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FACTORS POTENTIALLY INFLUENCING THE ABUNDANCE OF MOSQUITOES IN LOUISIANA RICE FIELDS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfilment of the
Requirements for the degree of
Master of Science

in

The Department of Entomology

by
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ABSTRACT

The rice stink bug (*Oebalus pugnax*) is the major pest of late-season rice in Louisiana. Prior studies indicate that large populations of mosquitoes are often present in rice fields during the time that rice fields are infested with rice stink bugs. Lambda-cyhalothrin (Karate®) is widely used to control rice stink bugs. The purpose of this study was to determine if application of the insecticide Karate® at a rate of 0.033 kg/ha, for rice stink bug control had an effect on the population of mosquitoes in rice fields. This effect was assessed by monitoring natural populations of mosquitoes before and after applications of Karate® in simulated rice paddies at the LSU AgCenter Rice Research Station (Crowley, Acadia Parish, Louisiana) during the summers of 2003, 2004 and 2005. In 2005, monitoring of native mosquito population was complemented by the use of sentinel cages with *Culex quinquefasciatus* Say larvae and exposure of *Cx. quinquefasciatus* larvae to treated rice field water in the laboratory. The number of larvae in Karate® plots decreased after Karate® applications but a significant overall effect was observed only after the first application of 2004 ($p=0.034$). Mortality of larvae in sentinel cages ($p=0.0386$) and of larvae exposed to rice field water at 48hrs ($p=0.0130$) was also observed in some cases. A test conducted in a large rice plot with sentinel cages confirmed the effect of Karate on mosquito larval populations ($p=0.0012$). An additional effect of foliar Karate residues on adult *Cx. quinquefasciatus* mosquitoes was observed in the laboratory (2004, 2005). Higher mortality was detected in adults exposed to rice foliage treated with Karate than the untreated control. Another factor that could influence the number of larvae present in rice fields is the oviposition response of mosquitoes to the presence of predatory insects. To test this response, *Cx. quinquefasciatus* adult mosquitoes were exposed in cages to water conditioned by previous exposure to potential aquatic predators. According to the Oviposition Activity Index (OAI), female mosquitoes preferred to lay eggs in water conditioned water rather than distilled water. The highest OAI was obtained with the Hydrophilidae-conditioned water.

CHAPTER 1. INTRODUCTION

1.1 Mosquitoes in Rice Fields and Diseases Transmitted

The human population is expanding worldwide; the twentieth century began with 1.6 billion people and ended with 6.1 billion (Population Reference Bureau 2003). Rice is one of the most important crops in the world, and it provides the main source of energy for more than half of the world population (Business Group International 2001). Because of the large number of people dependent upon rice, it has been estimated that annual production must increase by five million tons a year to keep pace with population growth (Heinrichs and Miller 1991). Dramatic population growth (Population Reference Bureau 2003) after World War II occurred in less developed countries including all countries in Africa, Asia (excluding Japan), Latin America and the Caribbean, as well as the regions of Melanesia, Micronesia, and Polynesia. It is also mainly in these developing countries that vector-borne diseases most adversely affect the health and the quality of life of millions of people (Garrity 1988). According to the World Health Organization - WHO (2007) a considerable portion of the diseases suffered by children less than five years of age (in Asia, Latin America, and Africa) was due to four key vector-borne diseases: malaria, schistosomiasis, Japanese encephalitis and dengue haemorrhagic fever. Therefore, these regions not only need to increase their food production but also improve their strategies against vector borne diseases. A well-established link has been provided in the past between agricultural practices, particularly in wetland rice production, and the increased prevalence of mosquito-borne diseases such as malaria, filariasis, Japanese encephalitis and a variety of other zoonotic arboviruses (Lacey and Lacey 1990). In the last decade, major global demographic, environmental and societal changes have contributed to the re-emergence of vector-borne diseases, such as West Nile Virus-WNV (WHO 2007). Developed countries, including the United States, are less affected by vector-borne diseases but since they are globally interconnected with developing countries the chances of being invaded by potential hosts or vectors of diseases like malaria or West Nile Virus has become a constant risk that needs to be surveyed. Map 1 (Appendix) shows the number of human WNV disease cases reported to the Centre for Disease Control and Prevention's (CDC) ArboNET system by state and local health departments for public notification (Centre for Disease Control and Prevention, 2007).

The Agricultural Research Service-ARS (Agricultural Research Service 2003) has estimated that 460 million tons of rice are grown annually on more than 145 million ha. worldwide. Over 90 percent of the production occurs in Asia, while the remainder is divided among Latin America, Africa, Australia, Europe, and the USA (Agricultural Research

Service 2003). Rice has been produced commercially in the USA for more than 300 years. Nearly all rice grown in the United States is produced in Arkansas, California, Louisiana, Mississippi, Missouri and Texas (US Rice Producers Assoc. 2002). According to the January National Agricultural Statistics Service-NASS monthly newsletter in 2006, the average yield per acre for all U.S. rice is an estimated 6,636 pounds per acre. This high yield is achieved in part by the use of efficient management practices. Among these practices is the control of rice pests. There are more than 70 species of insect pests worldwide known to feed on rice, and at least 20 of them can seriously affect rice production (Agricultural Research Service 2003).

Rice farmers worldwide have become increasingly aware of the potential health hazards associated with flood irrigation in rice. Riceland mosquitoes arising from their fields are a potential hazard for farmers and their livestock (Dame et al. 1988). The survival of immature mosquitoes and subsequent emergence of adult mosquitoes depend on the continued presence of water. Over 135 pest and vector anopheline and culicine mosquito species have been found in association with riceland habitats (Lacey and Lacey 1990). Rice insect pests and haematophagous insects that breed in the rice fields continue to be a limiting factor for both the production of rice and public health. Therefore, these insects need to be studied and controlled in an integrated fashion in order to increase rice production and decrease vector problems to both human and domestic animals. Table 1 lists the mosquitoes associated with rice fields and their roles as potential vectors of human disease.

As in other regions, rice fields in Louisiana are potential breeding sites for mosquitoes. The major rice producing areas in Louisiana are in the southwestern region and in the northeastern part of the state. Previous studies (Chambers et al. 1979, Chambers et al. 1981, Andis et al. 1983, Andis and Meek 1984, McLaughlin et al. 1987) have shown larvae of different mosquito species belonging to the genera *Anopheles*, *Culex*, *Psorophora* and *Uranotaenia* present in rice fields in Louisiana The flooding of rice fields for rice production promotes the emergence of eggs present, e.g. *Ps .columbiae* (Dyar and Knab) and attracts other species as it becomes a permanent source of water. The development of mosquito larvae in rice fields, their abundance and species composition, could be an important factor in maintaining the transmission of some arboviral diseases among humans and/or their animals, e.g. horses. Our objective was to investigate interactions between rice production and potential mosquito vectors in Louisiana. In particular, we sought to understand the effect that insecticides used to control rice stink bugs had on the mosquito populations and the effect on oviposition of mosquitoes when exposed to conditioned water (water previously exposed to potential predators of mosquitoes).

1.2 Objectives

1. Determine the effect of lambda-cyhalothrin (Karate®) applied to manage rice stink bugs in late season rice on populations of mosquitoes in Louisiana rice
2. Determine the effect of the presence of potential predators on the oviposition of *Culex quinquefasciatus* in the laboratory

CHAPTER 2. REVIEW OF LITERATURE

2.1 Rice Fields as Sources of Mosquitoes

Worldwide there are about 3450 known species and subspecies of mosquitoes (Service 1993a), of which about 200 species representing 13 genera and three subfamilies (Anophelinae, Culicinae and Toxorhynchitinae) occur in the United States (Darsie and.Ward 2000). The Louisiana Department of Health and Hospitals' Office of Public Health has stated that over 60 species are present in Louisiana (LDHH and OPH 2001).

The immature stages of mosquitoes are aquatic, and need water for their development. A wide range of aquatic habitats are used such as natural tree-holes, streams, ponds, swamps, and artificial water-holding containers. Most of these different habitats are found in Louisiana. The practice of flooding rice fields to grow the crop and to control weeds encourages the presence of populations of immature mosquitoes. The relative abundance of mosquitoes in Louisiana rice fields varies extensively from year to year and field to field, as different species prefer different habitats within rice fields. In studies conducted by Chambers et al. (1979, 1981), mosquito larvae appeared from the end of May through September in rice that had been planted and flooded permanently by April in the southern and middle regions of Louisiana. They also found that the number of larvae increased in fields that were reflooded to produce a second crop of rice (Chambers et al.. 1981). *Anopheles crucians* (Weidemann) and *Ps. columbiae* appeared earlier in the growing season and *Culex erraticus* (Dyar and Knab) increased in numbers during the second crop season.

In another study conducted during the first crop season (May to July) in Louisiana (McLaughlin et al. 1987), larvae of *An. quadrimaculatus* Say were predominant over *An crucians* in recently flooded fields. Later, as the rice fields became more eutrophic, *An .crucians* became dominant. Sampling was mainly performed at the edges of the rice fields. Andis and Meek (1984) observed that *Ps. columbiae* appeared primarily by the edges of the rice plots when initially flooded but, during the second flooding, there was no significant difference in larval populations between the edges and the middle.

Most mosquito species of concern in the temperate regions are facultatively polyvoltine, with several generations in the summer months, and over-winter in an inactive state (Holck and Meek 1991). Most female mosquitoes usually mate only once but produce eggs at intervals throughout their life, and to do so they require a blood meal (Rozendaal 1997). This behaviour can turn them into a nuisance, but also into a potential vector of diseases for humans and their livestock. For mosquitoes to act as vectors they must be able to multiply and successfully transmit a pathogen. Vector incrimination as described by Reisen

(2002) involves field and laboratory data that quantifies field infection rates, vector competence and vectorial capacity. Field infection rate is finding a number of infected mosquitoes in the field which indicates that they have fed on vertebrate hosts carrying the parasite (Reisen 2002). Their ability to transmit infection must still be proven in order to be considered true vectors. This ability to transmit infection is defined as vector competence, the physiological ability of a vector to support the development of a particular parasite (Lane and Croskey 1993), susceptibility to infection with the parasite and ability to transmit this acquired infection (Reisen 2002). To prove transmission or vector competence the vector “candidate” has to feed on an infected host, incubate the pathogen and then infect a non-infected host after feeding. Finally, to establish the role that a mosquito may have in the transmission of a particular infectious disease, there is vectorial capacity, the number of new infections per bite per day, which quantifies the basic ecological attributes of the vector relative to parasite transmission (Reisen 2002). Vectorial capacity is an estimate of the transmission rate by the vector; it represents the average number of potentially infective bites that will be delivered by all the vectors feeding upon a single host in one day (Eldridge and Edman 2000).

A major concern about the rice habitat is that it may serve as a breeding site for potential vectors of diseases such as malaria (*Anopheles spp*), which was present until the 1940s in the southeastern United States, and other arboviruses, especially West Nile Virus (*Culex* spp). Malaria affected colonization along the East Coast and was not effectively controlled until the 1940s when the *Anopheles* vectors were controlled (AMCA 2005). *Anopheles quadrimaculatus* was the primary vector of *Plasmodium vivax* (protozoa) in the United States. After the 1999 outbreak of West Nile in New York City, this disease spread across the United States. In California the mosquitoes that seem to play the primary role in the enzootic maintenance and transmission of WNV are *Cx. tarsalis* (Goddard et al.. 2002), which is also known to transmit SLEV and WEEV. *Culex tarsalis* feeds mainly on birds and immature stages can be found in rice fields.

The Centre for Disease Control and Prevention has reported a list of 60 species that have been found positive for the West Nile Virus (CDC 2007) since 1999. This list includes Louisiana rice field species: *Ps. columbiae, An. crucians, An. quadrimaculatus, Cx. erraticus, Cx. salinarius* Coquillett, and *Uranotaenia sapphirina* (Osten Sacken) (Chambers et al. 1979, Chambers et al. 1981, Andis et al.1983). Table 1 lists the species found in the United States (the Louisiana species are marked with an asterisk) rice field habitats as presented by Lacey and Lacey (1990), including those cited by Chambers et al. (1979) and Andis et al. (1983).

Table 1 also provides the potential vectors, as listed by Lacey and Lacey (1990), including those positive infected species reported by the CDC, for West Nile Virus as of August 2005.

Table 1. Mosquitoes associated with rice fields in the United States and their roles as potential vectors of human diseases or pests.

Mosquito species	***Medical importance/pathogens vectored***[d]
Aedes melanimon Dyar [a]	CE, SLE, and WEE viruses
Aedes scapularis (Rondani) [a]	ILH, KRI, MEI, LUK, MAG, WYO, SLE and VEE viruses
Aedes vexans (Meigen) * [b]	EEE, SAG, SF, JC, TAH, WEE, CE, GET, TNT, WN and BAT viruses
Anopheles albimanus Weidemann [a]	TLA virus, malaria
Anopheles crucians Wiedemann * [a, b, c]	Malaria, CV, TEN, EVE, KEY, CE, EEE, SLE, VEE, WN and SR viruses
Anopheles franciscanus Mc Cracken [a]	Malaria, MD virus
Anopheles freeborni Aitken [a]	Malaria, WEE and VR viruses
Anopheles pseudopunctipennis Theobald [a]	Malaria, SA and VEE viruses
Anopheles quadrimaculatus Say * [a, c]	Malaria, CV, WN and TEN viruses
Culex erraticus (Dyar and Knab) * [a, b, c]	WN virus
Culex salinarius, Coquillett * [a, b, c]	Pest, EEE, FLA, SLE and TEN viruses
Culex tarsalis Coquillett [a]	WEE, SLE, LOK, TUR, HP, VEE, CE, FLA, GLO, LLS, WN and UMA viruses
Culex territans Walker [a]	FLA virus occasional pest and WN virus
Psorophora ciliata (Fabricius) *[a, b]	Pest, VEE and WN virus
Psorophora columbiae (Dyar and Knab) *[a, b, c]	Veterinary pest, VEE, TEN, WN and CV viruses
Psorophora discolor (Coquillett) *[a, c]	Pest, VEE virus
Uranotaenia sapphirina (Osten-Sacken) *[b, c]	WN virus

[a] From: Lacey and Lacey 1990, [b] From: Andis et al. 1983, [c] From: Chambers et al. 1979, [d] The complete names of these arboviruses are listed in appendix 2, * species found in LA.

As shown in Table 1 some of these species are potential vectors of multiple pathogens. In Louisiana, besides West Nile Virus (WNV), three arboviruses transmitted by mosquitoes have been detected: St. Louis Encephalitis (SLE), Eastern Equine Encephalitis (EEE) and LaCrosse Virus (LADHandH, 2004). Different species have different preferences for their hosts. In order to maintain WNV epizootic transmission mosquitoes must be avian feeders, but in order to transmit WNV to humans, they should be general feeders and include mammals among their hosts. Although different mosquito species have different host preferences for their blood meal (e.g. *Ae. vexans, An. quadrimaculatus* and *Psorophora* prefer mammals; *Cx. salinarius* and *Cx. erraticus* prefer birds), man can become an accidental host when present, thereby getting involved in the transmission cycle of an enzootic disease. Therefore, it is important to determine the species of mosquitoes present and their potential as vectors.

Some of the main factors that encourage oviposition by females are proximity and number of host animals, water quality (ionic and organic content), presence or absence of a water current, degree of shading and plant composition, and the density and height of the crop (Lacey and Lacey 1990). Rice fields in Louisiana could be good breeding sites for mosquitoes as they provide water with organic content, still water, nearby pastures (with mammal hosts), shade and plant biomass for protection from predators. The mosquitoes that rice fields produce are mainly a nuisance for the farmers and human populations working in these areas but some species may become vectors of diseases as they feed on infected animals and later feed on man.

Lacey and Lacey (1990) suggested that although low numbers of larvae were found in rice fields, there were certain species that could be significant potential vectors, especially when found near human populations. Increases in rice production could enhance the abundance of mosquito species or lead to the introduction of new potential vectors into the area. An important assumption of this project is that rice fields are an important source for potential vectors of human arbovirus diseases like SLE, EEE, WNV or LaCrosse, in Louisiana. Holck and Meek (1991) stated that "the rice-cattle agroecosystem in Louisiana provided an ideal environment for mosquito populations". Previous studies had found the species breeding in rice fields to be potential vectors (Table1) of some pathogens. In addition the emergence of a new vector borne-disease arbovirus in this country, West Nile Virus (which is usually transmitted in urban areas), has been detected in some rice field mosquitoes (CDC 2007).

2.2 Control Methods of Immature Mosquitoes

2.2.1 General Discussion of Control Methods

The aims of mosquito control measures are to reduce the biting nuisance and prevent or stop disease transmission (Service 1993b). Control measures can be directed either against the adult stage, the immature aquatic stage, or both. A variety of techniques have been used (source reduction, water management, personal protection, biocontrol and insecticides) to obtain the desired suppression (Dame et al. 1988). In general a combination of methods is used rather than focusing on a single method.

2.2.1.1 Integrated Control

Integrated control is generally known as integrated pest management (IPM) or integrated vector management (IVM). Integrated pest management is a concept based on ecological principles that integrates multidisciplinary methodologies in developing ecosystem management strategies which are practical, effective, economical and protective of both public health and the environment (Axtell 1979). Because it is usually easier to control the immature stages of mosquitoes, which are located in a known area, in this study we focused on larval control. The three basic methods of larval control are insecticidal, biological and physical control (Service 1993a). All of these methods are part of the integrated pest management approach. As described by Rozendaal (1997) the use of insecticidal and biological control with other measures that are either long-lasting or permanent, referred to as environmental modifications, are incorporated into control programs to prevent or eliminate the breeding of mosquitoes.

2.2.1.2 Larvicides

Some of the advantages of application of larvicides are: mosquito larvae can be destroyed before adults disperse to human habitations, operations can sometimes be carried out in a shorter time, many of the effective larvicides are widely available, and application of larvicides can be done by hand (small scale) or by different types of sprayers (larger scale) (Rozendaal 1997). Petroleum oils and the arsenical powder Paris green were the first frequently used chemical larvicides (Rozendaal 1997). Later organochlorines (e.g. DDT, lindane, dieldrin all no longer used) appeared and reduced the use of oils and Paris green (Service 1993a). Other chemical products developed to control larvae are organophosphate (e.g. temephos, fenthion, malathion), carbamate (e.g. propoxur) and pyrethroid (e.g. deltamethrin, permethrin) classes of insecticides.

Two of the main bacterial larvicides used against mosquito larvae are *Bacillus thuringiensis var israelensis* (*Bti*), and *B. sphaericus*. Both affect the larvae with the toxins they release once ingested. They can be effective against insects that have developed resistance to chemical larvicides (Rozendaal 1997). *Bacillus sphaericus* is more effective against *Culex* mosquitoes in polluted water, while *Bti* is more successful against *Anopheles* species.

Besides the larvicides mentioned above insect growth regulators can also be used. These products interfere with mosquito development into adults. The best known are methoprene, which mimics juvenile hormone and interferes with metamorphosis and emergence, and diflubenzuron, which inhibits chitin formation of the pre-adult stages (Service 1993b).

All of these products can be found in different types of formulations (e.g. dust, powder, water-soluble liquid, emulsion, oil-soluble liquid, granule, pellet, briquette). Their application depends on the target organism, breeding habitat, methods available and non-target specimens present (Woodbridge and Walker 2002)

2.2.1.3 Biological Control

Biological control is mainly directed at the larval stage, and consists of the introduction of natural enemies into the breeding sites. Some of the most effective organisms used against mosquito larvae are categorized as predators, pathogens and parasites. The most effective predators as reported by Holck (1988) are the larvivorous fish (e.g. *Gambusia afinis* and *Poecilia reticulata*) and predatory mosquitoes of the genus *Toxorhynchites*. Other attempts, with limited effectiveness, have been with the nematode *Romanomermis culicivorax*, the ciliates *Lambornella* and *Tetrahymena*, the gregarine sporozoan *Ascogregarine*s, and the microsporidian *Nosema* (Woodbridge and Walker 2002).

2.2.1.4 Physical Control

Also known as cultural, mechanical or environmental control, this method promotes the reduction of water bodies as sources for breeding habitats and of resting sites for adults by harborage alteration (Woodbridge and Walker 2002). Knowledge of mosquito behaviour and its life cycle is necessary to interrupt their development. For larval control, water is altered or eliminated, for example by placing plastic foam beads on sewages, disposing of water from containers in a cemetery, draining water from ditches, removing plants in ponds, or using intermittent irrigation in rice fields. For each of these procedures it is important to know the biology of the species being controlled.

2.2.2 Control of Immature Mosquitoes in Rice Fields

2.2.2.1 Rice Fields as Sources of Potential Vectors

In the past, the occurrence of malaria in the U.S. was strongly correlated with rice cultivation in parts of the southeast and in the central valley of California. Now, rice field mosquitoes have been found to be potential vectors of other arboviral diseases (Table1). Rice fields could also extend the survival rates of adult mosquitoes through provision of nearby hosts, harborage and an environment of elevated humidity (Lacey and Lacey 1990). Rice fields in Louisiana could be productive sources of mosquito larvae because of permanent flooding, adequate organic matter in the water, sufficient shade in which to hide, and raised temperatures. Whether either just as a nuisance or as potential vectors of diseases, it is important to determine the abundance and increase control of the mosquito species present in Louisiana's rice fields.

2.2.2.2 Larvicides Used to Control Mosquitoes in Rice Fields

Several tests have been conducted in rice fields using a variety of biological and chemical insecticides to control mosquito populations. Although control methods are either directed against adults or immature stages, the focus in this study was the larval population. Controlling immature mosquito populations can reduce the need for further insecticides against the adult population. The presence of the larvae, concentrated in an aquatic habitat makes them an easier target than flying adults. The main control products available are chemical larvicides, microbial larvicides and insect growth regulators.

A study conducted in Arkansas, in rice field plots, tested two experimental chemical compounds (RH-0994 and FMC-45806), potential larvicides, against *Ps. columbiae*. In that study, Roberts et al. (1983) showed that both compounds (treatment rates of 27 g AI/ha each), as well as the standard (temephos at the rate of 35 g AI/ha), were effective (100 percent mortality) until 2 days after treatment. In another study, Dennett et al. (2003) reported that at 24 hr post-treatment a higher control was obtained for *An. quadrimaculatus* when using fipronil (69% control) than lambda-cyhalothrin (10% control). In Arkansas, Fipronil was used as a rice seed treatment to reduce damage to the roots of rice plants by rice water weevil larvae and lambda-cyhalothrin is applied aerially against adult weevils shortly after permanent flood to reduce egg oviposition (Dennett et al. 2003).

Several studies have been conducted to control rice field mosquitoes in Louisiana. Craven and Steelman reported (1968) that the organophosphates Abate and Fenthion, in combination with Propanil (herbicide) applied to rice plots 2 days before flooding, gave 100 and 99% control respectively against *Ps. confinnis* (Lynch Arribalzaga) larvae, and was not phytotoxic. Gifford et al. 1969, reported that Dasanit and Carbofuran (1lb and 0.5lb AI/ha respectively), both insecticides used to control rice water weevil, provided 100 percent control of the dark rice field mosquito larvae in outfield tests, when applied within 4 days after flooding. Another test in 1969 (Steelman and Poche 1970), showed that the application of the larvicides Abate, Fenthion and Dursban applied with fertilizer after flooding, caused 100% mortality to 1^{st} stage larvae in 24hr at rates of 0.5, 0.1, 0.005, and 0.0025 lb AI/acre respectively.

Biological insecticides used include the bacterial larvicides *B. thuringiensis* var. israelensis H-14 (Bti) and *B. sphaericus*. Results of laboratory tests for selected bacterial and chemical pesticides in Louisiana against 4th instar *An .quadrimaculatus*, *Cx. salinarius* and *Ps. columbiae* by Holck and Meek (1987), showed that Bti and Resmethrin + PBO were the most toxic. Experimental floating formulations of Bti provided up to 100% control of 3rd and 4th instar *Anopheles* larvae within 24-48 hrs., whereas water-dispersable granule formulations containing *B. sphaericus* required 48-72 hrs to yield >75% mortality (Dennet and Meisch 2000). Vectolex WDG, a *B. sphaericus* formulation, applied to small rice plots in Arkansas, resulted in a 90 and 97% control of *Ps. columbiae* 24 and 48hrs after treatment, respectively, while poor control was obtained against *An. quadrimaculatus* for the same times (Dennett et al. 2001).

The impact of the insect growth regulators, diflubenzuron and methoprene, on non-target aquatic populations in Louisiana were studied by Farlow et al. (1978) and Breaud et al. (1977). Six applications of each growth regulator, over an 18 month period, caused statistically significant differences in the population density of aquatic organisms (i.e.: scuds, opossum shrimps, coenagrionids, noterids, hydrophilids, chironomids). Some were reduced and others were increased. Additional studies with a slow release briquet or a sustained release pellet formulation of methropene have also been conducted (Weathersbee and Meisch 1991; Kramer and Beesley 1991). Bearden and Steelman (1971) showed that a surface film FLIT® MLO could cause high degree mortality to 1st-stage larvae of *Ps. confinnis* within 24hr under field conditions.

2.3 Control Methods of Rice Pests

2.3.1 Chemicals Used against Rice Pests

Probably the most effective means of controlling rice water weevil, the main pest during the early crop season, and rice stink bug, the main pest during the late crop season, is the use of insecticides (Stout et al. 1999). The only treatment against rice water weevil larvae after flooding for most of the past 30 years was the application of the carbamate insecticide Carbofuran, but this insecticide was removed from the market in the late 1990s after its registration in rice was revoked by the U.S. Environmental Protection Agency (Stout et al.. 1999). The insecticides currently available against rice water weevil are: Karate® (lambda-cyhalothrin), Mustang-Max (Zeta-cypermethrin), Prolex (gamma-cyhalothrin) and Dimilin (diflubenzuron) (Stout et al. 1999). The registered insecticides available to control rice stink bug are: Karate® Z, Mustang-Max, malathion, methyl parathion, Penncap-M, Prolex and Sevin. Fury and Karate® have a little longer residual activity than methyl parathion.

2.3.2 Impact of Rice Management Practices on Mosquitoes

The agricultural system requires the use of herbicides and insecticides to protect the crop from weeds and insects, respectively. Previously it had been shown that carbofuran, applied to control the rice water weevil, and molinate, used for aquatic weed control, also controlled rice field mosquitoes (Chambers et al. 1981). Since the registration of carbofuran was revoked, new insecticides became available to control rice water weevil and rice stink bug. Some of these insecticides were fipronil (Icon) and lambda-cyahothrin (Karate®). Icon (Fipronil) treated rice seed was extensively introduced into the Louisiana market in 1999 to combat the rice water weevil, after 2004 Icon was removed from the market (St. LA. 2004). Lab studies to determine the toxicity of fipronil against mosquito larvae were conducted by Ali et al. (1998). Larvae of colonised mosquitoes *Ae. aegypti* (Linnaeus), *Ae. albopictus* (Skuse), *Oc. taeniorhynchus, An. quadrimaculatus, Cx. nigripalpus* Theobald, and *Cx. quinquefasciatus* proved to be highly susceptible with a 48 hrs median lethal concentration (LC_{50}) ranging from 0.00043 to 0.023ppm. This toxicity was similar to the most effective insect growth regulators (diflubenzuron, pyriproxyfen, UC-84572 and abamectin) (Ali et al.. 1998).

Additional field studies on the effects of fipronil and lambda-cyahlothrin on *An. quadrimaculatus,* and the non-target predators, *Tropisternus lateralis* (Fabricius) and *Notonecta indica* Linnaeus, in small rice plots in Arkansas determined that, at 24-hrs. post-treatment, control for *An. quadrimaculatus* was 48% in fipronil plots and 10% in lambda-cyhalothrin plots (Dennett et al.. 2003). In both studies, fipronil appeared to be relatively safe or less harmful to non-target chironomids and insect predators (Ali et al. 1998, Dennett et al. 2003).

Earlier studies done in Louisiana found *Gambusia affinis* to be more effective in rice plots which received herbicide applications than in those that received no herbicide treatments. This increased efficiency was probably a result of less decumbent vegetation in these plots (Craven and Steelman 1968). In addition, the study indicated that effective control of 1st stage *Ps. confinnis* larvae could be obtained with low insecticide concentrations premixed in fertilizers, routinely applied to rice fields after flooding, therefore eliminating the cost of an insecticide application (Steelman and Poche 1970).

2.4 New Insecticides for Mosquito Control

The introduction of other insecticides like Novaluron (Rimon EC, Makhetshim, Beer-Sheva, Israel) used to control whiteflies, thrips and leafminers (Pesticide Fact Sheet), and new formulations of *Bt* larvicides, like WS-Bti, provides new tools to reduce the population of mosquito larvae present in rice fields. Novaluron is an insect growth regulator that acts by ingestion and some contact activity. It has been shown, in the lab, to have a high level of activity against 2^{nd} and 4^{th} instar larvae of *Ae. aegypti* (Mulla et al. 2003). WS- Bti is a combination of a patented evaporation reduction powder (WaterSavr) and the bactericide Bti. The combination of WS's surface active, self-spreading and biodegradability might increase the efficacy of Bti's control on mosquito larvae.

2.5 Larval Distribution and Oviposition Habitat Selection

The distribution of larvae depends mainly on the selection performed by the female mosquito for an appropriate oviposition site, an oviposition habitat selection (OHS). Kramer and Mulla (1979) mention that the distribution of larvae is controlled not by their survival potential in different habitats but by the selective discrimination of the ovipositing females. Gravid female mosquitoes use a combination of physical factors and chemical cues to locate suitable oviposition sites (Millar et al.. 1994). Some components of habitat quality, as mentioned by Angelon and Petranka (2002), that may influence offspring fitness, are the density of competitors and the risk of predation.

The number of larvae found in the rice fields is therefore affected by several factors, one of which could be oviposition habitat selection. Within each genus of mosquito, there is considerable variation among species. Individual species tend to oviposit, and therefore develop, in sites with characteristic physical and chemical properties. Among these properties the presence of predators could be an influencing factor.

CHAPTER 3. EFFECT OF KARATE® APPLIED TO MANAGE RICE STINK BUGS, ON MOSQUITO POPULATION IN LATE RICE SEASON IN LOUISIANA

Arthropod herbivores are important constraints on rice yields in Louisiana. A number of insect pests attack rice in Louisiana throughout the growing season. The most important pest attacking the rice crop during the late season is the rice stink bug, *Oebalus pugnax*. This insect attacks after panicles emerge, mostly between July and September. The rice stink bug reduces both grain yield and grain quality by removing the liquid contents of grains as they develop. Rice stink bugs infest nearly every rice field in Louisiana every year.

Applications of insecticides are used as part of an integrated pest management approach to manage rice stink bugs in Louisiana rice. Pyrethroid insecticides, including lambda-cyhalothrin (Karate®, Syngenta, Basel, Switzerland) are widely used for this purpose. Because pyrethroids are broad-spectrum insecticides, application of these insecticides may also affect mosquito larvae or adults present in rice fields when they are applied. In Arkansas, Dennett et al. (2003) determined that, at 24-hrs. post-treatment, populations of *An. quadrimaculatus* were reduced by 10% in plots treated with lambda-cyhalothrin. In this experiment the pest being targeted by the insecticide applications was the adult rice water weevil, *Lissorhoptrus oryzophilus*, the major early-season pest of rice in the United States. Application was conducted when rice, of the Cypress variety, was approximately 30in. (0.76m) in height. Similarly, in California Lawler et al. (2007) demonstrated that lambda-cyhalothrin (Warrior® TM) applications made in July, when *Culex* mosquitoes peak in northern California, affected feral mosquito populations for as long as 21 days after application, although numbers of mosquitoes were low and variable. Warrior is typically used in California to control rice water weevil at plant emergence.

In this study, we investigated the effect of Karate® (lambda-cyhalothrin) on populations of rice field mosquitoes in Louisiana when applied against rice stink bug during the late season. We reasoned that applications of insecticides made late in the season had greater potential to impact mosquitoes in Louisiana than early season applications, because larval mosquito populations in Louisiana rice fields are typically higher later in the growing season than earlier.

3.1 Material and Methods

Experiments were conducted from 2003 to 2005 to determine if application of the insecticide Karate® for rice stink bug control had an effect on populations of mosquitoes in simulated rice paddies. All field experiments were conducted at the LSU AgCenter Rice Research Station, Crowley, Acadia Parish, Louisiana.

3.1.1 Small Plot Field Studies

3.1.1.1 Summer 2003 An area measuring approximately 30.5m x 91.4m was divided into two blocks (East and West). East and West blocks were further divided into plots, with each plot measuring approximately 6.1m x 30.5m. Each plot was surrounded by earthen levees to prevent movement of water and insecticides between plots, and each plot had separate access via pipe to a water source (lateral). In each of the 4 plots used in this study, 8 small areas (1.5m x 6.1m) of rice were planted, four areas with the rice variety 'Bengal' and four with 'Cocodrie' (randomly distributed). Planting was conducted on April 17 and flooding on May 15 for all plots. Lambda-cyhalothrin (Karate®) at a rate of 0.033 kg/ha and an untreated control were assigned randomly to the two plots in each block.

The two plots assigned to the Karate® treatment were first treated when rice reached the early milk stage of grain maturation. This timing was chosen to simulate the timing most often used for control of rice stink bugs. Rice stink bugs were present in all plots at the time of Karate® application. Karate was applied with a backpack CO_2 sprayer over the top of the heading rice. Karate® was first applied on July 29, 2003 and later on September 30, 2003.

To determine the effect of Karate® on populations of mosquitoes, larvae were sampled before and after application with a standard 400ml dipper (Table 2). For each plot, a total of eight sites were chosen haphazardly along the edges of plots. At each dipping site, 5 separate dip samplings were made, covering a $1m^2$ area, for a total of 40 dippings per plot on each date. Sampling (Table 2) was first conducted on the day of Karate® application, two to three hours before application (pre-treatment sampling) and then again one to two days later (post-treatment sampling).

Table 2. Schedule for pre- and post-treatment larval samplings in 2003, 2004 and 2005 for lambda-cyhalothrin (Karate®)application in small plot experiments

	2003		**2004**		**2005**	
Application	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
1st application	July 29, 2003	July 30, 2003	September 7, 2004	September 9, 2004	July 28, 2005	July 29, 2005
2nd application	September 30, 2003	October 2, 2003	September 21, 2004	September 24, 2004	August 4, 2005	August 5, 2005

3.1.1.2 Summer 2004 In the summer of 2004, three blocks (A, B, and C in Figure 1) were each divided into three plots with each plot measuring 14.6m x 17.7m (259m^2). These nine plots were defined as experimental units and were separated physically from one another by levees to prevent insecticide, water and insect movement among plots. Water was supplied separately through pipes for each plot. Within each plot four small areas (1.5m x 6.1m), located near the center of plots, were seeded with the rice variety Cocodrie. The three plots in each block were randomly assigned to three treatments: Karate®, an experimental *B. thuringiensis* (BT) product and untreated control. Results from only the Karate® and control plots are presented here.

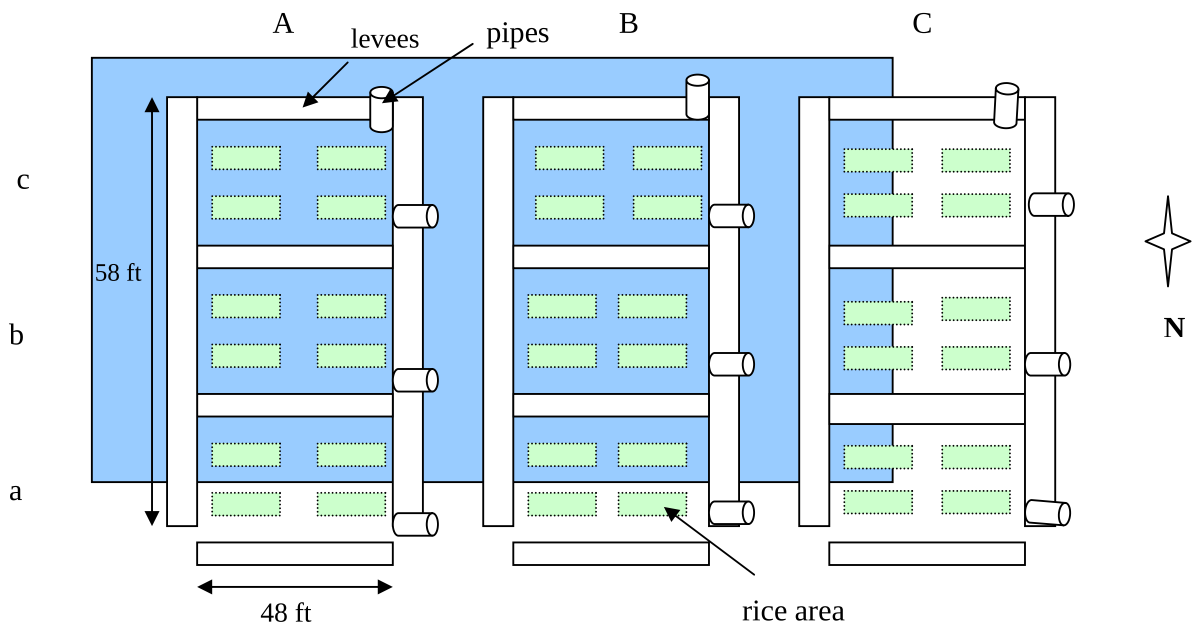

Figure 1. Small-plot design at Crowley during 2004: distribution of blocks (A, B, and C), plots (a, b, and c) and small rice areas.

Karate® was applied to appropriate plots when rice was in the anthesis stage of grain development (ca. 75% heading) and stink bugs were observed in plots. The first application of Karate was made on September 7, 2004 and a second application was made on September 21, 2004. Karate® was applied at a rate of 0.033 kg/ha and was sprayed over the top of rice plants with a backpack sprayer.

To determine the effect of Karate® on mosquitoes we sampled mosquito larvae using a standard 400ml dipper, before and after application (Table 2). The sampling plan was as follows: the sampling device was randomly dipped five times in each of the four areas of rice in each plot by dividing the 1.5m x 6.1m area of rice into 25 “cells”, then choosing five sites for dipping randomly by reference to a random number table. In addition, forty dippings were conducted on the edges of each plot at eight different sites. Thus, a total of 60 dip samplings were conducted per plot on each sampling date. Sampling was conducted the day of application (two to three hours before application) and then two to three days after application (Table 2).

3.1.1.3 Summer 2005 During the summer of 2005, three blocks were each physically divided with levees into 4 plots (a, b, c and d). Each plot measured approximately 10ft x 100ft and plots were assigned as experimental units. Each plot had separate access to a water source (lateral) and each plot was seeded with four small rice (variety Cocodrie) areas (1.5m x 6.1m). In each block, two plots were randomly assigned to control and Karate® treatment (the other two plots in each block are not discussed further here). Applications of Karate® at a rate of 0.033 kg/ha were made once stink bugs appeared in plots on July 28, 2005 and August 4, 2005 (Table 2). Two of the four areas of rice in each plot were randomly selected for sampling of mosquito larvae. In each of these areas of rice three different sites were chosen and five dip samplings were conducted at each site. In addition, the edges of plots were sampled by choosing eight sites at which five dip samplings were conducted. Thus, a total of 70 dip samplings were taken on each sampling date from each plot. Larvae were sampled the day of Karate application (two to three hours before application) and the day after.

Data analysis**:** An analysis of variance was conducted to test the hypothesis that the number of larvae present in each plot was affected by Karate® treatment. PROC MIXED in SAS was used for the analysis. Insecticide treatment (Karate® or control), sampling date (pre- or post- treatment) and the interaction of treatment and sampling date were entered into the model as fixed effects, and block was a random effect.

3.1.1.3.1 Sentinel Cages and Exposure of Larvae to Water Samples, 2005.

Because native populations of mosquito larvae were very low throughout these studies (Appendix 3), two additional methods were employed in 2005 to further evaluate the effects of Karate® treatment on mosquito populations. The first method involved the use of sentinel cages. Cages (Figure 2A) were constructed by modifying nets from CDC gravid traps and attaching them to PVC tube frames. Cages were placed within a meter of the edge of the plot (Figure 2B) a few hours before application and removed the next day. Thirty larvae of *Cx. quinquefasciatus* were introduced into the sentinel cages immediately after Karate® applications were made. Mortality of larvae in cages was evaluated the next day.

The second method involved exposure of mosquito larvae to water samples from control and Karate®-treated plots in the laboratory.

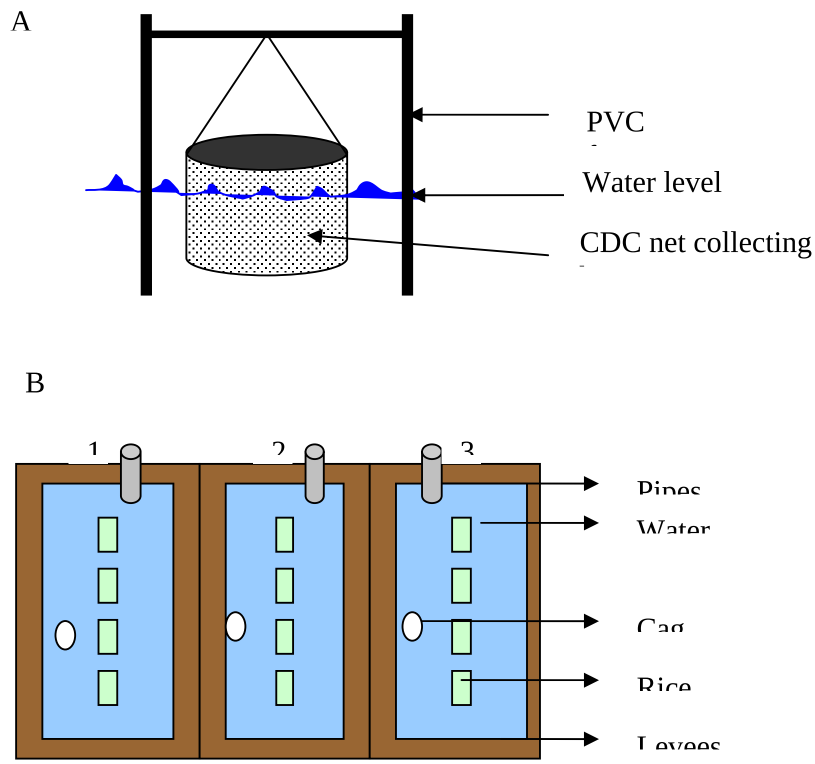

Figure 2. During 2005, sentinel cages were placed along the edge of the plots
(A) Sentinel cage. (B) Setting of cages in rice plots.

Water samples were collected from the edges of Karate®-treated and control plots within a few hours of application and transported back to the laboratory in plastic specimen cups. Water samples (250ml) were sieved in the laboratory to remove debris and other insects and then placed in clean plastic cups. *Culex quinquefasciatus* larvae were introduced (25 per cup) and fed with 5% liver powder solution. Because only low numbers of larvae were available, only three cups were prepared per treatment (one cup per plot) per sampling date. The six cups were then placed in a controlled environmental room with a temperature of approximately 26.7° C and a photoperiod of 13:11. The effect of the treatments on the larvae was observed 48 hrs later. Assays were conducted on July 28 and August 4 2005.

Mosquito larvae used in all assays (both for sentinel cage experiment and water exposure experiment) were *Cx. quinqefasciatus*, which came from two sources: 1) Larvae reared from eggs in an insectary located in the Department of Entomology, LSU and 2) Larvae obtained from the East Baton Rouge Mosquito Abatement District (EBR-MAD). For the assays conducted on July 28, 2005 larvae came from LSU and for the assays conducted on August 4, 2005 larvae were provided by the EBR- MAD.

Data analysis: Mixed-model analyses of variance were conducted to test the hypothesis that the mortality of larvae in sentinel cages or in cups were affected by treatments. Insecticide treatment (Karate® or control), was enered into the analyses as fixed effect, and block was a random effect.

3.1.2 Large Plot Field Studies

In the summer of 2005, two large plots (approximately 0.202 ha each) were selected for an additional study of the effects of Karate® on populations of mosquito larvae in 2005. One plot was assigned as a control plot and the other plot received an aerial application (August 11, 2005) of Karate® at 0.033kg/ha on August 11, 2005. A few hours before Karate® application, natural populations of mosquito larvae were sampled using a standard dip sampler. Three sites within one meter from the edge of each plot were selected randomly. At each site 5 dip samplings were conducted. In addition, three pairs of two sentinel cages were placed in each of the two plots; one cage of each pair was placed approximately one meter away from the edge of the plot and the second cage was placed in the interior of the plot, within the rice canopy.

Data analysis: A one-way analysis of variance was conducted to test the hypothesis that the number of larvae present in the plots and mortality in sentinel cages were affected by the application of Karate®. PROC GLM in SAS was used for all our analysis.

3.1.3 Effect on Adult Mosquitoes of Residual Karate®

An assay was conducted in summers 2004 and 2005 to test for possible effects on mosquito adults of Karate® residues present on rice foliage. Foliage was obtained from plots used for the small-plot studies of the effects of Karate® on larval populations (section 3.1.1.3). Immediately after applying the insecticide to appropriate plots (three plots of Karate®-treated foliage), foliage was removed from 3 plants randomly selected from each of the treated and control plots. The foliage was transported to the laboratory on ice and placed in modified 2 litre plastic bottle (Figure. 3). Bottles were open at the top and the openings were covered with mosquito mesh. A hole was cut into the side of the bottle and closed with a stopper. Twenty adult mosquitoes (a mix of females and males) were introduced through the hole with a mouth aspirator into each bottle. There were three bottles per plot, one bottle for each foliage sample from each treatment plot, a total of 9 bottles per treatment, with 180 mosquitoes per treatment. There was a total of 18 bottles per assay per date. Mosquitoes were fed by placing cotton humidified in a 10% sugar solution on top of the mesh. Bottles were kept in a controlled environmental room with approximately 80°F and a photoperiod of 13:11. A recording of mosquito survival was conducted at 24 and 48 hrs after introducing the mosquitoes into the bottles. This assay was performed twice in 2004 (September 7 and 24, 2004) and once in 2005 (August 4, 2005).

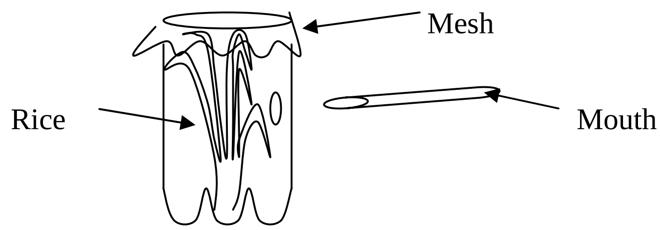

Figure 3. Indirect exposure of mosquitoes to lambda-cyhalothrin (Karate®).

Data analysis: Mixed-model analyses of variance (Proc Mixed) were used to test for differences in the mortality of adults, when exposed to Karate®-treated or control foliage.

3.2 Results

3.2.1 Small Plot Field Studies

3.2.1.1 Field Mosquitoes The number of larvae found in the experimental rice plots was generally low and varied among the three years of this study and also among sampling dates within years (see Appendix 3). On 2003 some larvae collected from the field were reared to adults to identify to species, we found *Anopheles quadrimaculatus* and *Culex erraticus* throughout August and October present in the rice fields. Two applications of Karate® at a typical rate (0.033 kg/ha) were made to rice plots each of the three years, one application early (at approximately 50% to 75% panicle heading) and one application later. Natural larval populations were sampled before application (one to two hours pre-spray) and one to three days after application. Thus, a total of six separate comparisons were made between pre-spray and post-spray larval populations over three years.

Throughout these experiments a consistent pattern was observed in which numbers of mosquitoes collected from Karate®-treated plots were lower than numbers colleted from control plots in five of six post-treatment samplings (Figures 4, 5, 6). Also, in all six experiments, the densities of mosquito larvae sampled from Karate® plots declined after Karate® application. However, in only one of our six experiments (September 7- 9, 2004) was a significant overall treatment effect (p=0.009) observed (Table 3); although a marginally significant treatment*sampling date interaction also observed in the September 30/ October 1, 2003 experiment.

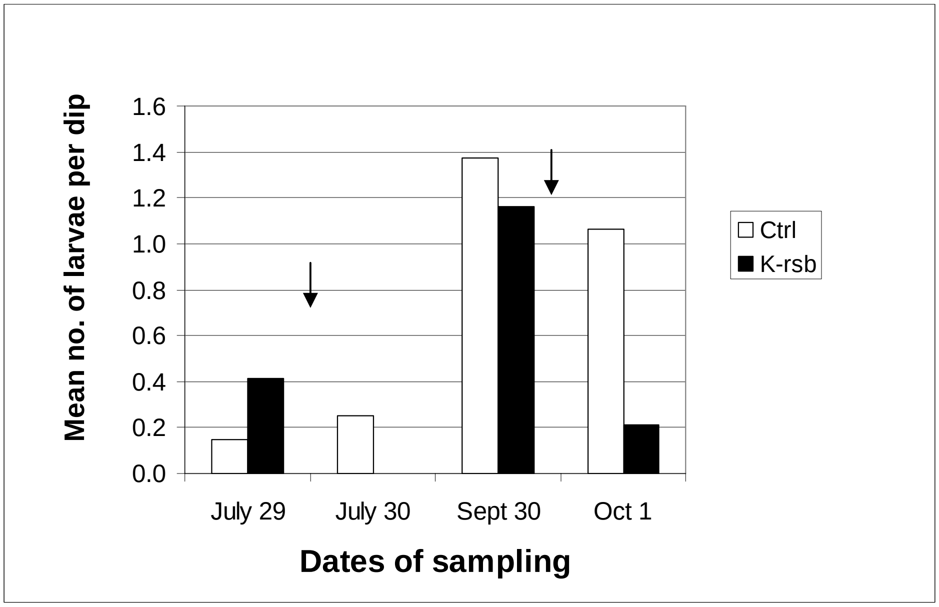

Figure 4. Densities of mosquito larvae (# larvae per dip sample) in Karate®-treated and control plots before and after application of Karate® to appropriate plots during 2003. Arrows denote timings of Karate® application.

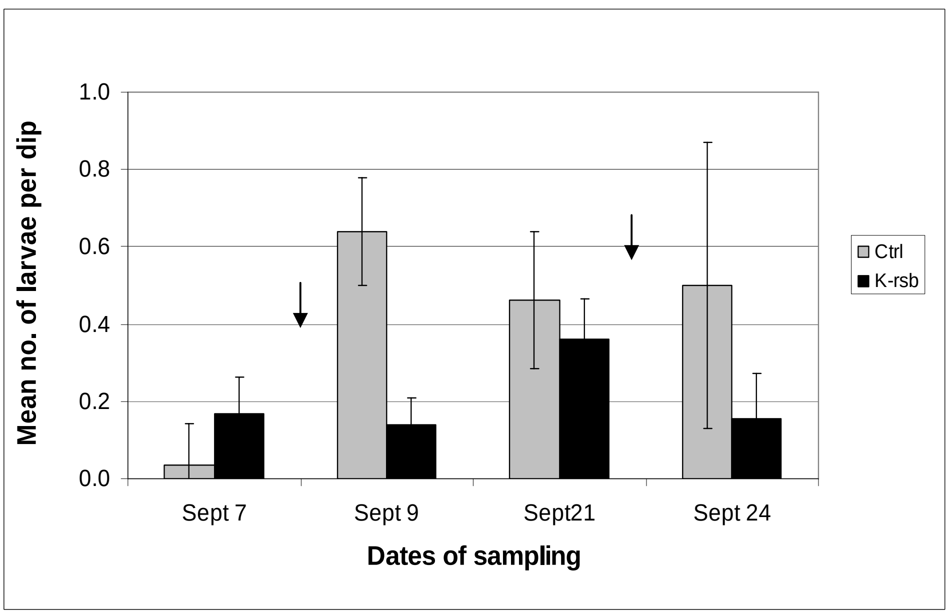

Figure 5. Densities of mosquito larvae (# larvae per dip sample) in Karate®-treated and control plots before and after application of Karate® to appropriate plots during 2004. Arrows denote timings of Karate® application.

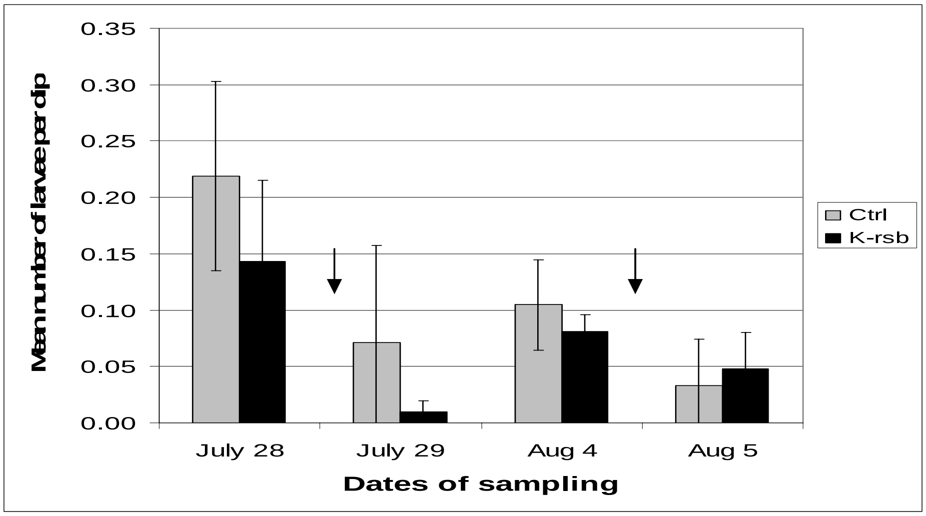

Figure 6. Densities of mosquito larvae (# larvae per dip sample) in Karate®-treated and control plots before and after application of Karate® to appropriate plots during 2005. Arrows denote timings of Karate® application.

In addition, in the July 28-29, 2005 experiment (Table 3), a significant date effect (p=0.0329) was observed

Table 3. Results of the analysis of variance of the effect of lambda-cyhalothrin (Karate®) treatment on natural populations of mosquito larvae in experimental rice plots at Crowley, Louisiana. Densities of larvae (larvae per dip sampling) in Karate®-treated plots were compared with densities in untreated plots

Year	Dates samplings	Type 3 Tests of Fixed effects			
		Effect	Df	F	p
2003	July 29 - July30	Trmt	1,3	0	0.9537
		Date	1,3	2.48	0.0815
		Date*trmt	1,3	6.68	0.0815
	Sept 30 - Oct 1	Trmt	1,4	3.16	0.1501
		Date	1,4	4.46	0.1023
		Date*trmt	1,4	1.14	0.346
2004	Sept 7 - Sept 9	Trmt	1,8	11.73	**0.009**
		Date	1,8	1.82	0.2148
		Date*trmt	1,8	2.65	1.1421
	Sept21 - Sept 24	Trmt	1,2	2.75	0.1481
		Date	1,4	0.39	0.5566
		Date*trmt	1,4	0.83	0.3966

Table continue

2005	Jul 28 – Jul 29	Trmt	1,2	1.84	0.2240
		Date	1,4	9.09	**0.0329**
		Date*trmt	1,4	0.02	0.8931
	Aug 4 – Aug 5	Trmt	1,8	0.02	0.8805
		Date	1,8	2.92	0.1261
		Date*trmt	1,8	0.39	0.5519

3.2.1.2 Sentinel Cages and Exposure to Water Samples. In 2005, in addition to sampling natural populations of mosquito larvae, mosquitoes were placed in sentinel cages in treated and untreated plots (Figure 2a) to asses the effects of Karate® application on mosquitoes. Also, mosquito larvae were exposed to water collected from treated and untreated plots to further evaluate the effects of Karate® on mosquitoes.

In sentinel cages following the first Karate® application (July 28), high mortality (93%) was observed in larvae in one of the control plots. This obscured any statistical effect of Karate® treatment (df $_{1,4}$, F=1.75, p= 0.2576) but the overall mortality of mosquitoes in Karate® plots on this date was twice as high as mortality of mosquitoes in control plots (Figure 7). For the second application on August 4 a marginally significant difference in the mortalities of mosquitoes in cages placed in control and Karate®-treated plots was observed (df $_{1,2}$, F=10.47, p=0.0837).

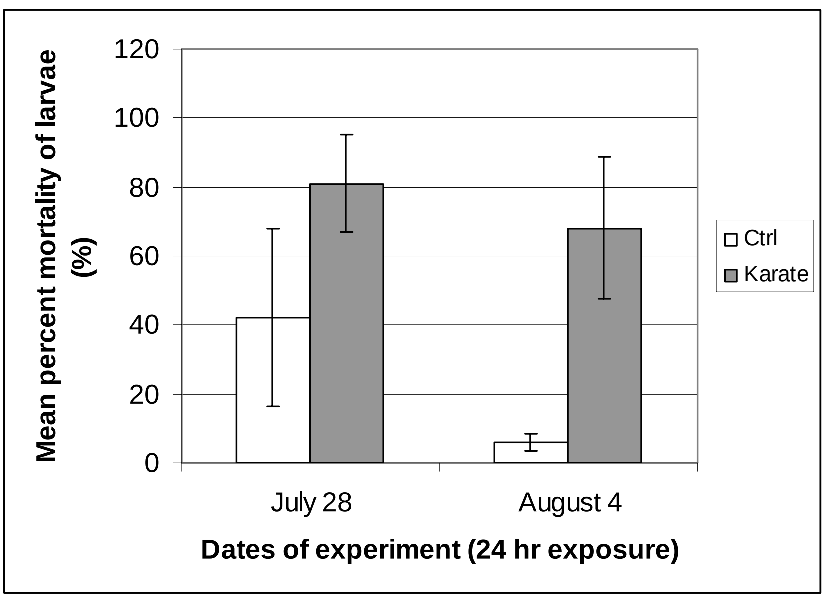

Figure 7. Mean percent mortality of larvae in sentinel cages when placed for 24 hours in Karate® treated and untreated small rice plots.

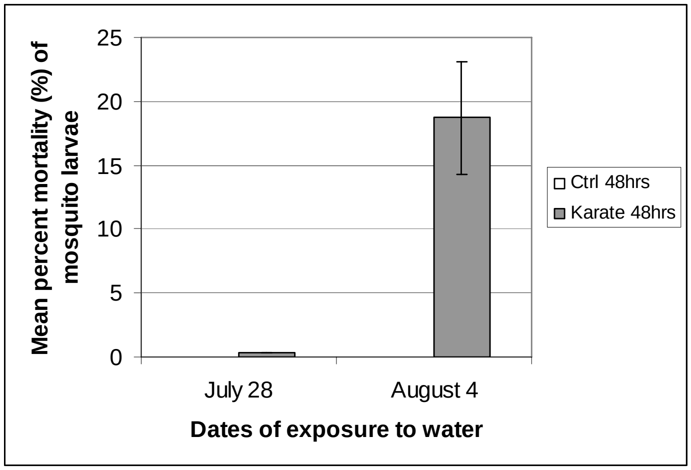

Figure 8. Mean percent mortality of larvae (%) in the laboratory when exposed (48 hours) to Karate® treated and untreated water from small rice plots

3.2.2 Large Plot Field Studies on Summer 2005

In the large rice plot experiment at Crowley (Figure 9) smaller numbers of larvae were collected in dip samplings from the Karate® plot than from the control plot, but the effect of treatment was not significant (df $_{1,8}$ F=1.05 p=0.3360).

In these same large plots, mortalities of mosquitoes placed in sentinel cages were higher in the Karate®-treated plot than in the control plot (df $_{1,8}$ F=24.36 p=0.0011). Mortality was also higher in cages placed on the edges of plots than in cages placed in the canopy (df $_{1,8}$ F=6.39 p=0.035), but there was no significant interaction between cage location and treatment. Overall mortality in cages in the Karate® plot averaged 64%, whereas mortality in control plots averaged 30% (Figure 9).

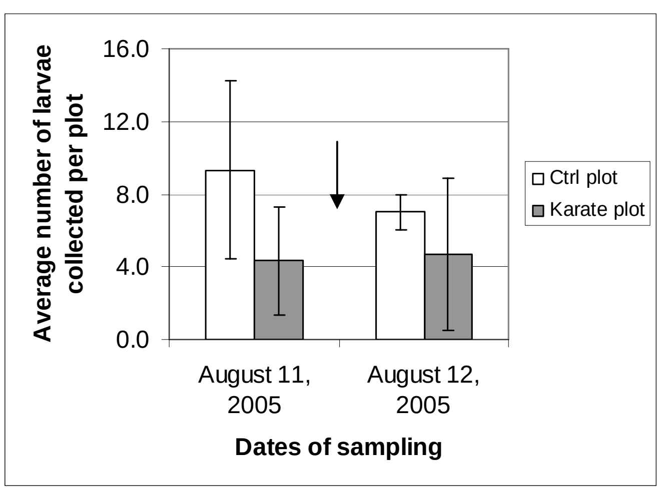

Figure 9. Average number of mosquito larvae collected in large plots (15 dippings per plot) before and after the application of Karate®. Arrow denotes Karate® application.

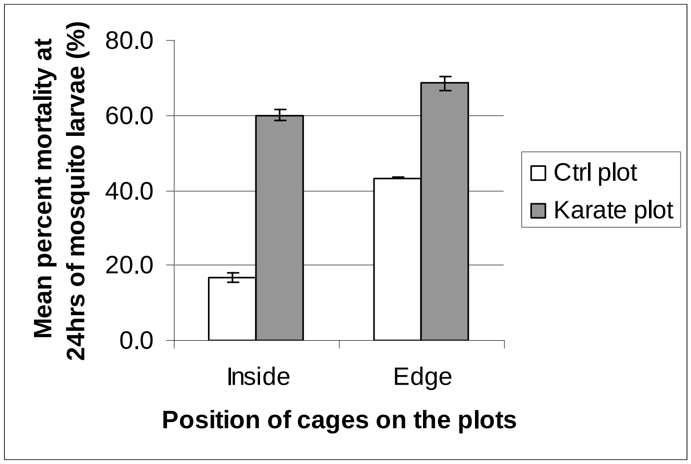

Figure 10. Mean percent mortality of sixty *Culex quinquefasciatus* larvae in sentinel cages at 24hrs exposure in large rice plots.

3.2.3 Effect of Residual Karate® on Adult Mosquitoes

The effect of Karate® on adult *Cx. quinquefasciatus* mosquitoes was tested in the laboratory using foliage collected from plots treated in the field in 2004 and 2005. Adult mosquitoes exposed to foliage from rice plots treated with Karate® experienced higher mortalities than mosquitoes exposed to untreated foliage in both 2004 and 2005 (Figure 11). Table 4 shows mean percent mortalities of adults and the treatment effect obtained for 2004 and 2005 after a 48-hour exposure to foliage.

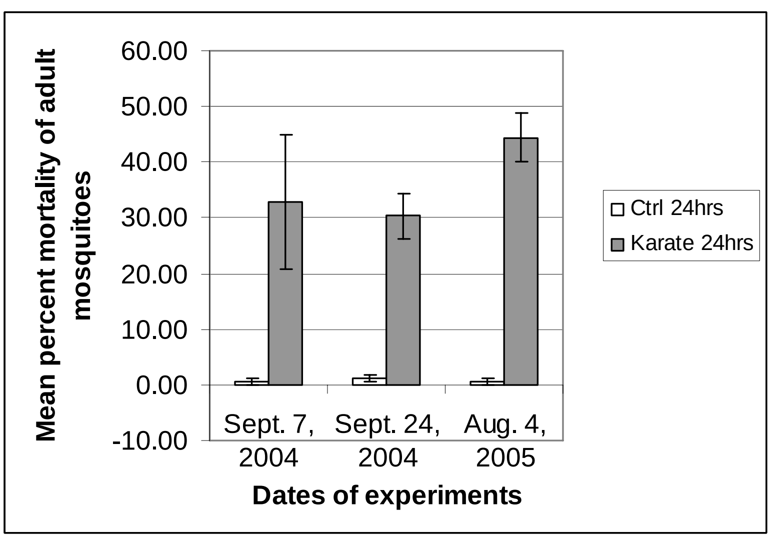

Figure 11. Mean percent mortality of adult mosquitoes when exposed to Karate® treated and untreated foliage.

Table 4. Mean percent mortality of adults exposed 24 hours to untreated and treated foliage.

Year	Dates of application	Mean % adult mortality Ctrl / Karate	Df	F	p
2004	Sept. 7	0.57 / 33	1,4	6.77	0.0599
	Sept. 21	1.11 / 30	1,4	55.18	0.0018
2005	Aug. 4	0.56 / 44	1,16	102.87	<0.0001

CHAPTER 4. EFFECT OF THE PRESENCE OF PREDATORS ON THE OVIPOSITION OF *CULEX QUINQUEFASCIATUS* IN THE LABORATORY

Mosquitoes, like other organisms, engage in activities conducive to their success and the success of their progeny to ensure species continuity. The choice of an appropriate oviposition site (oviposition habitat selection, OHS) is therefore considered an important aspect of maternal reproductive success. As mentioned by Kramer and Mulla (1979), the distribution of larvae is not controlled primarily by their survival potential in different habitats but by the selective discrimination of the ovipositing females. Gravid mosquitoes use a combination of sensory modalities and a combination of physical factors and chemical cues to find and determine the suitability of water-containing sites for egg deposition (Braks et al. 2007, Millar et al. 1994). Some components of habitat quality, as mentioned by Angelon and Petranka (2002) which may influence offspring fitness are the density of competitors, the risk of predation, and the seasonal duration and productivity of the habitat.

It has been observed that invertebrate predators can directly and indirectly influence mosquito population dynamics. Natural selection should favor females that avoid ovipositing where risk of predation is high for their progeny (Blaustein et al. 2004). Chesson (1984) observed that backswimmers *Notonecta* spp. (Hemiptera: Notonectidae) are highly predaceous and their presence within a water body can significantly reduce oviposition by adult mosquitoes. In a study conducted by Munga et al. (2006), *An. gambiae* mosquitoes laid significantly fewer eggs in rainwater conditioned by prior exposure to backswimmers than in the unconditioned rainwater.

The study described in this chapter was conducted in the summer of 2006. In an effort to determine the factors that elicit or deter oviposition by mosquitoes in rice fields, several aquatic insects commonly found in rice fields, many of them potential predators of mosquito larvae, were selected for use in oviposition bioassays. Water was exposed to these aquatic insects and female mosquitoes were the exposed to insect-conditioned water in cages to determine the effect conditioned water had on the oviposition behaviour of *Cx. quinquefasciatus*, the southern house mosquito, a common species often used in laboratory bioassays.

4.1 Material and Methods

In order to investigate the possible influence of predators on the oviposition behaviour of female mosquitoes, assays were conducted in which field-collected female *Cx. quinquefasciatus* mosquitoes were exposed to distilled water (control) and water conditioned by exposure to aquatic insects. Conditioned water was obtained by placing different aquatic insects in distilled water for a period of time, while the control, distilled water, was not exposed to aquatic insects but otherwise treated identically (Table 6). Choice assays were conducted in cages which allowed gravid females free access to conditioned and control water. Several assays were conducted on different days with different specimens, depending on availability. For each assay the number of eggs laid in each treatment were counted and compared the following day.

4.1.1 Laboratory Studies

Mosquitoes: Adult female *Cx. quinquefascitus* mosquitoes were collected using CDC gravid mosquito traps. This trap is considered to be selective for females that have already taken at least 1 blood meal and thus were preparing to oviposit (Reiter et al. 1986). Traps were set up during the early afternoon, at a site suggested by the personnel with the East Baton Rouge Mosquito Abatement District and picked up the following morning. The number of adults collected throughout the season varied but was usually low. Once collected, adults were separated and placed into cardboard cups with a mouth aspirator and kept from 0 to 4 days until they were used in oviposition assays (Table 5). Adults were provided with 10% sugar solution on cotton as their food source. Unlike many other studies using mosquitoes from colonies, we used mosquitoes directly from the field.

Table 5. Dates of collection and use of female *Culex quinquefasciatus* mosquitoes in choice laboratory trials

Date mosquitoes collected	Date of assay (days from collection to assay	No. mosquitoes introduced into each cage
May 25	May 25 (0)	10
June 01	June 02 (1)	10
June 07	June 08 (1)	10
July 14	July 17 (3)	13
July 27	July 31 (4)	20
August 04	August 7 (3)	22
August 18	August 22 (4)	7

Aquatic insects and conditioning of water: The insects used to condition water were collected from experimental rice plots at the LSU Ag Center Rice Research station at Crowley using a standard aquatic collection net. They were later transported to the laboratory, separated by family and placed in 500ml glass beakers filled with 400 ml of distilled water (DI). After the assay was conducted aquatic insects were identified by Stephanie Gil to the genus or species level. This taxonomic information is shown in table 6.

Table 6. Insect families and species used in bioassays

Family	Genus
Dytiscidae	*Laccophilus* sp
	Cybister fimbriulatus (Say)
Hydrophilidae	*Tropisternus lateralis nimbatas* (Say)
Belostomatidae	*Belostoma lutariun* (Stal)
Notonectidae	*Buenoa margaritacea* (Torre-Bueno)
Aeshnidae	*Anax junius* (Drury)
Coenagrionidae	*Coenagrion* sp.

Water was conditioned with predators by keeping the aquatic insects in 400 ml of distilled water from 1 to 7 days (Table 7). The number of insects placed in each beaker varied with species as did duration of conditioning. Table 7 shows the family identities, the number of insects used to condition the water (the number varied according to availability) and the durations of exposure for the ten experiments conducted. Distilled water was our control and was allowed to stand in a beaker for the same number of days as the conditioned water.

Table 7. Specimens used for conditioned water and type of cages used on bioassays.

Expt.	Date specimens placed in beaker	Date of assay	Treatments: specimens placed in beaker[a]	No. specimens used per beaker	Type of cage
1	May 22	May 25	Notonectidae	2	Small
2	June 01	June 02	Notonectidae	5	Small
3			Coenagrionidae	3	Small
4	June 01	June 08	Notonectidae	2	Small
5			Coenagrionidae	1	Small
6	July 13	July 17	Hydrophilidae	5	Small
7			Coenagrionidae	3	Small
8	July 26	July 31	Hydrophilidae	3	Small
			Belostomatidae	2	

Table continue

			Dytiscidae sm	4	
			Dytiscidae big	1	
9	August 04	August 7	Hydrophilidae	6	Large
			Hydrophilidae	20	
			Rice water		
10	August 17	August 22	Aeshnidae	2	Large
			Belostomatidae	1	
			DI + net *		

[a] distilled water (DI) was the second or fourth treatment depending if a two or a four choice assay was conducted, * net was rinsed in rice field water and then rinsed in distilled water.

Oviposition test: On the first day of the assay 100 ml of the conditioned water was removed from each of the beakers (along with distilled water from control beakers) and placed into glass dishes (10 cm diameter and 3 cm height). The glass containers were randomly placed inside screened cages at the rear corners. Two types of collapsible cages (Bioquip®, Rancho Dominguez, CA, USA) were used. Small 30.5 x 30.5 x 30.5 cm screen cages were used when a two-choice bioassay (distilled water (DI) and another treatment) was conducted (Figure 12A, Table 7) and large screen cages (61 x 61 x 61 cm) when comparing four treatments at the same time (Figure 12B, Table 7). Assuming that a gravid female deposits her eggs in a single raft (Clements 1992) each egg raft corresponded to a single female oviposition event. The number of egg rafts deposited was counted after 24 and 48 hrs in each container. Each treatment had three cages. Cages were placed in a controlled environment room with an approximately 27.5°C temperature and a photoperiod of 13:11 (L: D).

A total of 10 assays were conducted. Table 7 lists the different families of aquatic predators used as well as the number of days they remained in the beakers to condition the water. Table 7 provides the date insects were placed in the beaker with the distilled water and then also the day that water was used in an assay.

An Oviposition activity index (OAI) was calculated by the formula:

$$OAI = \frac{(Nt - Ns)}{(Nt + Ns)}$$

Nt = number of ovipositions (egg rafts in Culex sp.) in the treated sample (conditioned water)

Ns = number of ovipositions in the standard (distilled water)

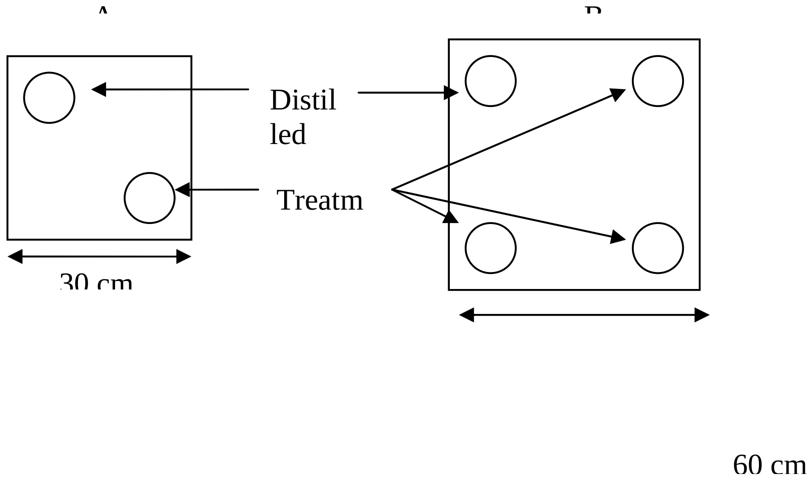

Figure 12. Example of the spatial arrangement of glass containers in cages. (A) two choice assay (B) Four choice assay.

All index values lie within the range of +1 (100% attraction) to -1 (100% repellency). Positive values indicate that more oviposition activity was observed in the treatment than in the distilled water (control) and is an indication that the material is an attractant in the broad sense. Conversely, more oviposition in the distilled water (control) than in the treatment dish results in a negative OAI (indicating the material is repellent in the broad sense). An OAI of zero indicates no preference.

Data analysis: The numbers of eggs laid in the conditioned water (treatment) and in distilled water (control) were compared. An analysis of variance was conducted to test the hypothesis that the number of egg rafts oviposited in each cage was affected by conditioned water treatment. PROC MIXED in SAS was used for the analysis. Water treated was entered into the model as fixed effect, and cage was a random effect. Means were compared using a Tukey means comparison.

4.2 Results

Figures 13, 14 and 15 show the results obtained for the oviposition activity index (OAI) of *Cx. quinquefasciatus* when tested with water conditioned by exposure to common aquatic insects in the small cages in two-choice bioassay. Figures 16 and 17 are the results for the larger cages where four-choice bioassays were conducted.

In all but one assay, the number of eggs laid on the surface of water conditioned by prior exposure to aquatic insects was greater than the number of eggs laid in control dishes. The graphs show a greater attraction of female mosquitoes for oviposition on the surface of conditioned water than on the surface of distilled water.

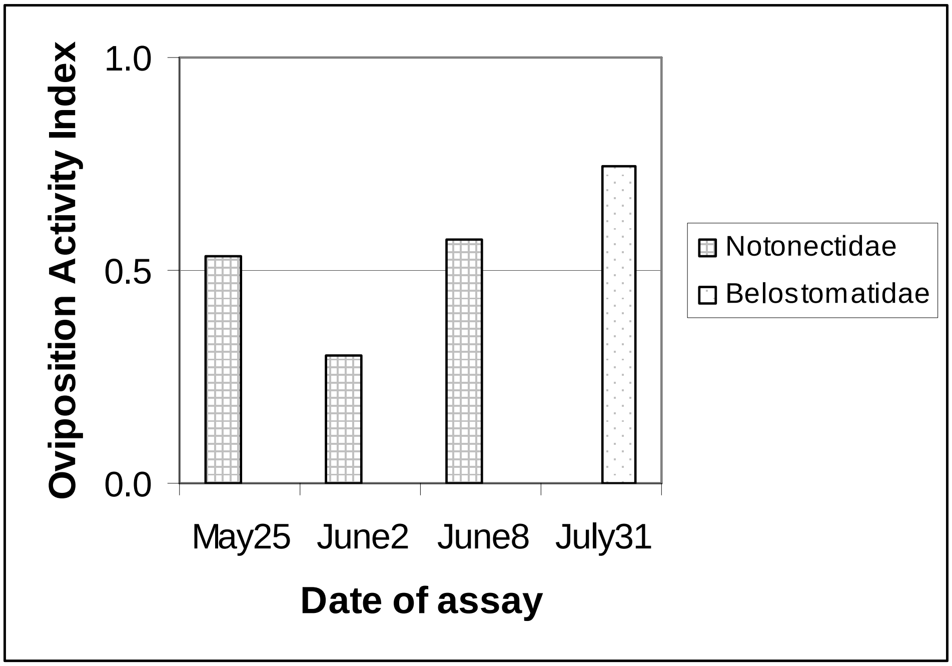

Figure 13. The Oviposition Activity Index of *Cx.quinquefasciatus* when exposed to water conditioned by insects in the families Notonectidae and Belostomatidae.

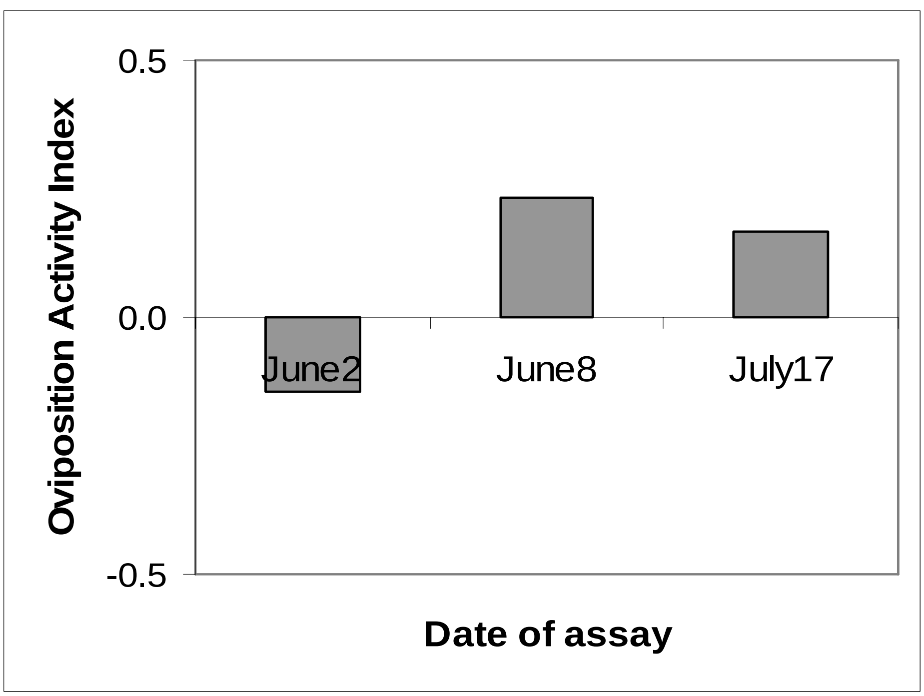

Figure 14. The Oviposition Activity Index of *Cx.quinquefasciatus* when exposed to water of conditioned by insects in the family Coenagrionidae.

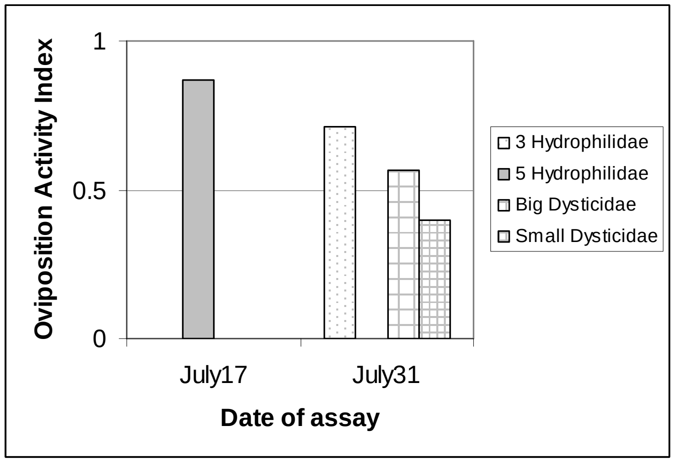

Figure 15. The Oviposition Activity Index of *Cx.quinquefasciatus* when exposed to water conditioned by insects in the families Hydrohilidae and Dysticidae.

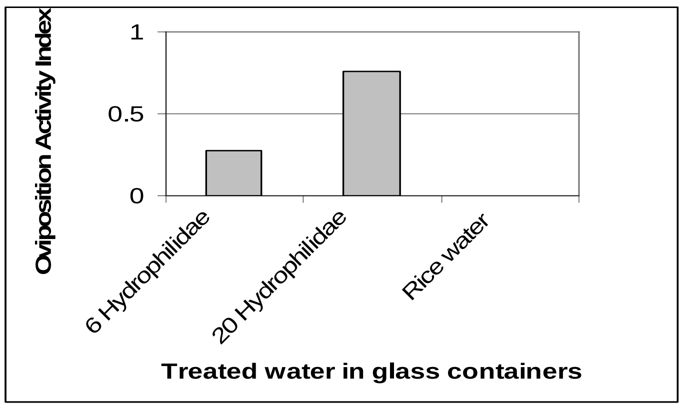

Figure 16. The Oviposition Activity Index of *Cx.quinquefasciatus* when exposed to water conditioned by six and twenty insects in the family Hydrohilidae and rice water in a four-choice assay on August 7, 2005.

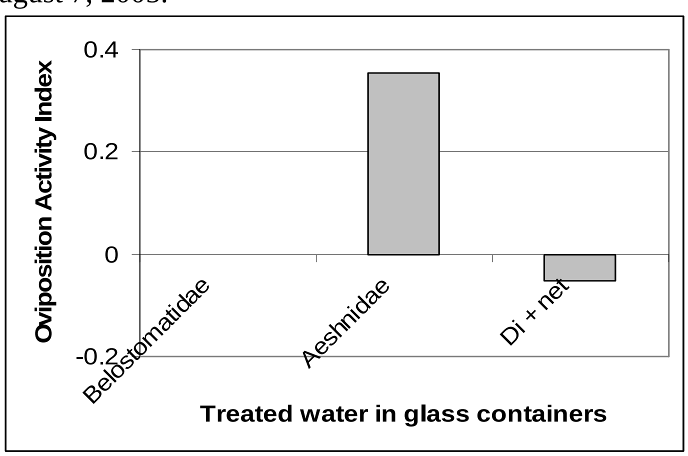

Figure 17. The Oviposition Activity Iindex of *Cx.quinquefasciatus* when exposed to water conditioned by insects in the families Belostomatidae and Aeshnidae, and distilled water with the rinse of a net previously introduced into rice water in a four-choice assay on August 22, 2005.

In the two- choice assays (Table 8) a significantly greater number of egg rafts were laid in water previously exposed to the large Dytiscidae specimen, to Hydrophilids and to the Belostomatidae. The greatest difference was found with Hydrophilids.

Table 8. The oviposition response of *Cx. quinquefasciatus* to conditioned water and distilled water (DI) in two-choice assays

Treatments	F	df	P
DI vs Dytiscidae - large	15.21	1,4	0.0175 *
DI vs Dytiscidae - 4 small	6.05	1,4	0.0697
DI vs 5 Hydrophilidae	145.8	1,4	0.0003 *
DI vs 3 Hydrophilidae	306.25	1,4	< 0.0001 *
DI vs Belostomatidae	24.05	1,4	0.0080 *
DI vs 2 Notonectidae	3.13	1,4	0.1518
DI vs 5 Notonectidae	0.5	1,4	0.5185
DI vs 2 Notonectidae	6.4	1,4	0.0647
DI vs 3 Coenagrionidae	0.25	1,4	0.6433
DI vs 1 Coenagrionidae	1.13	1,4	0.3486
DI vs 3 Coenagrionidae	0.5	1,4	0.5185

* indicates p< 0.05   Proc mixed SAS

Table 9 shows the results obtained in the four-choice assays. In the first trial we found some significance difference among distilled water and 20 hydrophilids, among hydrophilids and 20 hydrophilids and rice water. On the second trial we had no significant difference among the treatments.

Table 9. The oviposition response of *Cx. quinquefasciatus* to conditioned water and distilled water (DI) in four-choice assays. Means were compared using a Tukey test

Date	Treatment	Adj p
August 7, 2005	Di – 20 Hydrophilidae	0.0037 *
	Di – 6 Hydrophilidae	0.9137
	Di – Rice water	1.0
	6 Hydrophilidae – 20 Hydrophilidae	0.0083 *
	20 Hydrophilidae- rice water	0.0037 *

Table continue

	6 Hydrophilidae – rice water	0.9137
August 22, 2005	Aeshnidae - Belostomatidae	0.6551
	Aeshnidae - Dysticidae	0.9303
	Aeshnidae - Di + net	0.9303
	Belostomatidae - Dysticidae	0.9303
	Belostomatidae - Di + net	0.9303
	Dysticidae - Di + net	1.00

* indicates p< 0.05   Proc mixed SAS

CHAPTER 5. SUMMARY AND CONCLUSIONS

Mosquitoes are probably the most important group of arthropods from a medical/veterinary perspective as they can be a nuisance or transmit arthropod-borne diseases such as malaria, yellow fever, dengue and filariasis in humans or heart worm disease, Eastern Equine Encephalomyelitis and West Nile Virus in animals. Moreover, rice fields are important habitats for larval mosquitoes. Thus, it is important to understand the factors that influence mosquito distribution and abundance in rice fields. Two factors that may influence the distribution and abundance of mosquitoes in rice fields are the use of insecticides and the presence of other aquatic insects, including predatory species.

5.1 Effect of Lambda-Cyhalothrin (Karate®) Applied to Manage Rice Stink Bugs, on Mosquito Population in Late Rice Season in Louisiana

Commercial rice production in Louisiana begins in March and April and harvesting of the first crop is mostly complete by September. In southwest Louisiana a second (ratoon) crop is also produced from the stubble of some early planted fields. Although massive larval mosquito populations were observed during the months of May and June in 1965 (Craven and Steelman 1968), it is during the late rice season that the numbers of mosquitoes are typically higher. For example Chambers et al. (1979, 1981) observed that larvae appeared in rice fields from the end of May through September, and that some species increased in number when ratoon fields were re-flooded following the first harvest.

Prior studies of the effects of insecticides, used against rice pests on mosquitoes in rice fields have generally focused on the effects insecticide applications made for the control of the rice water weevil. In Arkansas, Dennett et al. (2003) applied the insecticides fipronil and lambda-cyhalothrin, at rates labelled for control of the rice water weevil, during August of 2000 to observe their effect on *An. quadrimaculatus* and nontarget aquatic mosquito predators. These insecticides are typically used early in the rice growing season to control rice water weevils. They found that when using lambda-cyhalothrin to control rice water weevils, populations of *Tropisternus lateralis* (Fabricius) and *Notonecta indica* Linnaeus were reduced (93 and 53% reductions, respectively, at 48 hours), while *An. quadrimaculatus* was less affected (7% redution at 48 hours). The use of fipronil controlled *An. quadrimaculatus* to a greater degree and was less harmful to the other two species (Dennett et al. 2003). In another study conducted in California, Lawler et al. (2007) observed that a single application of lambda-cyhalothrin at a rate of 5.8 g AI/ha (a rate typically used to control rice water weevil) killed 80-90% of pyrethroid-susceptible mosquitoes for 21 days (*Cx. pipiens s.l.*). The application of insecticides against rice water weevil is part of the normal management strategy used in early-season rice production in the U.S. Because the abundance of mosquitoes is typically greater later in the season in Louisiana (Chambers et al. 1981), and because Dennett et al. (2003) and Lawler et al. (2007) had already studied the effect of insecticide applications used to control rice water weevils on mosquito larvae, we focused our study on late season rice when rice stink bugs appear and insecticide applications are made for their control.

To monitor native mosquito larval populations after the application of lambda-cyhalothrin dip samplings were conducted. Throughout our study natural populations of mosquito larvae in small experimental rice fields in Crowley were low. This was also true at another site at which sampling was conducted (commercial rice fields at Angelina plantation in Tensas Parish, Louisiana). Dip sampling was conducted at this site on three dates during the summer of 2004. The first collection was made on June 29, 2004 and a total of 45 larvae were collected from 90 dip samplings. On July 8, 25 larvae were collected in 300 dip samplings taken from three separate fields. On July 20, 33 larvae were collected in 360 dip samplings from four fields.

The application of Karate to control rice stink bugs does appear to affect populations of mosquito larvae and adults. From the six trials conducted in small experimental rice plots at Crowley, mosquito populations were consistently lower in Karate®-treated plots than in control plots. The overall treatment effect was nevertheless significant in only one of the trials (Sept. 7 2004, p=0.034). Some significant sampling date * treatment interactions were also observed during these experiments (Table 3). In the same small rice plots the application of Karate® caused a significant mortality to *Cx. quinquefasciatus* larvae (p=0.0386) placed in sentinel cages in one of two trials in 2005. An effect of Karate® treatment on mosquito larvae in sentinel cages (p=0.0012) was also observed in the large rice plot experiment conducted in 2005.

In the laboratory, some results were obtained that confirmed the effect of Karate® on mosquito larvae and adults. *Culex quinquefasciatus* larvae exposed to water from Karate®-treated small plots showed significant mortality at 48 hs (p=0.0130) in one of two trials (August 4, 2005). The other experiment performed in the laboratory measured the survival of adult *Cx. quinquefasciatu*s on Karate®-treated foliage. The results (Figure 11, Table 5) demonstrated a higher mortality in adults exposed to foliage treated with Karate® than those exposed to non-treated foliage. Adult mosquitoes experienced 30 to 45 percent mortality when exposed to rice foliage from treated rice plots (Figure 11).

In conclusion, the application of Karate® to rice plots for control of rice stink bugs appears to affect populations of mosquito larvae and adults. Farmers who use pyrethroids to control rice stink bugs may also indirectly affect populations of mosquitoes in rice fields. Similar results have been found in California (Lawler et al. 2007) and Arkansas (Dennett et al. 2003). The lack of statistical significance in some trials might have been the result of inadequate replication, and low and variable mosquito populations. This study was the first to show an effect of treated foliage on adults which may increase the probability of the development of insecticide resistance in mosquitoes.

5.2 Effect of the Presence of Aquatic Insects on the Oviposition of *Culex quinquefasciatus* in the Laboratory

The sites or habitats where immature *Culex quinquefasciatus* usually develop are often rich in organic materials. These mosquitoes deposit their eggs in the form of egg rafts on the water surface. Infusions of organic matter have long been used as oviposition attractants or stimulants for *Culex* mosquitoes (Hwang et al.. 1977, Beehler et al.. 1993). Previous studies demonstrated that volatile chemicals produced by the infusions of organic substances in mosquito breeding sources influence the oviposition behaviour of many species of mosquitoes (Hwang et al. 1977). In fact, we used an organic attractant, fish oil, to attract female mosquitoes to our traps.

A gravid or oviposition trap detects the presence of female mosquitoes that usually have taken a bloodmeal and are looking for an oviposition site. The type of traps we selected was the CDC gravid traps, an oviposition trap, which are useful for virus surveillance programs because they attract a much higher proportion of gravid, blood fed mosquitoes that may have acquired disease organisms from a blood meal, than do CO_2, light or vertebrate-baited traps (Reiter et al.. 1994). With these traps we had a higher chance to get female mosquitoes searching for a place to oviposit and therefore be used in the assays. Although the numbers of mosquitoes present throughout the season were not as high as we had expected there were sufficient numbers to conduct these assays.

The aquatic insects that we used in our assays are commonly found in rice fields and are possible predators of mosquito larvae. From our assays, and according to the OAI's, female mosquitoes preferred to lay eggs in water conditioned by previous exposure to aquatic insects than in distilled water. The highest OAI observed for *Cx.quinquefasciatus* females was with the Hydrophilidae-conditioned water. This result was contrary to our hypothesis, that oviposition would be reduced in water exposed to predators. The significance of these findings is not understood at this time, and further investigation is needed. Further studies could be conducted to evaluate new predators and other mosquito species as well as to identify the chemicals involved in oviposition behaviour.

Elucidating the cues governing oviposition behavior may provide a tool for behavioural manipulation of mosquitoes in the field (Munga et al. 2006). Discovery, characterization, and assessment of oviposition attractants or deterrents operating in mosquito-breeding sites would provide a basis for an understanding of the distribution and abundance of various species of mosquitoes (Ikeshoji and Mulla, 1970). To learn where mosquitoes might prefer to oviposit, according to the presence or lack of certain aquatic insects, may provide us a way to identify their preferred habitats, apply a control method and reduce their numbers in the field.

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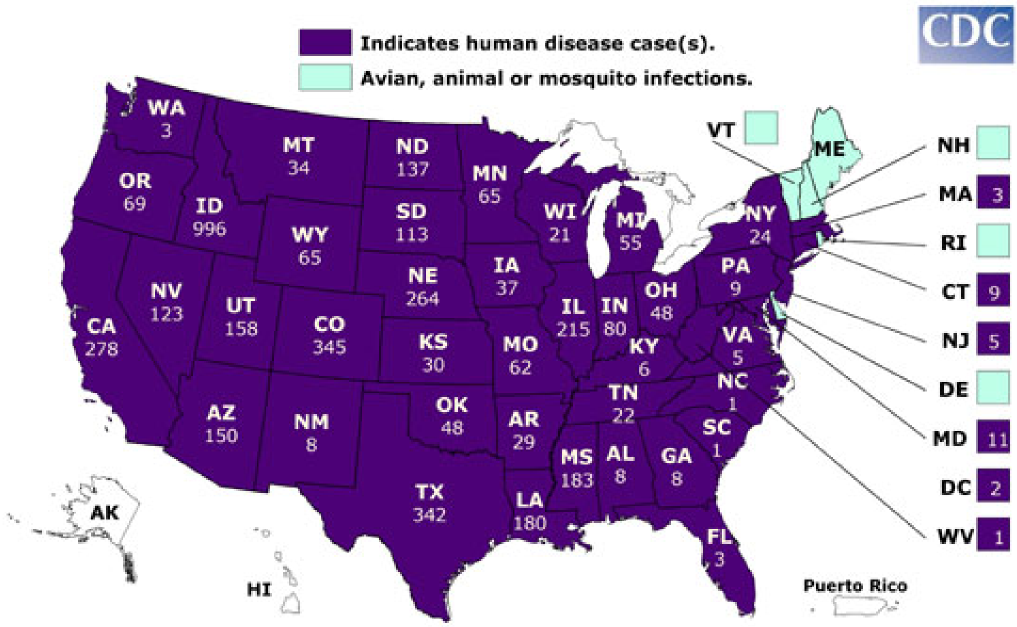
APPENDIX 1. MAP OF 2006 WEST NILE VIRUS ACTIVITY IN THE UNITED STATES (REPORTED TO CDC AS OF MARCH 6, 2007)*



Found in:

http://www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/survandcontrol06Maps.htm on March 14th 2007.

APPENDIX 2. ARBOVIRUS NAMES FOR ABBREVIATIONS CITED IN TABLE 1 ON PAGE 6.

Name	Abbreviation
Barmah Forest	BAT
California Encephalitis	CE
Cache Valley	CV
Eastern Equine Encephalitis	EEE
Everglades	EVE
Flanders	FLA
Getah	GET
Gray Lodge	GLO
Hart Park	HP
Ilheus	ILH
Jamestone Canyon	JC
Keystone	KEY
Kairi	KRI
Llano Seco	LLS
Lokern	LOK
Lukuni	LUK
Maguari	MAG
Middleburg	MD
Melao	MEL
San Angelo	SA
Sagiyama	SAG
Semliki Forest	SF
St. Louis Encephalitis	SLE
Shark River	SR
Tahyna	TAH
Tensaw	TEN
Tlacotalpan	TLA
Triniti	TNT
Turlock	TUR
Umatilla	UMA

Table continue

Venezuelan Equine Encephalitis	VEE
Virgin River	VR
Western Equine Encephalitis	WEE
West Nile	WN
Wyeomyia	WYO

APPENDIX 3. MOSQUITO LARVAE (40, 60 AND 70 DIPS PER PLOTS IN 2003, 2004 AND 2005, RESPECTIVELY) DURING THE SECOND CROP SEASON (JULY -OCTOBER).

Year	**Treatment**	**Number of Larvae**									
2003	Plots	Jul 29	Jul 30	Aug 14	August	Sept 5	Sept 12	Sept 18	Sept 30	Oct 1	Oct 10
	Ctrl	8	19	8	40	97	27	30	78	42	42
	Ctrl	4	1	1	17	35	14	19	32	43	57
	K-rsb	22	0	16	22	61	24	59	41	12	36
	K-rsb	11	0	13	12	15	2	47	52	5	0
2004	Plots	Aug 26	Aug 31	Sept 7	Sept 9	Sept 14	Sept 21	Sept 24	Sept 28	Sept 30	Oct 5
	Ctrl	30	5	8	42	17	43	66	132	45	44
	Ctrl	42	46	25	48	7	27	9	17	40	14
	Ctrl	17	20	29	25	30	13	15	65	31	37
	K-rsb	-	-	0	17	9	24	23	59	12	30
	K-rsb	-	-	20	4	2	31	0	13	59	25
	K-rsb	-	-	10	4	10	10	5	23	16	9
2005	Plots	Jul 28	Jul 29	Aug 4	Aug 5	Aug 8	Aug 17				

Table continue

Ctrl	25	15	12	0	4	47
Ctrl	16	0	6	7	0	18
Ctrl	5	0	4	0	1	6
K-rsb	14	0	5	0	6	48
K-rsb	16	0	4	8	0	24
K-rsb	0	2	8	2	1	36

Karate was applied on: July 29 and September 30, 2003; September 7 and September 21, 2004; July 28 and August 4, 2005

VITA

Ana Maria eldest daughter of Temistocles Sanchez and Susana Zavaleta de Sanchez was born in Lima, Peru on April 6, 1967. Her early education took place in various countries of South America (Paraguay, Guatemala, Colombia, and Peru). In 1992 she earned a Bachelor of Science degree with a major in biology from the Universidad Peruana Cayetano Heredia and a Masters degree in medical entomology in 1995 from the London School of Hygiene and Tropical Medicine. The degree of Masters of Science will be conferred on Ms. Sanchez de Cuadra on the May 2008 commencement.