the response to insecticides at this level (LC_{50}, RR_{50}) by any given insect population are less variable than for the response at the RR_{95} (LC_{95}) level (Likitvong 1996). The insecticide resistance ratios of the field collected mosquitoes were determined by dividing the lethal concentrations calculated in Chapter IV for the field collected mosquito populations by the lethal concentrations of the Sebring laboratory strain also calculated in the Chapter IV of this dissertation.

This objective was conducted as a preliminary study to determine if the resistance status of the mosquito populations varied between the months insecticide resistance testing was performed. A single resistance ratio was produced for each month the mosquito population was sampled with a total of six data points recorded for the entire study per insecticide for each population. Due to fact that only a single data point was recorded per month there was no way to run statistical tests on the results produced. With no statistical analysis run the trends observed on fluctuation in the resistance ratios between months are described for each mosquito population.

Results

Results of this study include the mosquito populations from areas 51, 109, 225, 530, and 904 in Harris County had resistance ratios for malathion that ranged between the 0 and 4, with considerable overlap of the resistance ratios occurring for the mosquitoes in the various areas from month to month (Fig. 41). There was only minor fluctuation between the monthly resistance ratios with the largest change observed in the first two months of the study in area 109. Populations in this area had an increase in their resistance ratios from June (RR_{50} = 0.70) to July (RR_{50} = 2.95) 2005 and then leveled off at higher ratio of resistance for the duration of the study (Fig. 41). The area 11 mosquito populations had a higher resistance ratio when it was initially tested in June 2005, thus separating it from the other five areas in terms of the baseline of resistance detected in this particular mosquito population. For June 2005 only slight changes in the resistance ratios were detected in the mosquito populations sampled and tested from this area (Fig. 41).

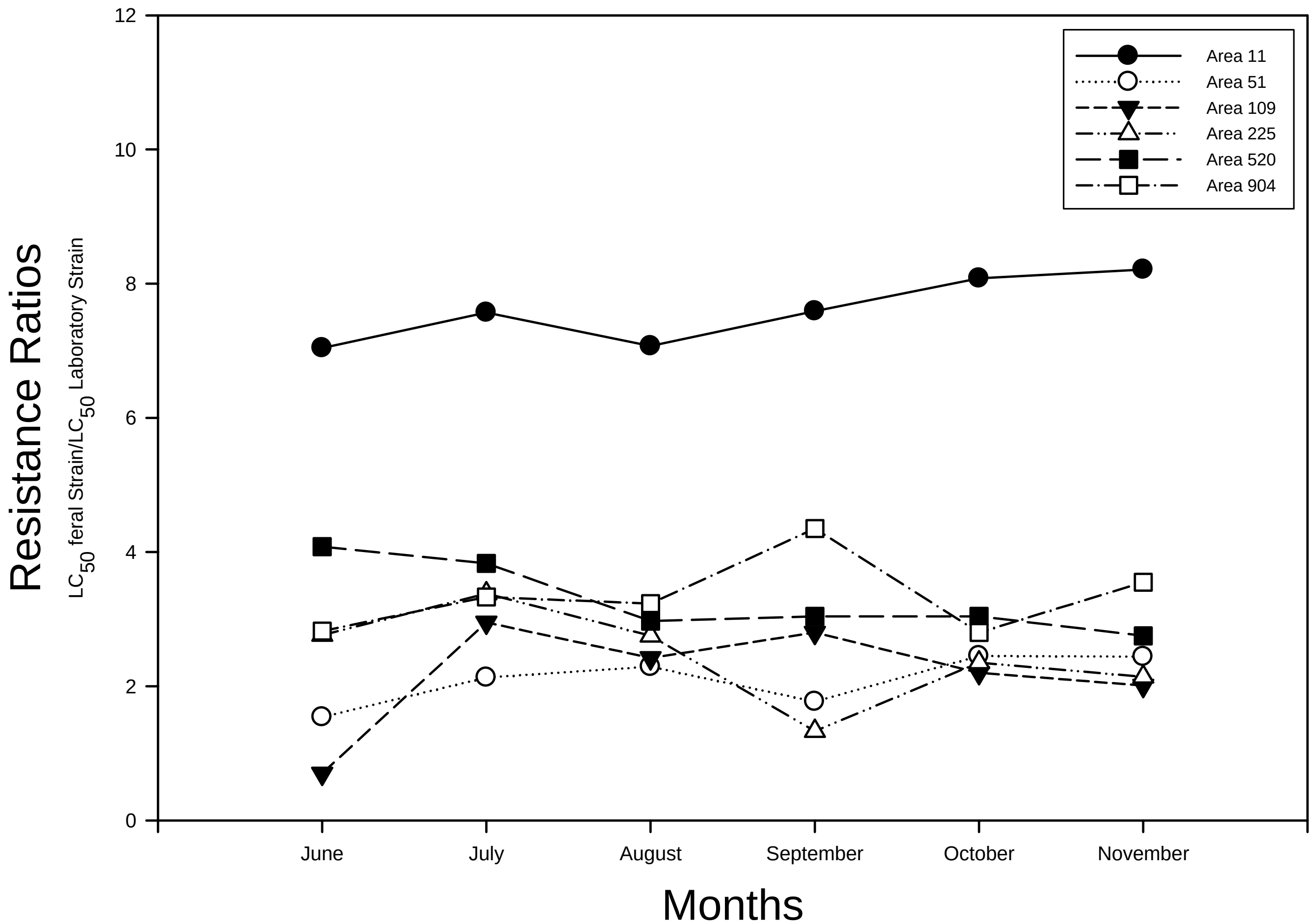

Fig. 41. Malathion resistance ratios for female *Culex quinquefasciatus* from six select operational areas sampled and tested over a six month period in Harris County, Texas, during 2005.

The resistance ratios for the mosquito populations from all six Harris County areas tested against naled ranged between 0 and 1 and had little variation between testing dates, and considerable similarity of the resistance ratios between the various populations tested in these areas each month of the testing period (Fig. 42). Only the mosquitoes from area 520 showed a minor increase between June (RR_{50} = 0.42) and July (RR_{50} = 0.82) 2005 in their population's resistance ratios. There particular ratios subsequently leveled off and stayed constant at the higher value for the remaining months of the testing period (Fig. 42).

The mosquito populations from the Harris County areas that were tested against resmethrin fell into three distinct groups based on their resistance ratios. Area 225 mosquito populations demonstrated the highest resistance ratios of the Harris County populations tested against resmethrin (Fig. 43). This separated the mosquitoes from this area from all other populations tested, but only minor fluctuations were observed over the remainder of the research period. Mosquito populations in areas 11 and 51 fell in between those in area 225 and those in the group of three areas whose resistance ratios for resmethrin ranged between 0 and 3. Area 11 populations demonstrated a gradual increase in their resistance to resmethrin over the first 5 months (June RR_{50} = 4.40 to September RR_{50} = 5.18), with a final spike occurring between October (RR_{50} = 5.18) and November (RR_{50} = 6.64) 2005 (Fig. 43). Populations from area 51 exhibited resistance ratios for resmethrin that remained fairly constant for the first four months (RR_{50} = 3.07 to RR_{50} = 2.94), then these ratios gradually increased over the last two months, finishing with a resistance ratio of 4.46 (Fig. 43).

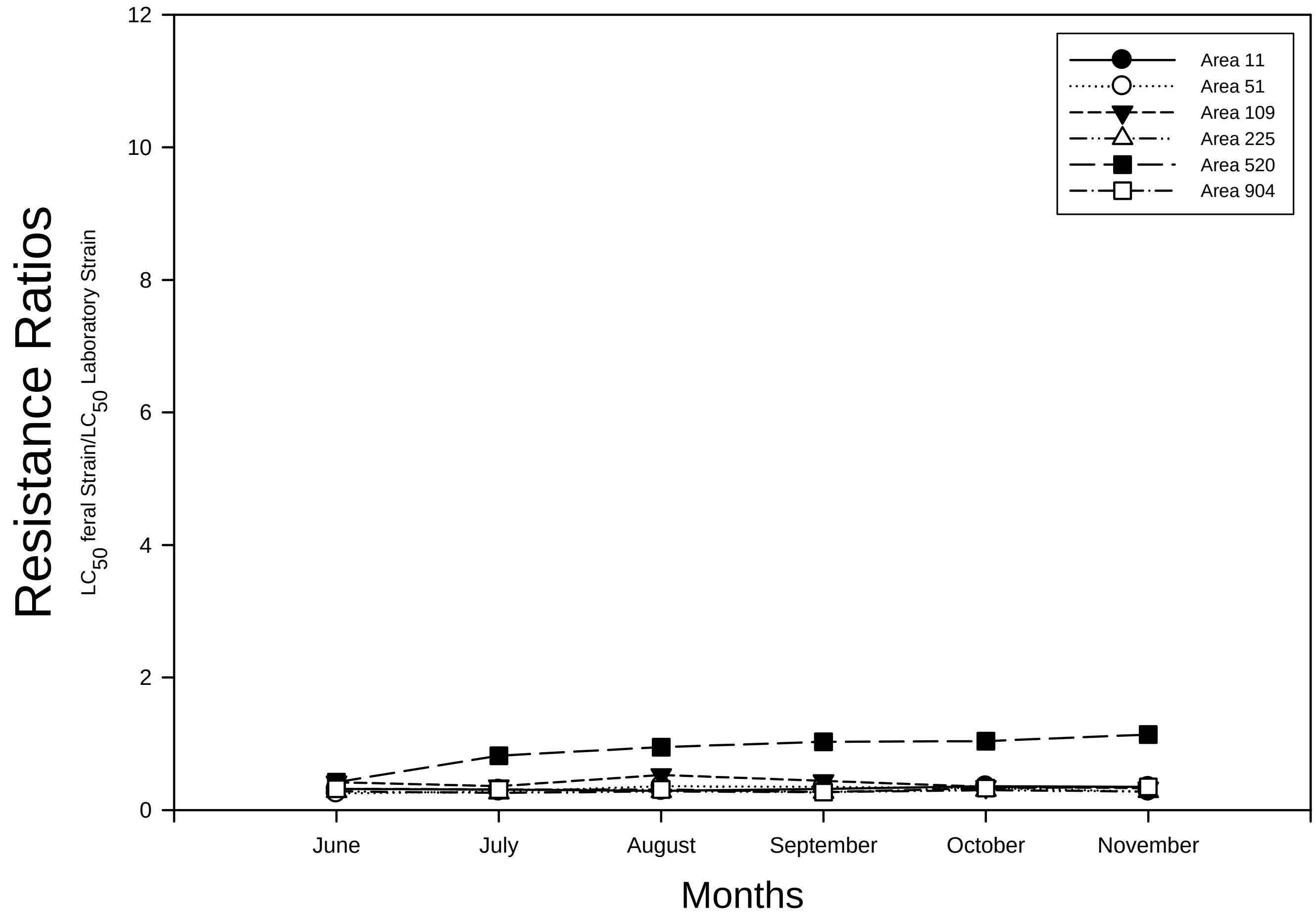

Fig. 42. Naled resistance ratios for female *Culex quinquefasciatus* from six select operational areas sampled and tested over a six month period in Harris County, Texas, during 2005.

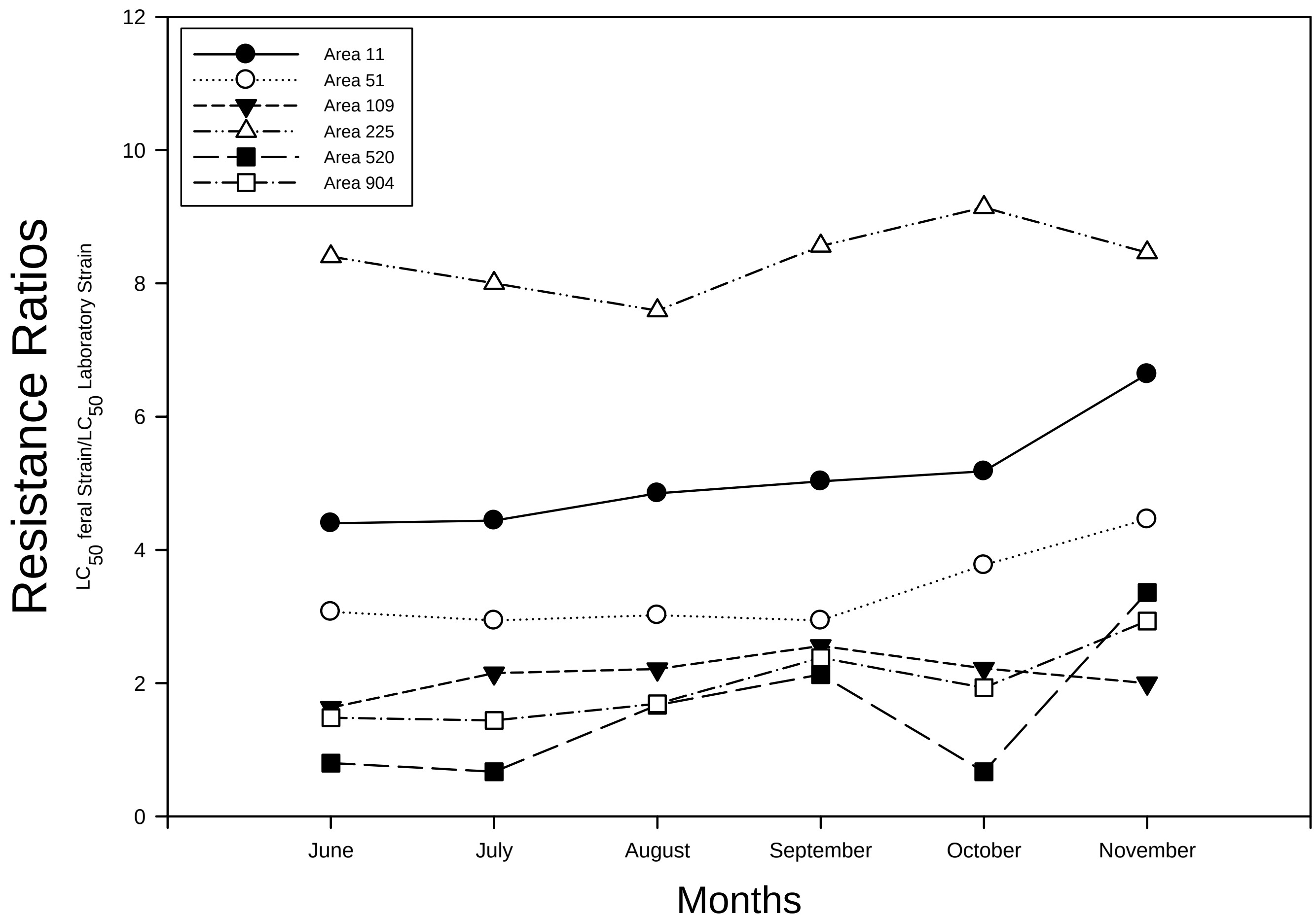

Fig. 43. Resmethrin resistance ratios for female *Culex quinquefasciatus* from six select operational areas sampled and tested over a six month period in Harris County, Texas, during 2005.

Mosquitoes from the remaining three areas in Harris County (109, 520, and 904) were grouped together with regard to their resistance ratio values for resmethrin, had fairly similar resistance ratios over the entire length of the study period (Fig. 43). Populations from areas 109 and 904 exhibited little variability in their resistance ratios for resmethrin from month to month. Mosquitoes from area 520 showed the greatest fluctuation in their month to month resmethrin resistance ratios over the duration of the study period. The populations had an initial low resistance ratio over the first two months (June RR_{50} = 0.80 and July RR_{50} = 0.67) then gradually increased over the next two months (September RR_{50} = 2.13) before declining in October back to the initial resistance ratio recorded (RR_{50} = 0.67) before peaking the following month at the highest recorded resistance ratio recorded for the population of the area at 3.36 (Fig. 43).

All six areas tested against permethrin where clustered between 0 and 4 with some overlapping of the resistance ratios (Fig. 44). The mosquito populations had minor increases and decreases in the populations from month to month with populations from areas 11, 51, 225, and 904 having slightly higher beginning resistance ratios.

The mosquito population exhibited minor oscillations between the monthly sumithrin resistance ratios recorded with the exception of two populations which demonstrated large decreases in resistance to sumithrin. Area 225 mosquito populations demonstrated a high initial sumithrin resistance ratio (RR_{50} = 8.47) which sharply declined between July (RR_{50} = 8.52) and August (RR_{50} = 5.21) 2005 before stabilizing and remained constant at the lower value for the remaining months of the testing period (Fig. 45).

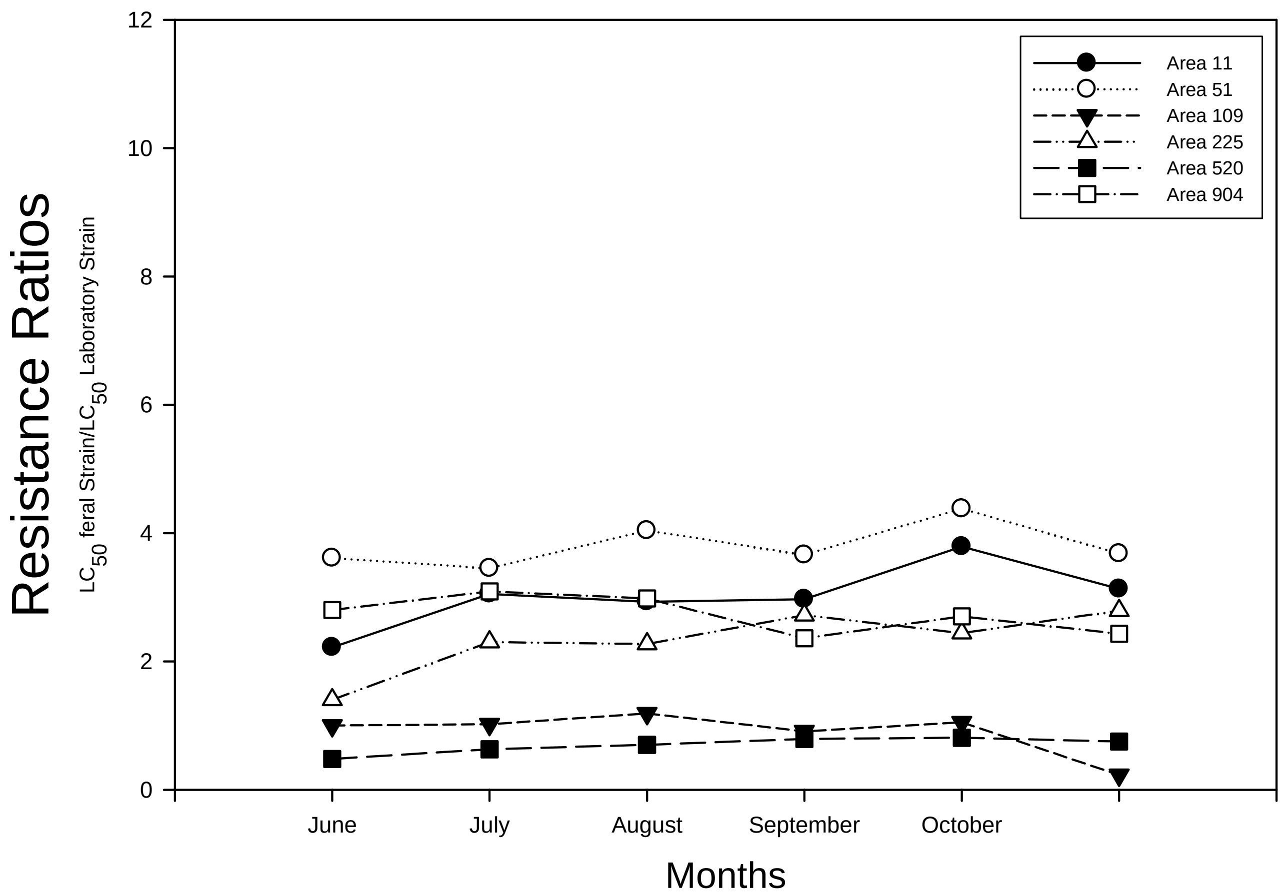

Fig. 44. Permethrin resistance ratios for female *Culex quinquefasciatus* from six select operational areas sampled and tested over a six month period in Harris County, Texas, during 2005.

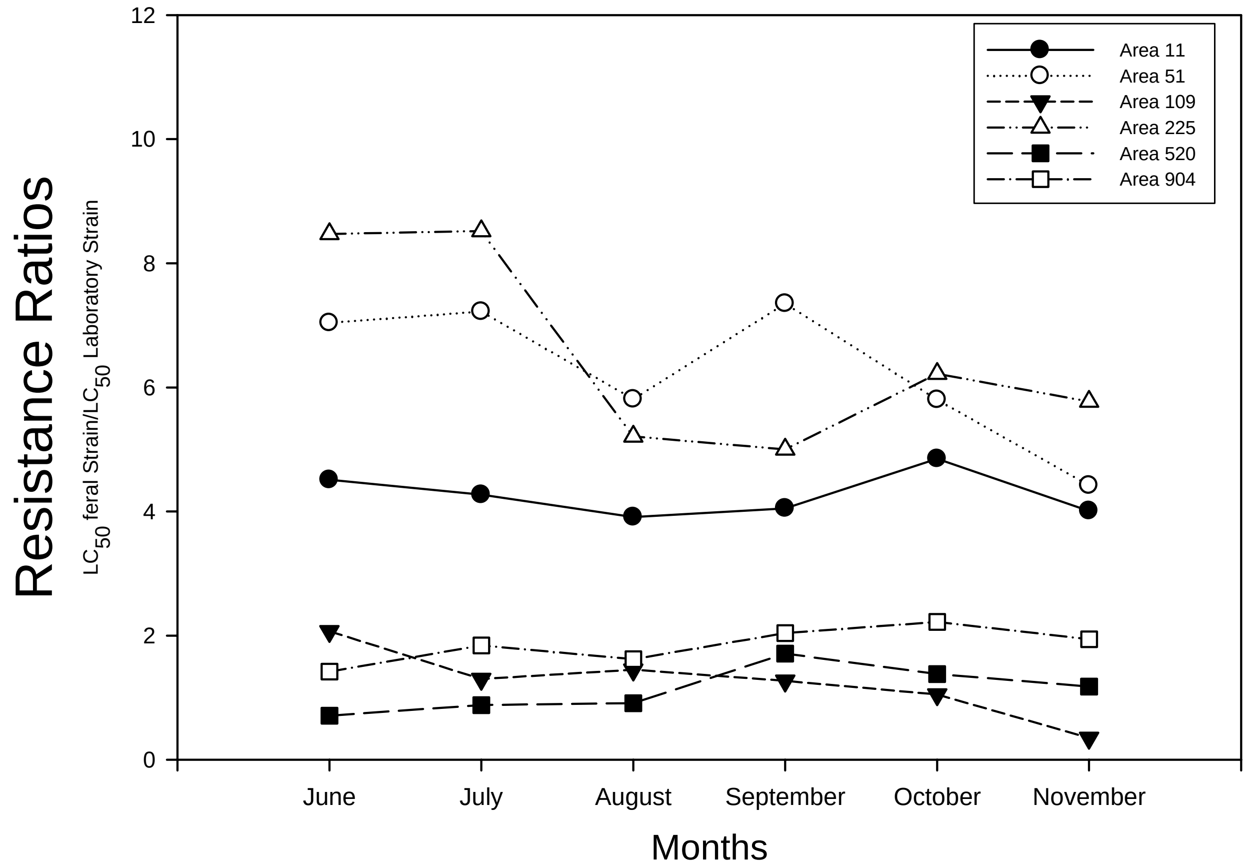

Fig. 45. Sumithrin resistance ratios for female *Culex quinquefasciatus* from six select operational areas sampled and tested over a six month period in Harris County, Texas, during 2005.

Populations from area 51 exhibited sumithrin resistance ratios that remained fairly constant peaking in September (RR_{50} = 7.35) 2005 before declining over the final two months ending with a resistance ratio of 4.42 (Fig. 45). Populations from areas 11, 109, 520, and 904 had only minor fluctuations in their sumithrin resistance ratios with populations from area 11 had higher resistance sumithrin when tested in 2005, thus separating it from the other three areas in terms of the baseline of resistance detected in this mosquito population (Fig. 45). The mosquito populations of the three other areas (109, 520, and 904) tested had sumithrin resistance ratios that exhibited overlapping and bunching that ranged between 0 and 2 over the entirety of the testing period (Fig. 45).

The resistance ratios for mosquito populations from five (11, 109, 225, 520, and 904) of the six areas tested against pyrethrum ranged between 0 and 2 and had little variation between the monthly testing, and considerable grouping of the pyrethrum resistance ratios for the duration of the study (Fig. 46). The mosquitoes from area 51 had higher resistance to pyrethrum, thus separating it from the grouping of the other five populations tested. Populations from area 51 demonstrated a gradual increase in their resistance to pyrethrum over the first four months peaking in September (RR_{50} = 3.79) before decreasing over the final two months, finishing with a resistance ratio of 2.66 (Fig. 46).

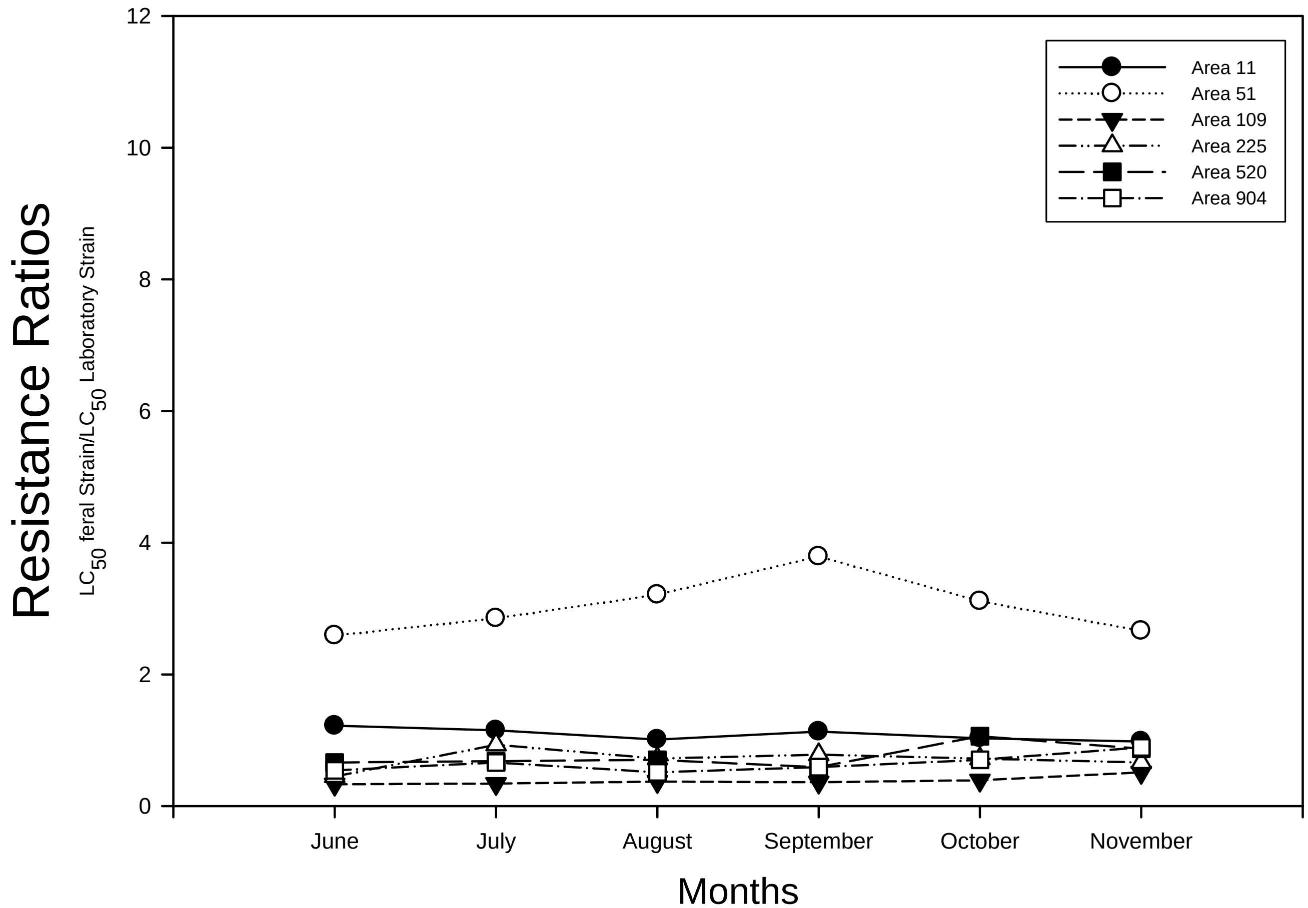

Fig. 46. Pyrethrum resistance ratios for female *Culex quinquefasciatus* from six select operational areas sampled and tested over a six month period in Harris County, Texas, during 2005.

The results acquired from the monthly bioassay testing in Brazos County demonstrated minor variations in the monthly insecticide resistance ratios of the *Cx. quinquefasciatus* populations as was previously demonstrated in *Cx. quinquefasciatus* populations from Harris County. The mosquito populations from the three collection areas in Brazos County had fairly similar insecticide resistance ratios and exhibited minor variations with considerable overlapping and grouping of the monthly resistance ratios over the duration of the study period when tested against resmethrin (Fig. 47), permethrin (Fig. 48), sumithrin (Fig. 49), pyrethrum (Fig. 50), and naled (Fig. 51).

Brazos County mosquito populations had a slight separation between malathion resistance ratios of the populations sampled and tested from the three collection areas. Populations from area 1 had malathion resistance ratios that separated it from the grouping of the two areas whose resistance ratios ranged from 0 to 2. The malathion resistance ratios for the mosquito populations from area 1 remained fairly constant with minor fluctuations that peaked in August (RR_{50} = 4.20) and October (RR_{50} = 4.14) 2005 but decline in the final month (RR_{50} = 3.34) (Fig. 52). The mosquito populations from areas 6 and 7 were grouped together with regard to their resistance ratio values for malathion, which ranged between 0 and 2 with considerable similarity of the resistance ratios observed each month of the testing period (Fig. 52).

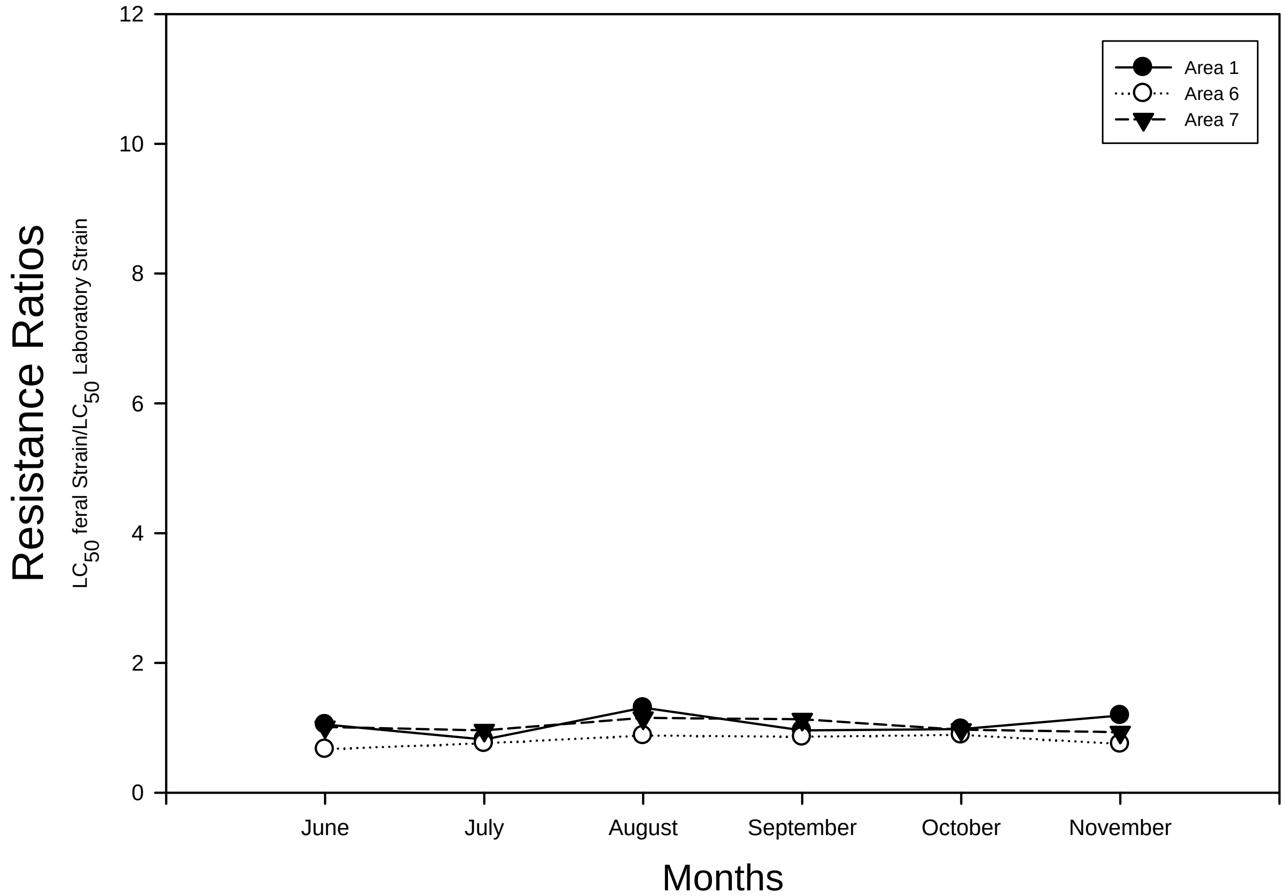

Fig. 47. Resmethrin resistance ratio for female *Culex quinquefasciatus* from three areas sampled and tested over a six month period in Brazos County, Texas, during 2005.

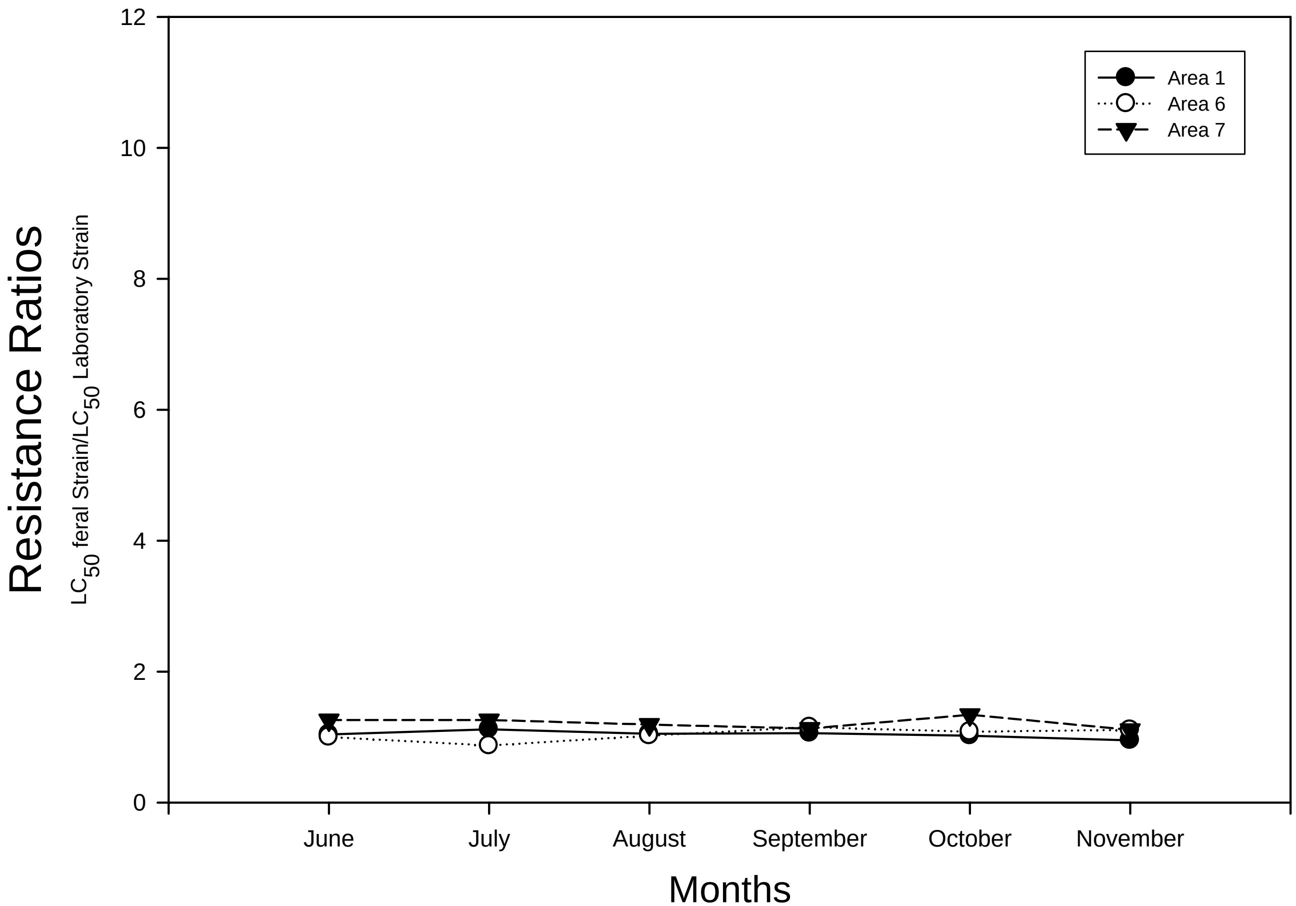

Fig. 48. Permethrin resistance ratio for female *Culex quinquefasciatus* from three areas sampled and tested over a six month period in Brazos County, Texas, during 2005.

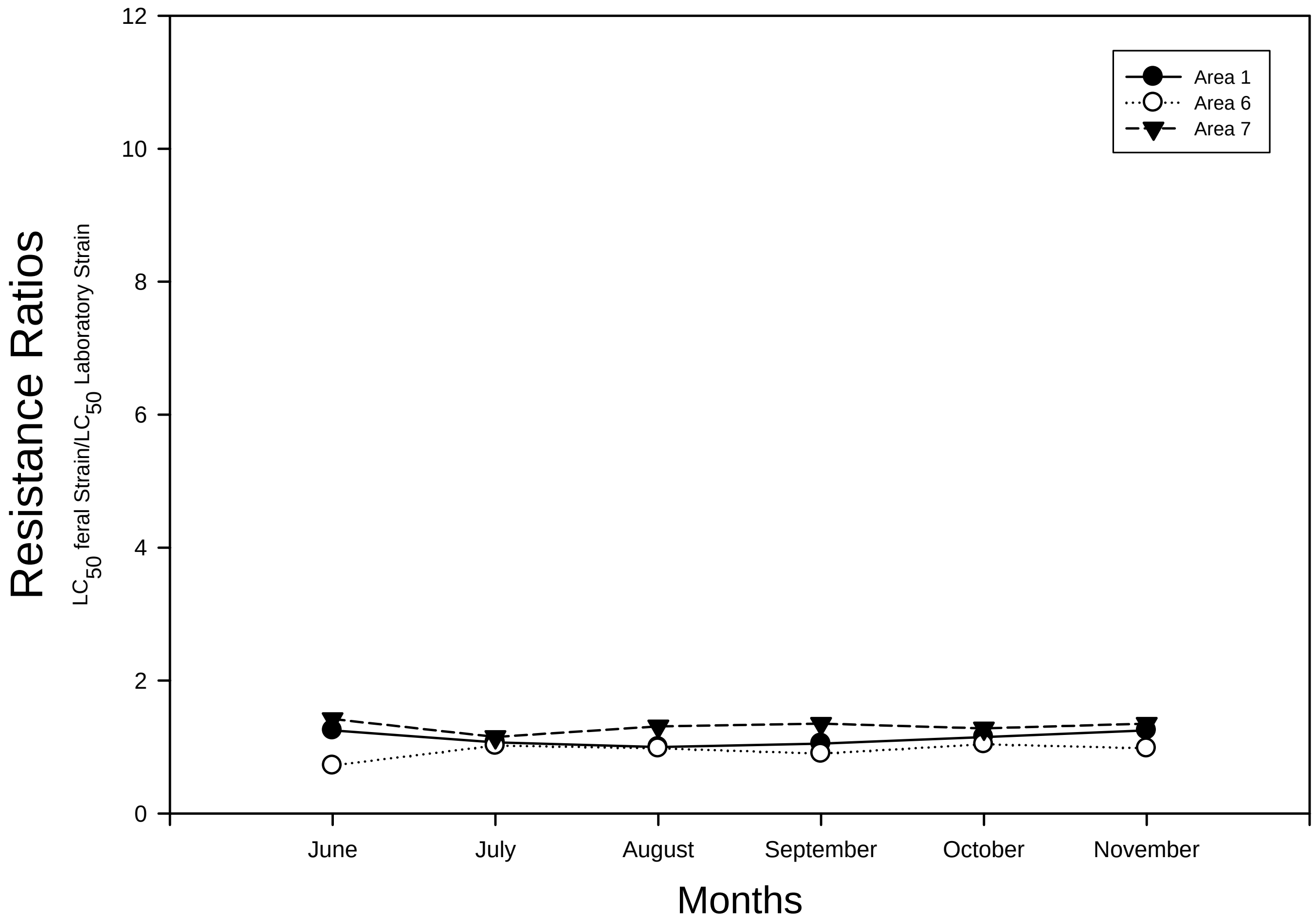

Fig. 49. Sumithrin resistance ratio for female *Culex quinquefasciatus* from three areas sampled and tested over a six month period in Brazos County, Texas, during 2005.

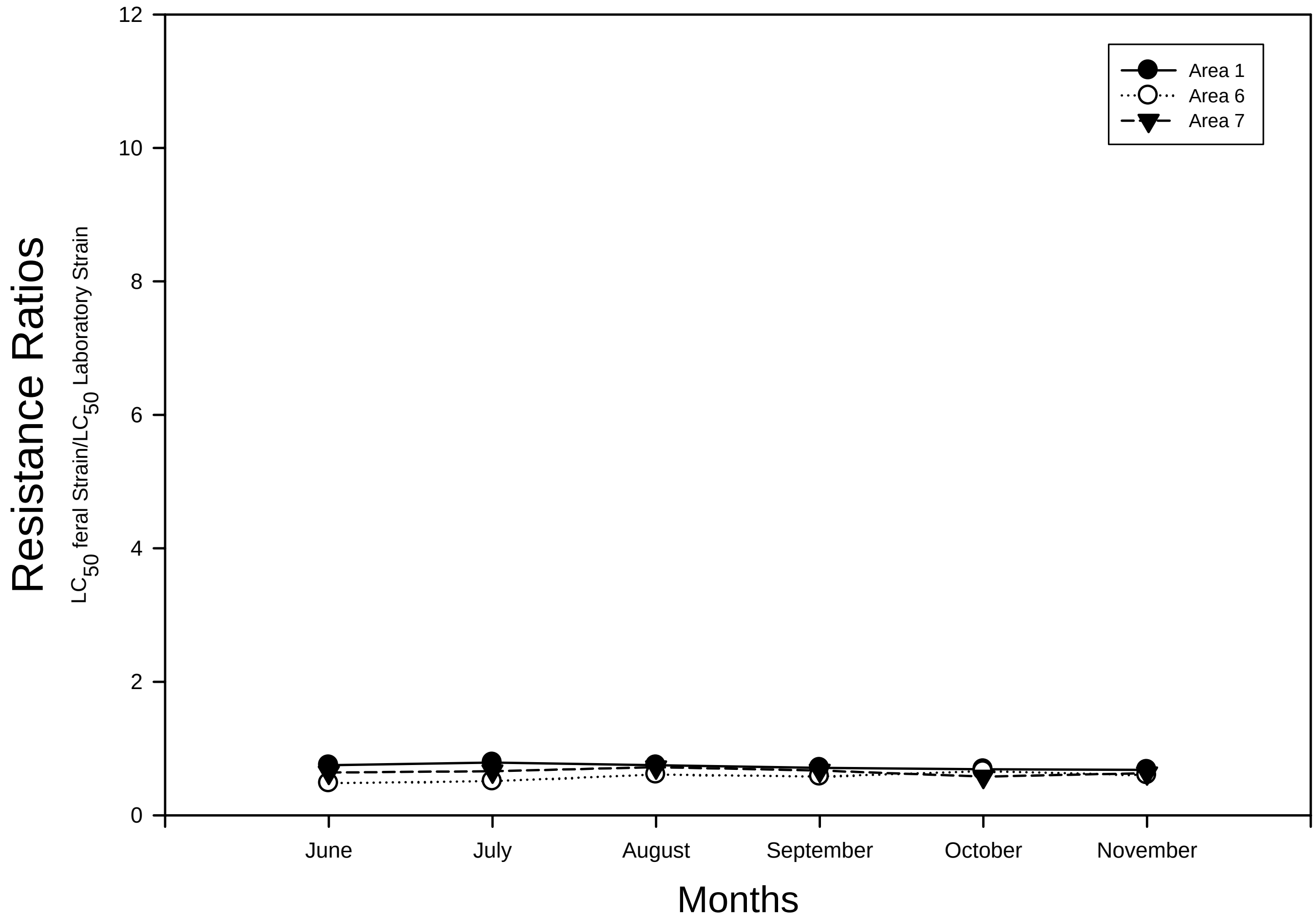

Fig. 50. Pyrethrum resistance ratio for female *Culex quinquefasciatus* from three areas sampled and tested over a six month period in Brazos County, Texas, during 2005.

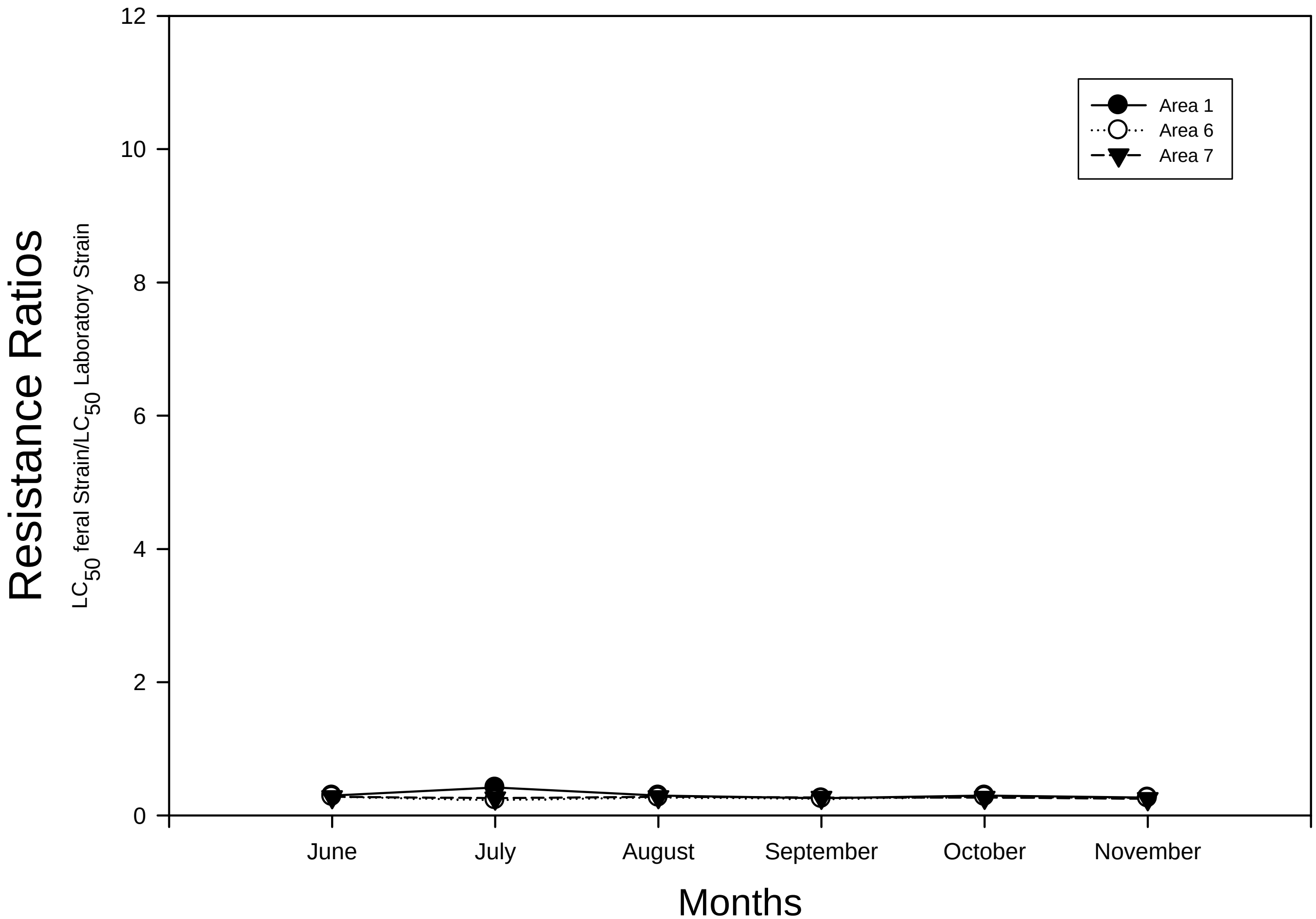

Fig. 51. Naled resistance ratio for female *Culex quinquefasciatus* from three areas sampled and tested over a six month period in Brazos County, Texas, during 2005.

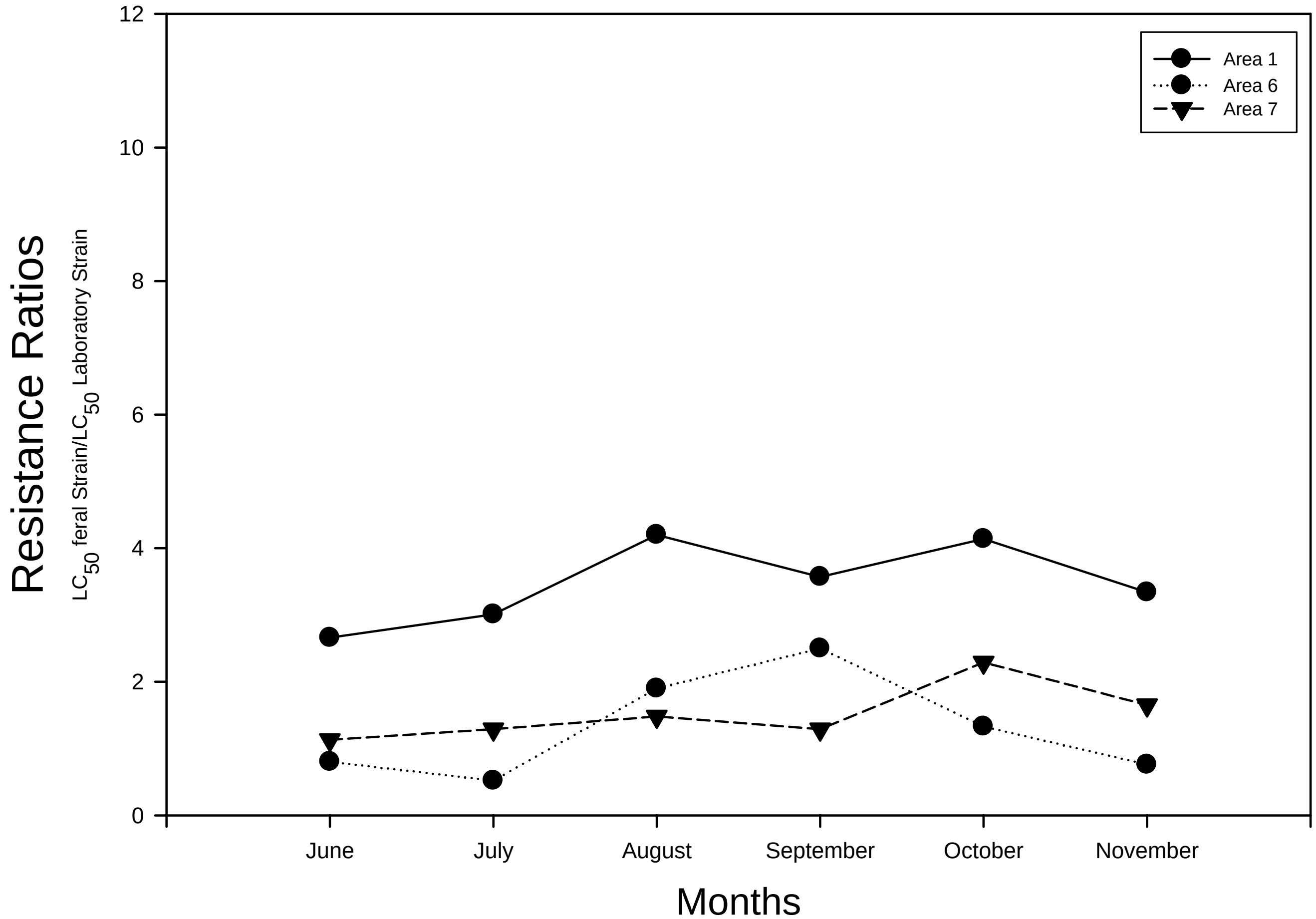

Fig. 52. Malathion resistance ratio for *Culex quinquefasciatus* from three areas sampled and tested over a six month period in Brazos County, Texas, during 2005.

Mosquito populations sampled and tested from southern Brazos County (area 6) had a gradual increase in their resistance to malathion from July (RR_{50} = 0.52) until it peaked in September (RR_{50} = 2.50) before declining over the final two months finishing with a resistance ratio of 0.76 (Fig. 52). Mosquitoes from area 7 had only minor fluctuations in their month to month resistance ratios over the duration of the study period. The population's malathion resistance ratios ranged from its highest peak October (RR_{50} = 2.29) to its lowest point in June (RR_{50} = 1.13) 2005 (Fig 52).

Discussion

The vial bioassay results from the six month study period had, that the *Cx. quinquefasciatus* populations had a trend that was denoted by grouping, overlapping, and minor fluctuations in the monthly level of resistance from the six areas (11, 51, 109, 225, 520 and 109) located throughout Harris County. These fluctuations may be attributed to natural genetic variability in the population, immigration of susceptible individuals into the population, suppression of the resistance mechanisms by the alternation of chemicals, or a combination of these factors. There was also a separation between the mosquito populations of the six operational areas by the level of resistance initially detected in this study. This finging may be attributed to greater selection pressure on the population from chemical control or possibly the composition of aquatic media in which the larvae developed. An example of this is found in the resistance ratios recorded over the six month study for Harris County populations to resmethrin (Fig. 43). Area 225 whose mosquito populations exhibited the highest resmethrin resistance ratios over the test period (Fig. 43) were subjected to heavy spray treatments with Scourge®

(resmethrin) by HCMCD due to the multiple cases of West Nile virus mosquito isolates in this area (Unpublished HCMCD data). Area 225 has also been consistently one of the areas in Harris County where WNv mosquito isolates were confirmed early each year, which in turn led to an extended period of treatment time and number of chemical treatments occurring in this area over a given year (Unpublished HCMCD data).

Areas 11 and 51 are both located within Interstate Highway Loop 610 (Fig. 5) and have historically received more chemical treatments because their proximity to downtown Houston and their history of having numerous arbovirus isolates recorded from their mosquito populations in the area (Baylor et al 1965, Lauderdale 1969, Chandler 2001, Lillibridge 2004, unpublished HCMCD data). The primary chemical used by the HCMCD for adult mosquito disease abatement for the past 12 years was the synthetic pyrethroid, Scourge® (resmethrin). This reliance on a single insecticide contributed to high resmethrin resistance ratios in the *Cx. quinquefasciatus* populations, of areas located inside Interstate Highway Loop 610 (Figs. 15-16) in Houston, Texas. Prior to 1994, the primary chemical used by HCMCD for adult mosquito disease treatments was Fyfanon® (Cythion®) or malathion, which may account for the regional resistance to organophosphates noted in the *Cx. quinquefasciatus* populations located inside Interstate Highway Loop 610 in Harris County (Figs 28-29). The insecticide pressure applied by adult mosquito control spray treatments manifests itself in the higher resistance ratios recorded for the populations in areas 11 and 51 as opposed to those in the other four areas (109, 225, 520, and 904) that were chosen for this study. This result would explain the separation of the resistance ratio lines from one another (Figs. 41, 43).

The selection pressure caused by resmethrin applications in recent years on the insects could also explain the high resistance ratios recorded for mosquito tested to permethrin and sumithrin to some extent, in Harris County because these chemicals belong to the same class of insecticides as resmethrin (pyrethroids) and work on the same target site in the mosquito even though they have never been used on the mosquito populations tested. The lack of major fluctuations in the monthly resistance ratios of the *Cx. quinquefasciatus* populations in the six areas tested in Harris County is a positive trend and demonstrated that the mosquito populations did not develop resistance as the HCMCD proceeded with its disease abatement activities throughout the year.

This study was conducted in 2005 which coincided with the implementation of an insecticide resistance management program by HCMCD which consists of alternation of malathion and resmethrin during the course of the operational year. No mosquito population was subjected to more than three treatments in a row with the same chemical during the 2005 calendar year which was based on the criteria determined by the insecticide resistance management program (Unpublished HCMCD data). The change of chemicals can be responsible for the lack of major variations observed between the monthly resistance ratios by suppressing the resistance mechanism of the mosquito populations and not giving them a change to adapt to the new chemical in use. These results provides anecdotal evidence that the resistance management program instituted is effectively suppressing the development of resistance in the mosquito populations in these six areas.

The variation in the level of resistance for the Brazos County mosquito populations from the three collection areas demonstrated only minor fluctuations in the monthly resistance ratios over the six moth study period. The fluctuations in the level of resistance in the *Cx. quinquefasciatus* population from Brazos County is can be attributed to natural genetic variation in the population. The lack of substantial change in the population is most likely because of the lack of chemical selection pressure on the *Cx. quinquefasciatus* mosquito populations in Brazos County.

The Brazos County mosquito populations had the most variation in their monthly resistance ratios when tested against malathion during this study period. The higher level of resistance may be attributed to use of this chemical in agricultural pest eradication programs (i.e., Boll Weevil Eradication Program) in the rural portions of Brazos County. Another possibility that may have contributed to the higher resistance ratios recorded in area 1 mosquito populations could be attributed to cross resistance from previous organophosphates (Dursban®) used in control programs by city officials from Bryan and College Station. This practice has since been abandoned.

The data produced from this study developed a resistance baseline for the City and County Health Departments of Brazos County and exhibited how the level of resistance naturally fluctuates from month to month. The results have no evidence that selection pressure was being applied to the mosquito population through pest control programs.

Future research is needed to help determine the significance of the results presented in this study. To help determine what effect the resistance management program initiated by HCMCD in 2005 had on the results obtain during the testing period, a duplicate study needs to be conducted in a county that conducts regular mosquito control with only a single insecticide. This will provide data that will support or refute the theory that the alternation of chemicals was suppressing the development of resistance in the mosquito populations.

Another modification to this research study was to extend the testing period from 6 months to a year. Extending the study period could determine what occurs to the level of resistance in the *Cx. quinquefasciatus* populations during the winter months in Harris County where no active mosquito control is practiced. This is the time period when it is hypothesized that susceptibility is bred back into the population from immigration of susceptible insects from surround areas (Taylor and Georghiou 1979). This study will provide a better overview of the status of resistance in the *Cx. quinquefasciatus* populations and the monthly fluctuations to occur in the populations that occurs over the whole year when subject to mosquito control tactics for a portion of the year.

The study was broken down further to test the mosquito populations weekly. This provided more data points for each month and allowed for statistical analyses to be performed on the data. This type of study was time and labor intensive and was focused on a minimal number of areas selected for their unique characteristics based on operational data.

CHAPTER VI

CORRELATION OF THE ANNUAL RESISTANCE RATIOS OF *Culex quinquefasciatus* IN BRAZOS AND HARRIS COUNTIES, TEXAS

The development of resistance can not be attributed to a single factor but is a process that depends on several independent dynamic factors that each contribute to the level and speed at which resistance arises in the population. These factors have been identified and classified into three broad categories designated genetic, biological, and operational factors (Georghiou and Taylor 1976). The recognition and manipulation of these factors may help retard resistance and should be an integral part of any control program. These aspects have also been combined into models for use in predicting the evolution of resistance in the population (Georghiou and Taylor 1977a,b). The additional predictive data derived in the accomplishment of the study described in this chapter provides yet another tool to help mosquito control agencies plan future activities.

Factors affecting insect population's resistance to insecticides in the genetic and biological categories are inherent qualities of the population and therefore, are beyond human control; but, their assessment is essential in determining the "risk for resistance" of a target population. Genetic factors include the frequency of the resistance alleles present and if the alleles are dominant or recessive in the population. These factors are difficult to determine and require intensive laboratory testing to measure their presence in an insect population. Biological factors are more easily measurable and include the bionomics and life histories of the particular insects targeted for control, as well as the environmental situation they develop in.

Operational factors affecting resistance are those related to the application of pesticides and are thought of as being under human control (Georghiou and Taylor 1986). Operational factors can be altered to an extent, depending on the risk of resistance that is revealed by laboratory testing of genetic and biological factors as long as it is operationally and economically feasible. This approach to managing resistance provides the greatest opportunities for countering the evolution of resistance by limiting the degree of selection pressure on the target population. Operational decisions include the timing, dose, mode of application, previous chemicals used, and formulation of pesticides used (Georghiou and Taylor 1976, Georghiou 1983, Georghiou and Taylor 1986) and should be taken into account when planning a control program and when assessing the effectiveness of the program.

This study was undertaken to determine if there was an association between the resistance ratios derived for mosquitoes in Harris and Brazos Counties, Texas, during the two-year resistance monitoring effort conducted in these counties as described in Chapter IV of this dissertation. If an association between these resistance ratios proves to be significant, then future status of resistance can be predicted for the mosquitoes in that particular area. This will give the operational agency a predictive value of the potential resistance status of the mosquito populations in the operational areas, giving the agency a head start on planning future control strategies including chemical choice.

Materials and Methods

The resistance ratios calculated in Chapter III were ranked from smallest to largest for each county and year. Only mosquitoes from areas that were tested both years were included in the correlation. A Spearman's rank correlation was run on the ranks from 2004 and 2005 for Harris County and from 2005 and 2006 for Brazos County. *A priori* the significance level was set at p = 0.100. The resistance ratio 50s (RR_{50}) were used in this study, since the responses to insecticides at this level (LC_{50}, RR_{50}) by any given insect population are less variable than for the responses at the RR_{95} (LC_{95}) level (Likitvong 1996).

Results

The correlation between the 2005 and 2006 resistance ratios for mosquitoes in Brazos County were significant for malathion (p = .007), naled (p = .036), resmethrin (p = .001), permethrin (p = .003), and sumithrin (p = .007) (Figs. 53-57). A definite trend was established between the resistance ratios for mosquitoes in Brazos County for the majority of chemicals tested. Pyrethrum was the only chemical for which the correlation of the mosquito resistance ratios was not significant (p = .147) (Table 4).

Correlation between 2004 and 2005 resistance ratios for mosquitoes in Harris County were significant for resmethrin (p = .063), permethrin (p = .007), and sumithrin (p = .091) (Figs. 58-60). The association of the resistance ratios between the two years for Harris County mosquitoes was most obvious in the results for permethrin and less apparent in the two other synthetic pyrethroids. The correlation was not significant for malathion, naled, and pyrethrum (Table 4).

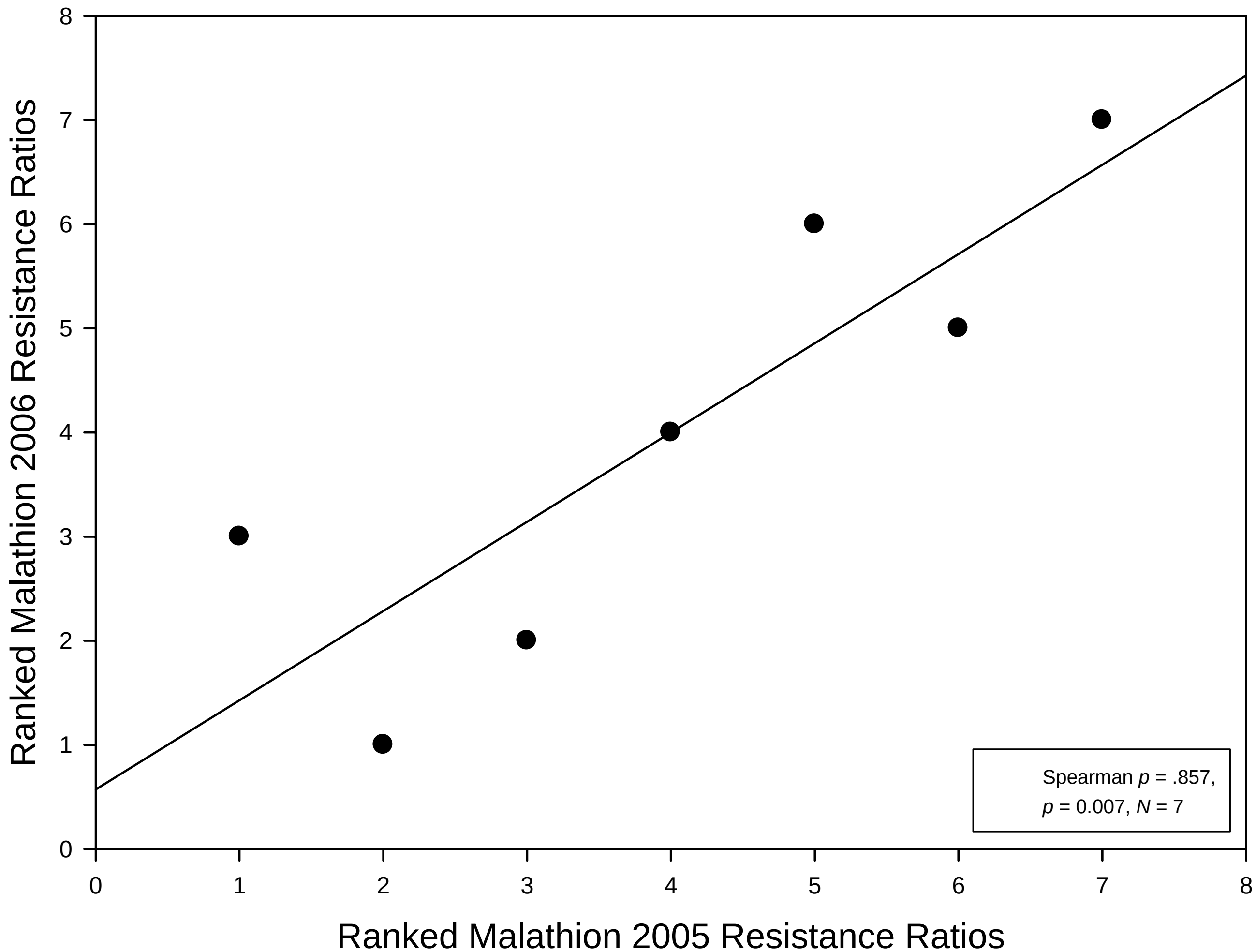

Fig. 53. Spearman correlation of 2005 vs. 2006 Brazos County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for malathion.

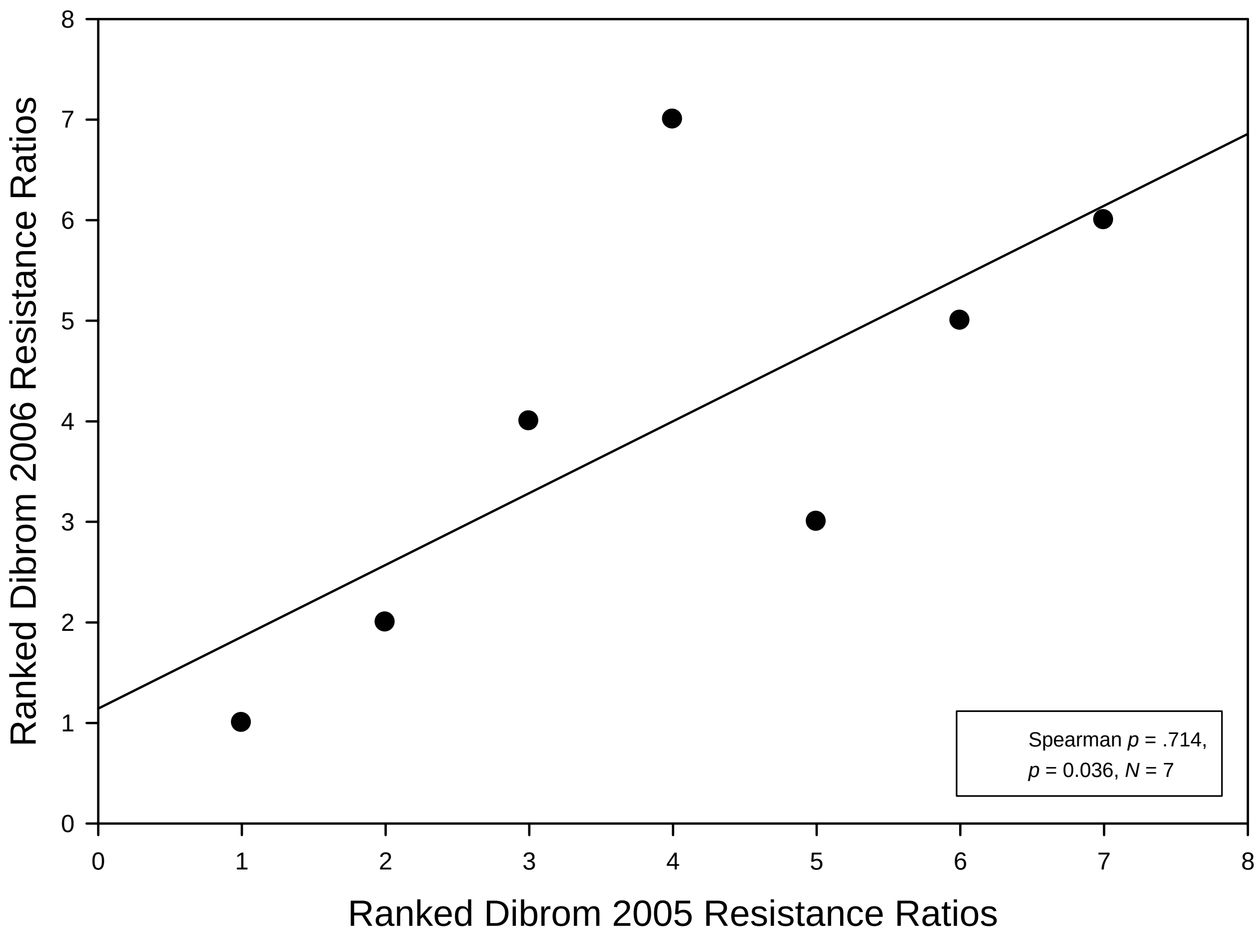

Fig. 54. Spearman correlation of 2005 vs. 2006 Brazos County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for naled.

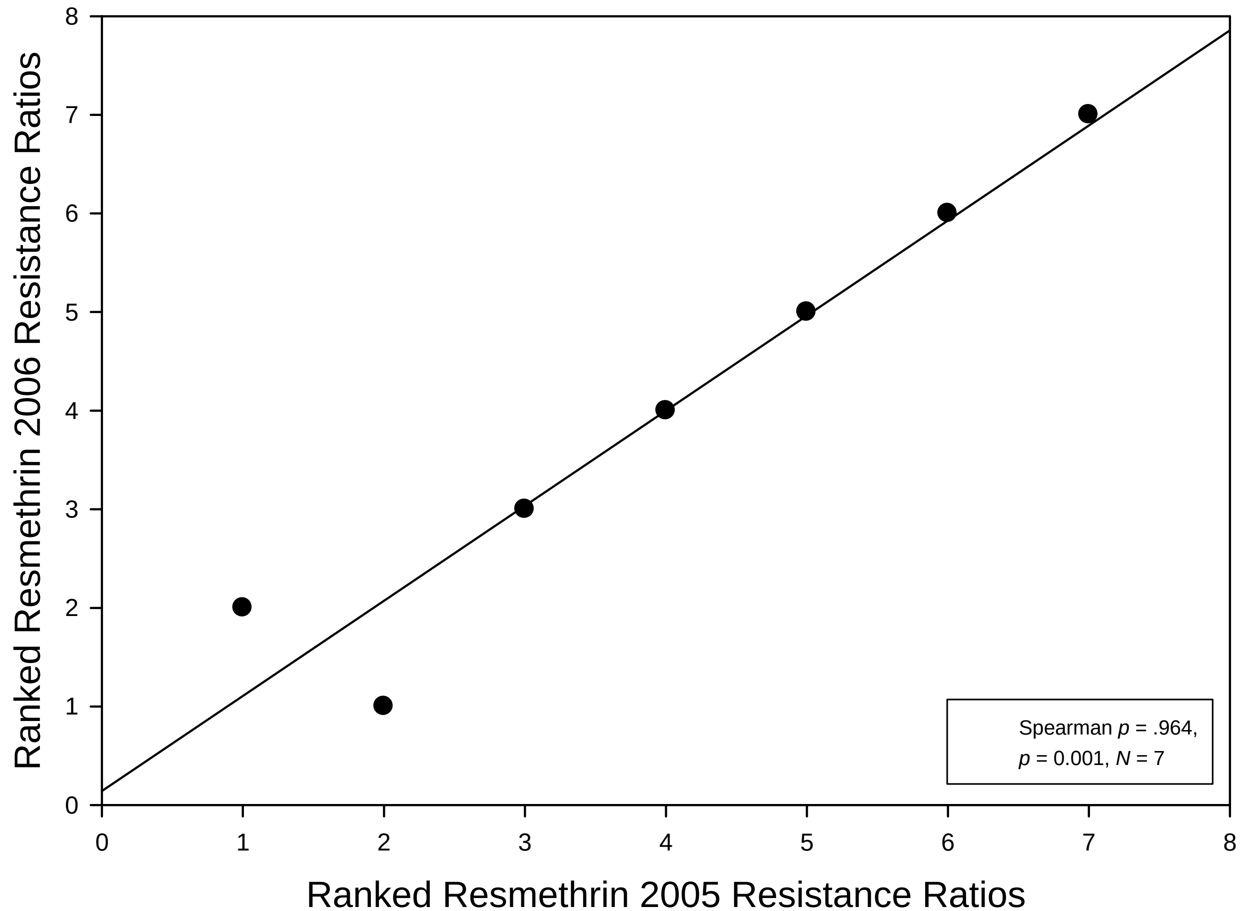

Fig. 55. Spearman correlation of 2005 vs. 2006 Brazos County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for resmethrin.

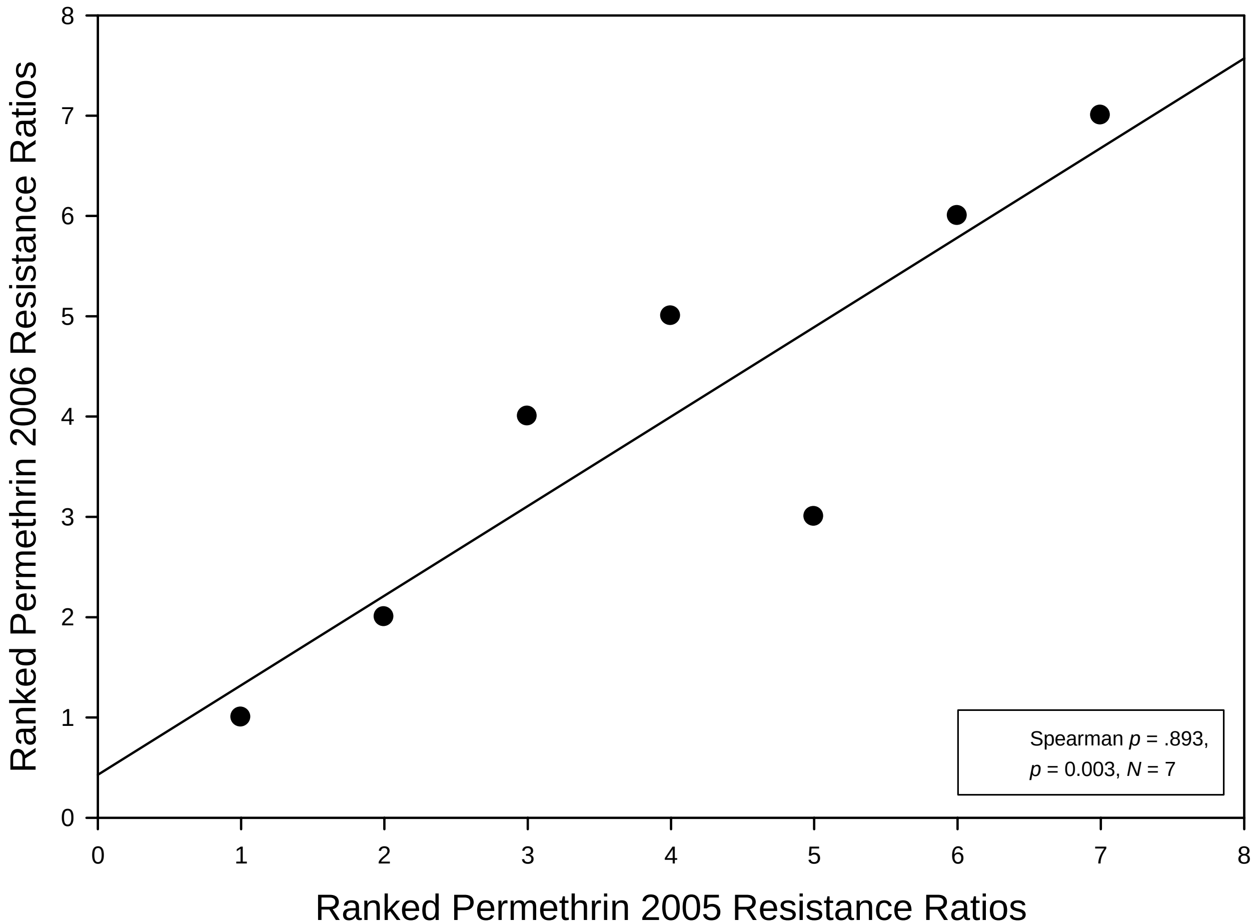

Fig. 56. Spearman correlation of 2005 vs. 2006 Brazos County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for permethrin.

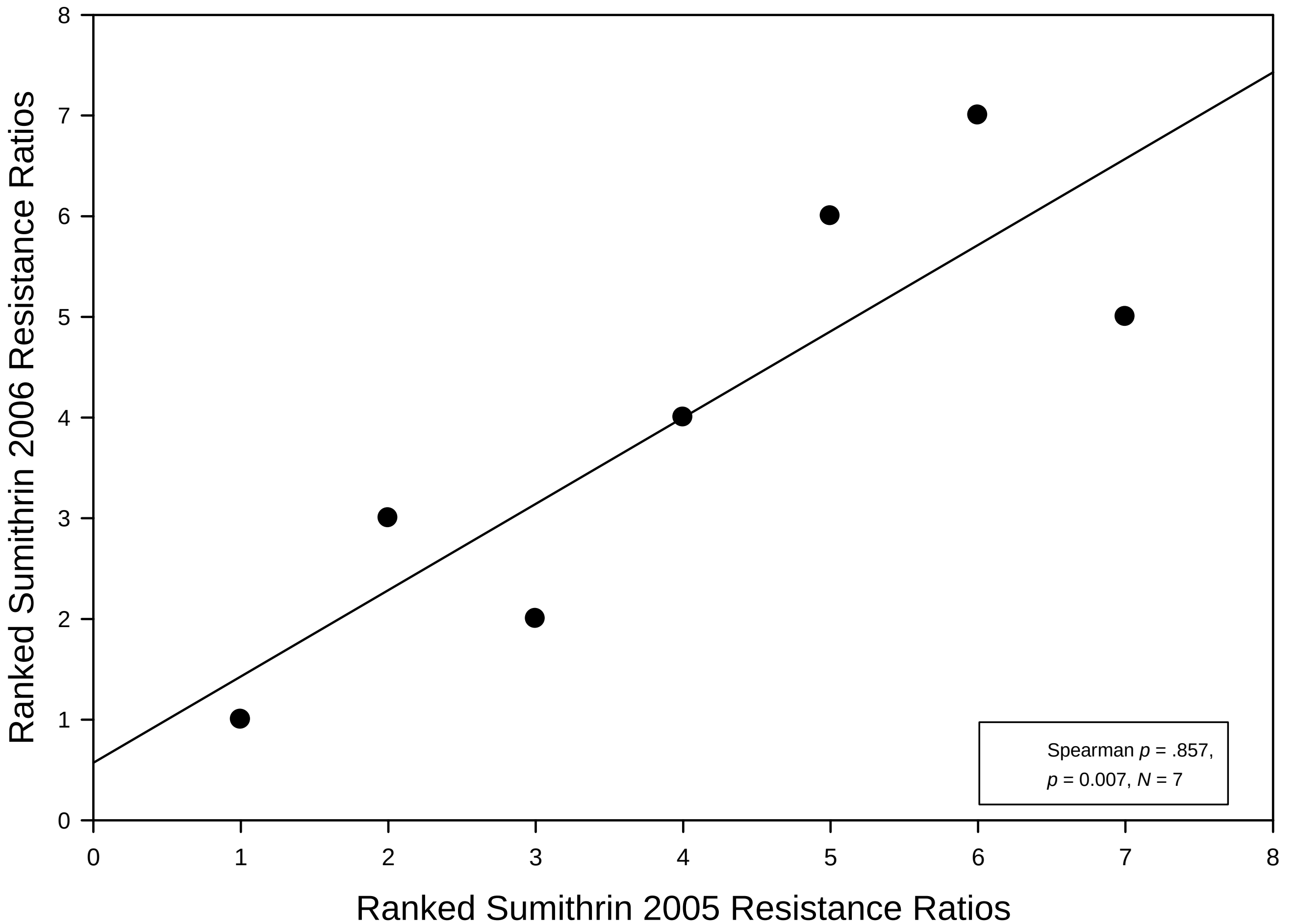

Fig. 57. Spearman correlation of 2005 vs. 2006 Brazos County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for sumithrin.

Table 4. Spearman rank correlations for female *Culex quinquefasciatus* in Harris and Brazos Counties that demonstrated no significance between the insecticide resistance ratios recorded over a two year test period (Harris County 2004-2005, Brazos County 2005-2006).

County	Chemical	Spearman's Correlation Coefficient	Significance	N
Harris	Malathion	.215	.157	24
Harris	Naled	-.171	.271	15
Harris	Pyrethrum	.200	.290	10
Brazos	Pyrethrum	.464	.147	7

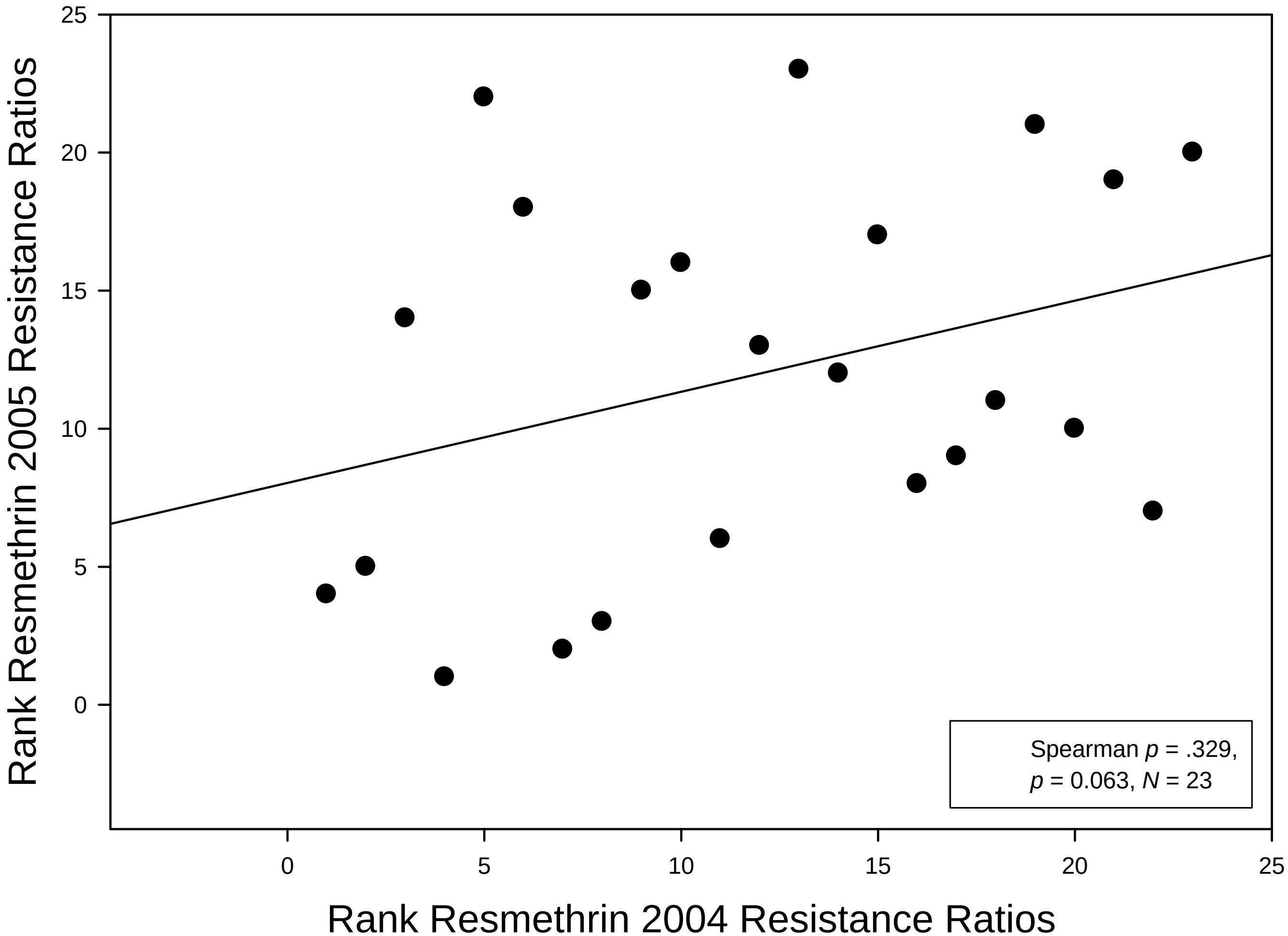

Fig. 58. Spearman correlation of 2004 vs. 2005 Harris County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for resmethrin.

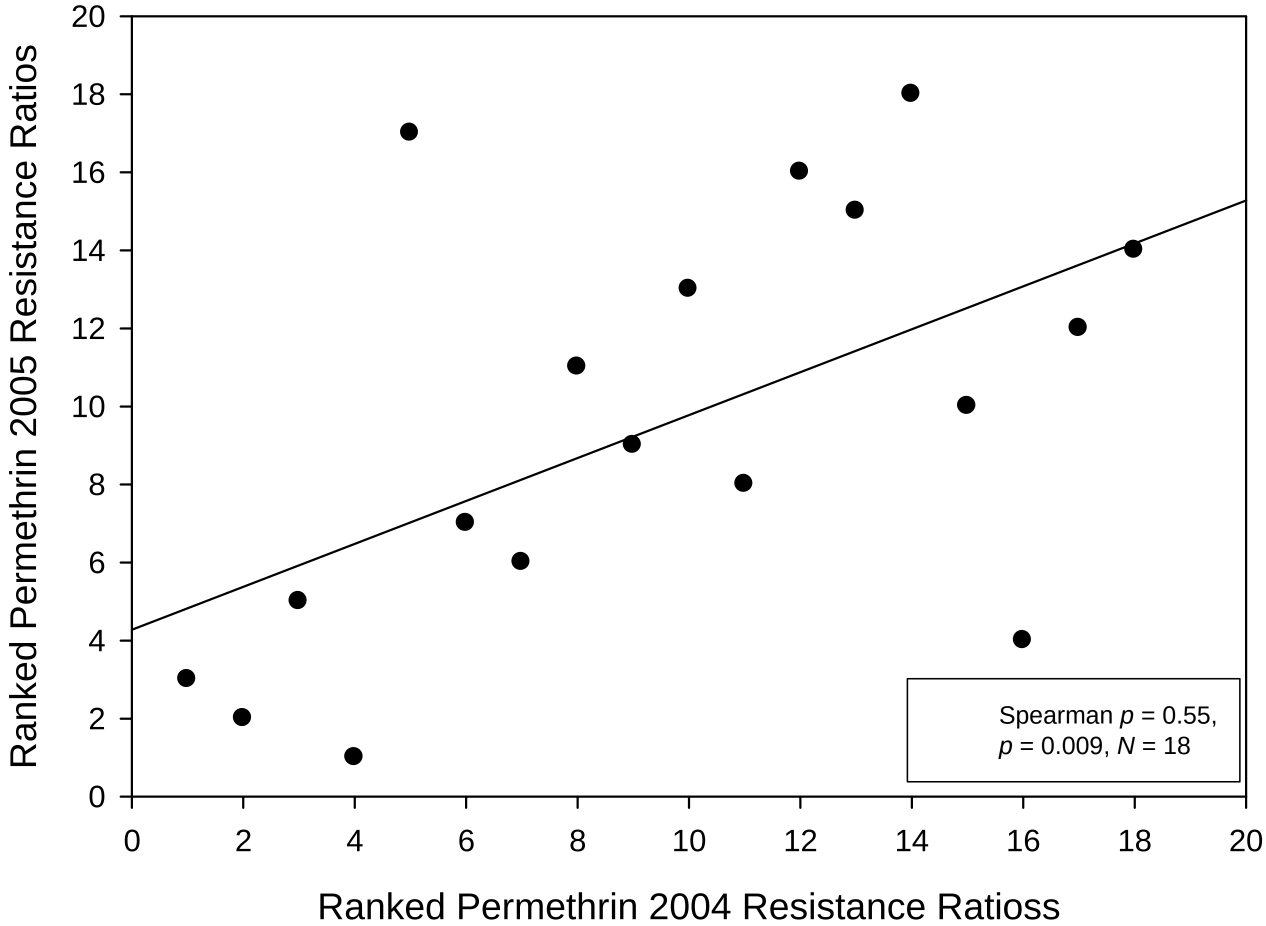

Fig. 59. Spearman correlation of 2004 vs. 2005 Harris County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for permethrin.

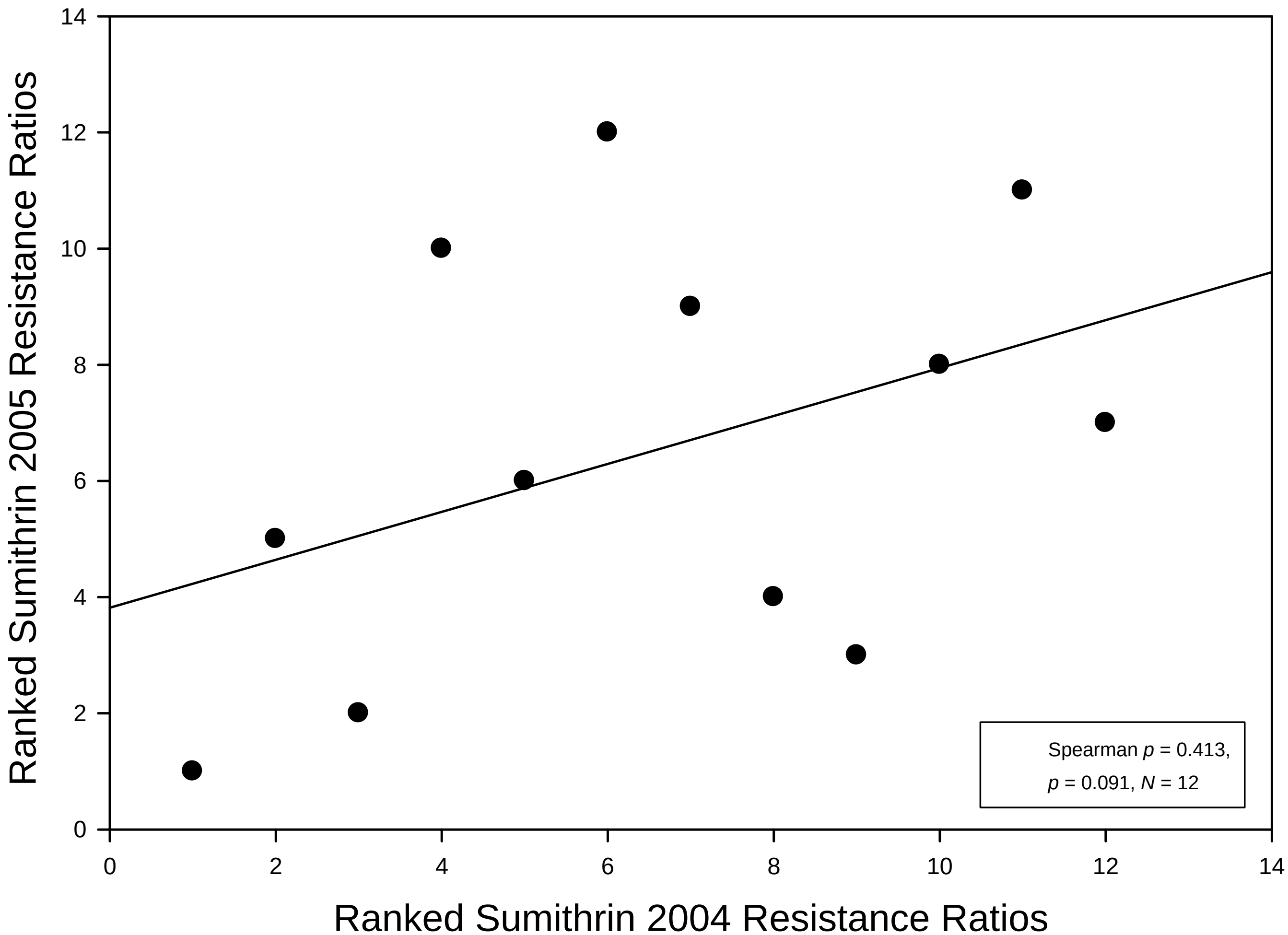

Fig. 60. Spearman correlation of 2004 vs. 2005 Harris County, Texas, female *Culex quinquefasciatus* insecticide resistance ratios (RR_{50}s) for sumithrin.

Discussion

The strong correlation between most of the insecticide resistance ratios for Brazos County mosquitoes over the two-year study period was expected because of the lack of insecticidal pressure on these particular populations of mosquitoes. The resistance ratios had little variation between the two years, thus a high association exists. This information can be used to develop future operational strategies, if an organized control program is established in Brazos County. If a correlation results in a positive association between the resistance ratios of the mosquito population between years the level of resistance in the subsequent year can be estimated and chemical choice can be made to select against the resistance mechanism present in the mosquito population.

The only chemical for which an association between the mosquito resistance ratios failed to be obtained during 2005 and 2006 in Brazos County was pyrethrum. County and local city agencies perform only minimal amounts of mosquito control in Brazos County, placing only slight selection pressure on their *Cx. quinquefasciatus* populations. However, pyrethrum is an active ingredient in several agricultural and commercial chemicals available on the market. It is possible that the use of one or more of these chemicals by private contractors could lead to selection pressure being placed on the Brazos County populations of *Cx. quinquefasciatus*, thus selecting of the portion for a populations that can survive the insecticide. The subsequent progeny of the individuals that survived the chemical selection inherits the genetic makeup of their resistance parentage. Also, since the rank correlation is based on resistance ratios from only seven mosquito populations in Brazos County a major change in a just a few of these populations can throw off the association between the two years.

Recently, the pest control industry has begun to market backyard spray systems that discharge a chemical treatment on a predetermined timetable. One chemical that has been advertised for use in the spray system is that of pyrethrum, because it is labeled as a “natural” chemical. These systems expose populations of mosquitoes to regular applications of insecticide and have the possibility of applying heavy select pressure on the target mosquito populations.

It has been shown that there is cross resistance in insects for pyrethrum and DDT (dichloro-diphenyl-trichoroethane) (Malcolm 1988). Prior to its being banned for us in the United States in 1972, DDT was the primary chemical used in agricultural and vector control programs (Lauderdale 1969, WHO 1970, WHO 1980, Georghiou 1990). Populations of mosquitoes in Brazos County have had minimal exposure to pyrethrum but have no doubt been exposed to agricultural application of DDT. This prior selection for resistance against DDT may be still present at some level in the populations of *Cx. quinquefasciatus* in the county and can translate to some degree of increased pyrethroid resistance in the county’s mosquito populations.

Mosquito populations in the operational areas of Harris County showed a greater fluctuation in their resistance ratios from one year to the next. These variations were somewhat expected due to the mosquito control operations conducted by the HCMCD division in these areas. The selection pressure placed on the populations through the application of chemical control tactics can increase the resistance ratio between years or with the implantation of an insecticide resistance management program (as occurred in Harris County in 2005), could lead to a decreases in resistance ratios for the pyrethroids tested. The fluctuations in resistance ratios caused by the activities of the HCMCD most likely attributed to large variation between the rankings for resistance ratios derived in 2005 as opposed to those derived in 2004 and caused the correlation of ratios for malathion, naled, and pyrethrum to be insignificant. There was a significant correlation between the two years worth of resistance ratios gathered on Harris County mosquito populations tested against the three pyrethroids (resmethrin, permethrin, and sumithrin) Again, this information can be used as a tool in planning future control activities and choosing the chemical to use in the various operational areas of Harris County. With the estimated level of resistance in the population for the subsequent year calculated from this correlation the proper chemical for alternation purposes (i.e., malathion or resmethrin) can be chosen to select against the resistance mechanism present in the mosquito population.

HCMCD’s use of natural pyrethrum is primarily limited to thermal fogging the storm sewer systems in operational areas located inside Interstate Highway Loop 610 in Harris County and in Houston. The absence of proper storm sewer systems because of insufficient size of storm sewer systems required for adequate treatment, lack of manpower and equipment (one thermal fog truck), and the location of the storm sewer systems are all factors entering into why such systems are not treated in other areas of the county. The threshold necessary to elicit a thermal fog treatment of storm sewers in Harris County in the absence of disease (e.g., West Nile virus, St. Louis encephalitis) are storm sewer trap collections being more than 1000 female *Cx. quinquefasciatus* per trap. When the trap count threshold is not exceeded, storm sewer treatments will still be carried out if a virus positive mosquito pool (group of 50 mosquitoes for virus testing) is acquired from a storm sewer trap. This leads to different operational areas receiving varying amounts of insecticidal pressure on the population of mosquitoes. This variation might explain the difference in the rank of resistance ratios in the Harris County mosquito populations included in this study for pyrethrum in the various Harris County operational areas and the lack of association between them. As in Brazos County, the backyard spray systems are being heavy pushed by pest control operators in Harris County for control of biting flies. These systems apply a periodic application of pesticide on a predetermined schedule, which also may be contributing to the selection pressure on the population in certain areas of Harris County when spray systems are concentrated in some areas.

Based on the test results from studies conducted in Harris County in 2004, malathion has been reinstituted as a chemical control option by HCMCD. The vial bioassay resistance ratio baselines determined for malathion resistance in Harris County *Cx. quinquefasciatus* populations during 2004 indicated low resistance to malathion existed in mosquito populations throughout the county. Harris County mosquito populations received selection pressure from control tactics utilizing malathion in 2005 for the first time in five years thus affecting the resistance ratios gathered in 2005. The change in chemical use in 2005 resulted in an increase in some of the resistance ratios of the mosquito populations thus increasing their assigned rank in the 2005 data set. The changes between the assigned rank of the 2004 and 2005 resistance ratios resulted in insignificance of the correlation.

Naled is a chemical that is only applied by private contractors through aerial applications in rural portions of Harris County, which lack significant infrastructure (i.e. roads) to successfully conduct spray operations from ground based truck mounted spray units. Correspondingly, resistance ratios in Harris County mosquito populations for naled were consistently low for all areas tested for both years. Small increases or decreases in the resistance ratios can severely alter the rank leading to insignificance in the correlation even though the susceptibility is maintained in the target mosquito population to this chemical.

The results of the bioassay tests on the field collected mosquito populations demonstrated a significance association between the resistance ratios of all three synthetic pyrethroids collected over the two years. This association allows the possibility of predicting the future resistance ratios for the areas. This could be a useful tool for the Operations Branch of HCMCD when planning future control activities for the various areas. If the area has a consistently high resistance rank for the two years, this should lead to a modification in insecticide choice from a pyrethroid to malathion to begin the following year.

A possible cause of the variation in the Harris County mosquito resistance ratios for resmethrin occurring between 2004 to 2005 is very likely the result of the implementation of the resistance management program of insecticide rotation by HCMCD. With the change of chemical classes, the populations that had high resistance ratios to resmethrin became susceptible, thus selecting against mosquitoes with resistance mechanisms primed against pyrethroids. The reduction of number of individuals of expressing resistance genotypes leads to progeny with reduced resistance ratios when collected and tested.

Future resistance testing will be necessary to determine if the trends noted during this study are maintained over a longer period of time. Since malathion was only used in 2005, future testing is needed to determine if the resistance ratios can be used in a predictive nature. Data collected from bioassay tests in 2006 should correlate with the data from 2005 due to the operational situations being more comparable. Another modification to future testing might be the addition of more operational areas to the correlation matrix, which possibility would help validate the trend that is described from the results described herein.

CHAPTER VII

CORRELATION OF THE INSECTICIDE RESISTANCE RATIOS FOR *Culex quinquefasciatus* TO THE NUMBER OF SPRAY EVENTS CONDUCTED IN AN OPERATIONAL AREA IN HARRIS COUNTY, TEXAS.

In 1949, the Texas State Legislature gave legal recognition to the fact that mosquitoes pose a threat to the health and well-being of the citizens of the state by passing enabling legislation allowing for the establishment and funding of organized mosquito control districts along the Gulf Coast region of Texas (Micks 1965). This legislative action provided the justification for the development of organized mosquito control programs whose primary concern was the control of salt marsh mosquitoes that migrate into the cities from the surrounding coastal marshes along the Gulf Coast of Texas. Harris County was one of the last Gulf Coast counties amongst those establishing districts to establish a mosquito control district; but, it finally did, and this was due largely to a St. Louis encephalitis (SLE) outbreak in Houston in 1964, which resulted in 711 human cases and 33 deaths (Baylor et al. 1965). Because the district was founded as a result of an outbreak of disease, the primary mission of the Harris County district as established by its charter, has been the surveillance for mosquito-borne encephalitides and the control of mosquitoes that vector these diseases, with *Cx. quinquefasciatus* being the species of main importance in this regard (Bartnett et al. 1969).

Since the inception of organized mosquito control in Texas, the principle reliance has been placed upon adulticiding (Micks 1965). In the past, when resistance developed to the point of operational failure, a district would change to another chemical and use it until the target mosquito populations developed resistance to the new chemical and then, another chemical change would be made. This trend was viable as long as the chemical industry was able to keep producing novel insecticides at a rate faster than resistance was developing in the mosquito populations. This approach to handling insecticide resistance problems related to mosquito control has become undesirable because of the deficiency of new insecticides being developed by industry for mosquito control, due to the financial burden of bringing new chemistry through the discovery, development, efficacy and safety assessment phases to market and the small market that vector control represents on a global basis. Since adulticiding is still a major part of any given mosquito control district's vector control program, the development of resistance by its target mosquito populations is a devastating blow to its operational effectiveness. To combat the development of resistance, most of the mosquito control districts in Texas have now begun to turn to insecticide resistance management programs to preserve mosquito susceptibility to the few chemicals that are still labeled for adult mosquito control in the United States.

HCMCD has employed a variety of insecticides over its history (Fig. 61), with the primary chemicals used being the synthetic pyrethroid, resmethrin (Scourge®) and the organophosphate, malathion (Fyfanon®).

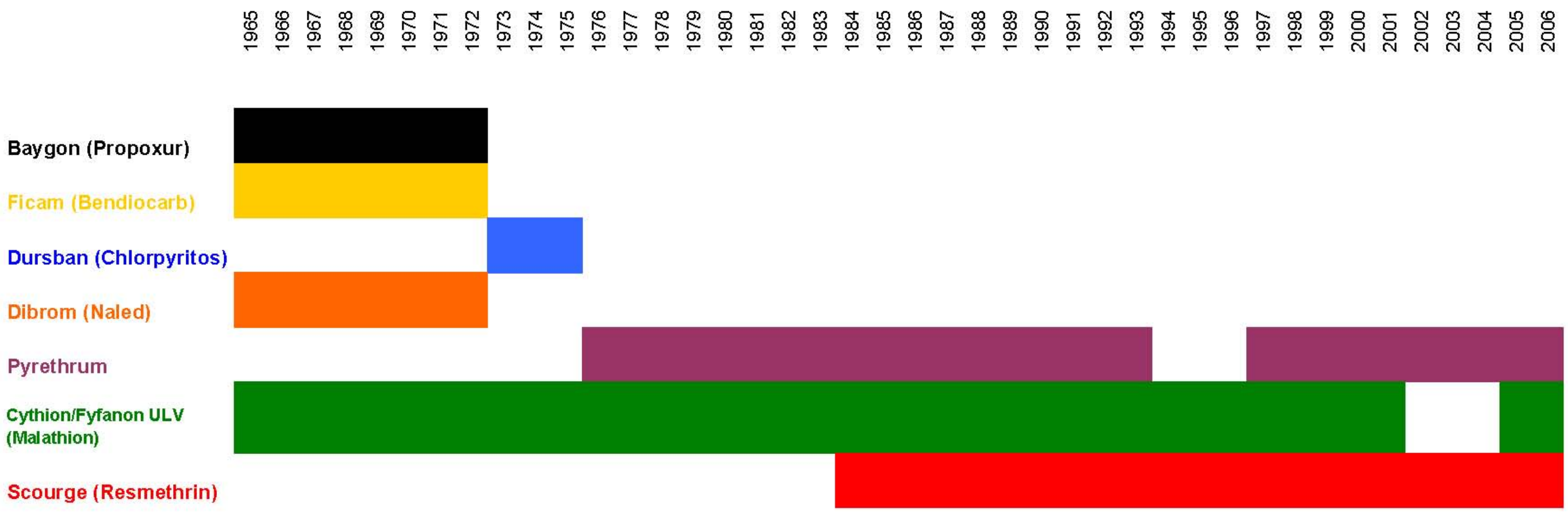

Fig. 61. Harris County Mosquito Control Division's mosquito adulticide chemical use history 1965-2006.

Since 2005, these two chemicals have been used in an insecticide resistance management program implemented in Harris County that is based on a rotation of these two chemicals throughout the year. In the immediate years prior to this program's implementation, the only chemical used for adult suppression was resmethrin which led to evidence of resistance developing in certain populations of the Harris County *Cx. quinquefasciatus* (Dr. Ray E. Parsons, personal communications). The resistance management program was begun by HCMCD in 2005 and was based on the bioassay data produced by this researcher and field cage test data produced by HCMCD personnel conducted in 2004. These data gave cause for a modification of the operational philosophy of the Harris County control district. The Field Operation Branch of HCMCD is always looking for novel predictive methods that can help HCMCD make better operational decisions so as to ensure the success of the program and the system of tests developed in 2004 lent themselves to the division's predictive needs.

The study described herein was undertaken to determine if there was a association between the insecticide resistance ratios (RR_{50}) for Harris County *Cx. quinquefasciatus* populations acquired from vial bioassays conducted in 2004 and 2005 and the number of chemical treatments (spray events) performed by HCMCD on an annual basis during a three year period (2003-2005). If a positive RR_{50} association exists, it may be possible to use this relationship to develop a method for predicting the level of resistance that might come to occur in target mosquito populations based on the number of spray events conducted on an annual basis in the area where these populations are located.

Materials and Methods

A Pearson's correlation analysis was run to determine if there was an association between the magnitude of the *Cx. quinquefasciatus* insecticide resistance ratios calculated from vial bioassays performed in Harris County on mosquitoes in 27 areas in 2004 and in 45 areas in 2005 and the number of insecticide spray events conducted in these operational areas by the HCMCD. Malathion, resmethrin, and pyrethrum were the three adulticidial chemicals included in the study due to their use for mosquito control activities in Harris County in the years just before and during the time when this study was conducted (Fig. 61). The data on spray treatments per area were provided by Ms. Christina Hailey Dischinger, GIS Coordinator and Trainer at HCMCD. Two correlation analyses were preformed on the resistance ratios from 2004 and 2005 to test for an association between the number of spray events conducted in the year prior to when the resistance ratios were performed (i.e., 2004 resistance ratios vs. 2003 spray events) and the number of spray events in the same calendar year (i.e., 2004 resistance ratios vs. 2004 spray events). The only chemical treatments included in the correlation were applications performed prior to the mosquitoes used in the resistance ratio tests being removed from each given area. The spray missions that occurred after this point were excluded from the data set.

The correlation analyses were then run including and excluding "zeros" from the data matrix. These "zeros" represent operational areas that did not receive an application of insecticide during the time period prior to the removal of the mosquito egg rafts from the environment which ultimately gave rise to the adults that were used in a given resistance ratio test. This was done to determine if these placeholders affected the association between the two variables (resistance ratios vs. spray events) positively or negatively. The result of the correlations with and without zeros were included in the final results to demonstrate the effect these placeholders have on the association ultimately derived.

A priori, the significance level was set at p = 0.050. The resistance ratio 50s (RR_{50}) were used for this objective, since the responses to insecticides at this level (LC_{50}, RR_{50}) by any given insect population are less variable than they are for the responses at the RR_{95} (LC_{95}) level (Likitvong 1996).

Results

The results of the correlation analysis of the Harris County *Cx. quinquefasciatus* 2004 resmethrin resistance ratios and the number of spray events using Scourge® performed by the HCMCD for 2003 and 2004 are summarized in Fig. 62 and Table 5. A significant correlation (p = 0.033) was observed between the 2004 *Cx. quinquefasciatus* resmethrin resistance ratios and 2003 spray events performed by the HCMCD (Fig. 62). However, the results obtained from the correlation analysis run between the 2004 resistance ratios and the 2004 spray events demonstrated no significant association between the variables when both of the data sets (including and excluding zeros) were analyzed (Table 5).

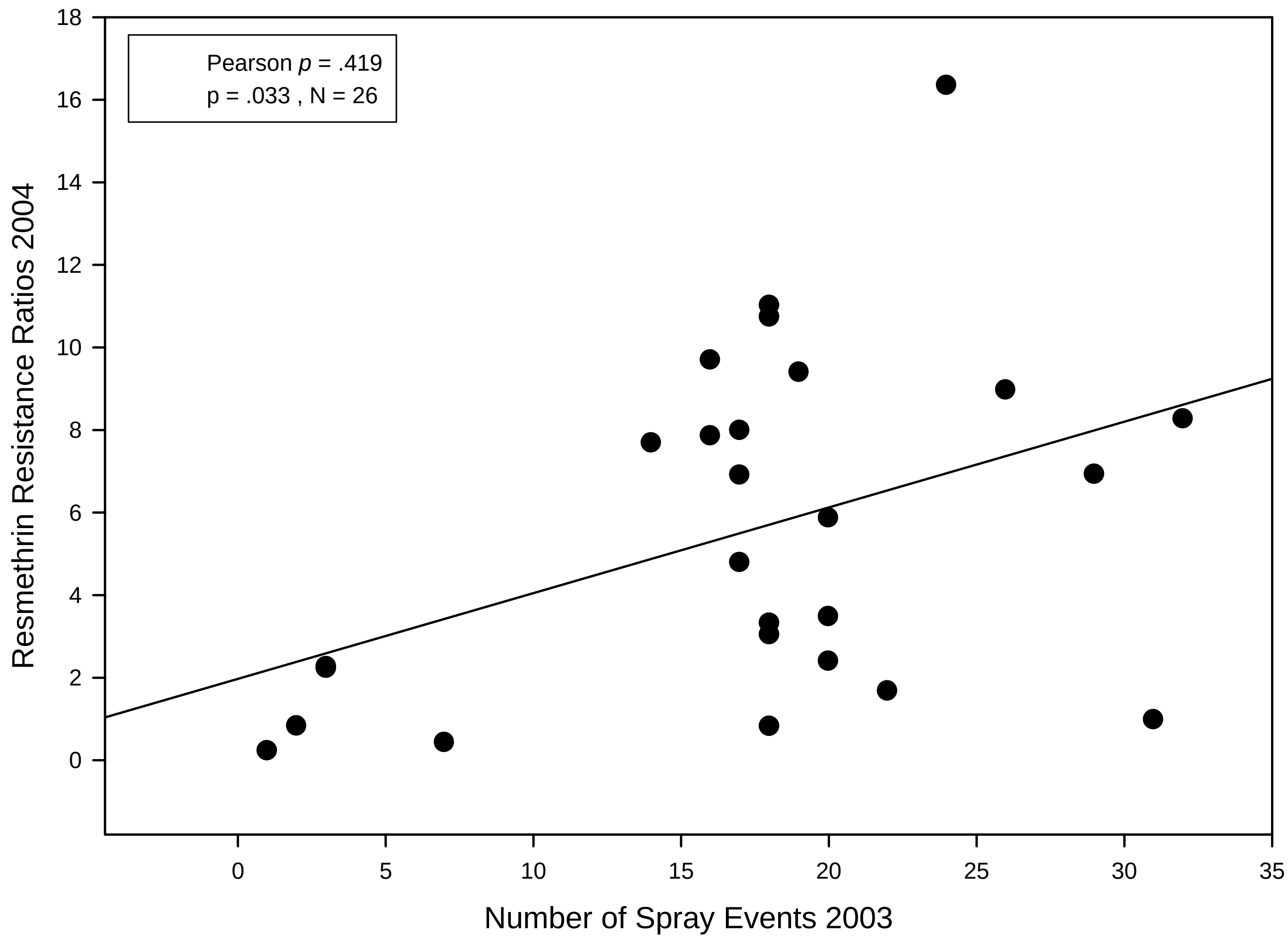

Fig. 62. Pearson correlation of resmethrin resistance ratios for female *Culex quinquefasciatus* in 2004 to the number of spray events conducted during 2003 in select operational areas in Harris County, Texas.

Table 5. Results for chemicals found not to be significant in the Pearson's correlation analysis run between *Culex quinquefasciatus* resistance ratios and the number of spray events conducted over a two year period in selected areas of Harris County, Texas.

	Pearson Correlation	Significance	N
Resmethrin			
2004 resistance ratios vs. 2004 spray events with zeros	-0.047	0.818	26
2004 resistance ratios vs. 2004 spray events without zeros	-0.237	0.288	22
Pyrethrum			
2004 resistance ratios vs. 2003 spray events with zeros	-0.361	0.250	12
2004 resistance ratios vs. 2003 spray events without zeros	-0.428	0.217	10
2004 resistance ratios vs. 2004 spray events with zeros	-0.007	0.982	12
2004 resistance ratios vs. 2004 spray events without zeros	-0.67	0.887	7
2005 resistance ratios vs. 2004 spray events with zeros	0.139	0.381	42
2005 resistance ratios vs. 2004 spray events without zeros	0.038	0.868	21
2005 resistance ratios vs. 2005 spray events with zeros	0.300	0.054	42
2005 resistance ratios vs. 2005 spray events without zeros	0.220	0.470	13

The correlation analysis performed on the 2005 data produced a significant association between the 2005 *Cx*. *quinquefasciatus* resmethrin resistance ratios (with the zeros included) and the number of spray events using Scourge® in 2004 (p = 0.006) and 2005 (p = 0.003) (Figs. 63-64). When the zeros were excluded from the data set, the significance was lost (p = 0.182) between the 2005 resmethrin resistance ratios and the number of spray events in 2005 (Fig. 65). However, the correlation with the 2004 spray events with the 2005 resmethrin resistance ratios still proved to be significant (p = 0.018) with the zeros excluded from the data set (Fig. 66).

The correlation analysis demonstrated a significant association between the 2005 Harris County *Cx*. *quinquefasciatus* malathion resistance ratios and the number of spray events using Fyfanon® performed by the HCMCD in 2005 for both data sets analyzed (Fig.67-68). The significance observed in the correlation between the 2005 malathion resistance ratios and the 2005 spray events with zeros included in the data set (p = 0.003) decreased but still held when the zeros were excluded (p = 0.013). There was no significant correlation detected in any of the eight combinations of resistance ratios to spray events run for the *Cx*. *quinquefasciatus* populations against pyrethrum (Table 5).

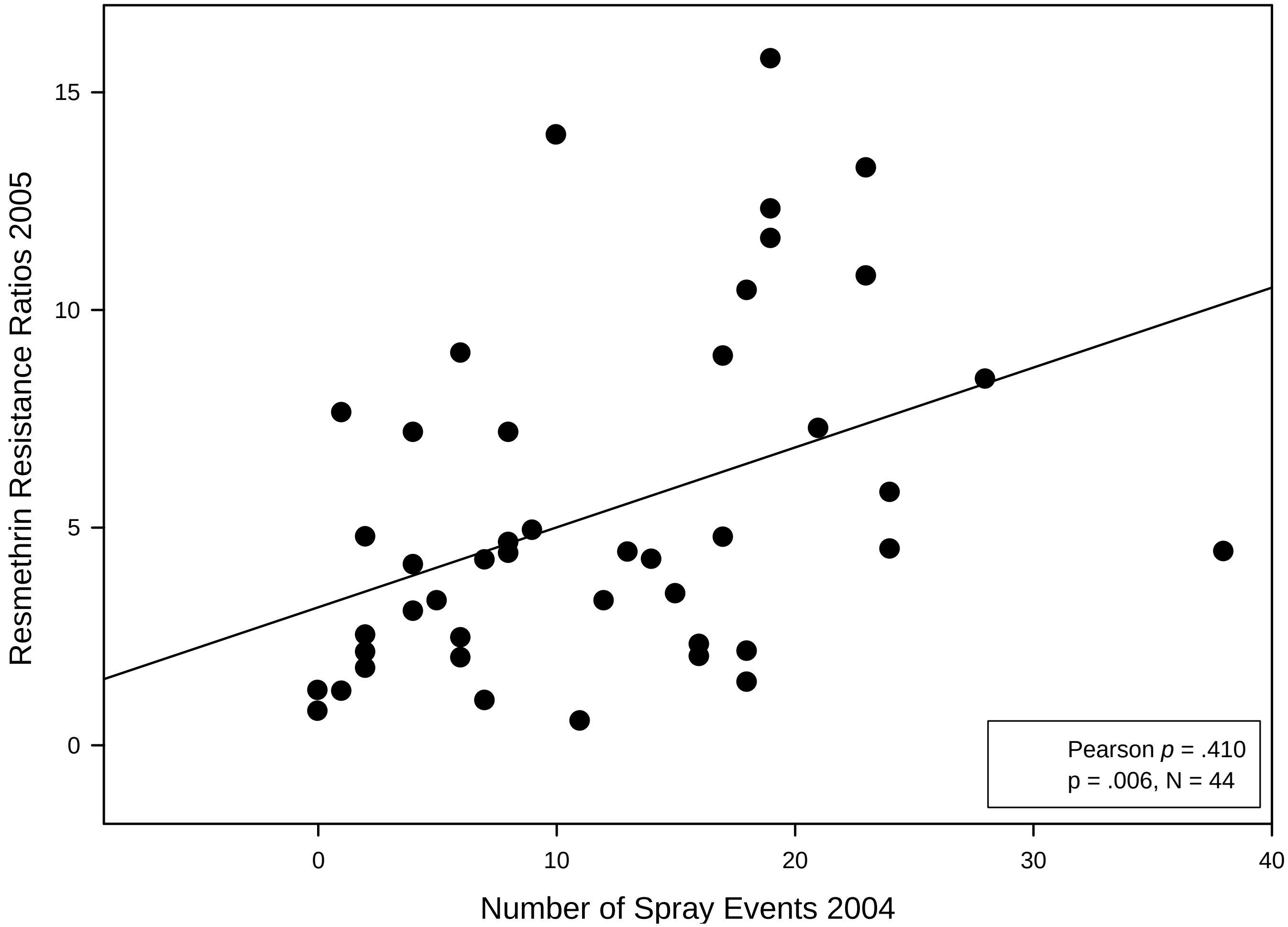

Fig. 63. Pearson correlation of resmethrin resistance ratios for female *Culex quinquefasciatus* 2005 to the number of spray events conducted during 2004 in select operational areas in Harris County, Texas (with zeros included).

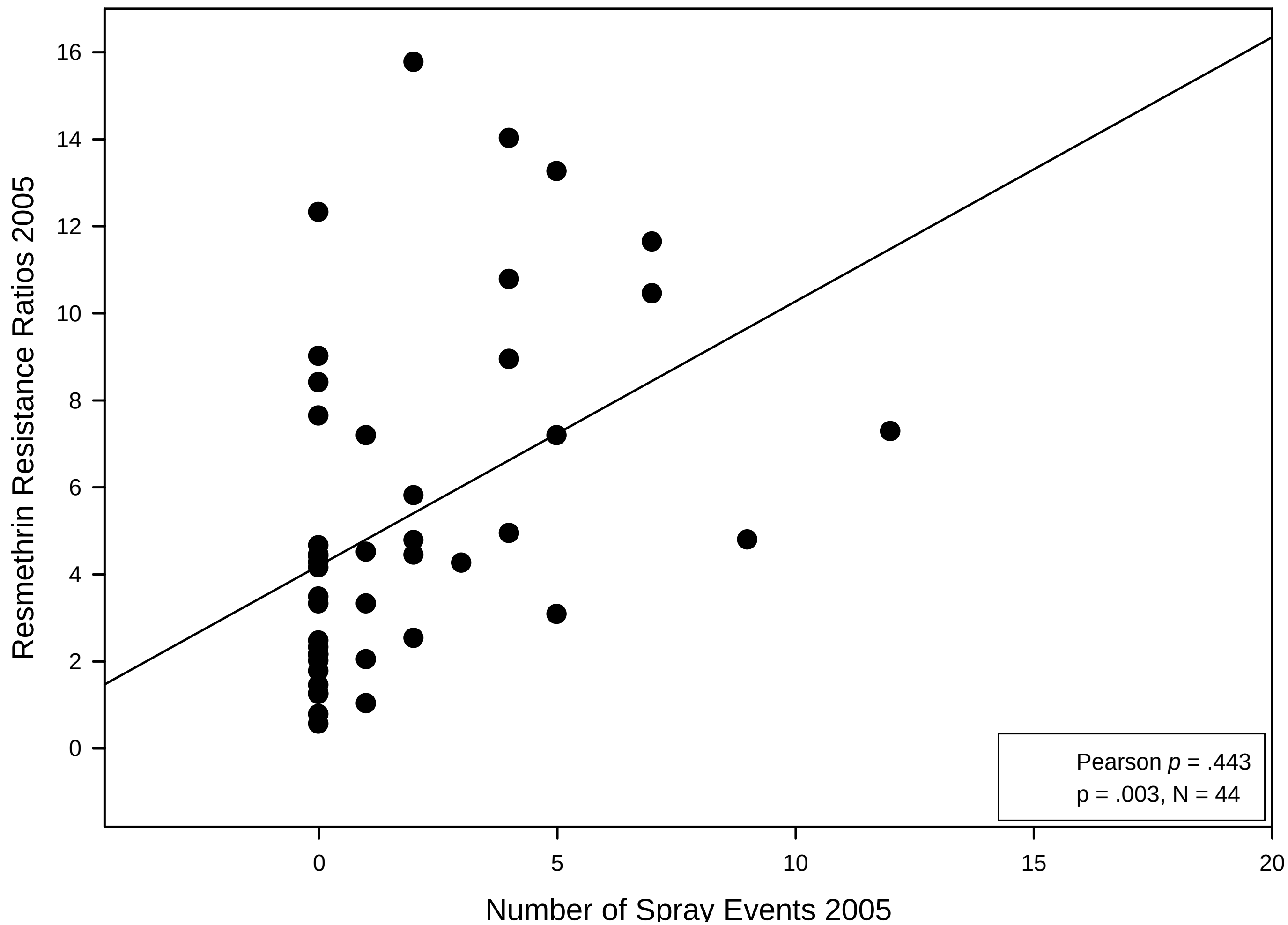

Fig. 64. Pearson correlation of resmethrin resistance ratios for female *Culex quinquefasciatus* in 2005 to the number of spray events conducted during 2005 in select operational areas in Harris County, Texas (with zeros included).

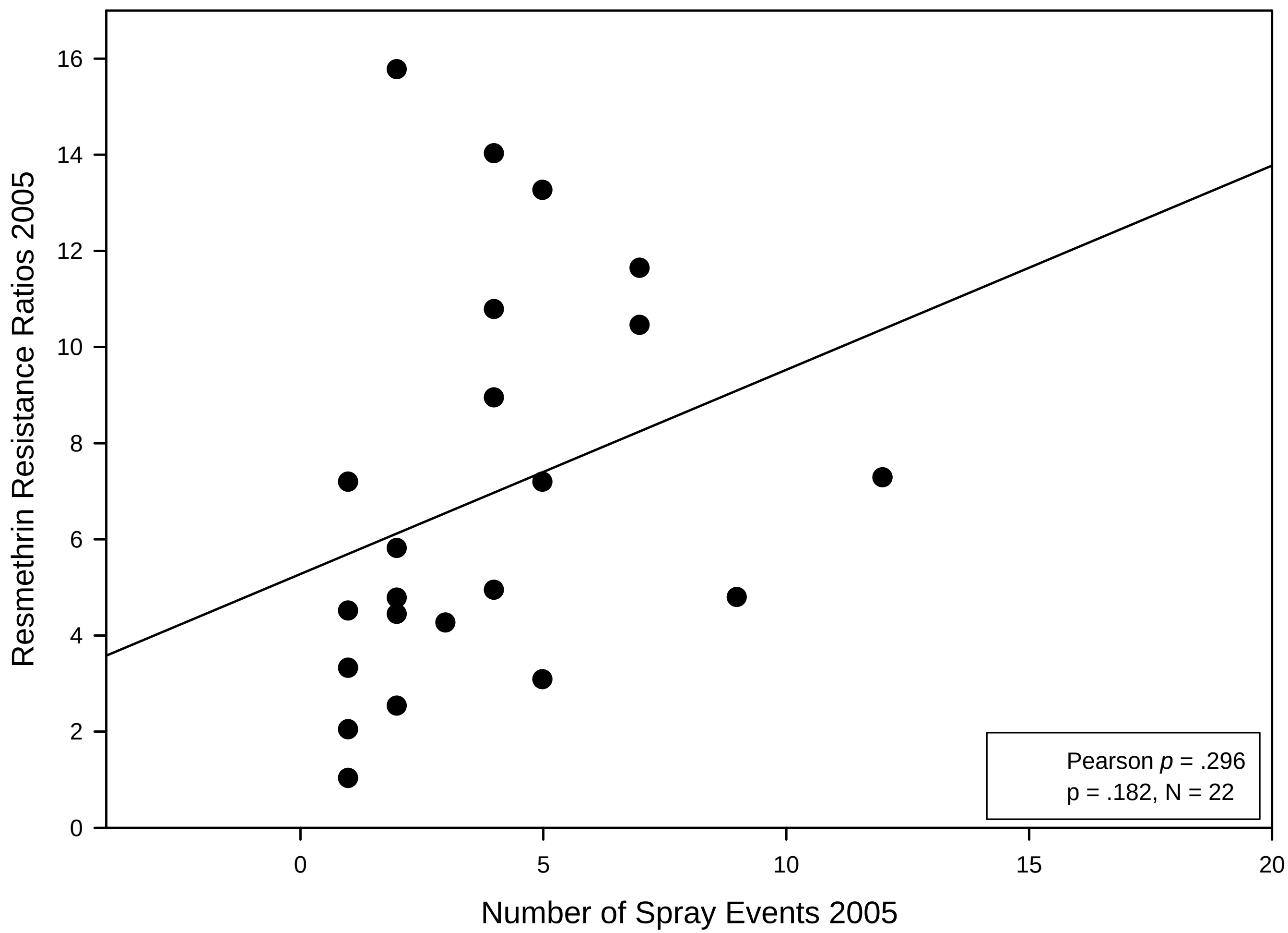

Fig. 65. Pearson correlation of resmethrin resistance ratios for female *Culex quinquefasciatus* in 2005 to the number of spray events conducted during 2005 in select operational areas in Harris County, Texas (with zeros excluded).

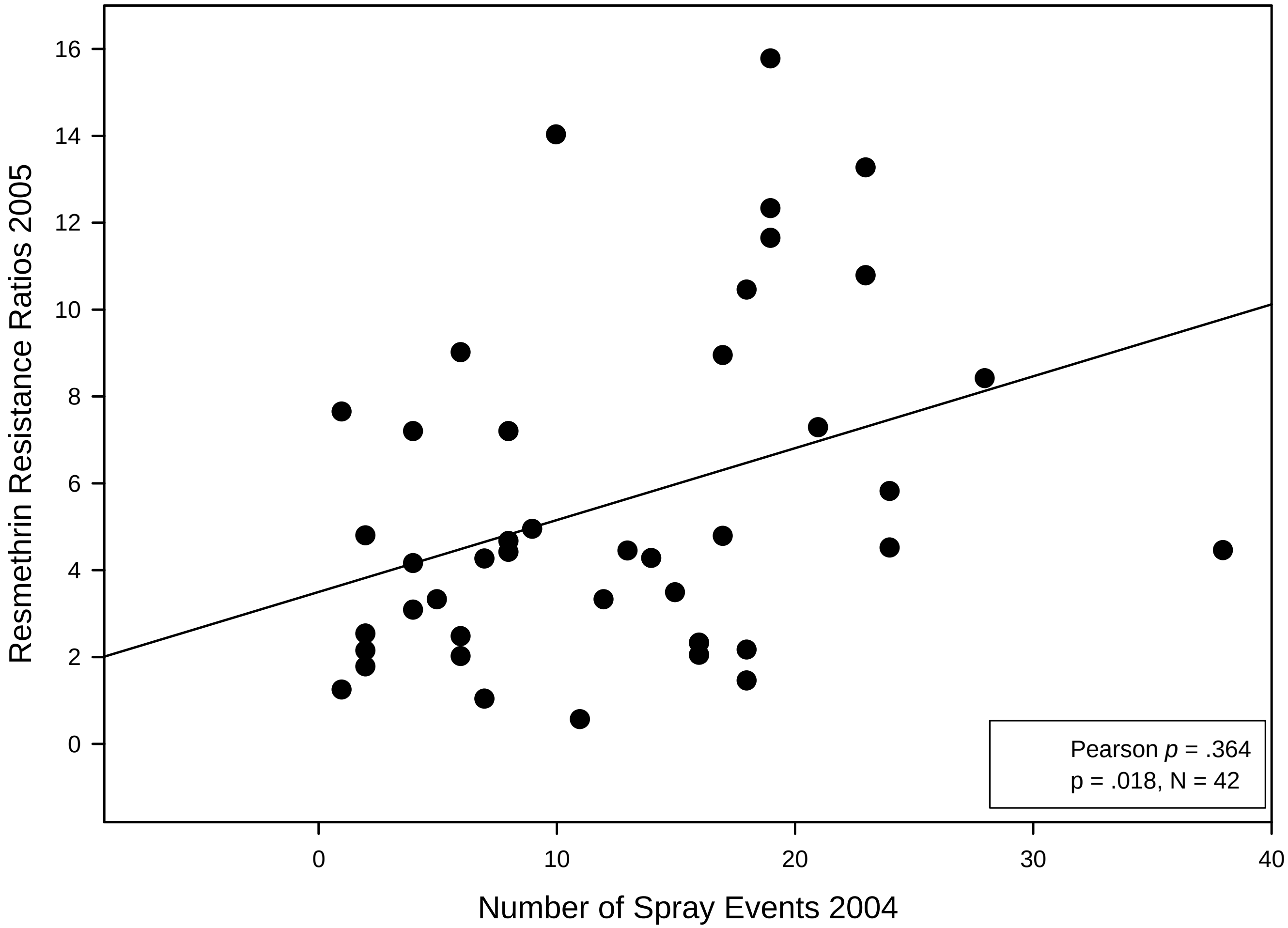

Fig. 66. Pearson correlation of resmethrin resistance ratios for female *Culex quinquefasciatus* in 2005 to the number of spray events conducted during 2004 in select operational areas in Harris County, Texas (with zeros excluded).

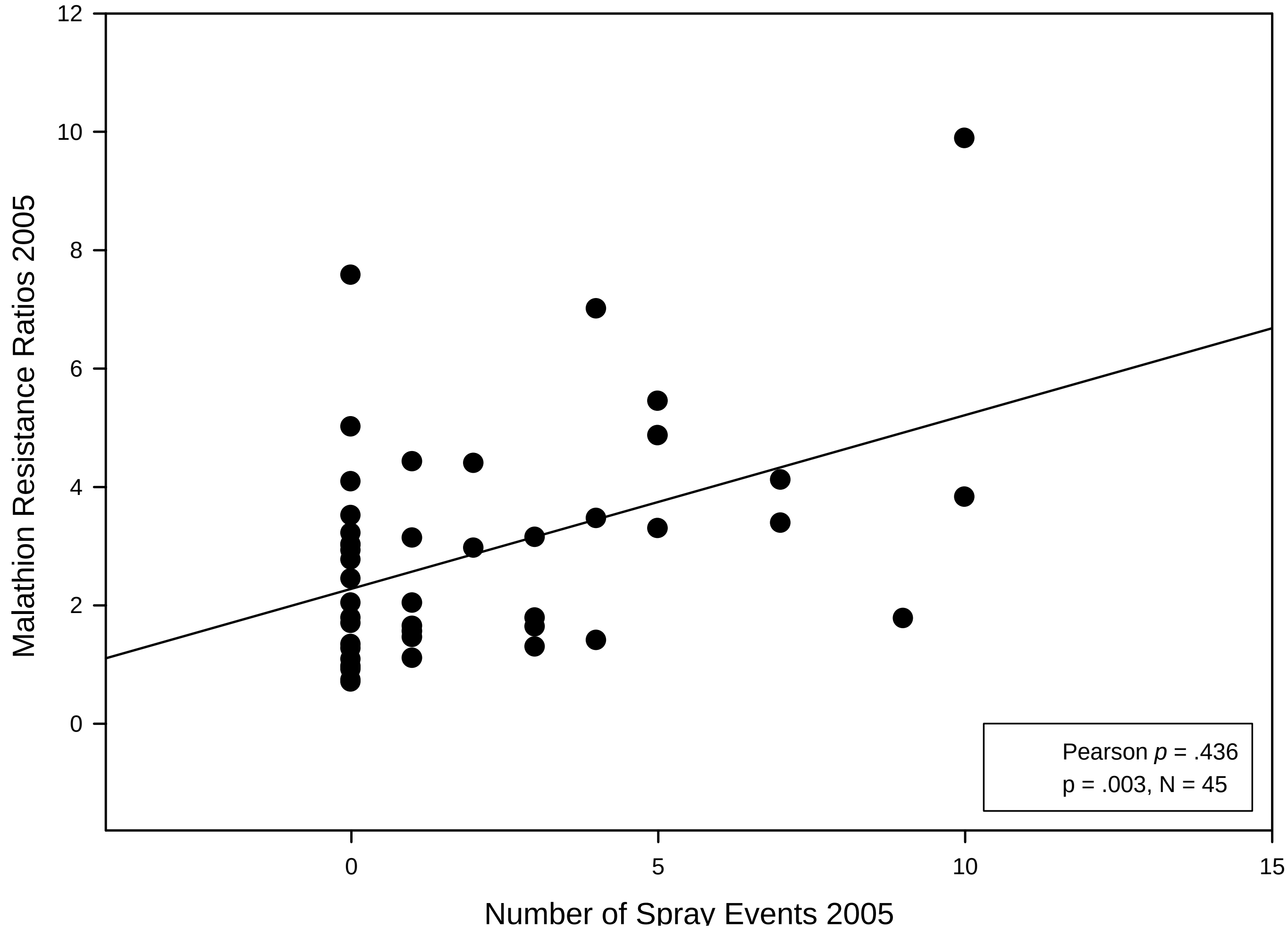

Fig. 67. Pearson correlation of malathion resistance ratios for female *Culex quinquefasciatus* in 2005 to the number of spray events conducted during 2005 in select operational areas in Harris County, Texas (with zeros included).

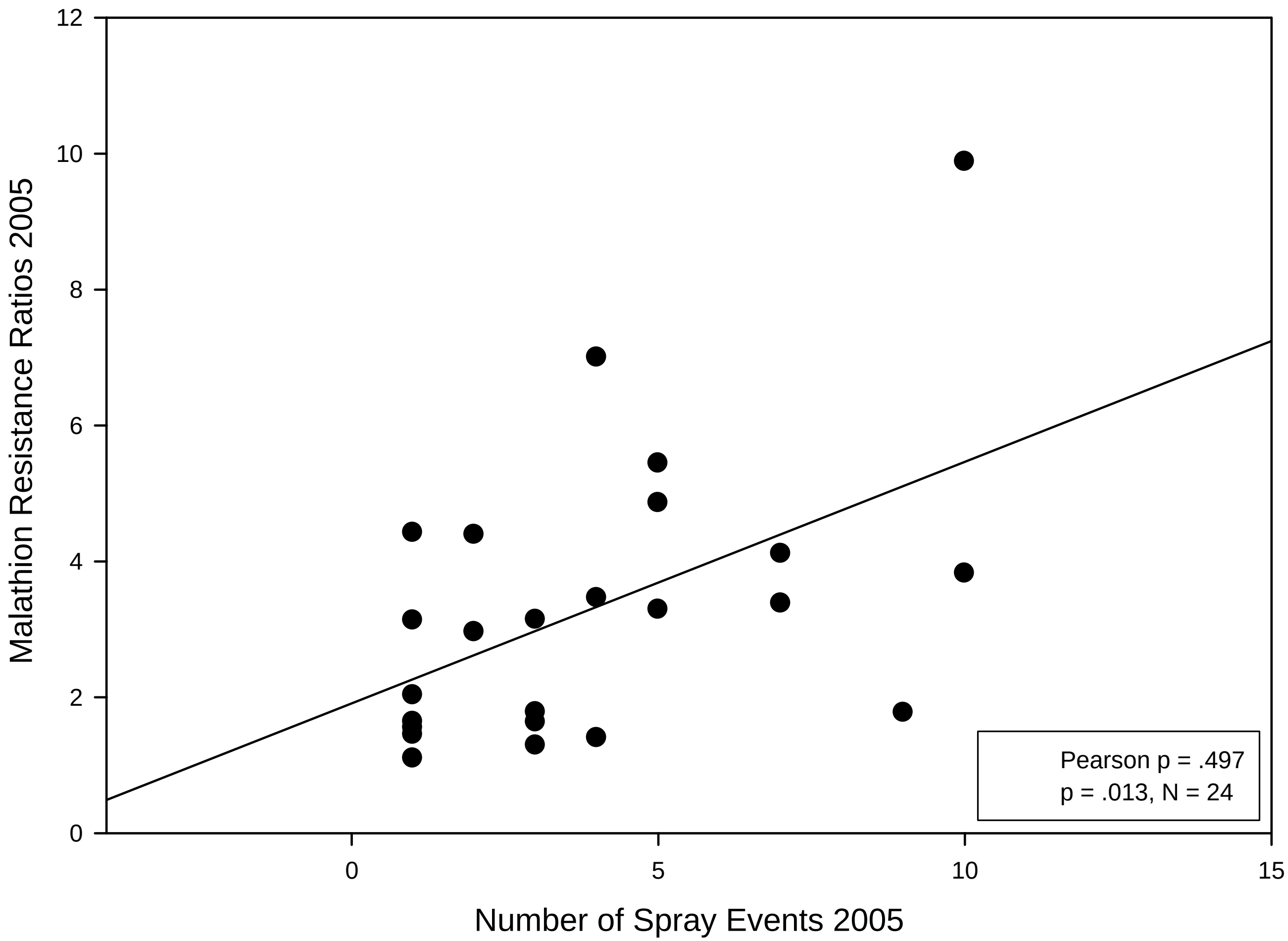

Fig. 68. Pearson correlation of malathion resistance ratios for female *Culex quinquefasciatus* in 2005 to the number of spray events conducted during 2005 in select operational areas in Harris County, Texas (with zeros excluded)

Discussion

The correlation analysis demonstrated positive associations for Harris County *Cx. quinquefasciatus* for the malathion and resmethrin resistance ratios with the number of spray events performed by the HCMCD. This association was especially evident when correlating the resistance ratios to the number of spray events from the year prior to the one for which the bioassay data was collected (i.e., 2004 resistance ratios vs. 2003 number of spray events). The resistance ratios for malathion and resmethrin in 2005 also had a positive correlation with the number of spray events conducted in the same year as the bioassay results were collected (i.e., 2005 resistance ratios vs. 2005 number of spray events). These correlations were conducted including and excluding "zeros" in the data set, which resulted in a change of the significance of the association.

Positive associations were determined to exist between the 2004 resmethrin ratios and the 2003 spray events and the 2005 resmethrin resistance ratios using both the 2004 and 2005 spray mission data (Fig 62-64). The association between the 2005 resmethrin resistance ratios and 2005 spray missions was significant when the zeros were included in the data set, but when they were excluded the significance was lost (Fig 64-65). However, the remnant of the trend that resulted in the significant association of the two variables is still present in the results. Many of the resistance ratios were collected early in the year before adulticiding operations were initiated by HCMCD, thus a zero was inserted into the data set grounding the correlation. If the mosquito populations were collected later in the year, the trend might have had a stronger association with the spray missions conducted in the same year. The correlations excluding the zeros in the data set are probably more accurate representations of what is actually occurring in the Harris County mosquito populations. The addition of zeros in the data set resulted in a grouping of points around the lower portion of the graph increasing the significance of the association.

The positive correlation with the previous year's spray events is logical because the populations sampled and tested were progeny of the generations that experienced the chemical treatments from the previous year, thus giving cause for their adapting to the selection pressure and developing the level of resistance detected by testing. The lack of correlation in the population's resistance ratios to the number of spray events conducted in the same year might be attributed to the small number of treatments to which they were exposed before being sampled thus, the stronger relationship with the previous year. The mosquito populations sampled at the end of the year demonstrated the development of an association between resistance ratios and spray events as seen in the 2005 data associated with the 2005 spray events.

A statistical correlation of malathion could only be conducted between the 2005 *Cx. quinquefasciatus* malathion resistance ratios to the 2005 spray events performed by the HCMCD due to malathion not being used as an adult mosquito chemical control tactic in the immediate years prior to 2005. The tests had a positive correlation between the variables for both data sets analyzed (Figs 67-68). The initial chemical treatment made by HCMCD in an operational area with a positive WNv mosquito isolate was with the chemical Scourge® (resmethrin). Malathion was then used as the second insecticide in the resistance management chemical alternation plan in 2005; so, several areas that received only a single treatment in 2005 did not receive exposure to this chemical. Further testing is needed to determine if the positive association observed between the malathion resistance ratios and operational treatments in 2005 continues or with the prolonged use of malathion, the significance of the correlation is lost.

None of the correlations were significant between the spray events performed by HCMCD and the Harris County *Cx. quinquefasciatus* pyrethrum resistance ratios collected in 2004 and 2005 (Table 5). This may because the small number of mosquito samples tested in 2004 (12 areas) for pyrethrum resistance. The lack of mosquito samples tested was due to problems in obtaining the chemical for testing from corporate suppliers until late in the year. Another factor the might contributed to the lack of significance of the correlation is how pyrethrum is applied in Harris County. Because of a lack of manpower (HCMCD has only one truck equipped with a thermal fogging unit). Thus, the number of areas treated with pyrethrum in the county is considerably less than is the case for the chemicals used in the truck-mounted ULV spray operations conducted in Harris County. Pyrethrum is only applied by HCMCD when positive West Nile virus-infected mosquitoes are detected in storm sewer traps or general trap collections that surpass 1,000 *Cx. quinquefasciatus* per trap. This resulted in the inclusion of only a few operational areas in the correlation which caused each point to weigh heavily in the association. Because of the low numbers included in each correlation, a few outliers can severely affect the significance.

These data offers a potential means for forecasting future resistance ratios in operational areas based on the number of spray treatments undertaken the pervious year.

This capability can be used in a resistance management program to determine which chemical to use at the start of a given season in each given operational area in Harris County.

This research needs to be continued in conjunction with the vial bioassay testing in Harris County. With additional results produced from future testing, it is possible to test if the correlations identified in this study hold true. If the association continues to exist between the resistance ratios and the number of spray events conducted during the previous year, a powerful means for prediction will be at the disposal of the operations branch of the HCMCD to plan future control operations. This research needs to be expanded to other mosquito control districts along the Texas Gulf Coast to determine if there is a differentiation between agencies that use only a single chemical in their control strategy as opposed to those that use more than one chemical in their strategy each season. Future testing needs to be conducted to determine if the use of a single insecticide in a control program will strengthen or weaken the correlation between the applications and resistance ratios. Other factors that need to be tested to determine how they affect the strength of the association are the insecticide formulations and application methods. If future testing does prove that the correlation is consistent from year to year, a predictive model can be built to help forecast the development of resistance in populations and make informed decisions on control tactics to employ to counter the development of resistance.

CHAPTER VIII

DEVELOPMENT OF INSECTICIDE RESISTANCE MAPS FOR *Culex quinquefasciatus* POPULATIONS IN BRAZOS AND HARRIS COUNTIES, TEXAS

Geographic information systems (GIS) are defined as a computer programs that carry out various management and analytical tasks on spatially referenced data or by means of geographical data collected (Heywood et al. 1998). In recent years, the use of GIS and related mapping software has become integrated in mosquito control operations. These computer programs have become a tool used by mosquito control organizations to distinguish patterns and trends represented by mosquito and disease surveillance data (Morrison et al. 1998, Mahadev et al. 2004, Gosselin et al. 2005, Sithiprasasna et al. 2005). In addition to its use in surveillance activities, GIS programs can also be used by Field Operations personnel to determine where to focus a treatment when the target mosquito species is active, how effective the control method, used were on the mosquito population, and their potential effects on non-target species (Spradling et al. 1998, Hailey and Nawrocki 2004, Barder 2006). The major advantage of this software is its ability to organize and display the data collected from various facets of the mosquito control operation on a multitude of spatial resolutions to allow for operational planning and assessment.

Harris County Mosquito Control Division has been one of the leading organizations in the development and integration of a GIS section into its overall organization structure. This section and its products have contributed greatly to the increased effectiveness of the other sections of the HCMCD and the execution of their responsibilities, to those of the disease abatement operation, mosquito surveillance, and public education and relations sections of HCMCD. One of the ways Harris County has incorporated GIS into its everyday operations is using it to map real time the positive isolation data for West Nile virus and St. Louis encephalitis collected in the county by the HCMCD the mosquito and bird surveillance sections. This provides data for the Field Operations section personnel to plan their control activities and also provides information to relay on to the residents in the county so they can take appropriate actions (Hailey and Nawrocki 2004).

When it is determined that the operational area is in need of insecticidal control, the GIS computer system plays an important role. Harris County has integrated geographic positioning systems (GPS) into their spray trucks which transmit the location of each spray unit and when they are in the process of spraying. This helps to determine the area that is covered by a specific truck and driver and where the insecticide is being applied at any point in time and can be transmitted into GIS maps which give a clear picture of control activities that have taken place. This data can also be important if there is ever legal action taken against the county for suspected misapplications of pesticide or other like information involving one of HCMCD’s spray units. The GIS mapping software has also been instrumental in planning and execution of aerial spray missions in Harris County. When it is determined that an aerial application is needed, the GIS officer at HCMCD develops a spray plan and inputs the data on a shapefile of the county, which, in turn, is sent to the private contractor who will perform the spraying mission. This file is downloaded and used by the pilot to complete the requested application. The spray data are then sent back to the GIS officer for validation and payment.

The current study was undertaken to demonstrate the spatial distribution of insecticide resistance in the *Cx. quinquefasciatus* populations in Harris and Brazos Counties, Texas, and how this resistance differed between the two years that resistance monitoring was conducted in each county. The maps produced will provide the local control agencies responsible for mosquito control in the two counties with an overview of any resistance problems they might have and identify patterns of resistance that are not apparent when using raw data. It will also point out areas in each county where future resistance problems may emerge and how they relate to areas that have confirmed resistance in their populations. To date GIS systems have not been utilized to show distributions of insecticide resistance in mosquitoes anywhere in the United States. The results of this study should also demonstrate that resistance maps can be a tool in determining the success or failure of an insecticide resistance management programs.

Materials and Methods

The insecticide resistance ratios obtained for *Cx. quinquefasciatus* populations in Brazos and Harris Counties from vial bioassay results as described in Chapter IV were mapped using ArcGIS 9.1© (ESRI, Redwood, California) to determine the spatial occurrence of insecticide resistance in *Cx. quinquefasciatus* in the two counties. Shapefiles from Harris and Brazos counties were obtained from Ms. Christian Hailey

Dischinger the GIS supervisor for the Harris County Mosquito Control Division and modified using ArcMap® to divide Brazos County into seven selectable operational areas. Colors were assigned to each chemical (red = resmethrin, yellow = permethrin, blue = sumithrin, green = malathion, orange = naled, and purple = pyrethrum) to denote the level of resistance recorded in the operational areas of each county. Each color ranged from light (low resistance ratios) to dark (high resistance ratios), with the range denoted in the legend.

Results and Discussion

The majority of the mosquito samples tested against malathion had resistance ratios that fell in the range between 1 and 5. The areas of Harris County that had mosquitoes with resistance ratios that exceeded 5 were clustered in the southeast corner of Loop 610 (Fig. 69). These areas include several of the locations where St. Louis encephalitis outbreaks have frequently occurred in Harris County over the past 42 years (Baylor et al. 1965, Lauderdale 1969, Tsai et al. 1988). Prior to 1994 malathion was the primary insecticide used for chemical disease abatement in Harris County and this historic use might account for the higher resistance ratios in these operational areas.

In 2005, resistance monitoring was greatly expanded to include 45 operational areas throughout Harris County. High resistance ratios were interdispersed within Interstate Highway Loop 610 with no apparent trend or pattern observable (Fig. 70). Areas 66 and 67 are made up of Bellaire, Southside Place, and West University Place, three very affluent communities that employ private contractors to provide mosquito control in addition to the control efforts of the HCMCD.

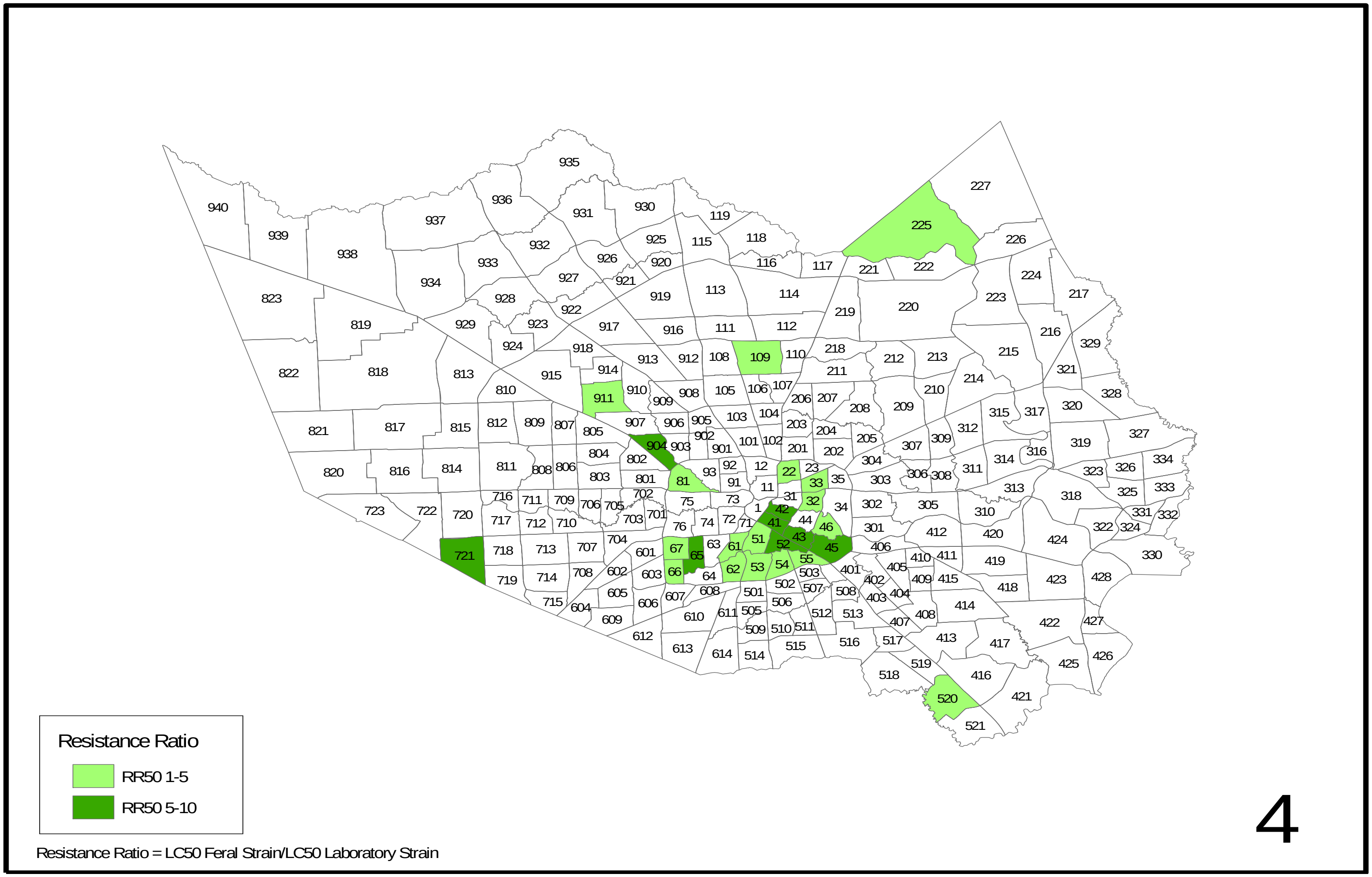

Fig. 69. Distribution of malathion resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2004.

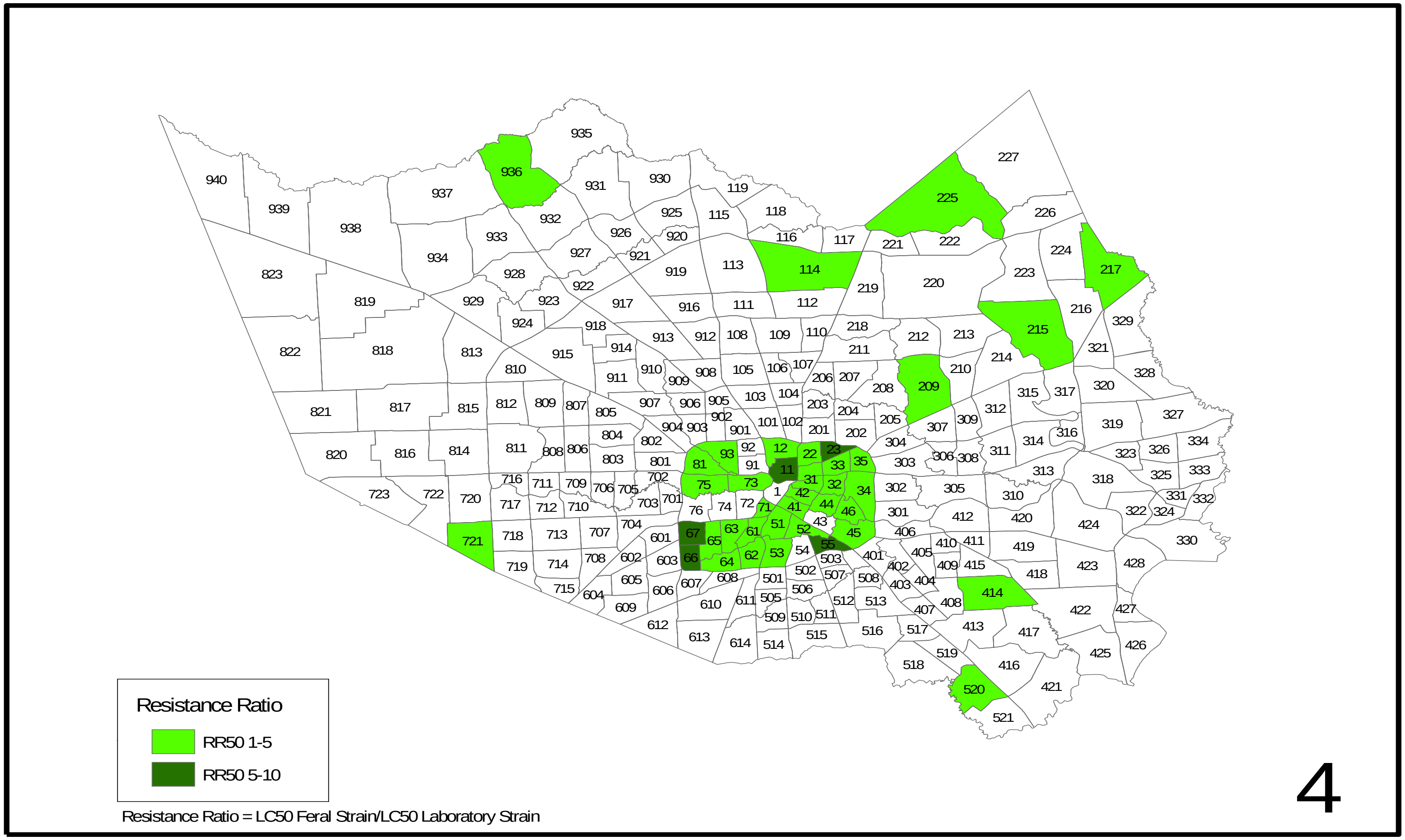

Fig. 70. Distribution of malathion resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2005.

It is not know what these pest control operators employ for their control operations; but, with malathion being a cheaper chemical to apply than synthetic pyrethroids, it is plausible that these other organizations are placing addition selection pressure on the mosquito populations thus accounting for the higher resistance ratios recorded.

As shown in Chapter IV, mosquito samples tested in 2004 and 2005 against naled have proven to be extremely susceptible to this chemical. In 2004, areas 22 and 53 were the only two areas that exceeded a resistance ratio of 1. These two areas are located inside Interstate Highway Loop 610 which has not received any treatments of this chemical (Fig. 71). Due to its corrosive natural, naled is applied in Harris County only by air and these applications are limited to rural areas where ground-based spraying is not practiced. With the location of these two areas being in the heart of Houston, the probability of the mosquito populations coming into contact with this insecticide are highly unlikely. The most probable cause of the elevated resistance ratios from naled in the mosquitoes tested from the two areas in question is natural variation in the population.

The results of the 2005 resistance monitoring program showed little change in the level of resistance in the Harris County mosquito populations to naled (Fig. 72). Mosquitoes in the two areas that had resistance ratios greater than 1 in 2004 decreased to a resistance level that was in similar to those in all other areas of Harris County tested in 2005. Mosquito samples from areas (200s and 900s) that received aerial treatments of naled in 2004-2005 displayed similar resistance ratios as mosquitoes from areas located in other parts of the county that did not receive treatments with this chemical.

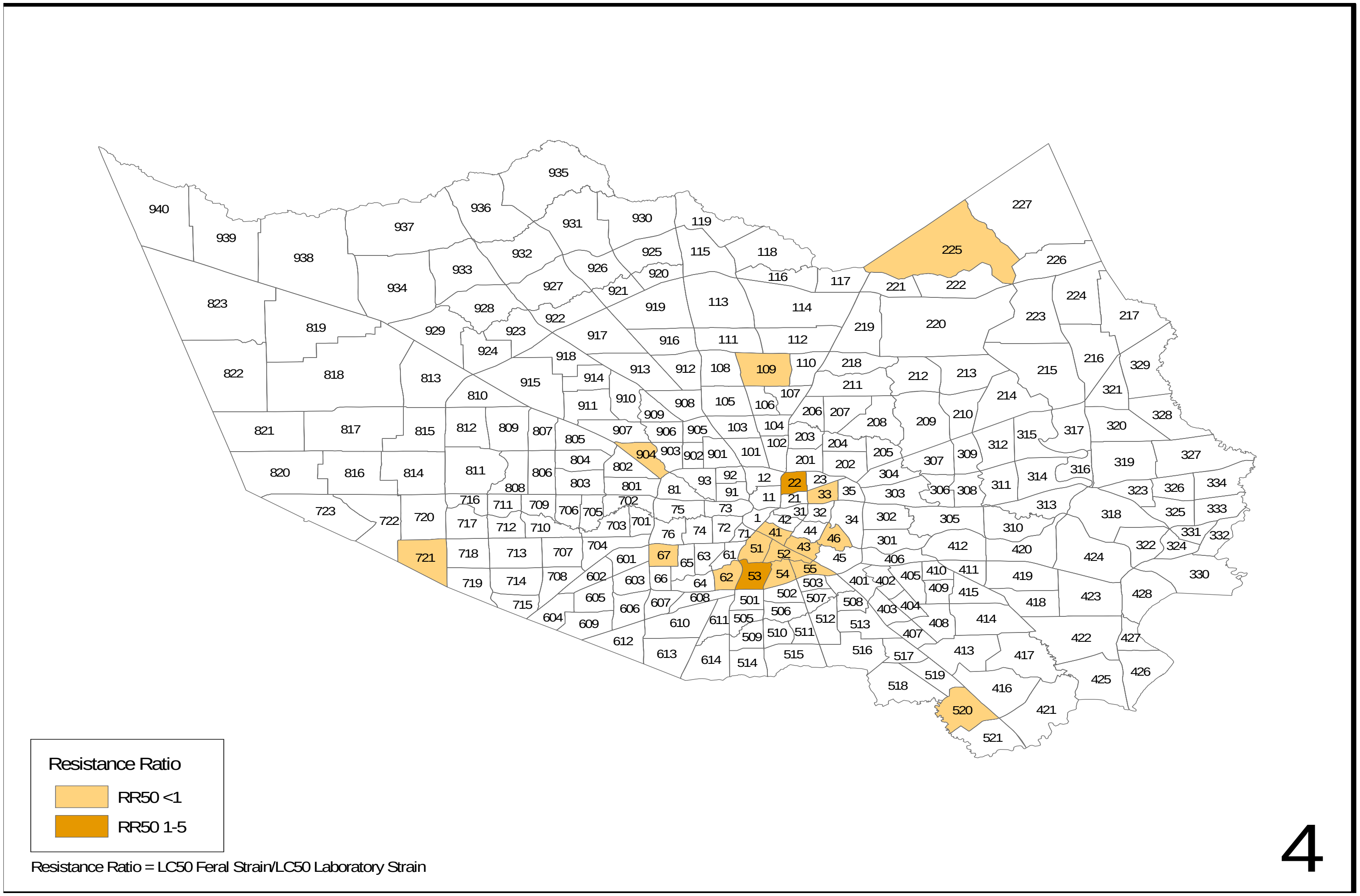

Fig. 71. Distribution of naled resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2004.

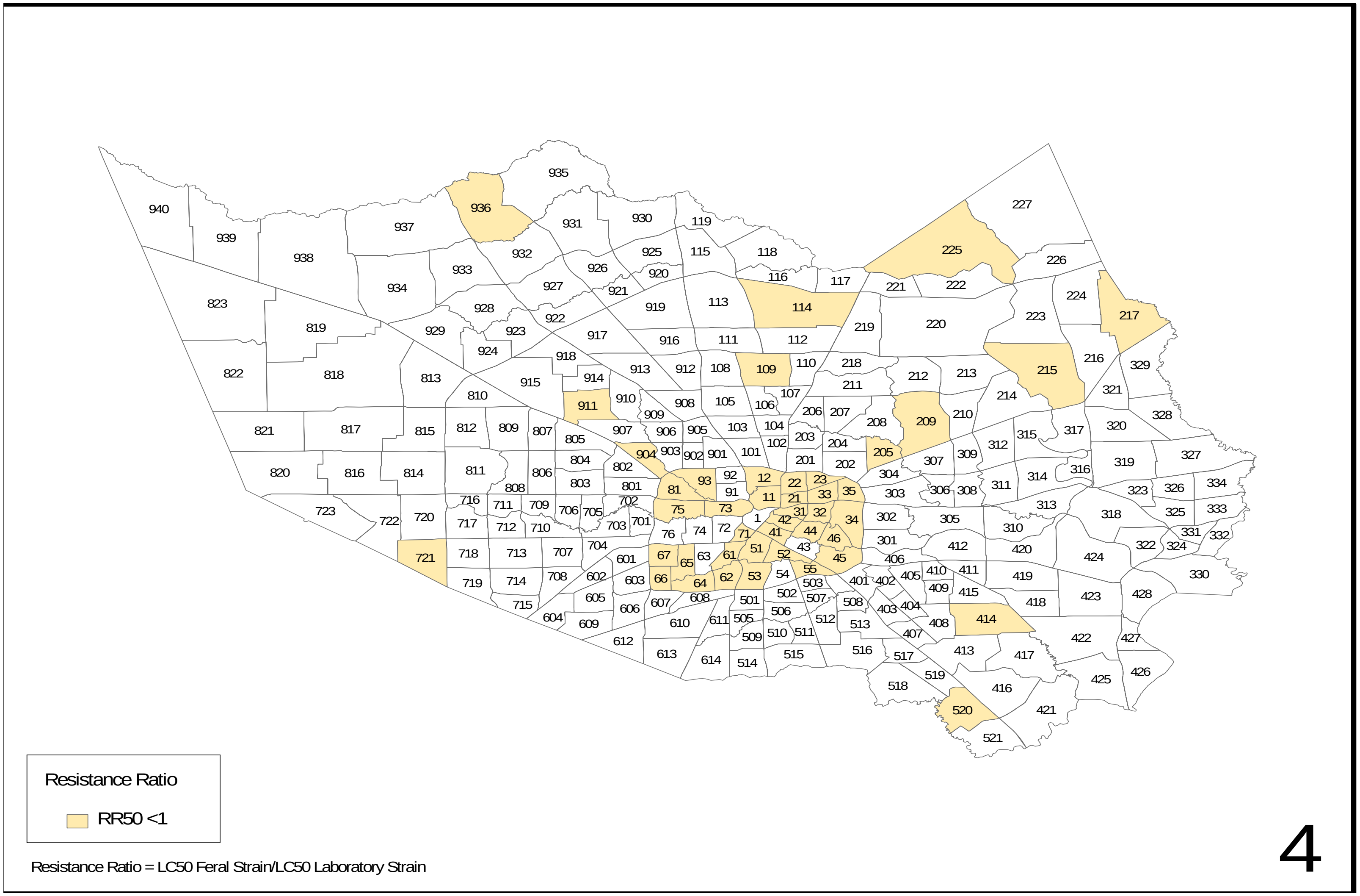

Fig. 72. Distribution of naled resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2005.

Mapping results for resmethrin are shown in Figs 73 and 74. As previously noted, resmethrin has been the primary chemical used by HCMCD for disease abatement since 1994 (Mr. Kyle Flatt, personal communication). With the advancement of West Nile virus into Houston, Texas, in 2002 (Lillibridge et al. 2004), the number and scope of spray missions performed by HCMCD to suppress the primary vector species for this disease (i.e., *Cx. quinquefasciatus*) increased to levels rarely seen before (Unpublished HCMCD data). This increase in control missions placed a level of selection on the county's mosquito populations that had not occurred in Harris County in decades. In May 2004, the Test and Evaluation Section of HCMCD conducted a quality assurance test on *Cx. quinquefasciatus* adult sample from its operational area 51 against three synthetic pyrethroids. The poor results of the field cage spray test for all three chemicals, especially the low rate of Scourge® (resmethrin), demonstrated the possible development of resistance in mosquito populations occurring inside Interstate Highway Loop 610 in Houston. These results led to the development of a multifaceted resistance monitoring program of which this research project was a part. The initial concern of the HCMCD was the status of resistance to resmethrin in the mosquito populations in its various operational areas in Harris County.

The resistance ratios for Harris County *Cx. quinquefasciatus* populations in 2004 indicated several mosquito samples to be resistant to resmethrin as confirmed by vial bioassay testing.

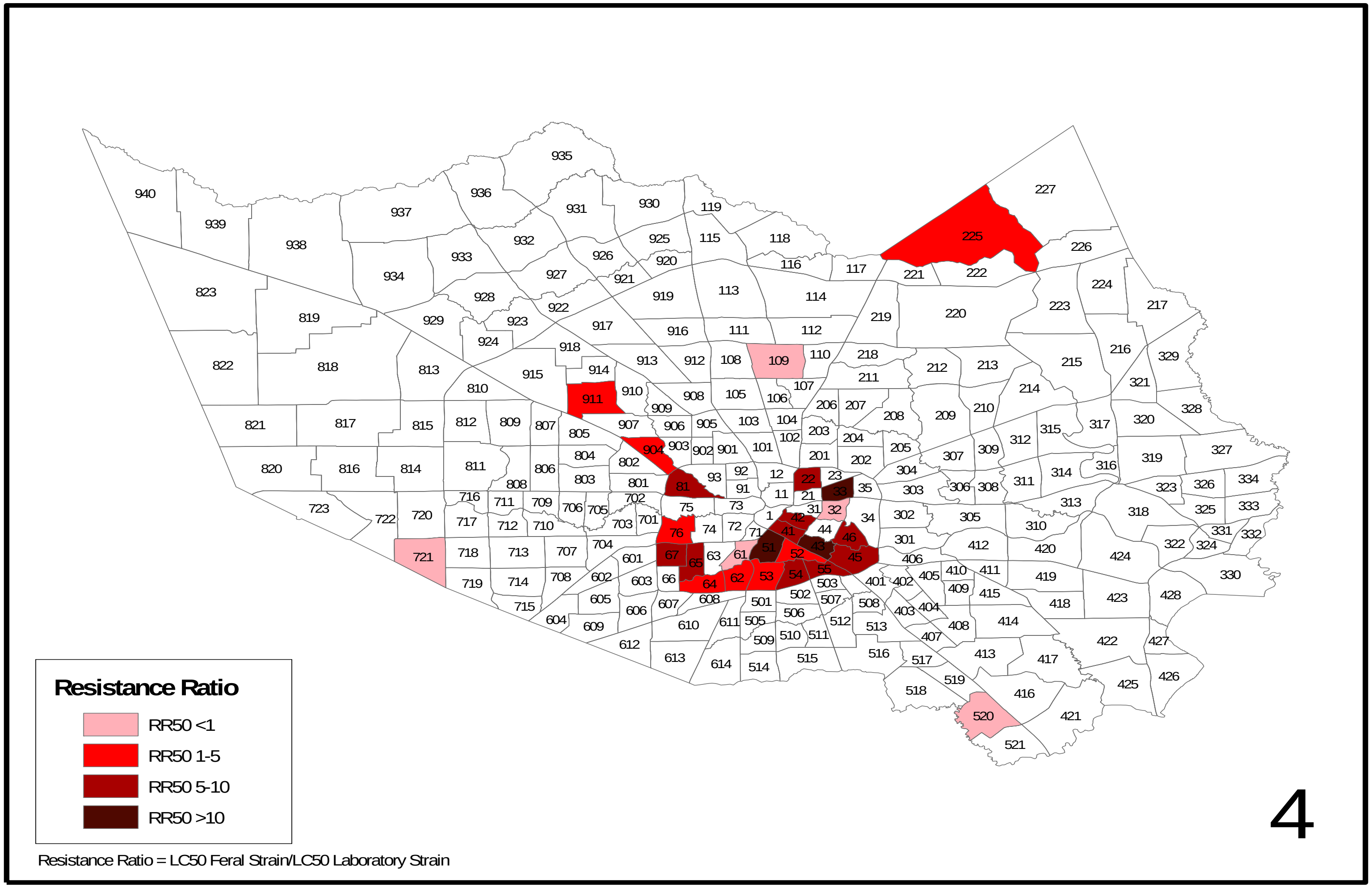

Fig. 73. Distribution of resmethrin resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2004.

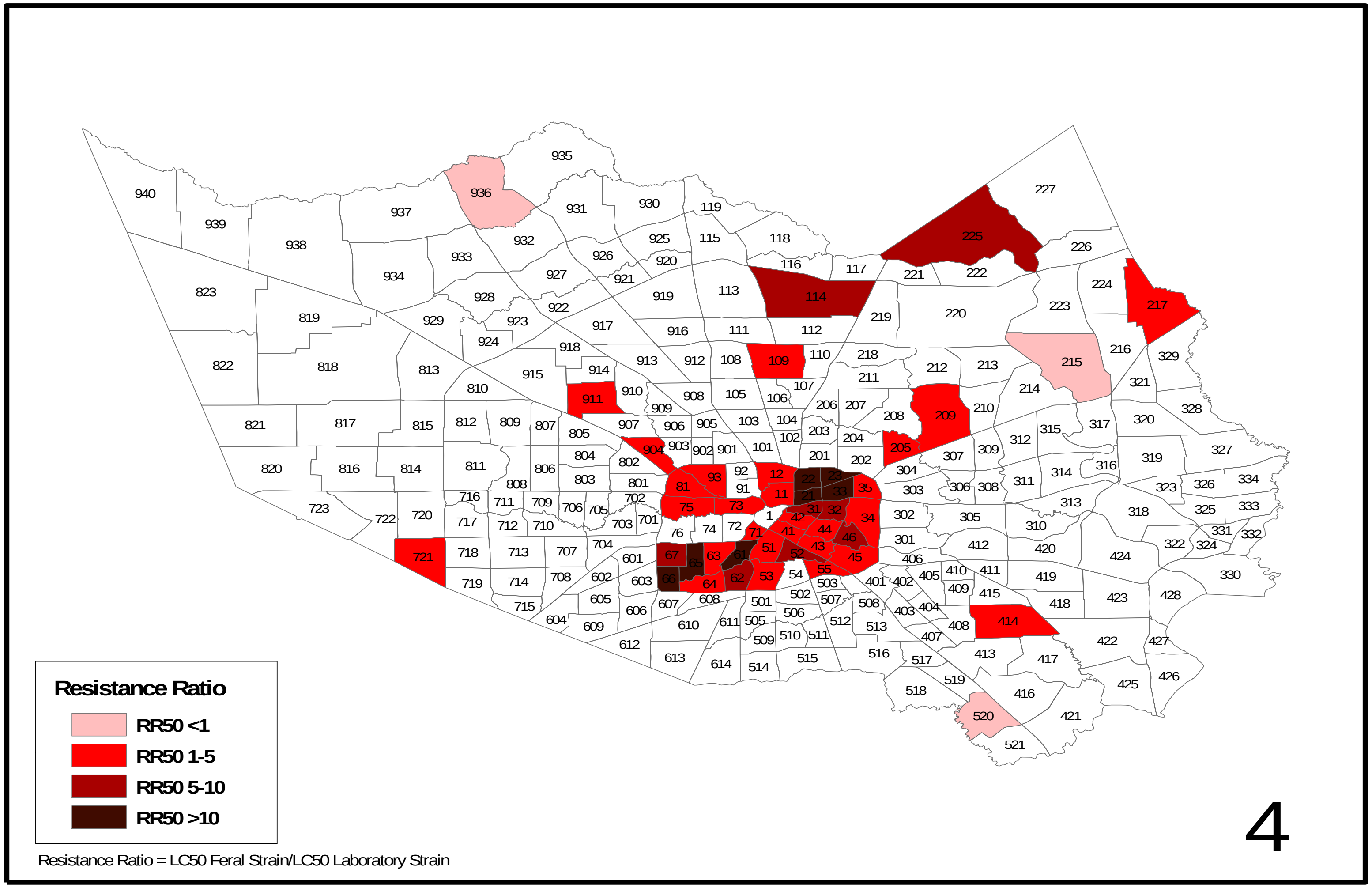

Fig. 74. Distribution of resmethrin resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2005.

When the resistance ratios were mapped using the ArcGIS® software, they revealed the majority of the *Cx. quinquefasciatus* populations in the operational areas inside Interstate Highway Loop 610 had high resistance ratios, with the three areas with resistant mosquitoes confirmed in them being located in the northeast and southeast corners of Interstate Highway Loop 610 (Fig. 73). All the areas located outside Interstate Highway Loop 610 had mosquito samples with low resistance ratios to resmethrin in 2004.

In 2005 the HCMCD began a resistance management program that was based on the alternation of insecticides, thus reducing the use of resmethrin and thereby, reducing its pressure on the target mosquito populations. The results of the mapping of the 2005 vial bioassays had a clustering of areas with established resistance in the mosquito samples in the northeastern and southwestern corners of Interstate Highway Loop 610 (Fig. 74). Areas 21, 22, 23, and 33 make up the northeastern focus of resistance and consist of heavy industry with low income housing interspersed throughout. The areas had received numerous treatments with resmethrin in recent years due to the multiple incidences of West Nile virus-positive mosquitoes being called in these areas (HCMCD unpublished data). The southwestern focus of resistance (areas 65, 66, and 67) was made up of the affluent communities of Bellaire, Southside Place, and West University Place, which had been known to employ private contractors (pest control operators) to provide mosquito control of annoyance mosquitoes in addition to the disease abatement activities conducted by the HCMCD. The mosquitoes tested from these areas also demonstrated high resistance to malathion which was the other chemical used by HCMCD in their resistance management program. The development of resistance on the part of Harris County *Cx. quinquefasciatus* populations to both organophosphate and pyrethroids could prove disastrous for control activities in the areas where such occurs, no other chemical classes to turn to, mosquitoes in these areas can not be effectively managed by HCMCD until susceptibility is bred back into the target mosquito population. Communication needs to be established between HCMCD and the private mosquito control contractors to determine what chemical they are using and the rates at which they are applying their insecticides. If possible, an attempt should be made to integrate these private operators into the resistance management program being implemented by the HCMCD. The mosquitoes in the majority of areas located outside of the Interstate Highway Loop 610 in Harris County demonstrated susceptibility to resmethrin with the exception of these in areas 114 and 225. Area 114 consists of the George Bush International Airport and the city of Humble which conducts its own mosquito control as a supplement to the HCMCD program. Area 225 is the Houston subdivision of Kingwood which has a long history of utilizing private contractors for control of annoyance mosquitoes and in recent years, this subdivision has had multiple incidences of West Nile virus in its mosquito populations increasing the control activities by HCMCD in the subdivision.

Mapping of the 2004 Harris County bioassay results for mosquito samples tested against permethrin resulted in a noticeable clustering of areas having mosquitoes with high resistance ratios in the southeastern corner inside Interstate Highway Loop 610 (Fig. 75). This pattern is similar to what was observed for the mosquito resmethrin resistance ratios that were mapped.

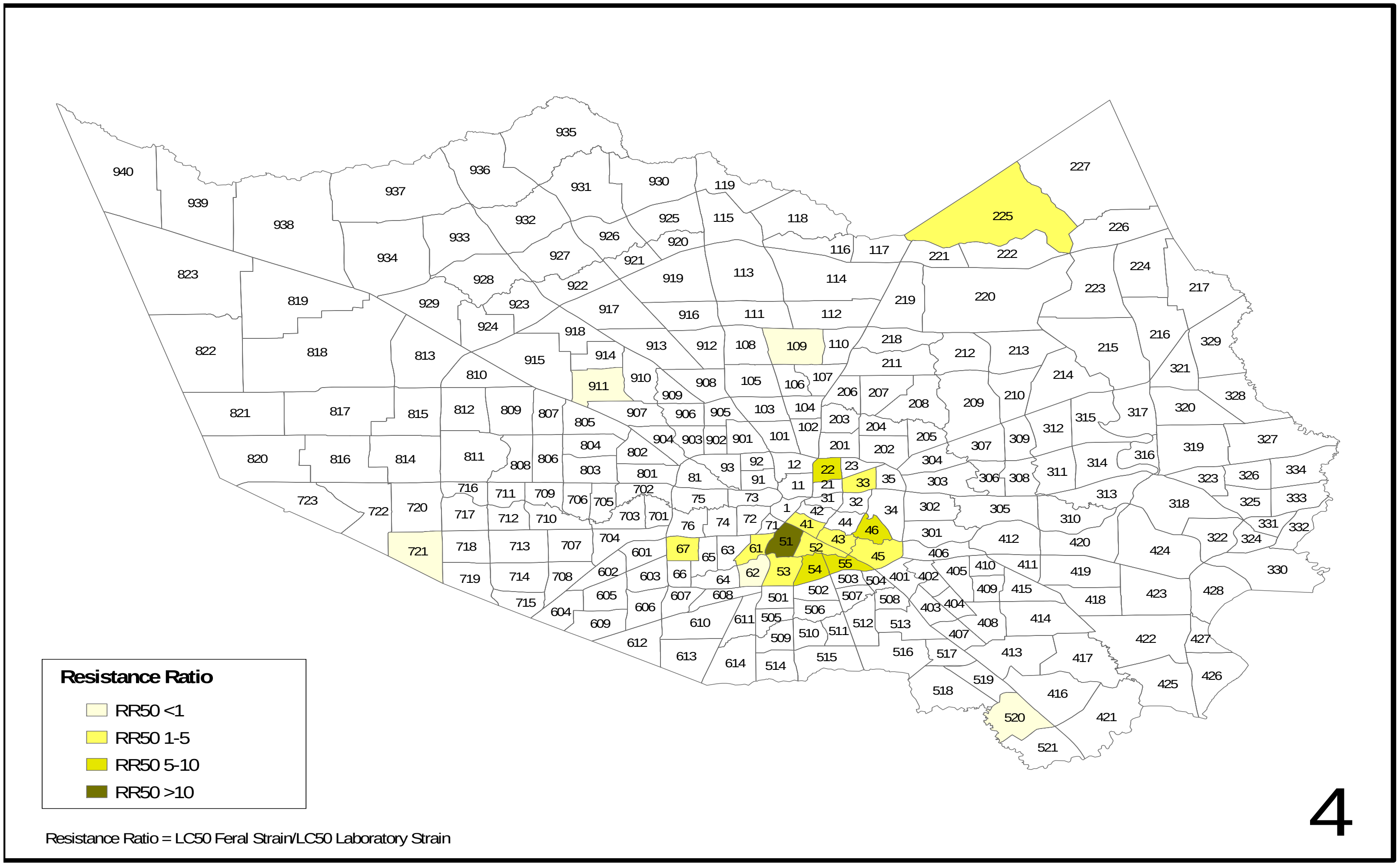

Fig. 75. Distribution of permethrin resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2004.

Since permethrin was not used in any abatement operations by HCMCD, it is possible that the resistance that developed in the mosquito populations for this chemical was due to cross resistance with resmethrin. The map of the 2005 mosquito permethrin resistance ratios had only three areas that had high resistance ratios recorded during the calendar year. These areas paralleled the locations where resistance was observed in the 2005 map for resmethrin resistance (Fig. 74) with foci for the permethrin resistance again being in the northeastern and southwestern corners of Interstate Highway Loop 610 (Fig. 76). The similarity between the resistance ratio distribution results for resmethrin and permethrin and the fact that permethrin was not used by HCMCD leads to the supposition that the high resistance ratios in mosquitoes are due to cross resistance between the insecticides. Mosquitoes in all other operational areas in Harris County where resistance monitoring was conducted against permethrin exhibited susceptibility to this chemical.

Sumithrin was the third synthetic pyrethroid tested on mosquitoes in Harris County as part of the resistance monitoring program, with maps for the resistance ratio produced for testing shown in Figs. 77 and 78. The 2004 map of sumithrin resistance ratios (Fig. 77) resembles the mapping results produced for the other two pyrethroids tested, with a grouping of mosquitoes with high resistance ratios being in the southeast corner of Interstate Highway Loop 610. Sumithrin is not a part of the chemical rotation used by HCMCD and as with permethrin the resistance in mosquitoes to this chemicals documented by bioassay test, is probably due to cross resistance produced by HCMCD's use of resmethrin for control activities.

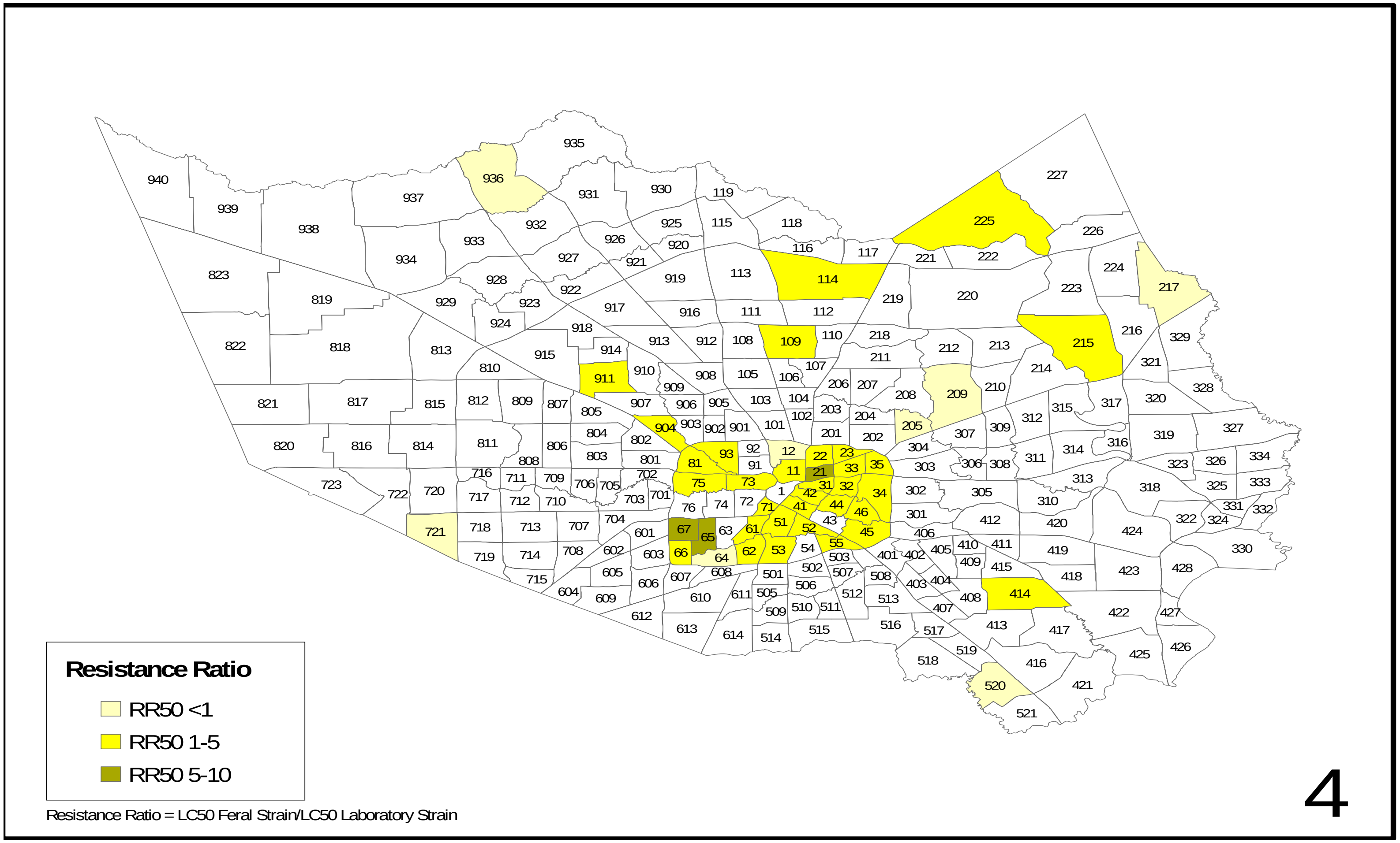

Fig. 76. Distribution of permethrin resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2005.

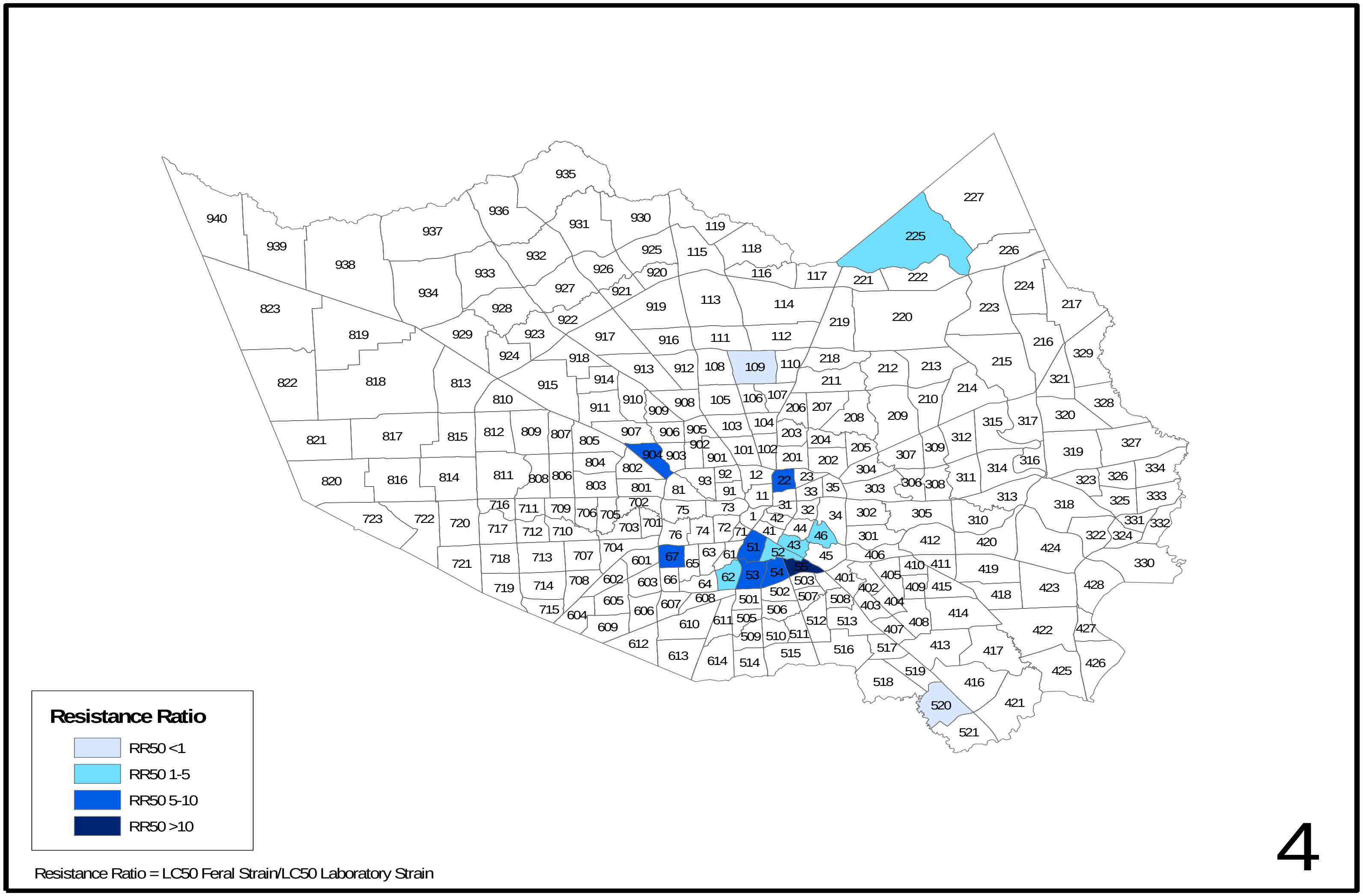

Fig. 77. Distribution of sumithrin resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2004.

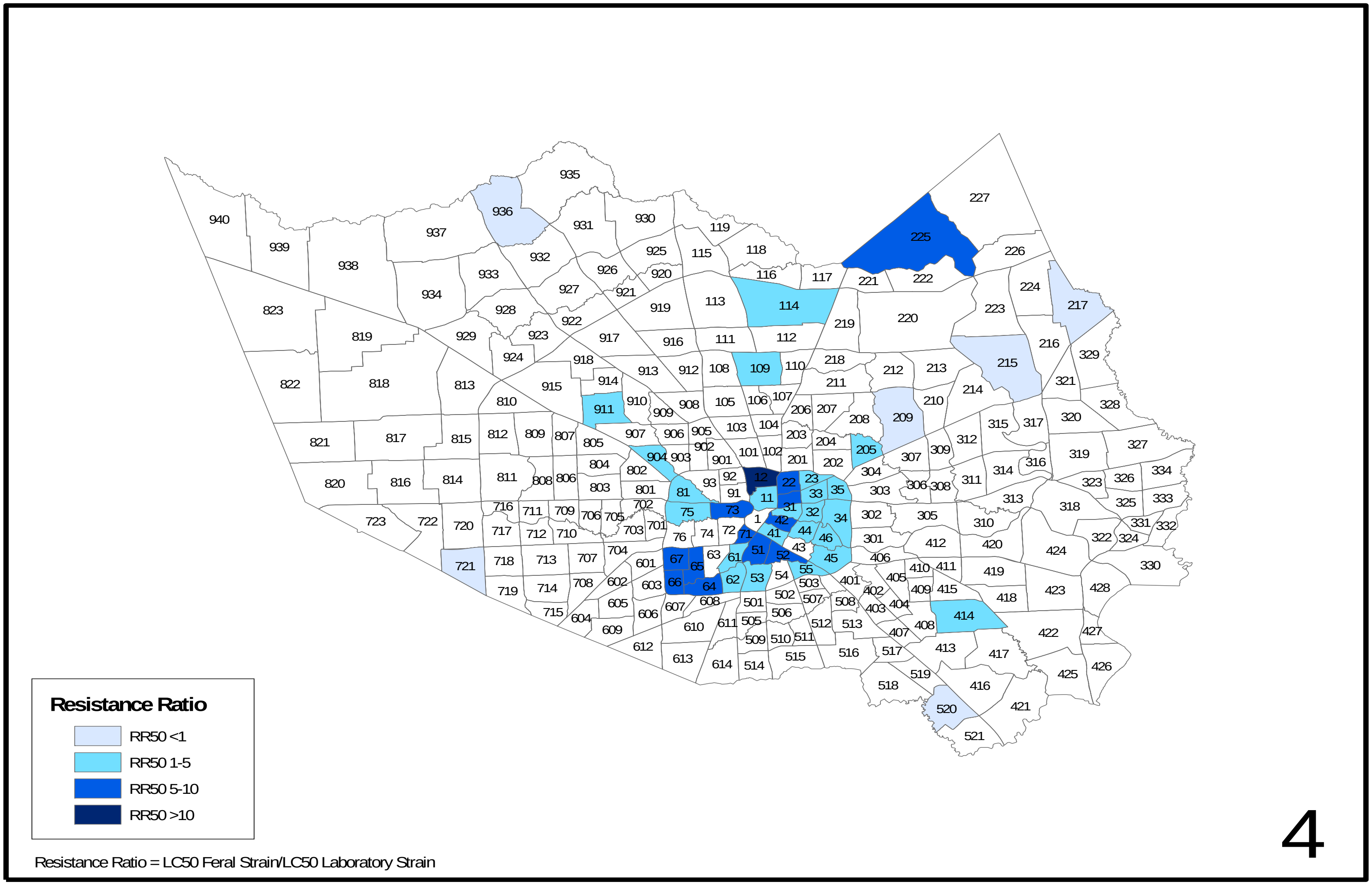

Fig. 78. Distribution of sumithrin resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2005.

Cross-resistance between the synthetic pyrethroids was also evident in Harris County when mapping the 2005 *Cx. quinquefasciatus* population's resistance to sumithrin (Fig. 78). The northeastern and southwestern corner of Interstate Highway Loop 610 had mosquitoes that had high resistance ratios similar to those recorded for mosquitoes to the other synthetic pyrethroids mapped in 2005 (Fig. 78). The majority mosquito samples tested from areas outside Interstate Highway Loop 610 against sumithrin proved to be susceptible to the insecticide, with the exception of those in area 225 (Fig. 78) which demonstrated mosquitoes with high resistance ratios to sumithrin as seen in the mapping of the 2005 resmethrin results (Fig. 74).

The number of areas in Harris County where mosquito samples were tested against pyrethrum in 2004 was limited, because of a lengthy period when the insecticide was on backorder from a chemical supplier; these tests could not be run. The 2004 resistance ratios that were mapped displayed susceptibility in all mosquitoes sampled for testing (Fig. 79). The limited use of this chemical by the HCMCD minimized the selection pressure on the population by this chemical, resulting in low resistance being detected in the samples tested. The 2005 resistance testing program was expanded to encompass mosquitoes in 45 areas and their resistance ratios where mapped for pyrethrum. The results were consistent with the level of resistance establish the previous year, with no mosquito population exhibiting a resistance ratio that exceeded 5 (Fig. 80). Due to the lack of variation in the resistance ratios of the mosquito samples tested, no patterns were apparent in the maps produced.

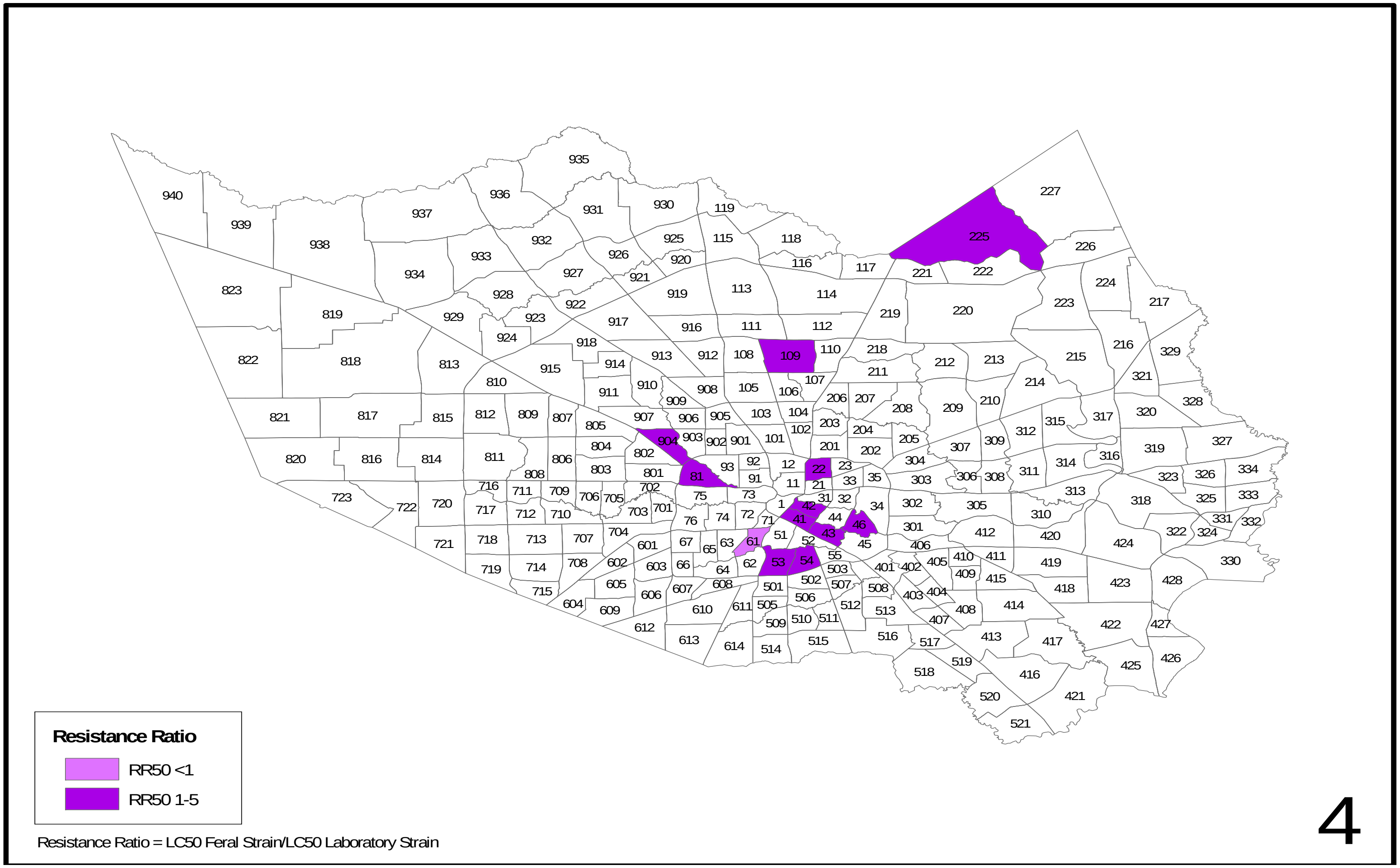

Fig. 79. Distribution of pyrethrum resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2004.

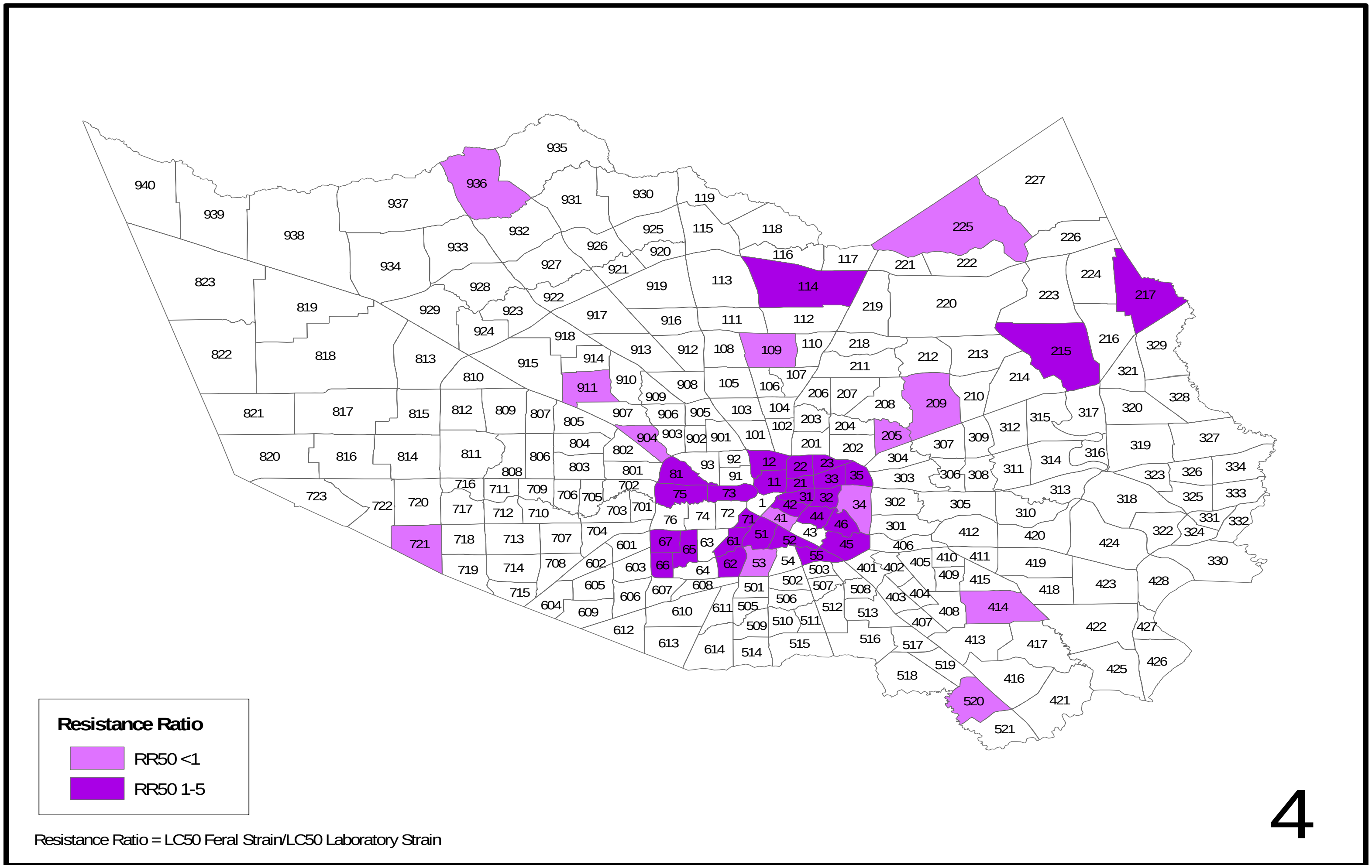

Fig. 80. Distribution of pyrethrum resistance in female *Culex quinquefasciatus* in operational areas in Harris County, Texas, 2005.

The 2005-2006 resistance ratios for *Cx. quinquefasciatus* population in Brazos County for the six insecticides commonly used in mosquito control programs were mapped on a shapefile of Brazos County to determine if any resistance trends are observable in the mosquito samples tested. The results were that all the mosquito samples tested to all six insecticides had a high level of susceptibility throughout the county. Because of to the low resistance ratios throughout the county, no patterns were detected when mapping the resistance ratios. If Brazos County does reactivate their mosquito control program, these maps can be beneficial in determining what insecticides to use and what parts of the county have higher resistance ratios before control starts.

In 2005 the results of the bioassay for malathion were mapped demonstrating the susceptibility of the mosquitoes tested to this insecticide. The areas that make up Bryan and College Station (areas 1-4) and northern Brazos County (area 7) have mosquito populations with slightly higher resistance ratios than do these in the southern and western portions of the county (areas 5 and 6) (Fig. 81). The elevated resistance in these mosquito populations can be attributed to the natural genetic variation in the population or remnants of resistance that developed to other organophosphates used by the general public and/or in pervious mosquito control programs. When the mosquito populations were sampled again in 2006 the results were similar to previous year. No disenable pattern was evident for malathion resistance, with only area 6 having a resistance ratio that was slightly less than those recorded in other six areas (Fig. 82).

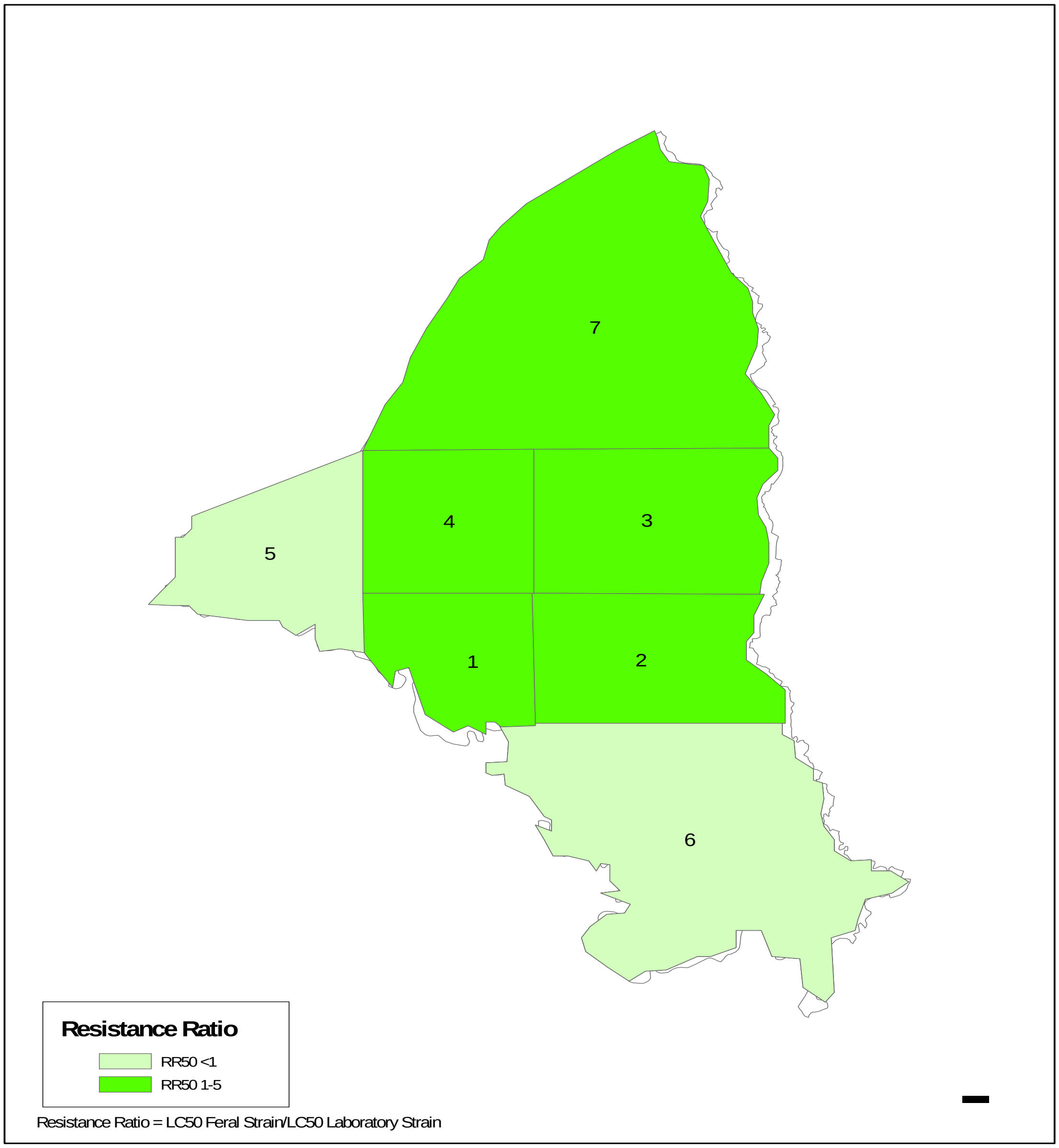

Fig. 81. Distribution of malathion resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2005.

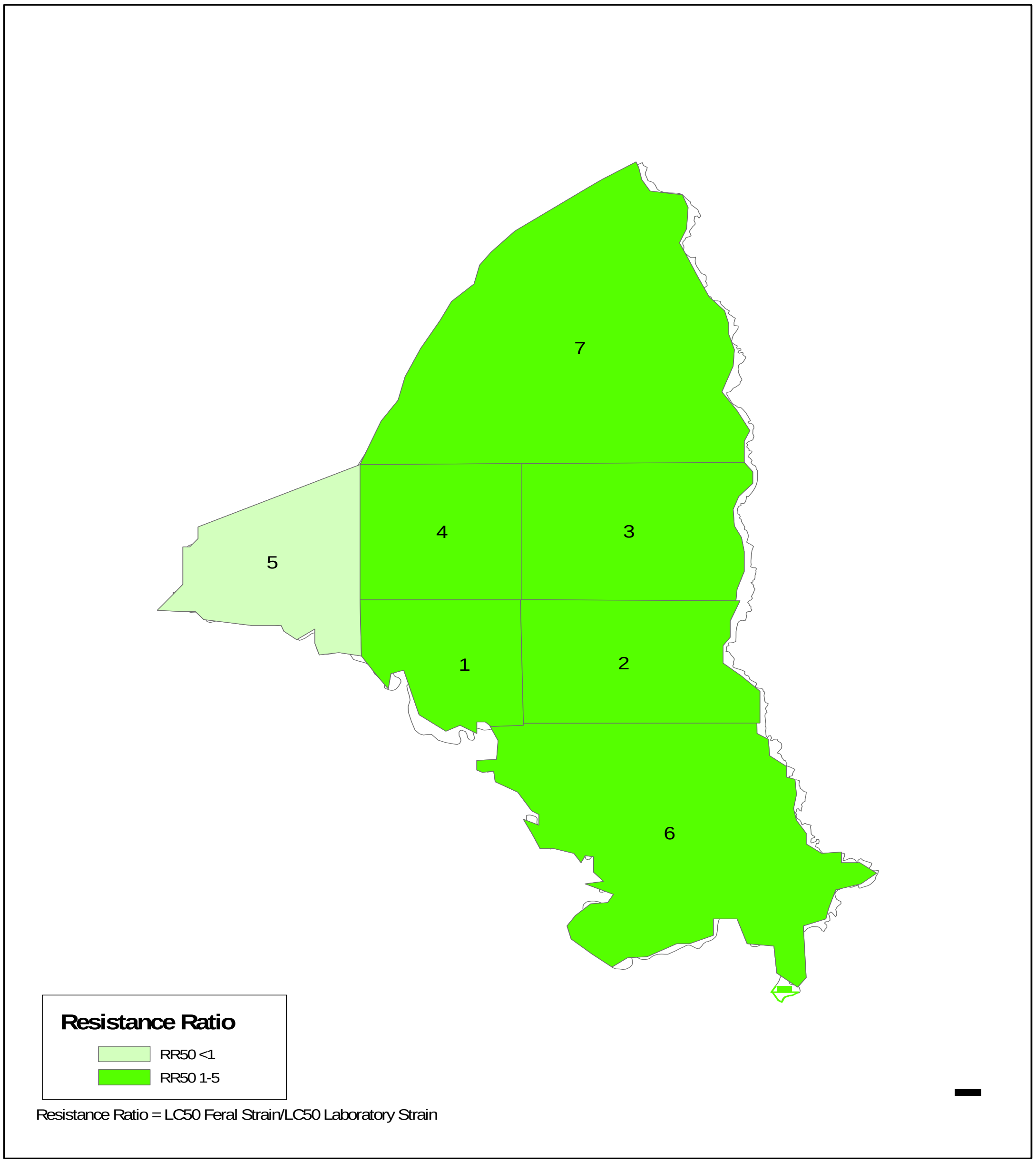

Fig. 82. Distribution of malathion resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2006.

The naled resistance ratios were mapped in 2005 and 2006, with the Brazos County mosquitoes had extreme susceptibility to the insecticide in all cases (Figs. 83-84). These results were consistent with the results for mosquito samples from Harris County to this insecticide.

The resistance ratios mapped for resmethrin, permethrin, and sumithrin had susceptibility to all three chemicals throughout Brazos County, with only minor differences occurring between the two years that monitoring was conducted in the county. Because of the low resistance ratios and lack of chemical control in the county, it is hard to determine if cross resistance played a part in the resistance ratios recorded or if it was the natural level of resistance in the populations tested.

The resistance ratio maps for resmethrin in Brazos County show the same distribution of resistance ratios in the county in 2005 and 2006 (Figs. 85-86). The areas that incorporate the western and southern portion of Brazos County had mosquito samples demonstrating resistance ratios slightly lower than these in the other areas where mosquitoes were tested. There were minor fluctuation in the resistance ratios from year to year, but the variations were not large enough to shift them into a lower or higher category. These fluctuations are attributed to the natural genetic variation in the populations due to the lack of chemical control in the county.

The greatest variation in the resistance ratios between the two years resistance monitoring was conducted occurred in Brazos County mosquito populations tested against permethrin.

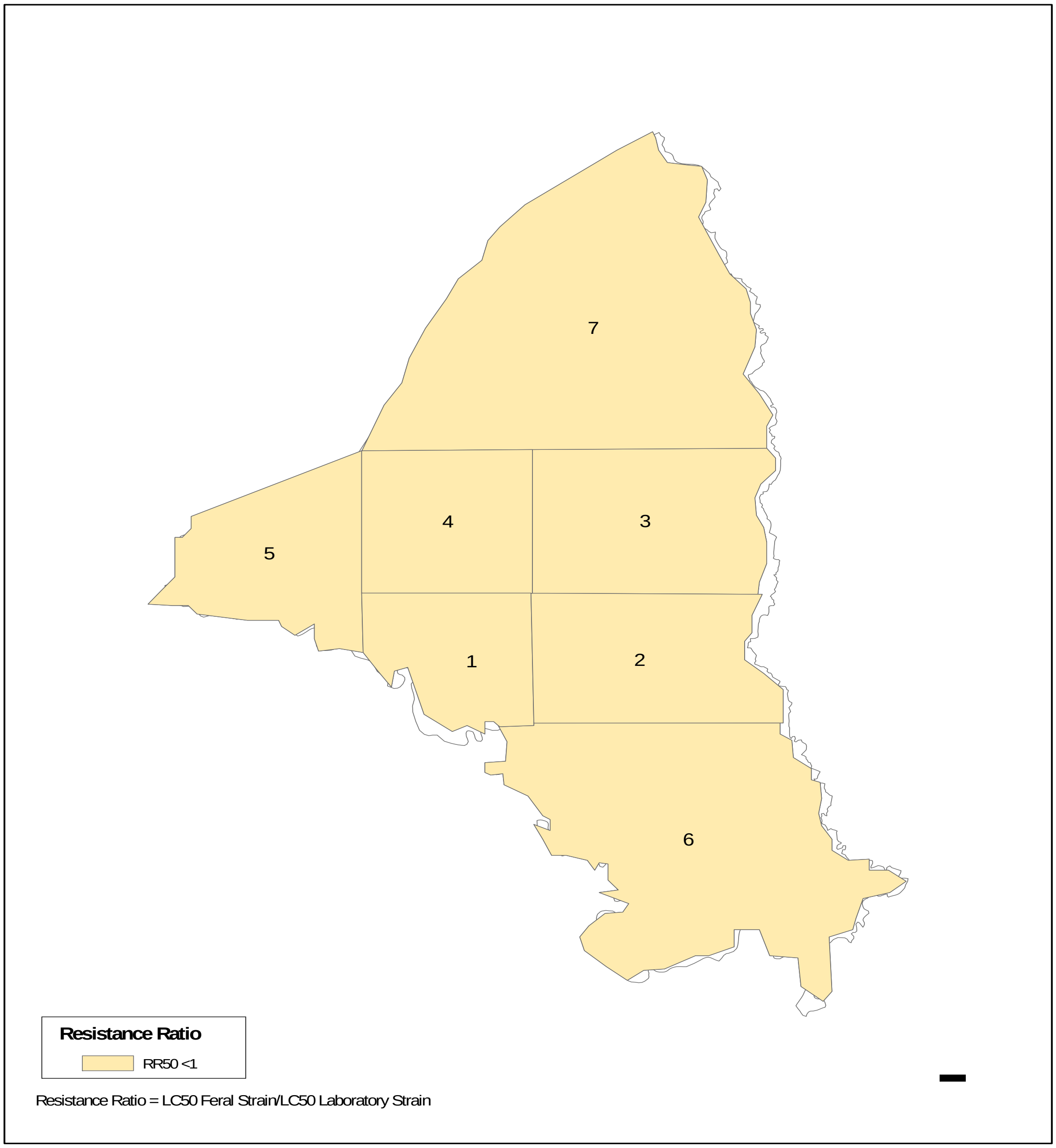

Fig. 83. Distribution of naled resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2005.

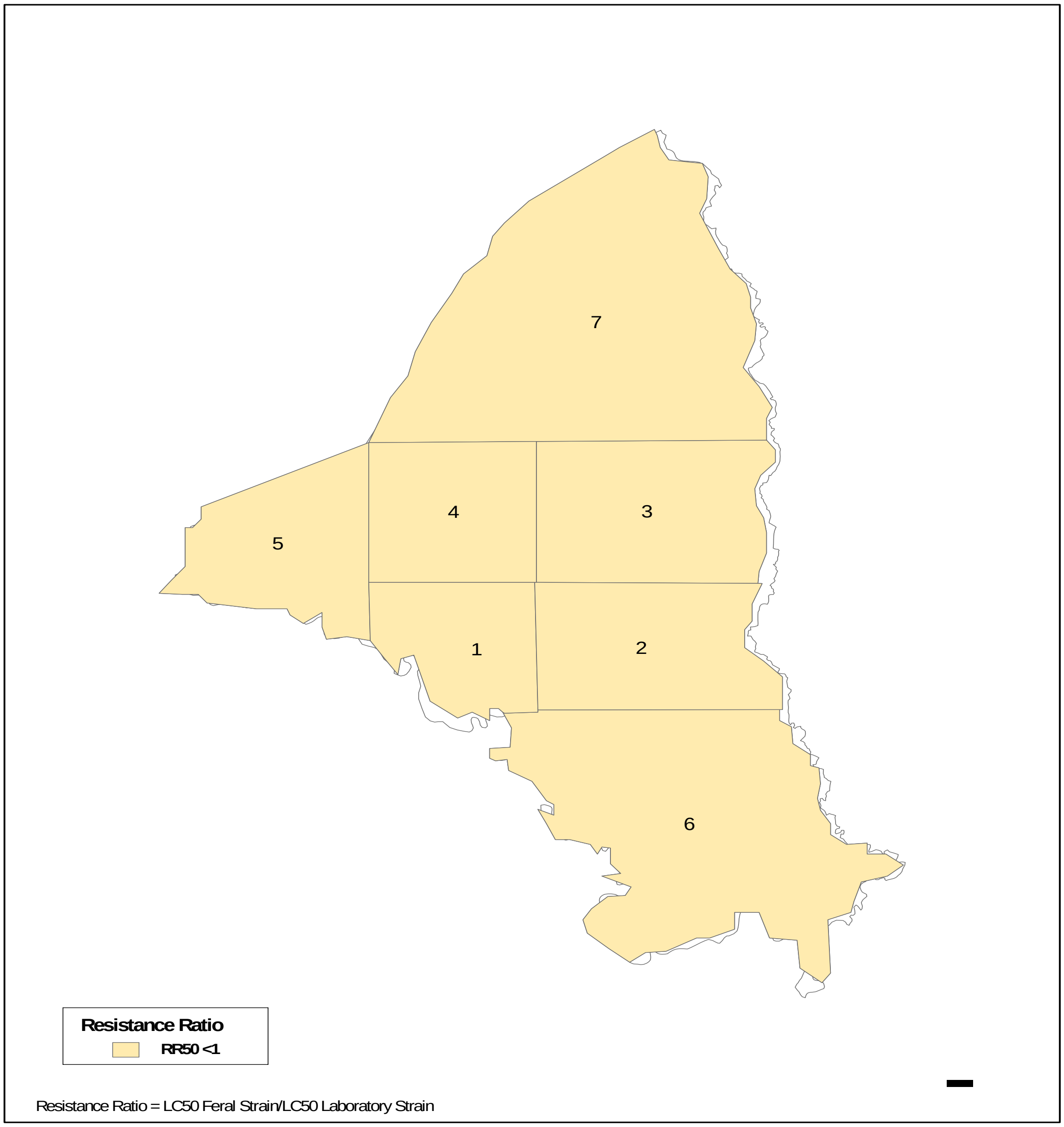

Fig. 84. Distribution of naled resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2006.

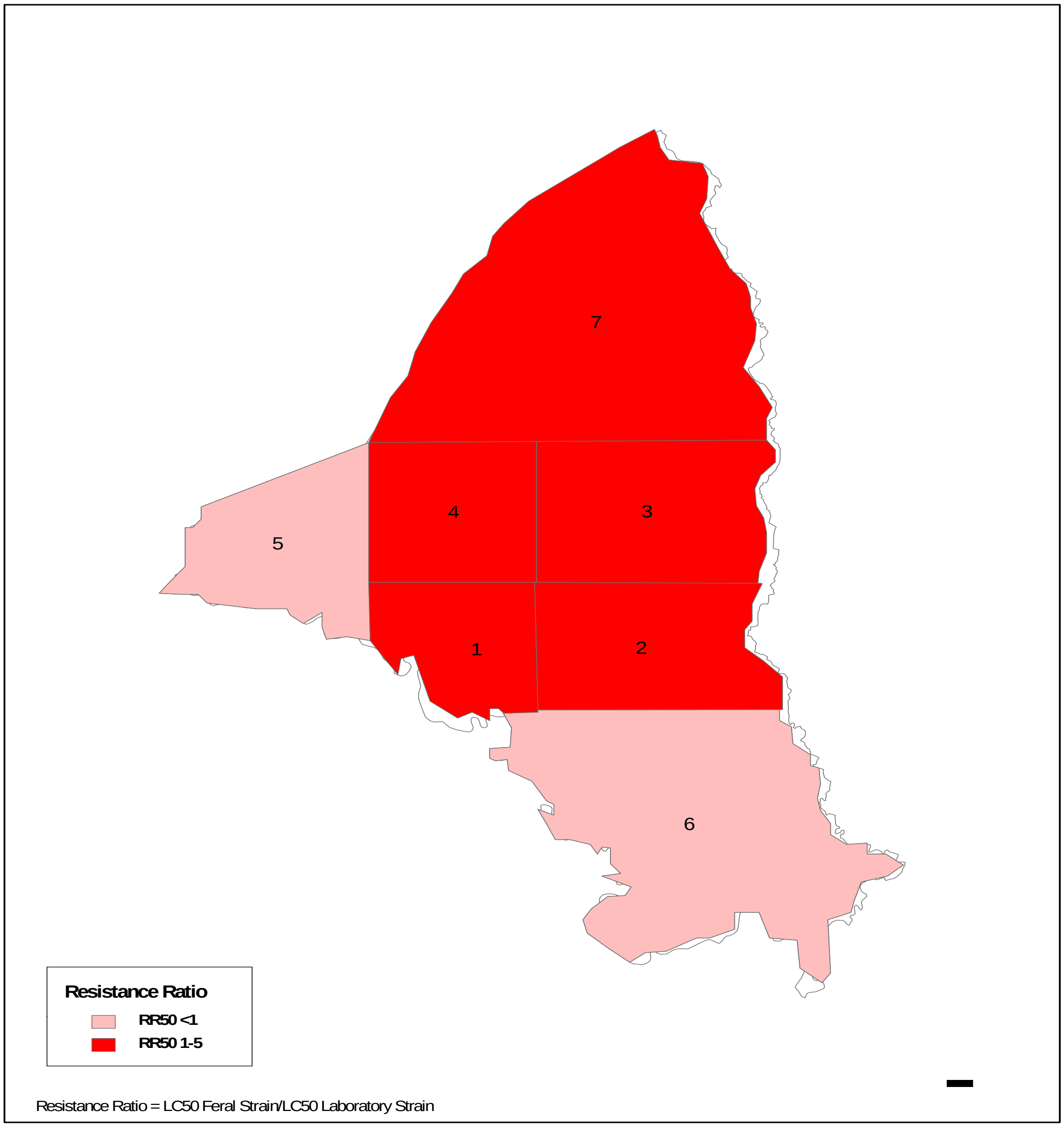

Fig. 85. Distribution of resmethrin resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2005.

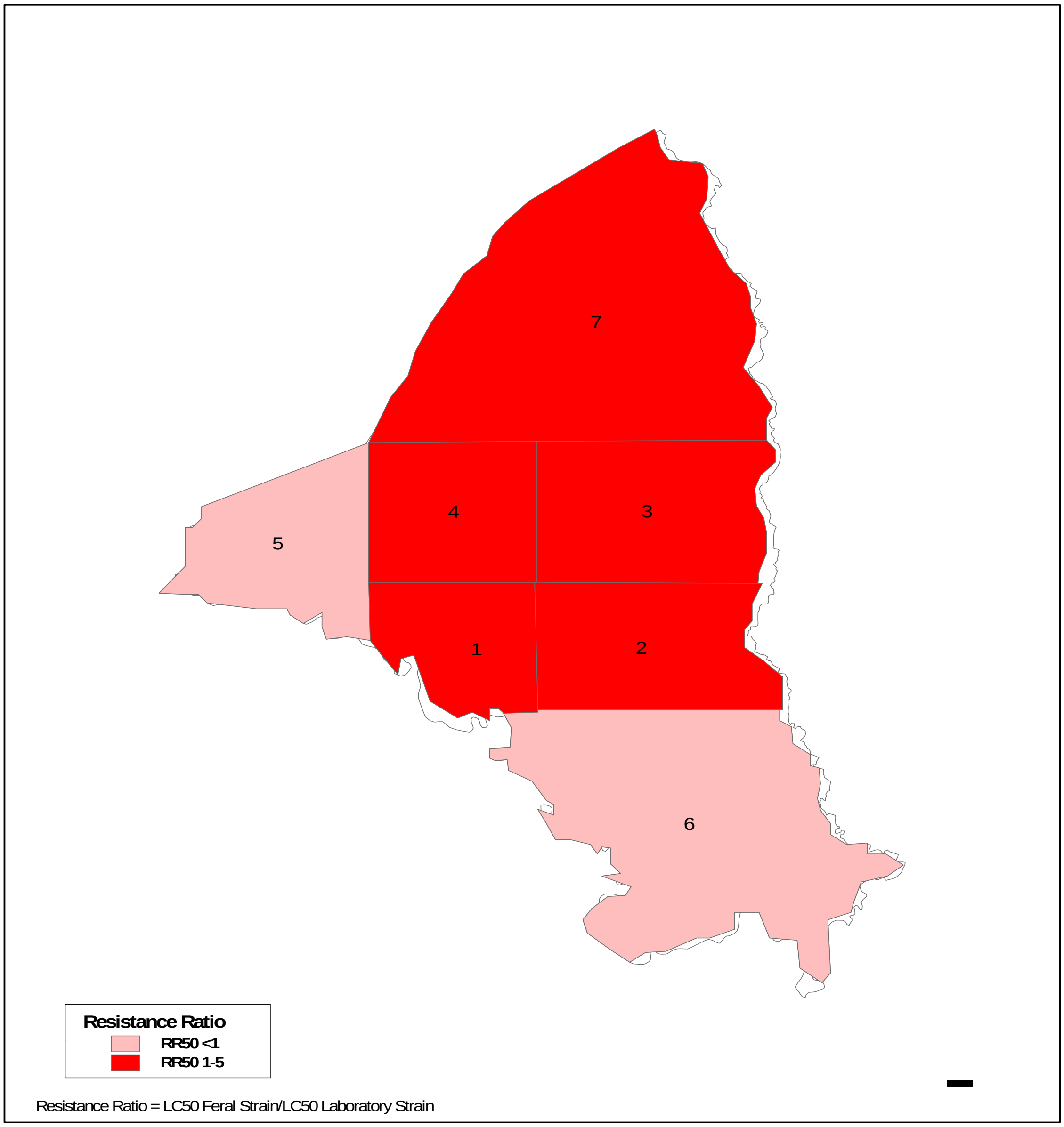

Fig. 86. Distribution of resmethrin resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2006.

The 2006 mapped resistance ratios demonstrated a reduction in the resistance to permethrin detected in the 2005 bioassay results in the mosquito populations tested from collections made in the northern and southern parts (areas 6 and 7) of Brazos County (Fig. 87-88).

Resistance patterns for sumithrin were similar to them for the other synthetic pyrethroids when the resistance ratios were mapped for Brazos County (Fig 89-90). Populations from area 4 exhibited a level of susceptibility to sumithrin that was only seen in the mosquitoes sampled from rural areas of Brazos County. This area consisted of sites in the city of Bryan and was present both years the population was sampled. The only difference between the results obtained was that mosquitoes sampled from area 5 demonstrated slightly more susceptibility to sumithrin in 2006 (Fig. 90). Overall, the mosquito populations sampled demonstrated a countywide susceptibility to sumithrin over the two years resistance monitoring was conducted, with only minor variations in the resistance ratios occurring between years (Figs. 89-90).

The pyrethrum resistance maps exhibited susceptibility in Brazos County mosquito populations to the insecticide in all mosquito samples tested in 2005 and 2006 (Figs. 91-92). These results were similar to those recorded for naled in Harris County.

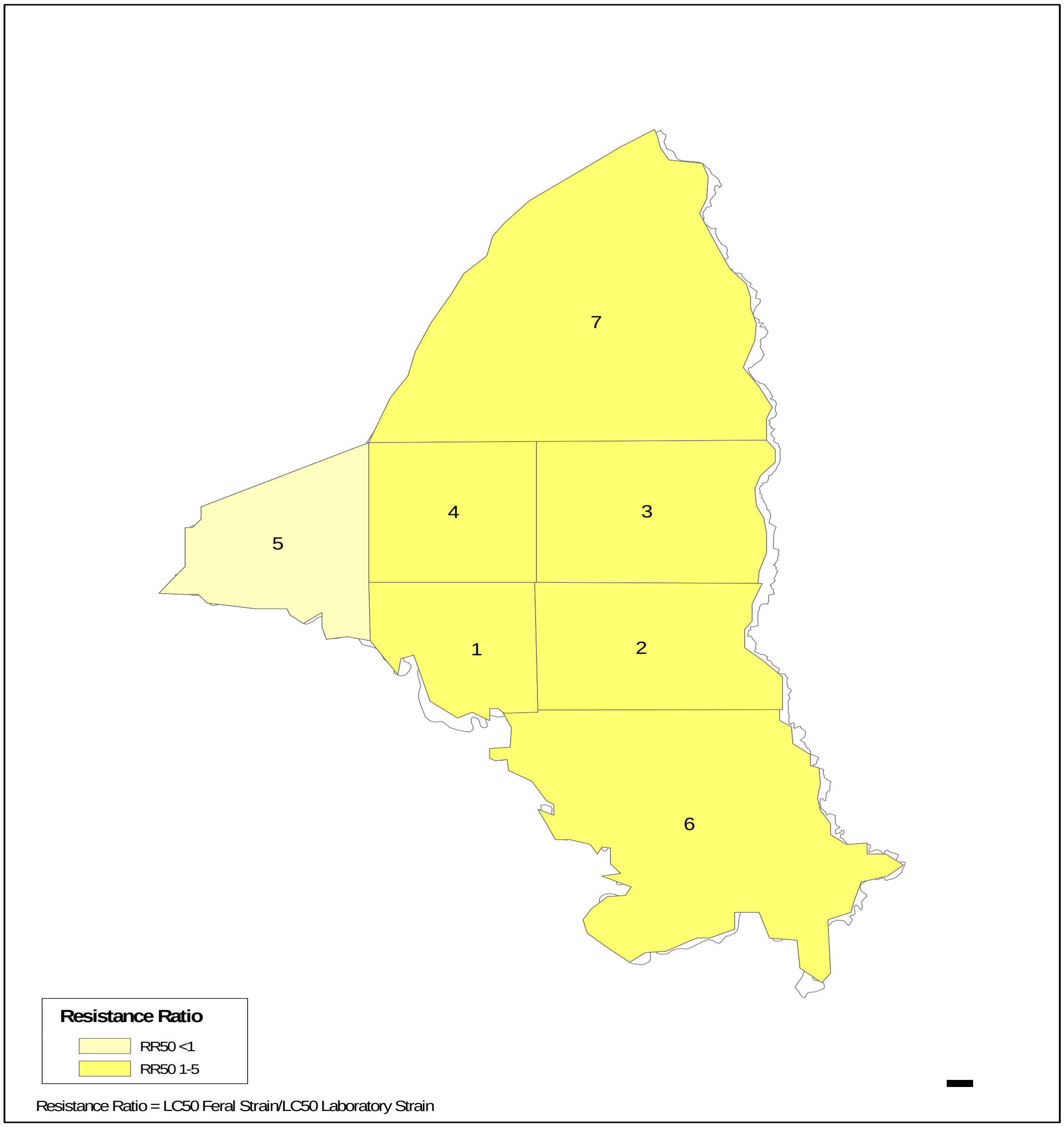

Fig. 87. Distribution of permethrin resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2005.

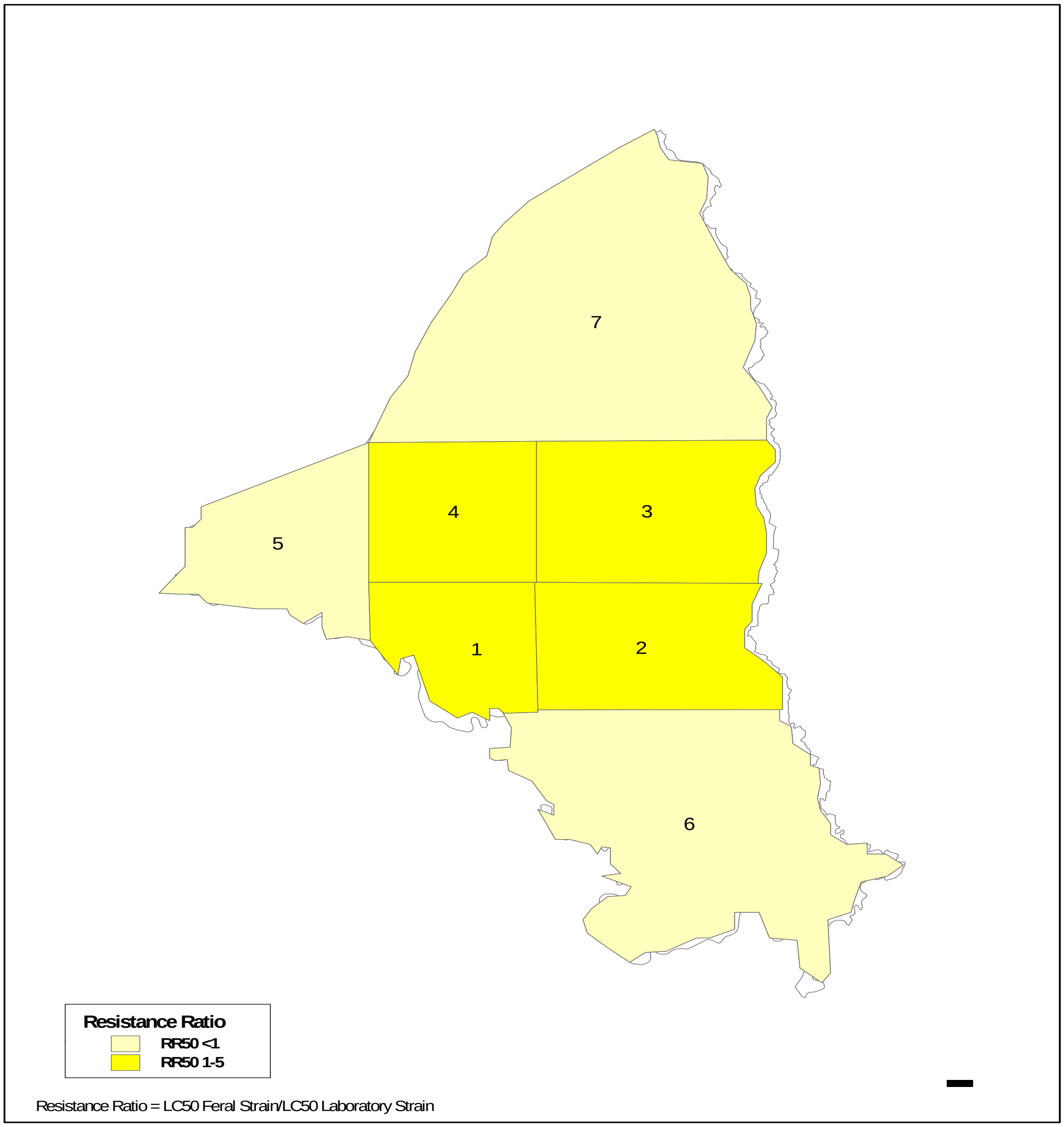

Fig. 88. Distribution of permethrin resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2006.

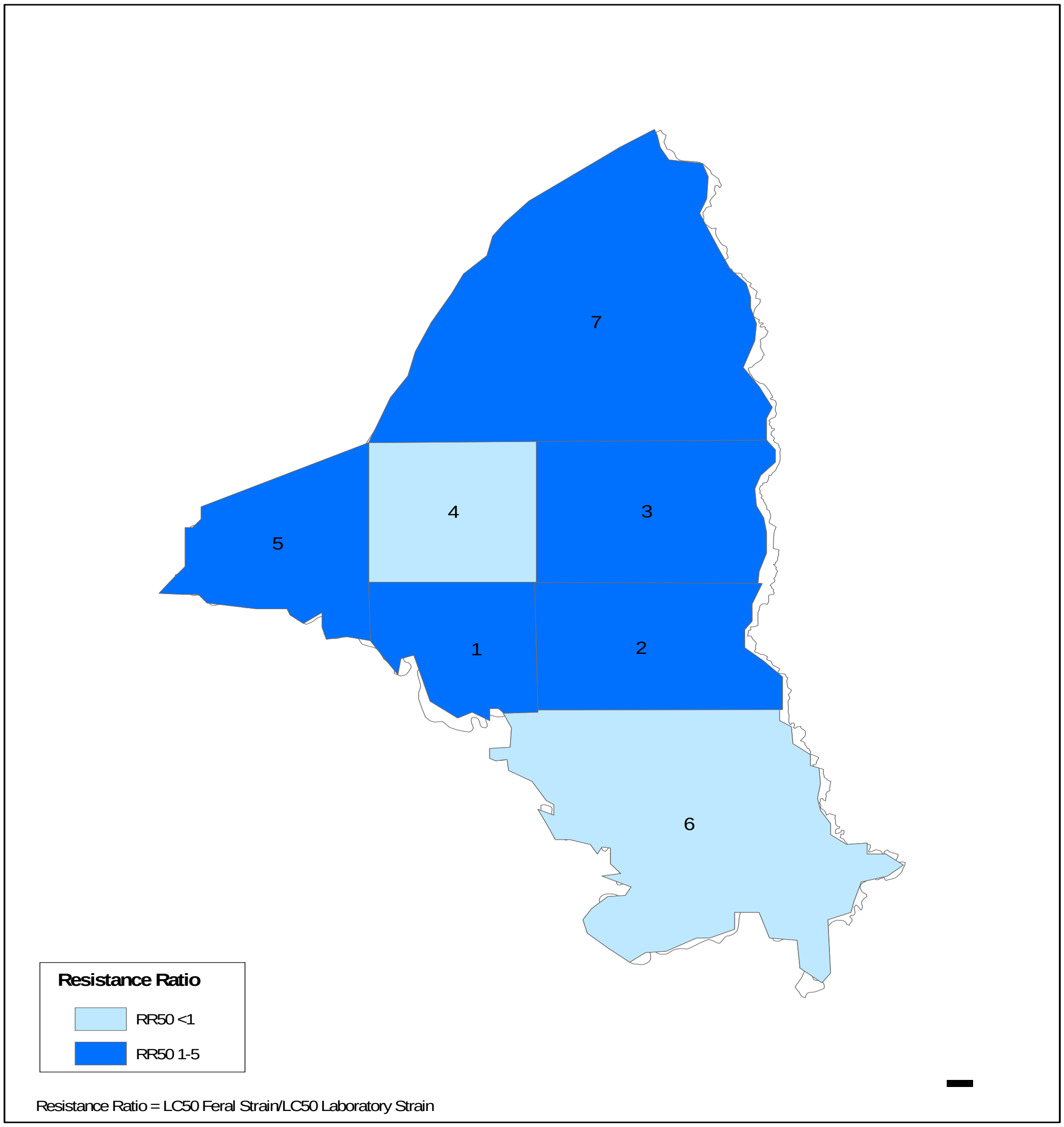

Fig. 89. Distribution of sumithrin resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2005.

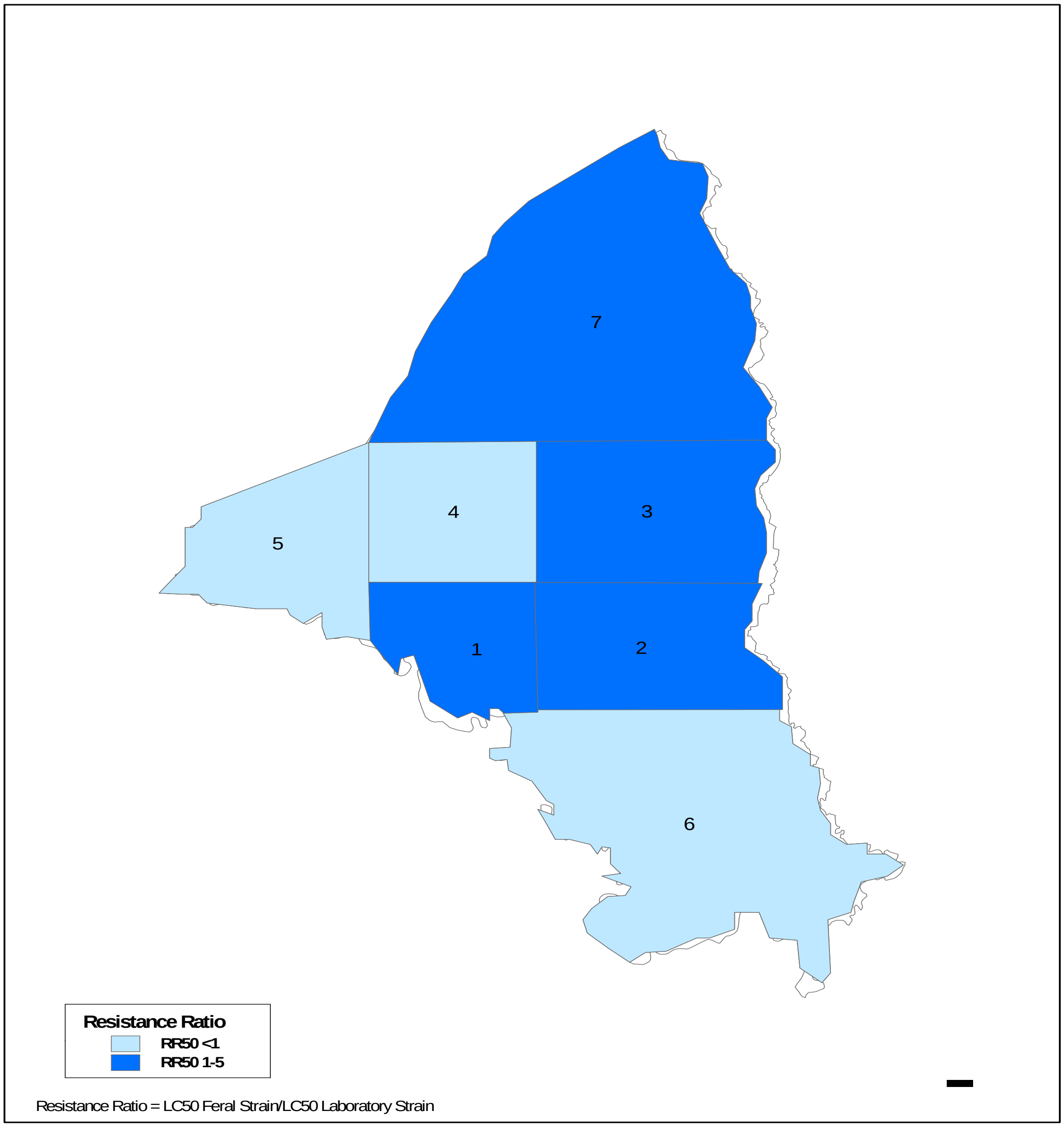

Fig. 90. Distribution of sumithrin resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2006.

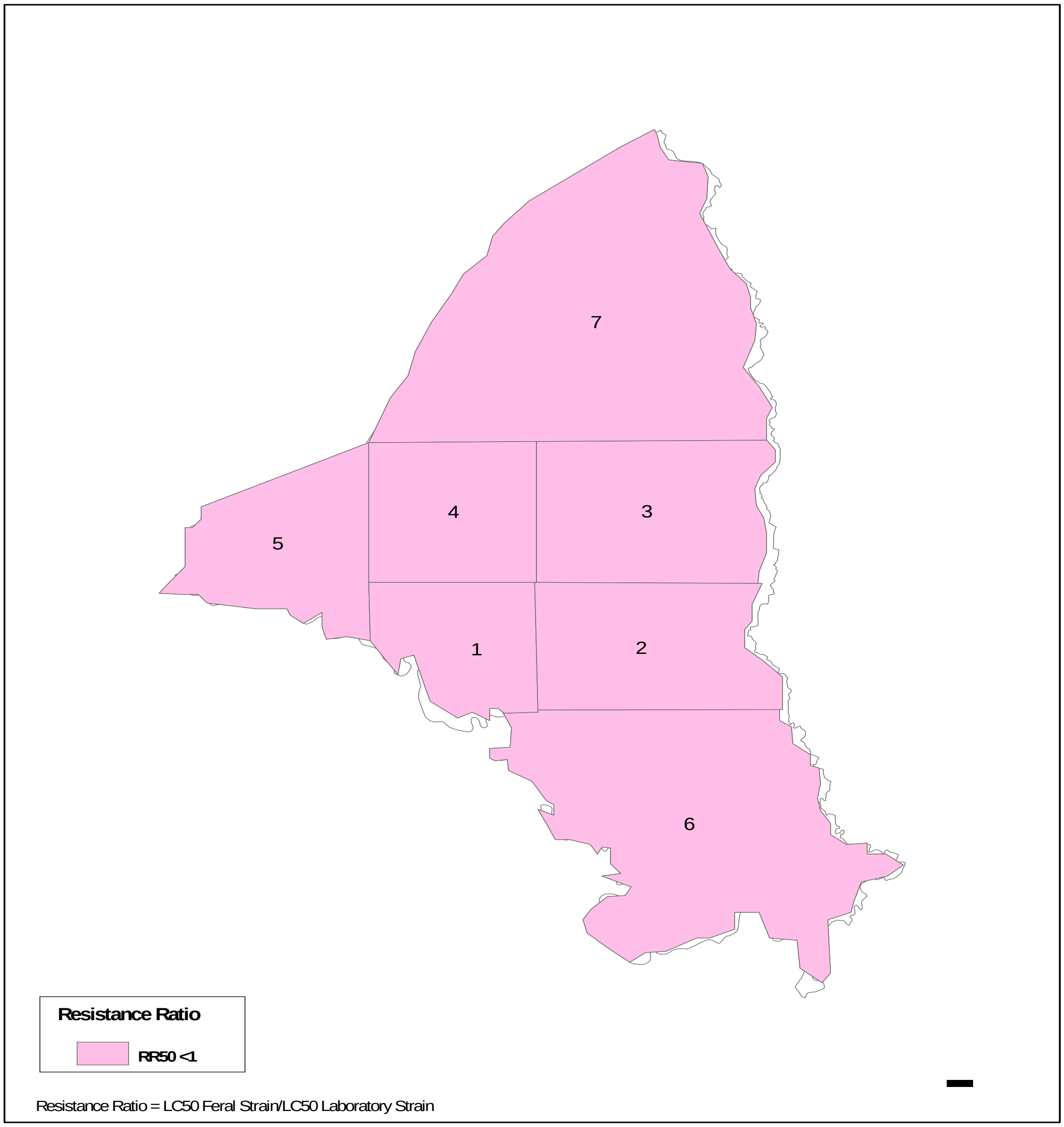

Fig. 91. Distribution of pyrethrum resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2005.

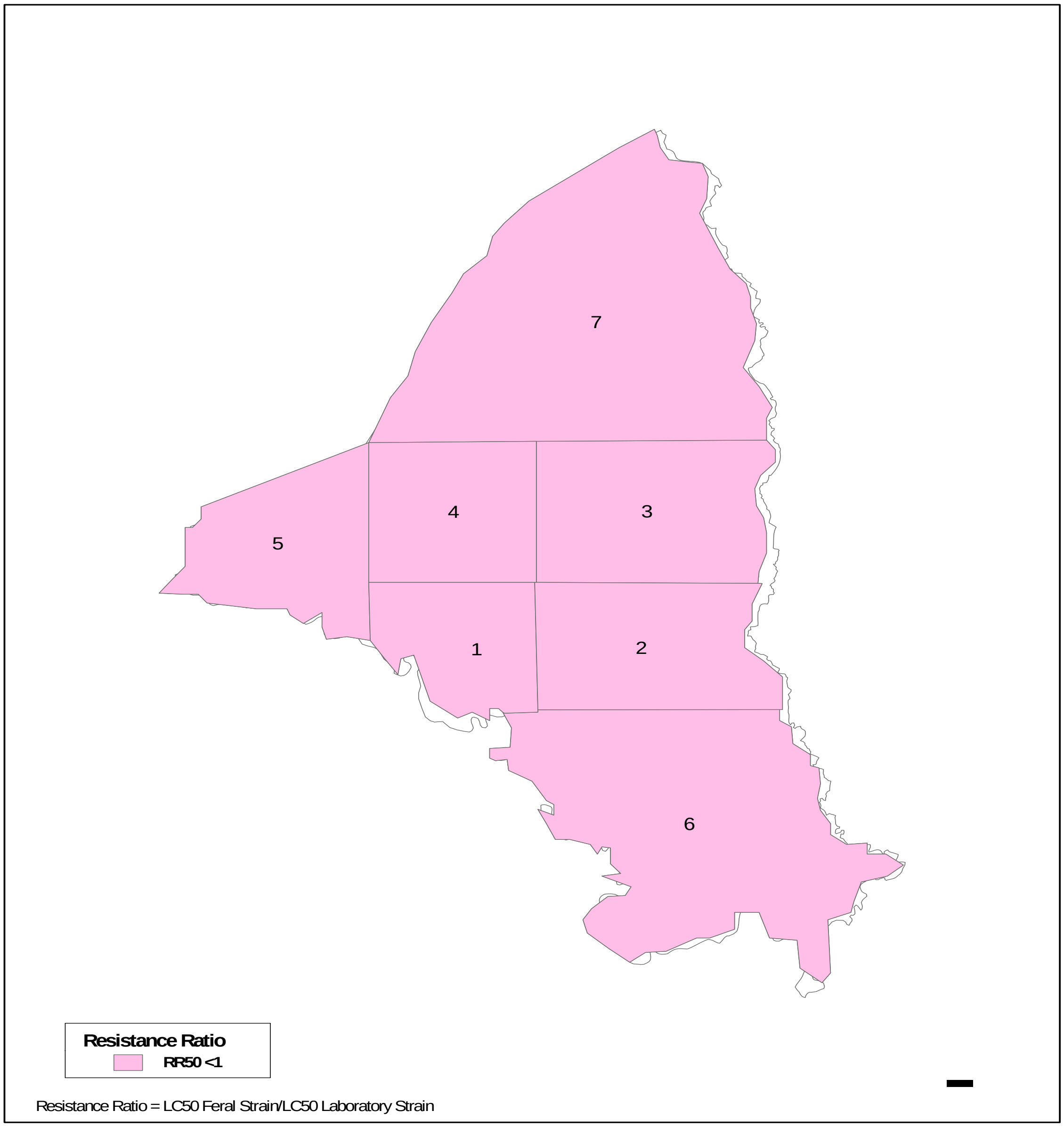

Fig. 92. Distribution of pyrethrum resistance in female *Culex quinquefasciatus* in Brazos County, Texas, 2006.

In the case of Harris County, future research should strive to sustain and expand on the use of mapping insecticide resistance in their monitoring program as demonstrated in this current study. This can be achieved by incorporating the mapping software used in other aspects of the HCMCD into the resistance monitoring program conducted by HCMCD's Test and Evaluation section. The mapping of resistance ratios from the scintillation vial bioassay tests must be continued and expanded to facilitate a greater overview of the resistance situation occurring in the county; and by using the same methods as were used in this study, it promotes increased confidence in the ability to compare the results between years. The expansion of the number of operational areas included will provide more data to determine if the resistance pattern described from the pervious two years are consistent or with the advent of the insecticide resistance management program in Harris County, will the mosquito populations were resistance was confirmed continue to decline to resistance with the influx of susceptible individuals.

With the help of the GIS computer program and related mapping software, the scope of the resistance situation needs to be conducted in different spatial resolutions to see the trends present on a smaller (single operational area) and a larger scale (Texas). The scope of the project can be reduced to a single operational area with bioassays run on multiple collections in the area and mapped to show the various levels of resistance in a smaller area. This small scale can also be used to measure the effectiveness of the control tactics in the local mosquito population and determine if there are pockets of susceptibility that can be used as refuges. The state of Texas (large scale) is in need of a statewide insecticide resistance initiative to determine what the status of resistance in the various parts of Texas. The state has funded a multitude of eradication programs that rely primarily on chemical control tactics to eliminate the pest species. The effect on the mosquito population by this judicious use of insecticides has yet to be determined or even actively considered. The development and implementation of a statewide mosquito resistance monitoring program can act as an early warning device for the various counties; and by mapping the results of the resistance testing, a resistance risk map can be developed for the state. However, it should be remembered that the maps produced are only as powerful as the information behind them. The only program that has attempted this type of research is Dr. Jimmy K. Olson's Mosquito Research Laboratory at Texas A&M University, which has provide resistance testing to counties with mosquito control operations for some 30 years; but, with his pending retirement, the state is in trouble of losing even this resource.

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CHAPTER IX

SUMMARY AND CONCLUSIONS

Insecticide resistance has been a serious cause of operational failures in insect pest control programs since first it was recognized by Melander in 1914. Insecticide resistance has provided a challenge to organized mosquito control efforts since the 1940s because of the dependence that developed on insecticides as being the primary control tactic used in such efforts (Smith 1949, Deonier and Gilbert 1950). With this threat to the effectiveness of a control program, the need for ways to monitor and predict the resistance in the mosquito population becomes paramount. This research project was undertaken to determine the levels of insecticide resistance in the mosquito populations in Harris and Brazos counties, Texas. These two counties have been severely impacted by the mosquito-borne disease, West Nile virus since its entry into Texas in 2002 and differ greatly as to their chemical application tactics that are used to control the mosquitoes vectoring this disease. Research on the five objectives explored in this study formed a base for the development of an insecticide resistance monitoring and management program in Harris County, Texas, that provides data for evaluation and modification of the mosquito control program managed by the Harris County Mosquito control Division (HCMCD)..

The first objective of this research project was to determine the level of resistance in the mosquitoes of Harris and Brazos counties to the six chemicals (resmethrin, permethrin, sumithrin, malathion, naled, and pyrethrum) labeled for use in mosquito adulticiding operations in Texas. The results of the scintillation vial bioassay tests demonstrated that there was no widespread resistance to any of the insecticides tested in either county, but a few localized cases of resistance were detected to specific chemicals in Harris County. The resistance detected in the Harris County mosquito populations was limited mainly to the synthetic pyrethroid class of insecticides, and most specifically, to resmethrin which is the primary insecticide used in disease abatement programs executed by the HCMCD. The resistance detected to the other two synthetic pyrethroids (permethrin, sumithrin) in Harris County can be attributed to cross resistance with resmethrin. All three of these insecticides work on the same target site which leads to the cross resistance seen. The variation in resistance between the pyrethroids as recorded in this study, can be attributed to the differences in chemical structure and where they specifically bind on target sites in mosquitoes.

The mosquito populations tested in both Harris and Brazos counties during this study demonstrated fairly high degrees of susceptibility to malathion, naled, and pyrethrum. These results contradicted the conventional thinking that the Harris County mosquito populations had a genetically-fixed resistance to malathion and thereby, would be ineffective for operational use. Because of these results, the HCMCD was able to initiate an insecticide resistance management program that is based on alternation of insecticides from different chemical classes, i.e. organophosphates (e.g., malathion or naled) with synthetic pyrethroids (e.g., resmethrin, permethrin, or sumithrin), to control their disease-vectoring *Cx. quinquefasciatus* populations.

The mosquito populations in the areas from Harris County that were verified by bioassays testing to have elevated levels of resistance in 2004 all dropped appreciably in their resistance when they were tested again in 2005. It is felt that the drop in these mosquitoes resistance ratios between the two years can be associated with the start of the HCMCD's insecticide resistance management program in 2005. The higher resistance levels that were detected the second year of the project in Harris County were in areas whose mosquito populations had not been tested the previous year.

The continuation of the insecticide resistance monitoring program that was initiated by this study in 2004 will be integral to the success of the HCMCD's insecticide resistance management program in future years. The monitoring program will work as an early warning device allowing for the detection of elevated levels of resistance in mosquitoes from the various areas in the county, which in turn, then will allow for modifications to be made to the control program before operational failures begin to occur. The data collected in Harris (2004-2005) and Brazos (2005-2006) counties provide an initial insecticidal susceptibility/resistance level baseline that the mosquito control personnel in both counties can use as starting points for the continuation of insecticide resistance monitoring in the future.

Six areas in Harris County and three areas in Brazos County were selected to perform a six month preliminary study to determine what variation in insecticide resistance occurs in target mosquito populations on a monthly basis in each area. The trend observed for the majority of the chemicals tested in this study was only minor fluctuations in the resistance demonstrated by the target mosquito population occurred from month to month. These minor fluctuations can be attributed to the natural genetic variability in the mosquito populations tested. In Brazos County, the mosquitoes insecticide resistance ratios tended to group together around a given value with a great deal of overlap between populations being noted for any given value. This was attributed to very little chemical pressure being applied to the populations in Brazos County by the mosquito control program being executed in this county. In Harris County the trend of resistance ratios grouping around a given value with a great deal of overlap between populations was also evident and expressed mostly in areas located outside Interstate Highway Loop 610. However, there was a noted separation of resistance levels between mosquito populations in some of the areas because of the initial level of resistance noted in certain parts of this county's mosquito populations caused chemical selection occurring prior to this study. This study was conducted in 2005 when the HCMCD implemented an insecticide resistance management program that consisted of alternation of chemicals. This program may have contributed to the suppression of large fluctuations in the monthly resistance ratios of the mosquito populations by preventing the mosquitoes from adapting to a single insecticide and developing resistance in the population.

A Spearman rank correlation analysis was performed on the resistance ratio data gathered during two year periods that insecticide resistance was assessed and monitored in mosquito populations in Harris and Brazos counties (i.e., in 2004-2005, and 2005-2006, respectively). The correlations between 2004 and 2005 resistance ratios were significant in Harris County *Cx. quinquefasciatus* populations for the three synthetic pyrethroids (resmethrin, permethrin, and sumithrin) only. In Brazos County a positive association was observed between the resistance ratios recorded from 2005 and 2006 for all the insecticides tested except pyrethrum.

The results of the correlations between the two years of recorded resistance ratios (i.e., 2004-2005 in Harris County and 2005-2006 in Brazos County) can be used as a predictive method program for the determination of what level of resistance can be expected in the mosquitoes from the various operational areas the following year. With this value determined, a modification in chemical used and/or rotated with each other can be determined. This correlation also identifies possible “mosquito populations of interest” that can be closely monitored throughout a given year as to the chance of their developing resistance.

A Pearson’s correlation analysis was run on the *Cx. quinquefasciatus* insecticide resistance ratios obtained from the vial bioassay tests and the number of spray events conducted in the operational areas by the Harris County Mosquito Control Division. The correlation analysis was performed on the three insecticides (resmethrin, malathion, and pyrethrum) used for adulticiding operations on a consistent basis by the HCMCD. The analyses were performed using the spray events conducted in the same calendar year as the resistance ratios were collected and the year prior to when the bioassay tests were conducted. This was done for the two years of bioassay testing done on mosquitoes in Harris County. The correlation analyses were then performed including and the excluding mosquito populations in areas that did not receive an insecticide treatment (zeros in the data set) to determine if the mosquitoes from untreated areas affected the significance of the association.

There was a positive association between the Harris County *Cx*. *quinquefasciatus* resistance ratios and the number of spray events conducted by HCMCD the previous year in both variations of the analysis performed. When the correlation analysis was performed including zeros in the data set the association between the 2005 resistance ratios and the 2005 spray events occurring was significant; but when the zeros were removed, the significance was lost. The significance was artificially inflated by the number of points grouped at the base of the correlation.

Because of the belief prior to this study that the *Cx*. *quinquefasciatus* populations in Harris County were resistant to malathion, there was only one year of that HCMCD used this insecticide in its control program; so, only a correlation analysis involving the 2005 spray events could be determined on malathion. The association between spray events and the 2005 resistance ratios for malathion was statistically significant when run including and excluding zeros. Further testing is needed to determine if this association holds as the chemical is used more extensively.

All correlations derived for the pyrethrum resistance ratios and number of spray events involving this agent were not statistically significant. The cause of the insignificance could be because of the small number of mosquito samples tested in 2004 or the limited use of the chemical by HCMCD, which led to multiple areas in the county never receiving an application of pyrethrum resulting in a large number of zeros in the data set.

The resistance ratios for Harris and Brazos county’s *Cx*. *quinquefasciatus* populations resulting from the scintillation vial bioassay tests were mapped on shapefiles of using ArcGIS® software to provide a spatial view of the distribution of resistance across the two counties. The resistance distribution maps produced provided an overview that revealed patterns and trends that were not evident in the raw data. This was especially apparent for the three synthetic pyrethroids tested in Harris County. The areas where high resistance ratios were recorded were located predominantly within Interstate Highway Loop 610 in Houston. The areas with confirmed resistance were grouped in the northeast, southeast, and southwest corners inside the Interstate Highway Loop 610. When the maps for resistance to the three pyrethroids were overlayed on each other, the locations of the areas with highest resistance ratios for each synthetic pyrethroid were found to primarily be in the same locations in Harris County. As previously noted, this phenomenon is thought to be due to these mosquitoes in these areas having developed cross-resistance to the three synthetic pyrethroids included in this study. In the areas of Harris County where resistance to the pyrethroids is grouped, many have factors other than the HCMCD’s control activities that may be contributing to the high resistance ratios recorded in the corresponding mosquito populations and many require further investigation.

The mosquito insecticide resistance maps developed for Brazos County indicate susceptibility to all the insecticides tested exists throughout the county. The mosquitoes in the three areas located outside the Bryan and College Station area tend to have slightly lower resistance ratios than those that occur in areas within the limits of either city. These maps will give those in Brazos County involved in mosquito control insight as to the status of resistance in the county and provides them with another means to help design an implement future mosquito control programs.

The mapping of the mosquito insecticide resistance ratios performed during this study provides further means by which insecticide can be managed in Harris and Brazos counties. The identification of the areas when mosquitoes tend to have elevated resistance levels will allow the control agency to modify the control program for that specific area. The maps also identify areas that may have extenuating circumstances contributing to the higher resistance other than a mosquito agency's control activities, and those identified problems can then be addressed.

This research study lead to the development of many methods which may be employed by control agencies when determining if there is resistance developing in the mosquito population, the effectiveness of the resistance management program, and what modifications can be made prior to control activities are conducted. With the implementation of the methods a mosquito control organization stands a better chance of conducting a successful disease abatement program and maintaining the effectiveness of the insecticides chosen for control methods.

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