FLORIDA
MOSQUITO CONTROL
2018

The state of the mission as defined by mosquito controllers, regulators, and environmental managers

Florida Coordinating Council on Mosquito Control

This report was funded in part by grants from the Florida Department of Agriculture and Consumer Services, the Florida Department of Health, and the Florida Mosquito Control Association. The report was initiated, reviewed, and accepted by the Florida Coordinating Council on Mosquito Control (FCCMC). The FCCMC was created and mandated by the Legislature in Chapter 388 Florida Statues in 1986 to develop and implement guidelines to assist the Florida Department of Agriculture and Consumer Services (FDACS) in resolving disputes arising over the control of arthropods on publicly owned lands, to identify and recommend research priorities and technologies, to develop and recommend to FDACS a request for proposal process for arthropod control research, to identify potential funding sources for research and implementation projects, and to evaluate and rank proposals upon request by the funding source. A final mandate is to prepare and present reports, such as this one, on arthropod control activities in the state to appropriate agencies.

To oversee the development of the report, the FCCMC appointed a Steering Committee that selected contributors and reviewers for this publication.

Cover Illustration: The artwork on the cover was designed and hand drawn by Lee "Leroy" Hansen of New Port Richey, Florida. Mr. Hansen captured a beautiful natural Florida swamp that mosquito control professionals across the state would inspect for mosquito larvae and adults.

Acknowledgments: The editorial assistance of Janice Broda is much appreciated.

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PREFACE

1st EDITION. During the Spring of 1994 at a meeting of the Florida Coordinating Council on Mosquito Control (FCCMC), a legislatively established interagency committee, Carlton Layne of the U.S. Environmental Protection Agency (EPA) requested that the Florida mosquito control community develop a "White Paper" on its control practices. Originally, it was intended that this document could help define the current state of mosquito control in Florida with the goal of developing recommendations on how mosquito control chemical use and risk could be reduced in the future. This goal of reduced chemical use and risk is a goal of the EPA's Pesticide Environmental Stewardship Program (PESP) of which the Florida Mosquito Control Association is a "PESP partner under the auspices of the American Mosquito Control Association". While this request probably was first intended as a brief overview of Florida's mosquito control programs and practices, it stimulated great interest in the mosquito control community and "took on a life of its own" resulting in the 1st Edition of the White Paper published by the University of Florida in 1998.

2ND EDITION. In the Spring of 2006, we saw that the inventory of the White Paper's 1st Edition was running low. Recognizing that some significant developments had occurred over the past eight years, we felt that an update was in order rather than simply a reprint of the document. At the June 2006 FCCMC meeting, the Committee authorized the development of a 2nd Edition. As was the case for the 1st Edition, a Steering Committee was formed, and Chapter Coordinators were solicited. Over the next approximately sixteen months with the expertise of numerous individuals, each chapter was revised and updated. The revisions were first reviewed by the Steering Committee, and any significant modification requests were forwarded to the Chapter Coordinators. Next, each chapter was peer-reviewed by several individuals knowledgeable about the chapter's specific topic. Again, any significant modification requests were forwarded to the Chapter Coordinators. In January 2008 the entire document was provided to the FCCMC for their review, and it was adopted at their February 2008 meeting.

3rd EDITION. In the spring of 2016, several Florida mosquito control leaders suggested that it was time to again update the White Paper given that many changes had occurred in the profession over the previous seven to eight years. At the May 2016 meeting of the Florida Coordinating Council on Mosquito Control, the question was brought to the Council as to whether they were interested in sponsoring the 3rd Edition of this document. The Council was in favor of moving forward so a Steering Committee was established, and shortly thereafter, Chapter Coordinators were chosen to start the process of updating each of the 13 chapters with drawing in additional expertise as they saw fit. By early 2018, each of the chapters was completed and prepared for printing by Text Editor Janice Broda. On June 1, the FCCMC accepted the document to move forward with finalizing and printing. This 3rd Edition was printed in mid-2018, and like the 2nd Edition, was placed on the website of the University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory: http://fmel.ifas.ufl.edu.

APPRECIATION. The Steering Committee would like to thank all the participants who contributed to this document which we believe is a fair, accurate and important look at mosquito control in Florida as we approach the 3rd decade of the 21st Century.

Aaron Lloyd, Douglas Carlson, and Roxanne Connelly
Co-Editors

Chapter 1

INTRODUCTION

Chapter Coordinators:
Doug Carlson, Dr. Roxanne Connelly, Peter Connelly, Dr. Larry Hribar, Aaron Lloyd, and *Dr. Marc Minno*

2009 Coordinators:
Doug Carlson, Dr. Roxanne Connelly, Peter Connelly, Dr. Larry Hribar, Dr. Ken Linthicum, and *Dr. Jack Petersen*

1998 Coordinators: *Dr. Richard Baker* and *Doug Carlson*

1.1 PURPOSE OF THIS DOCUMENT

The purpose of this document is to illustrate the intricacies of mosquito control and the relationships of mosquito control activities to other environmental management strategies. This project explores the benefits and risks of these strategies, particularly the various activities or environmental management strategies that may benefit or adversely affect each other.

1.2 MISSION STATEMENT

The Florida Legislature declares in Chapter 388 of the Florida Statues (F.S.) that it is the public policy of this State to achieve and maintain such levels of arthropod control as will protect human health and safety, foster the quality of life of the people, promote the economic development of the state, and facilitate the enjoyment of its natural attractions by reducing the number of pestiferous and disease-carrying arthropods. The Legislature also declares in the statute that it is the policy of the State to conduct arthropod control in a manner consistent with protection of the environmental and ecological integrity of all lands and waters throughout the State. In addition to the legislative declaration, mosquito control programs in Florida have established policies through the Florida Mosquito Control Association (FMCA), which enables mosquito control operations to offer maximum protection to the environment based on a need to control mosquitoes and recommendations of the Florida Department of Agriculture and Consumer Services (FDACS).

1.3 BRIEF INTRODUCTION TO MOSQUITO BIOLOGY

Mosquitoes are insects with long slender bodies, narrow wings with a fringe of scales on the hind margins and along the veins, and long, very thin legs. In females, the elongate proboscis is firm and usually adapted for piercing and sucking blood; The males cannot suck blood, but both sexes feed on nectar of various plants.

There are four life stages: Egg, larva, pupa, and winged adult. Eggs may be oviposited (laid) singly or in rafts, deposited in water, on the sides of containers where water will soon cover them, or on damp soils where they must undergo a maturing process before they can hatch when flooded by rainfall or high tides. After the tiny eggs hatch, the larvae (commonly called wigglers) begin to feed on very small plant and animal particles, going through four growth stages (called instars) before becoming pupae. Most larvae, except in the genera *Mansonia* and *Coquillettidia*, must breathe at the surface of the water. The two named genera have a sharp pointed siphon with which to pierce the roots and stems of aquatic plants to get their oxygen from the plant. The pupal stage is comparatively brief. The pupa does not feed and is active generally only if disturbed. When it has matured, the pupa remains at the surface, the chitinous pupal skin splits, and the adult emerges from the skin, briefly dries its wings, and flies away. Only the female mosquitoes bite, using blood protein for the development of their eggs. The flight range of mosquitoes varies greatly from several hundred feet in some species to more than twenty miles in others (FDACS 2011).

1.4 INTEGRATED PEST MANAGEMENT AS IT APPLIES TO MOSQUITO CONTROL

In order to accomplish long-range, intelligent, and environmentally sound pest control, the management and manipulation of pests must be conducted using not just one but all available pest control methods. This combination of methods into one thoughtful, ecologically-valid program is referred to as Integrated Pest Management (IPM) (paraphrased from Ware, 1994).

A typical mosquito control program employing IPM principles first determines the species and abundance of mosquitoes through larval and adult surveys and then uses the most efficient and effective means of control. In some situations, water management programs or sanitation programs can be instituted to reduce larval habitats. When this approach is not practical, a larviciding program then is used so that specific larval habitats can be treated. Where larviciding is not effective, adulticides are used. The choice of larvicides and adulticides used is based on the species targeted for control and environmental concerns.

An important part of an IPM program is public education. Public participation can do much to reduce the larval habitats of domestic mosquitoes. Public education can be most effective during disease epidemics to educate the public concerning mosquito habits and the ways individuals can protect themselves from mosquito attack.

Some mosquito control professionals prefer to use the term Integrated Mosquito Management (IMM) or Integrated Vector Management (IVM) in place of Integrated Pest Management. However, for the purposes of this publication, in particular because people outside of the mosquito control community who read this may not be familiar with the terms IMM or IVM, we have chosen to use the term IPM throughout this publication when appropriate.

We hope that the readers of this document will develop an understanding and appreciation of the challenges and importance of mosquito control in helping to make Florida a pleasant and safe place to live and recreate.

1.5 May 2010 Bioterrorism Workshop

Shortly after the publication of the 2nd Edition of the White Paper (2009) an important Florida meeting was held in May of 2010. It was a workshop sponsored by the University of Florida's Emerging Pathogens Institute, the Florida Medical Entomology Laboratory, and the United States Department of Agriculture's Center for Medical, Agricultural and Veterinary Entomology and was entitled: "Counteracting bioterrorist introduction of pathogen-infected vector mosquitoes". Participation in this two-day meeting was by invitation and dealt with the possibility of insect-based terrorism as a method of attacking Florida and beyond. Former Governor and U.S. Senator Bob Graham, who was a co-author of one of the most authoritative studies of terrorist threats against the U.S., provided the plenary address in which he provided an overview of the bioterrorism threat. This meeting brought together state and national experts to determine the threat of using mosquitoes as weapons and how to address it. Through a workshop format, preparations that Florida should take in anticipation of this threat were addressed in breakout groups and then discussed collectively. An impetus for this meeting was the fact that there is no organized plan at any level of government to develop the resources for mosquito control that could prevent an attack of this sort. It was acknowledged that terrorists with only basic scientific knowledge could potentially release infected insects thus possibly causing a serious public health problem. A publication resulting from this workshop (Tabachnick *et al.* 2011) identified possible steps to combat this threat including having each state create a "Council for Emergency Mosquito Control" with a source of dedicated funding to prepare for such an event (http://news.ufl.edu/archive/2010/05/workshop-at-uf-to-address-threat-of-insect-based-terrorism.php).

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Chapter 2

HISTORY OF FLORIDA MOSQUITO CONTROL

Chapter Coordinators: *Dr. Roxanne Connelly* and *Dr. Gordon Patterson*

2009 Coordinator: *Dr. Roxanne Connelly*

1998 Coordinator: *John Beidler*

Summary

Mosquitoes have long played a prominent role in Florida's history both as sources of pest and disease problems. Spanish, English, and French explorers told tales of mosquitoes in such abundance that they were forced to sleep on the beach covered with sand. In the 1870s and 1880s, outbreaks of yellow fever in the Panhandle, Jacksonville, Key West, Tampa, Plant City, and Manatee County took a tremendous toll in human suffering and death. These events led to the formation of the Florida State Board of Health in 1889. Dr. Joseph Y. Porter, a physician and a noted mosquito expert, was chosen as the first head of the Board.

The Florida Anti-Mosquito Association was formed in 1922. The Florida Legislature created the first mosquito control district, the Indian River Mosquito Control District, in 1925 in conjunction with the formation of Indian River County. The St. Lucie Mosquito Control District was formed a year later in 1926. Early permanent control efforts focused on hand ditching, some diking and dewatering, and proposed some dredge and fill work. The Work Projects Administration constructed 1,500 miles of ditches in Florida's salt marshes by hand or with explosives. Perhaps the most significant mosquito control event in Florida was the creation of State funds through the efforts of Dr. John Mulrennan, Sr. in 1953. This legislatively established program was designated for permanent control work which included dredge and fill, ditching, and impoundment and established the Entomological Research Center, now known as the University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory in Vero Beach. In 2004, Dr. Gordon Patterson published The Mosquito Wars: A History of Mosquito Control in Florida, a book that details the history of mosquito control in Florida.

2.1 INTRODUCTION

Mosquitoes have played a prominent role in Florida's history both as pests and carriers of disease from the beginning of human settlement. Various Spanish, English, and French accounts tell of mosquito abundance sufficient to force early explorers to sleep on the beach and cover themselves with sand. In the second half of the nineteenth century outbreaks of yellow fever (YF) in such widespread locations as Pensacola, Fernandina, Jacksonville, Key West, Tampa, Plant City, and Manatee County led to tremendous human suffering and death. In Jacksonville, with a population of 26,800, the 1888 epidemic killed 400 people, sickened 5,000 people, and caused 10,000 people to flee the city. Of the 16,400 people remaining in the city, 14,000 citizens were left unemployed as a result of the breakdown of commerce.

2.2 STATE INVOLVEMENT

These events, especially those in Jacksonville, led to the formation of the Florida State Board of Health (FSBH) in 1889. Joseph Y. Porter, a physician and noted YF expert from Key West, was chosen as the first head of the FSBH. The first efforts to prevent epidemics included fumigation of ships and quarantine of passengers. When the FSBH was created, the relationship between mosquitoes and YF was unclear. In 1881, Carlos Finlay, a Cuban physician, had proposed the role of *Aedes aegypti* mosquitoes in the transmission of YF. No one believed him at the time. More than a decade would elapse before the role of mosquitoes as disease vectors would be recognized. In 1898 it was determined that mosquitoes transmit malaria, and, in 1900, the same association was made for YF. In 1903 the FSBH issued Circular Number 1 identifying mosquitoes as disease vectors. In Florida, the last case of YF occurred in 1905. Until 2009, the last reported outbreak of locally-acquired dengue was in 1932 (see Chapter 8 for details on recent events). Malaria was eradicated from Florida by 1948. There was one locally acquired case of malaria in Gulf County in 1990, the first case in 42 years, and several cases were reported in Palm Beach County in 1996 and in 2003 by patients who had never lived in or visited a malaria endemic area.

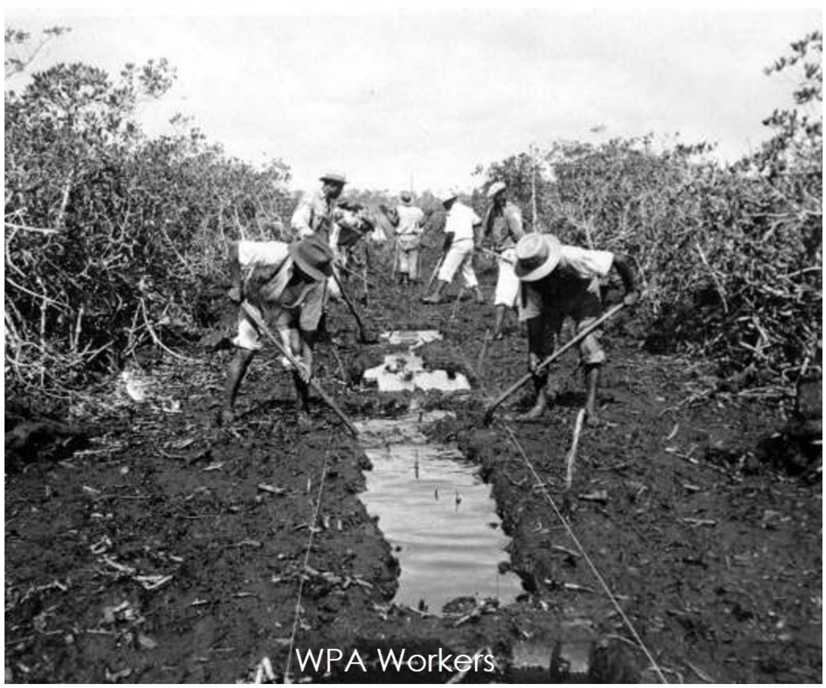
Although not documented, the first organized mosquito control efforts probably were directed at *Ae. aegypti*. During World War I, drainage and larviciding efforts were directed toward malaria control in the area that is now the Jacksonville Naval Air Station. The first FSBH involvement was a malaria control project in the city of Perry in 1919. The costs were born by the city, Taylor County, and the Burton-Swartz Cypress Company. The project was so successful that the manager of Burton-Swartz stated that it was the best money that the company had ever spent.

In 1941, the Bureau of Malaria Control was formed within the FSBH and was used primarily to train malariologists to serve Florida and other malarious areas during World War II. In 1946, the Bureau of Malaria was abolished, and the Division of Entomology was created within the Bureau of Sanitary Engineering, State Board of Health. In 1953, the Division of Entomology was upgraded to bureau status, and state aid to county mosquito control programs was established and administered by the new bureau. In 1976, the Bureau of Entomology became the Office of Entomology in the newly created

Florida Department of Health and Rehabilitative Services (FDHRS). In 1992 the office was transferred to the Florida Department of Agriculture and Consumer Services (FDACS) as a bureau.

2.3 ORGANIZED PROGRAMS

The Florida Anti-Mosquito Association (FAMA), now known as the Florida Mosquito Control Association (FMCA), was formed in 1922, followed shortly by legislation allowing the creation of mosquito control Special Taxing Districts. The first district formed was the Indian River Mosquito Control District (MCD) in 1925. The St. Lucie County MCD and Martin County MCD were formed shortly thereafter, and by 1935, five districts had been created. Early control efforts relied on hand and dynamite ditching, diking, and dewatering. Some districts proposed dredge and fill work, which was never implemented.



During the Depression, the Work Projects Administration (WPA) constructed 1,500 miles of ditches in Florida's salt marshes by hand or with explosives. Many of these ditches became a liability when the program ended and maintenance ceased. World War II brought a temporary end to Florida's organized mosquito control efforts. However, the State of Florida, through the Bureau of Malaria Control, helped to train malaria control workers for the armed forces. Mosquito Control in War Areas was established in Tallahassee and throughout malarious areas of Florida and the United States.

At the end of World War II, dichloro-diphenyl-trichloroethane (DDT) became available and was the material of choice for mosquito control. Almost all existing mosquito control districts embarked upon a program of aerial and ground use of DDT for both adult and larval control. Several new programs were formed to take advantage of this new insecticide. Beginning in 1949, the State provided funds, known as State I funds, on a dollar-for-dollar annual matching basis for the first $15,000 of the local budget for the purchase of chemicals and supplies. Results with DDT were amazing which led to the widespread belief that DDT had resolved Florida's mosquito control problems. This euphoria lasted only a few short years, long enough for resistance to develop to DDT and many of the other chlorinated hydrocarbon insecticides.

Early scientists and administrators, among them Drs. Maurice W. Provost and John A. Mulrennan, Sr., recognized that chemical control alone was doomed to failure for many reasons. Dr. Mulrennan, Sr. approached the legislature and in 1953 obtained additional funding, known as State II funds, to encourage permanent control (source reduction) with a money matching program in which the state provided $75 for each $100 in a program's local budget. State II funds were instrumental in eliminating thousands of acres of saltmarsh mosquito oviposition sites and prompted the creation of many new mosquito control programs. In addition, Dr. Mulrennan, Sr. obtained funds to build and staff a mosquito research laboratory subsequently constructed in Vero Beach and headed by Dr. Provost. State II funds were dropped in 1993.

2.4 RESEARCH

Several research facilities in Florida have been instrumental in the scientific guidance of mosquito control programs in Florida and the rest of the world. The first research facility was a United States Department of Agriculture (USDA) field laboratory established at New Smyrna (Volusia County) in early 1930. This laboratory emphasized the study of saltmarsh mosquito biology and the control of these mosquitoes by ditching.

In 1942, the USDA's Insects Affecting Man and Animals research group established a laboratory in Orlando with the responsibility of developing measures for control of and protection from insects of medical importance to the armed forces. During World War II and the Korean War, this laboratory furnished valuable information on the control of medically important insects including mosquitoes. This laboratory was the first to adapt DDT to medical entomology. Many of the methods that are used today for mosquito control, such as the Ultra Low Volume (ULV) adulticiding techniques and the development of the repellent N,N-diethyl-meta-toluamide (DEET), came from this USDA laboratory. In 1963, the laboratory was moved to Gainesville and in 1993 was renamed the Medical and Veterinary Entomology Research Laboratory. In 1996, it became the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE).

In 1947, Dr. Mulrennan, Sr., Chief of the Bureau of Malaria Control, State Board of Health, took the first step by the State of Florida toward research on the biology and control of mosquitoes by hiring Dr. Maurice W. Provost to organize such a program. This research effort was centered in Orlando and consisted entirely of field investigations into various problems in the Florida Keys, Lakeland, Leesburg, Ft. Pierce, New Smyrna Beach, and Panama City. The most significant of these studies was conducted on Sanibel Island, where the salt marsh and its role in mosquito production was investigated for several years. For seven years (1947-1954), this work was conducted without laboratory facilities. However, this deficiency was remedied in 1954.

In 1954 the state established the Entomological Research Center (ERC) at Vero Beach. It is now called the University of Florida, Food and Institute of Agricultural Sciences, Florida Medical Entomology Laboratory (FMEL). The ERC was created to study mosquito control problems with emphasis on mosquito biology and related subjects. All aspects of mosquito biology were studied and included such work as flight behavior, larval development, and salt marsh management. Now under the University of Florida's (UF) Institute of Food and Agricultural Sciences, FMEL is still operating in Vero Beach with many new facilities and programs designed to provide answers for and disseminate information to mosquito control agencies and the public.

In 1955, a Control Research Section was added to the ERC to study chemical and physical control problems. This section, headed by Dr. Andrew J. Rogers, was moved in 1964 to Panama City and named the West Florida Arthropod Research Laboratory as part of FSBH. The name was changed to the John A. Mulrennan, Sr. Arthropod Research Laboratory (JAMSARL) in 1986, while under FDHRS. In 1992, it was transferred to Florida Agricultural and Mechanical University (FAMU) and renamed the John A. Mulrennan, Sr. Public Health Entomology Research and Education Center (PHEREC). The Center's research involved adulticiding, larviciding, mosquito resistance, non-target effects, biological/alternative control, and arbovirus ecology designed to meet the needs of Florida mosquito control programs. The Center provided other useful information on mosquitoes and other arthropods of medical importance (*e.g.*, dog flies, yellow flies, and ticks). In 2011, FAMU closed the PHEREC laboratory.

Outbreaks of encephalitis in Hillsborough and Pinellas Counties led to the establishment in 1963 of the Encephalitis Research Center (ERC) in Tampa to study the epidemiology of arboviruses. This center has been renamed twice, first to the Epidemiological Research Center and finally, in 1991, to the Tampa Branch Laboratory, FDHRS. By the time of the last name change, the laboratory's mission had evolved to emphasize the diagnosis of more common infectious diseases that are not mosquito-borne. This laboratory still performs diagnostic serology for encephalitis viruses on sera from sentinel chickens.

In addition to the work of the formal governmental laboratories, a great deal of research is done by mosquito control programs. Most projects are on a cooperative basis with the above-mentioned laboratories and include such subjects as water management, chemical formulations, dispersal equipment, and surveillance. Some mosquito control programs have developed ideas of their own that have been quickly adopted by their colleagues, such as ULV application devices and formulas for more efficient insecticide dispersal. Cooperation and the sharing of ideas among mosquito control programs are a hallmark of the mosquito control profession, and many new advances have resulted from these efforts.

Private organizations also have studied mosquito control activities. Chief among these are the Harbor Branch Oceanographic Institution in St. Lucie County, the Mote Marine Laboratory in Sarasota County, and the Florida Institute of Technology in Brevard County. These institutions have conducted research on topics such as insecticidal effects on non-target organisms and ecosystem effects of salt marsh management practices (see Chapter 11 for more details).

2.5 TRAINING AND PROFESSIONALISM

In 1984, under the leadership of the late Glennon Dodd, former Assistant Director of the Indian River Mosquito Control District, a series of round-table discussions was held on topics such as surveillance, larviciding techniques, and adulticiding methods. With the help of Jim Robinson, Director of the Pasco County Mosquito Control District, and Bill Opp, then with the Office of Entomology, FDHRS, a formal set of courses was developed and used to train mosquito control personnel with the aim of certifying all workers with a prerequisite of the Environmental Protection Agency's (EPA) CORE examination in public health pest control.

Today, the Dodd Short Courses are sponsored by the FMCA and held annually. Courses are offered in fields as wide-ranging as personnel management and droplet size analysis. Twenty-seven topics were presented at the session. This program aims to assure that Florida mosquito control programs are staffed with well-trained individuals (see Chapter 12 for more details).

2.6 INTERAGENCY CONFLICT AND COOPERATION

In 1980, considerable disagreement concerning some mosquito control practices existed between mosquito control interests, represented by the Office of Entomology, FDHRS, and the Florida Department of Natural Resources (DNR), two state agencies with conflicting mandates. Governor Graham intervened to resolve this conflict and future problems by forming a committee representing these various concerns. This committee was the beginning of the Governor's Working Group on Mosquito Control, which had its first meeting in May 1980. It was from this beginning in 1986 that the Florida Coordinating Council on Mosquito Control (FCCMC) was created by the Legislature in Chapter 388 Florid Statutes (F.S.).

The membership of FCCMC essentially followed that of the Governor's Working Group. The original members represented each of the following organizations: DNR, FDACS, the Florida Department of Environmental Regulation (DER), the Florida Game and Fresh Water Fish Commission (FGFWFC), the UF, the EPA, the U.S. Fish and Wildlife Service (USFWS), and the USDA. Also included were two mosquito control directors and two representatives from FDHRS, an epidemiologist, and the Director of the Office of Entomology as chairman. Added in 1986 were two at-large environmentalists and two property owners whose lands were subject to mosquito control activities. The membership has been modified since 1986 to reflect changes in agencies, such as the creation of the Florida Department of Environmental Protection (FDEP) from the DNR and DER, the FGFWFC to Florid Fish and Wildlife Conservation Commission, the transfer of the entomology program from FDHRS to FDACS, the JAMSARL transfer to FAMU, and most recently the closing of the FAMU/PHEREC laboratory.

Currently, the FCCMC meets three times a year in January, May, and September and is specifically mandated to serve as an advisory board to public agencies involved in arthropod control activities to work together to reduce duplication of effort, to foster maximum efficient use of existing resources, to advise and assist the agencies involved in arthropod control in implementing best management practices and best available technologies for controlling arthropods, to assist FDACS in resolving disputes arising over the control of arthropods on publicly owned lands, to develop and recommend to FDACS a request-for-proposal process for arthropod control research, to identify potential funding sources for research on implementation projects, and to evaluate and rank proposals upon request by the funding source.

One of the most important activities of the FCCMC was the creation of the Subcommittee on Managed Marshes (SOMM), originally named the Technical Subcommittee on Mosquito Impoundments, in 1983. Like the FCCMC, it was formally established as SOMM in Chapter 388 F.S. in 1986. This interagency committee, with a membership makeup like that of the FCCMC, was established to provide technical assistance and guidance on salt marsh management plans and to develop and review research proposals for source reduction techniques.

2.7 CONCLUSION

This history of mosquito control in Florida and the institutions that have influenced it is all too brief and leaves out many facets of mosquito control history that should be recorded. Section 2.8 provides a reference for such a record. Additional history appears elsewhere in this report.

2.8 A PUBLISHED HISTORY OF FLORIDA MOSQUITO CONTROL

Dr. Richard Baker, during his tenure as Director of the FMEL, encouraged Dr. Gordon Patterson, Professor of History at Florida Institute of Technology, to write a history of mosquito control in Florida. Dr. Patterson was then introduced to John Beidler and a host of entomologists, mosquito control and public health workers, state officials, librarians, and others who provided assistance, information, and anecdotes about the early years of mosquito control in Florida. The result of Dr. Patterson’s research was the 2004 publication of The Mosquito Wars: A History of Mosquito Control in Florida. This work is an eloquent read that details the stories, names, and the faces of those who have influenced mosquito control in Florida.

2.9 REFERENCES

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Chapter 3

MOSQUITO SURVEILLANCE

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Summary

Mosquito surveillance is a prerequisite to an effective, efficient, and environmentally sound mosquito control program. Surveillance data is used to define the nature and extent of the mosquito problem, gauge the effectiveness of daily mosquito control operations, comply with State laws and regulations regarding the justification for treatments, and serve as a basis for evaluating the potential for transmission of mosquito-borne diseases. Commonly used methods to inventory mosquito habitats, collect immature and adult mosquitoes, and monitor environmental parameters that can be used to predict mosquito emergences are reviewed.

3.1 GENERAL APPROACH TO MOSQUITO SURVEILLANCE

Ideally, the structure and implementation of a mosquito surveillance program should be based on the needs of the local mosquito control agency. Moreover, these needs should define the components of the control program, as well as the required budget. In fact, this process is often reversed. Mosquito control programs are funded at a specific level, generally without a needs-assessment process. The program director is then required to meet all mosquito control needs within the constraints of a fixed budget. If funding for this process is inadequate, the result is incomplete mosquito surveillance with reliance upon undesirable and less effective control methods.

The steps in developing a mosquito surveillance program as part of an overall mosquito control effort are to:

1) Define area-specific mosquito problems
2) Design a mosquito surveillance program to be used as a decision-making aid to help determine when and where mosquito control efforts are needed

3.1.1 Defining Area-Specific Mosquito Problems

There are at least 80 mosquito species in Florida. Every Florida county has several species that are dangerous disease vectors and several more species that create a major nuisance during most months of the year.

The first step in determining which mosquito species must be monitored is to determine which species cause problems. Control efforts can be justified when a mosquito poses a nuisance or is an economic or health-related pest or vector. A nuisance mosquito bothers people, typically in and around homes or in recreational areas as the result of great numbers of biting adults that emerge from large tracts of untreated breeding sites. An economically important mosquito reduces property values, slows economic development, reduces tourism, and/or adversely affects livestock and poultry production. A health-related mosquito problem results when a mosquito species transmits pathogens that cause disease. In Florida, this definition currently includes certain mosquito species that transmit dog heartworm, St. Louis encephalitis virus, West Nile virus, eastern equine encephalitis virus, Zika, chikungunya, and dengue viruses and malaria. Disease surveillance is discussed in Chapter 8. Any mosquito that bites or annoys people can be considered a health problem, particularly for individuals who are allergic to mosquito bites or suffer from entomophobia (an unreasonable fear of insects).

A list of important nuisance and vector mosquito species can be compiled from a review of the literature. The geographic and temporal distributions of these species also can be found in the published literature (Darsie and Morris, 2000).

3.1.2 Surveillance Program Considerations

Carefully planned and executed mosquito surveillance is an important component of an integrated pest management program. Once target species have been identified, selected areas can be sampled to determine the abundance of adults and larvae of the species of interest. There are several considerations that should be evaluated when selecting the right surveillance methods.

- Examine if the purpose of the monitoring is to define the extent of the mosquito problem, record the presence or absence of species or document a specific species within a given area, justify control measures for compliance with state laws and regulations, gauge effectiveness of implemented control measures, or determine potential vector abundance and distribution.

- Identify habitat types within the area to assist in the identification of species that may be encountered, such as salt marshes or urbanized residential areas.

- Conduct a qualitative assessment of species present and consider the potential for problems based upon the species collected.

- Conduct a quantitative assessment of relative abundance of target species to aid in the development of appropriate control measures.

- Since mosquito collection methods differ in their effectiveness for sampling different species, multiple surveillance techniques for larvae and adult mosquitoes should be used to accurately quantify mosquito abundance.

- Other considerations may include workload/staffing requirements, employee skill level, and training requirements, all of which affect the duration, frequency, and types of monitoring that can be implemented.

- Mosquito surveys should be conducted throughout the mosquito breeding season. Surveillance data should be filed electronically and retained for historical purposes. Long term baseline weekly averages can be calculated and used to determine real time deviations from normal for all of the mosquito species that are monitored by a surveillance program.

- Surveillance programs should account for biological, operational, and environmental variables that affect methods utilized when conducting population comparisons. Variables may include, but are not limited to, mosquito species sampled, mosquito behavior, physiological state, sampling technique utilized, distance to breeding sites, food sources, as well as meteorological variables such as wind, rain and temperature. Meteorological conditions strongly affect mosquito activity. Therefore, the numbers captured typically will reflect weather conditions or seasonality rather than merely population changes (Bidlingmayer).

Surveillance methods vary by program due to environmental factors, such as habitat types, species distribution and abundance, as well as program needs and constraints, such as, resources and budgets. Most mosquito surveillance programs are usually a compilation of surveillance techniques that have been shown – usually by trial and error – to work for a specific program. In Florida, temporal and geographical changes in mosquito populations and the problems that mosquitoes cause are measured by monitoring the following:

1. Telephone or website service requests for mosquito control
2. Adult mosquito populations as measured by trapping and landing counts
3. Immature mosquito populations as measured by larval inspections

3.2 SERVICE REQUESTS

One method for identifying local nuisance mosquito problems is through telephone or website service requests. Most Florida mosquito control programs have a telephone number that citizens can call to request mosquito control services. Many programs have developed their own websites where citizens can go to enter a service request on-line.

Although service requests are an acceptable way to meet state requirements for monitoring mosquito problems to justify control, these requests must be verified by other surveillance techniques prior to any treatment (Chapter 5E-13, Florida Administrative Code (FAC)).

Service requests can be handled in a variety of ways. Most programs record the information on data sheets, while some programs record complaint data via software programs linked to a Geographic Information System (GIS). The service requests can be displayed on a map using a GIS software program to assist in displaying clusters or patterns of potential mosquito problems. The service request data are ultimately used to determine where to concentrate control efforts once the requests are verified. Typically, an inspector will be sent to verify the service request in areas where high mosquito population densities are not indicated by other surveillance techniques. In some cases, changes in the numbers of requests from one day to the next are used to evaluate the effectiveness of control operations.

Service requests that are generated by the presence of container-inhabiting mosquito species, such as the Asian tiger mosquito (*Aedes albopictus*), *Ae. aegypti,* or certain *Culex* species, may require an inspector to identify potential larval habitats. Inspectors also can assist homeowners with point-source reduction of containers that hold water. If the service request results from floodwater mosquitoes (saltwater or freshwater) or permanent water mosquitoes such as *Anopheles, Coquillettidia,* or *Mansonia*, mosquito control personnel inform the citizen of the steps that will be attempted to correct the problem. The inspector will assess the adult mosquito population and attempt to locate the source of larval development using techniques described in this chapter.

3.3 ADULT MOSQUITO SURVEILLANCE

There are a variety of surveillance techniques utilized to monitor adult mosquito populations. These include, but are not limited to, landing rates, light traps, baited traps, non-attractant traps, oviposition traps, resting populations, and emergence traps. The purpose of monitoring adult mosquitoes is:

- To determine where and when adults are most numerous.
- To substantiate service request claims of a mosquito problem.
- To determine the effectiveness of source reduction, larviciding, and adulticiding control methods.
- To provide data that satisfies Chapter 5E-13, FAC to ensure that applications of pesticides are made only when necessary.

Chapter 5E-13.036, FAC outlines the criteria that must be met and documented prior to the application of pesticides and focuses only on adult mosquito surveillance. According to this law, before adulticides can be applied, a monitoring program must detect an increase in the mosquito population above a predetermined baseline, collect more than twenty-five mosquitoes in a single trap night, or collect more than five mosquitoes per hour of operation during crepuscular periods. The law does not specify the type or number of traps or the species or sex of the mosquitoes captured, but they make the application of adulticides illegal when mosquitoes are not present. This law was initiated in 1987, and, for the first time, forced many mosquito control programs to use mosquito surveillance to justify spraying.

3.3.1 Landing Rates

The majority of the programs in Florida utilize landing rates (pers. comm., Florida Department of Agriculture and Consumer Services (FDACS) 2017). They are used for measuring adult mosquito activity, augmentation of existing mechanical trap collections, or assessment of service requests for making spot treatments with adulticides. The technique consists of counting the number of mosquitoes that land on a person in a given amount of time, often one minute. The Centers for Disease Control and Prevention (CDC) does not recommend conducting landing rates within areas of known arbovirus transmission, as they may be associated with potential health risks to field staff (CDC 2016).

The specific method used to determine a landing rate varies among programs. Important variables are the time of day at which observations are made, the duration of observations, the portion of the subject's body observed for landing mosquitoes, the number and type of nearby habitats, and the number of human subjects used. It is important to choose a landing rate protocol and avoid changing the variables to get meaningful data. Day-to-day changes in the biting population at a given site are best reflected when the same individual performs the landing rate at that site at the same time of day, as individuals differ in their attractiveness to mosquitoes and the time of day can significantly alter the landing rate counts.

Landing rates taken during the day can be effective for monitoring saltmarsh mosquitoes, which bite during the early morning and in the afternoon. Although many crepuscular species are found in well-shaded, moist canopied areas during daylight hours, it is best to assess their landing rate at the time of peak activity. The host-seeking females can be collected for identification purposes by using a battery powered aspirator.

3.3.2 Light Traps

The New Jersey Light Trap (NJLT) is traditionally green in color, uses a 25-watt bulb attached to a 110V AC power source, is placed 5½ feet off the ground, and is useful in measuring relative abundance of certain mosquito species. The light is the attractant, and many insects other than mosquitoes also are attracted to the trap. The NJLTs were first used in a statewide program in the mid-1950s by the Florida Board of Health. Local programs would operate the traps and send the collections to the Department of Health in Jacksonville for identification. Mosquito identification eventually became the responsibility of the local programs. During this transition, many programs that lacked expertise in mosquito identification stopped trapping.

Because NJLTs require 110V AC power, they have been operating in the same locations for decades, and the historical monitoring data have been valuable for documenting the long-term changes in mosquito populations at those locations. While NJLTs are usually operated overnight, the number of trap sites and the frequency of trapping vary among mosquito control programs. Currently, there are no rules of thumb, established standards, or State rules that apply to the operation of NJLTs.

Experimental light emitting diode CDC light trap using blue light to attract mosquitoes. Colored light LEDs use little power compared to incandescent lamps and are highly attractive to certain mosquito species.

Most Florida mosquito control programs use CDC light traps to monitor adult mosquitoes. The CDC light trap is a miniature version of the NJLT that operates on 6V DC power and can be used anywhere, as it is lightweight, compact, and battery-powered. It costs less than the NJLT and collects primarily mosquitoes. Although there are several manufacturers of CDC-like traps, they can be handmade by local mosquito control programs for about one-fourth of the retail cost. This cost differential has resulted in a proliferation of different designs of the trap. It is not important that all control programs use the same CDC trap design as long as the same model of trap is used within a program. CDC light traps can be supplemented with mosquito attractants and/or operated with the light off. Some mosquito control programs use carbon dioxide (either dry ice or bottled gas), octenol, or other mosquito attractants (such as lactic acid) as a supplement for the CDC trap. Most control programs run traps throughout the night, but some programs operate CDC traps for only a few hours a night due to budget constraints, not for entomological reasons. There is no standard protocol for placing or operating CDC traps.

3.3.3 Non-attractant Traps

Non-attractant traps sample airborne populations by use of truck traps, suction traps, or sticky traps. Two programs in Florida use methods other than light traps as their principal adult mosquito surveillance tool. Pasco County Mosquito Control District (MCD) uses permanently located un-baited suction traps, while Lee County MCD uses truck traps. A truck trap is a large screened funnel attached to the top of a pickup truck. Unlike the NJLT and CDC traps, suction and truck traps sample all airborne mosquitoes, which provides a better measure of mosquito density but does not measure the biting mosquito population. Suction and truck traps provide unbiased sampling; Chemical attractants and light play no part in collections and higher numbers of male mosquitoes are frequently collected. Lee County MCD has operated truck traps for more than 40 years. This data has been very useful for making control decisions for saltmarsh mosquitoes in Lee County.

Bidlingmayer truck trap used to sample airborne mosquitoes.

Pasco suction trap, originally called "mini-bidlies" because they are a mini version of the Bidlingmayer trap that was developed at FMEL.

3.3.4 BG Sentinel Traps®

Prior to the emergence of dengue, chikungunya, and Zika viruses in Florida, most programs were conducting adult mosquito surveys with light traps focused on pestiferous and nuisance mosquitoes and an endemic vectors species of West Nile and St. Louis encephalitis. With the public health threat of these viruses, programs have expanding expanded their focus to include surveillance for *Ae. aegypti* and *Ae. albopictus*. Light traps are inefficient in most cases for *Ae. aegypti* and *Ae. albopictus* surveillance (CDC 2016). The most widely used traps for these species are the BG Sentinel® (BG) trap, the gravid *Aedes* trap (GAT), and the CDC-autocidal gravid ovitrap (CDC-AGO) (AMCA 2017).

The BG trap is a ground-mounted suction trap used for surveillance of *Ae. aegypti* and *Ae. albopictus*, both daytime biting mosquitoes capable of transmitting dengue, yellow fever, Zika, and chikungunya viruses. These traps are lightweight, collapsible, and powered with either 110V AC electricity or a 12V DC battery. The BG trap makes use of a contrasting black and white coloring scheme, a visual cue known to be attractive to both *Aedes* spp. Traps can be baited with CO_2, BG Lure® (lactic acid, ammonia, fatty acids), a human skin mimic for *Ae.* mosquitoes, or octenol, which is attractive to several different *Culex* mosquitoes. Additionally, an optional UV light can be mounted atop the trap for collecting nighttime active mosquitoes and biting midges.

Mosquito Magnet Pro®, a propane-powered suction trap producing carbon dioxide, moisture, and heat.

3.3.5 Mosquito Magnets

Indian River MCD, St. Lucie County MCD, and Martin County Mosquito Control as well as most programs in the panhandle utilize the commercially available Mosquito Magnet® as their primary adult mosquito collection tool. The Mosquito Magnet® operates 24/7 by releasing a continuous stream of carbon dioxide and heat. It can be used with or without an attractant as a lure. The patented CounterFlow™ system vacuums the mosquitoes into a net instead of being collected by a fan.

3.3.6 Oviposition/Gravid Traps

Other specialized traps are used to trap either specific species or are used to augment collections as part of an arbovirus surveillance program and include the CDC gravid traps. The CDC gravid (Reiter) traps collect gravid females including species that transmit arboviruses. Essentially these traps use an “ovilure mixture” of organics in water that attract gravid females that are ready to oviposit. The hypothesis is that since these females have already blood fed at least once, the collected females have a greater probability of having been infected with an arbovirus, making public risk assessment easier. A number of different "ovilures" are used, and some attract different species, for example, hay infusion for *Culex quinquefasciatus,* alfalfa infusion for *Ae. aegypti,* and oak leaf infusion for *Ae. triseriatus* and *Ae. albopictus*.

Gravid traps use an "ovilure" mixture of organics in water that attract gravid females that are ready to oviposit.

The gravid *Aedes* trap (GAT) specifically targets *Ae. aegypti* and *Ae. albopictus*. Adult females are caught on glue boards when they rest on the side of the container. These traps are checked one to two times per week and can be effective indoors or outdoors, with or without infusions. It is recommended that the GAT and BG traps be used in a complementary way to monitor both sexes and all physiological stages of *Aedes* (AMCA 2017). The CDC-autocidal gravid ovitrap (CDC-AGO) also targets gravid females. It is inexpensive and easy to deploy. Mosquitoes can be counted in the field and the data correlates well with data from the BG traps. The GAT requires servicing every two months.

3.3.7 Resting Populations

Resting behaviors vary based upon species and gonotrophic stages and occur within natural or artificial resting sites, such as tree holes, vegetation, resting boxes, or shelters. Mosquitoes can be collected using vacuum aspirators, including hand held sweepers, hand-held battery-operated flashlight aspirators, or sweep nets. These devices will collect a number of resting mosquito species in dark areas and natural cavities. They are especially good for collecting in heavy vegetation around homes for assessing the mosquito problems of customer complaints calls if other methods are lacking or problematic. Collecting resting mosquitoes has an advantage by providing an accurate representation of the overall mosquito population, since adult mosquitoes are collected across various physiologic states, such as unfed, blood-fed, gravid females and males (CDC 2016).

Resting boxes are used for the collection of *Culiseta* and *Anopheles* spp. by programs interested in monitoring these vectors. Resting boxes are generally placed on the ground with the open end facing west to minimize the influence of direct sunlight during the early part of the day. A dark, forested habitat with high canopy yields the highest collections (Crans 1989). Mosquitoes utilizing resting boxes as diurnal resting sites enter the boxes during the morning hours, remain inactive during late morning and early afternoon, and then exit the boxes later in the day. The inside of the resting boxes is usually painted black or red, while the outside is painted flat black. The 12" x 12" x 12" plywood cubes have one open end and are usually positioned no closer than 10 feet from one another in either a line or grid design. Collection from these boxes is usually by aspirator and should be conducted from mid-morning to late afternoon.

3.3.8 Emergence Traps

Collection of *Mansonia* and *Coquillettidia* adults is difficult since the larvae are attached to the roots of aquatic vegetation. Both species are readily collected as adults in NJLT and CDC traps, but, to assess their population from aquatic plant habitats, a more direct trapping regime may be needed. Most workers use emergence traps to collect *Mansonia* species associated with water lettuce (*Pistia stratiotes*) mats and cattails (*Typha spp.*). The emergence traps cover a known surface area (typically 4 meter square), and adult collections are made on a weekly basis. Traps are generally spaced between 50-100 meters apart and number from 2-10 per site. Traps are periodically repositioned at 4-8 week intervals to compensate for the possible depletion of mosquito fauna.

3.4 IMMATURE MOSQUITO SURVEILLANCE

The number of devices and procedures that have been developed to sample mosquito eggs, larvae, and pupae is extensive (Service 1993). Unfortunately, little effort has been made to standardize the most frequently used methods. Each mosquito control program has its own version of the different sampling methods, which makes the comparison of data between programs difficult.

3.4.1 Collecting Eggs

There are many techniques available to sample mosquito eggs (Service 1993), but these methods are seldom used on an ongoing basis or as a primary surveillance system. While not employed regularly by mosquito control programs, a technique for egg sampling first reported by Bidlingmayer & Schoof (1956) has been used to identify the distribution of *Ae. taeniorhynchus* in Florida mangrove basin forests (Ritchie & Johnson 1991). Sampling mosquito eggs is too labor-intensive for practical purposes. A few programs have found egg sampling useful to initially describe or find mosquito habitats to be added to the inventory. Once documented, it is usually easier to sample larvae. One exception to this is the use of ovitraps, which monitor the distribution of the *Ae. albopictus* and *Ae. aegypti* in Florida. Using a network of highly attractive ovitraps to monitor this species is easier than searching for the small containers in which these species oviposit. However, egg surveillance may not always be an accurate measure of the adult population (AMCA 2017).

3.4.2 Collecting Larvae and Pupae

Mosquito larvae and pupae can be collected with dippers, nets, aquatic light traps, suction devices (turkey baster for bromeliad and container collections), and with

Dipper in use.

container-evacuation methods. The most commonly used apparatus is the dipper. The term "standard pint dipper" is used in the scientific literature, but, in practice, there is no standard dipper or standardized dipping techniques (Service 1993). The dipper consists of a white plastic cup, 400ml in volume, with a two to five-foot handle to allow for an extended reach. The dipper can be used as a surveillance tool to determine the presence or absence of larvae. Such a method usually involves taking several dipper samples from designated areas in a habitat and then counting the larvae captured in each dip. The dipping method will vary with water depth, presence of aquatic vegetation or other debris, and water clarity. Collectors must be aware of factors such as mosquito species difference in submerging behavior, stage differences (first and second instar stay under longer), and larval distribution or aggregation. Training, practice, and experience are important when control programs use larval density as a basis for larval control measures. Larval density is equal to the number of larvae collected per dip.

The collection of *Mansonia* and *Coquillettidia* larvae is difficult because the larvae do not breathe at the water surface but get their oxygen by piercing the stems and roots of aquatic plants. Collection of larvae is problematic since they quickly detach when disturbed and bury themselves in detritus. As a result, a mosquito dipper is an inappropriate sampling tool for this type of mosquito.

DeWald and Lounibos (1990) sampled water lettuce for *Mansonia* larvae and pupae in south Florida. Samples are taken of a known surface area using a stainless steel sampling tool. The tool had serrated teeth around the perimeter of the bottom to penetrate the water lettuce mat and a “trap door” to collect the underlying water column with the sample. In this system, five quadrants are used per site and sampled approximately every 30 days. Another method for collecting *Mansonia* larvae is to place a shallow pan under the floating aquatic vegetation. Care must be taken not to disturb the aquatic plants or surrounding area. Once the pan is in place, the pan and the aquatic plant must be lifted slowly out of the water. Clean water may need to be added to the pan to accurately view and count any mosquito larvae. This method requires a great deal of patience and practice.

To collect *Coquillettidia* larvae, a pump and wand system has been used (Morris *et al.* 1985) with good results. The eggs and larvae of *Cq. perturbans* are usually found in the detritus at the base of the aquatic plants. A mosquito dipper or siphon can be used to collect the larvae. However, the water may need to be placed in a pan containing clean water for accurate viewing and counting.

One of the objectives of larval surveys is to detect larval habitats and assess marked changes in larval densities as a result of control measures. Entomological indices were developed to assist mosquito control and public health officials to plan and implement *Aedes* control programs. This can be calculated after conducting immature stage surveys. The following indices have been utilized in Florida to quantify immature *Aedes* populations:

- House index: the percentage of houses that are positive for larvae
- Container index: the percentage of water holding containers that are positive for larvae
- Breteau index: the number of mosquito-positive containers per 100 houses
- Pupal index: the number of pupae per house per person or the number of pupae per hectare (2.47 acres)

There are some limitations with these indices. Most information collected is based upon a minimum of 100 houses or properties, which can be labor intensive. In addition, the basic larval indices do not correlate well with the adult abundance (Unlu *et al.* 2013). Surveys are reliant upon visual searches of containers and may miss or overlook cryptic habitats. The house and container indices fail to consider the actual mosquito population. The Breteau Index is best for estimating vector density as it combines the data on houses as well as the containers.

3.5 MONITORING ENVIRONMENTAL PARAMETERS

Mosquito surveillance is most effective when combined with an ongoing program for monitoring meteorological, climatological, and water table data that may influence mosquito population change. For example, rainfall, ground water levels, temperature, relative humidity, wind direction, wind velocity, tidal changes, lunar cycles, stormwater and wastewater management, and land use patterns are all factors that may influence mosquito population levels, as well as adult mosquito flight behavior and dispersal.
To maximize the usefulness of mosquito surveillance data, it is important to monitor certain environmental parameters such as rainfall and tidal events. Tide levels in coastal areas are monitored using charts and gauges. Tidal activity and rainfall dictate when high marsh sites will be flooded and when they will need to be inspected for mosquito larvae. Tide gauges also may reflect changes in the water level caused by rainfall and wind that often result in increased mosquito production in salt marshes and mangrove forests.

Rain gauges are important in both coastal and inland counties -- in fact, anywhere mosquito production is being monitored. Data from these instruments can be supplemented with data from the National Weather Service and local weather websites. Because rainfall in Florida is highly localized, it is important to collect rainfall data from many locations.

Knowledge of weather patterns is important during ground and aerial mosquito control applications. High winds, no wind, low temperatures, rainfall, and high humidity can influence the dispersal of the material applied and deter it from reaching its target, thereby affecting the efficacy of the application.

3.6 INVENTORY AND MAPPING SURVEILLANCE DATA

If the design of the mosquito control program includes source reduction or application of larvicides, both a mosquito habitat inventory and a larval surveillance system should be in place. The mosquito habitat inventory is a permanent collection of descriptions of all habitats. A larval surveillance system describes the abundance of mosquito larvae at each site. The information can be used to determine optimal times for use of larval control measures, including chemicals, biologicals, draining, or impounding. It also can be used to help forecast the need and timing for adult mosquito control and to help assess the effectiveness of both chemical and biological control measures.

As mosquito control programs evolve, topographical and aerial paper maps are being replaced by geo-referenced high resolution aerial images. These geo-referenced maps can show the location of potential larval habitats and the treatments that occurred within a specific time frame. These maps are used to develop and maintain a program for the surveillance of larvae and the application of larvicides. The maps provide an up-to-date record of the larval habitats within the jurisdiction of the control program.

The map inventory should be dynamic and updated on a routine basis. As new residential or commercial developments are created, the characteristics of mosquito habitats may change. In turn, the species composition of mosquitoes produced at each site also may change. Due to changes in rainfall patterns and intensity of tidal flooding, these habitats can vary greatly.

Deciding which characteristics of the larval habitat should be recorded in an inventory is difficult. Instantaneous measurements of rapidly or frequently changing variables, such as water depth, water temperature, and presence or absence of predators or parasites may be useful to help determine if control treatments are needed and should be included in a larval habitat inventory.

While the field work portion of the initial inventory is time consuming, creating and maintaining maps of larval habitats is even more difficult. It is highly desirable to use a computer-based mapping system using GIS technology to map these larval habitats if possible. A major advantage of a computerized mapping system is the ease with which data can be extracted and compiled. GIS and GPS tools provide programs with methods to locate, visualize, query, analyze, and interpret surveillance data. GIS is designed to capture and store data in various digital formats (*e.g.* geodatabases) that allow creation of maps, reports, and charts, which can then be utilized to reveal relationships, patterns, or trends in mosquito data.

3.7 EMERGING TECHNOLOGIES

Several new technologies are under development, including smart traps, such as the BG Counter® and Microsoft's Premonition® research, as well as the use of unmanned aerial vehicles (UAVs, *a.k.a.* drones). The BG-Counter® is the first commercially produced smart trap is a novel, automatic mosquito monitoring system. This trap is solar-powered and is usually baited with some source of carbon dioxide. The trap allows for precise monitoring of mosquito populations over wide areas and provides data in real-time, directly from the trap to a program's computer with minimal input. This real-time data on mosquito population activity allows for countermeasures to be initiated earlier and more precisely than traditional monitoring methods, and therefore control methods can be more effective. This real-time acquisition of mosquito population data also allows for a detailed assessment of the effectiveness of control measures. This is especially important in areas with active mosquito-transmitted disease activity.

Another smart trap currently under development is part of Microsoft's Project Premonition® research. This trap is designed to gather specific data on selected species and analyze them for disease pathogens and the impact that environmental parameters such as wind, rain, and temperature have on their activity. It minimizes time that manual sorting of a collection requires. These next generation traps use two small, battery-powered microprocessors, which gather data that can then be wirelessly downloaded and easily retrieved back at the program's office. These traps also rely on the latest advances in a branch of artificial intelligence called machine learning to distinguish target and non-target species.

The use of drones for mosquito surveillance is beginning to increase. Drones can collect data such as aerial images that can be analyzed to identify and map the location and abundance of larval developmental sites. These images can be aggregated into accurate maps to support more precise targeting of insecticide treatments. The use of drones for surveillance and control operations is likely to expand dramatically over the next decade.

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Chapter 4

MOSQUITO CONTROL THROUGH SOURCE REDUCTION

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Summary

Source reduction, also known as physical or permanent control, typically is one part of a mosquito control agency's Integrated Pest Management program. Source reduction is usually the most effective and economical mosquito control technique and is accomplished by eliminating mosquito habitats. This effort can be as simple as properly discarding water-holding containers capable of producing mosquitoes or as complex as implementing Rotational Impoundment Management or Open Marsh Water Management techniques, which control saltmarsh mosquitoes concurrent with significant habitat restoration or rehabilitation. Source reduction is important since it can virtually eliminate the need for insecticides in and adjacent to the affected habitat. The history of mosquito control source reduction efforts in Florida dates back to the 1920s when ditching of high marshes by hand or with explosives occurred. Since those early efforts, other source reduction projects include the filling of salt marshes and the creation of impoundments. While all of these techniques had mosquito control benefits, there were environmental impacts. Since the early 1980s, concerted efforts to restore or rehabilitate salt marshes impacted by mosquito control have been an ongoing management initiative.

Source reduction in freshwater habitats (e.g., floodplains, swamps, and marshes) typically involves constructing and maintaining channels. These channels or ditches can serve the dual functions of dewatering an area before mosquito emergence can occur and as harborage for larvivorous fish. Mosquito production from stormwater/wastewater habitats can be a problem but typically can be managed by keeping the area free of weeds through an aquatic plant management program and by maintaining water quality that can support larvivorous fish. Lastly, tires are a problematic mosquito producing habitat which can be managed by proper disposal.

4.1 INTRODUCTION

Source reduction, also known as physical or permanent control, is typically one part of a mosquito control office's Integrated Pest Management (IPM) program. Source reduction is usually the most effective of the mosquito control techniques available and is accomplished by eliminating larval mosquito habitats. This effort can be as simple as properly discarding water-holding containers capable of producing *Aedes aegypti, Ae. albopictus,* or *Culex* spp., or as complex as implementing Rotational Impoundment Management (RIM). RIM is a source reduction strategy that controls saltmarsh mosquitoes (*e.g.*, *Ae. taeniorhynchus, Ae. sollicitans*) concurrent with significant habitat restoration. Source reduction is important since it can virtually eliminate the need for insecticides in and adjacent to the affected habitat. Source reduction is appropriately touted for its ecosystem management effectiveness and economic benefits.

4.2 MOSQUITO PRODUCING HABITATS APPROPRIATE FOR SOURCE REDUCTION

4.2.1 Salt Marshes

In Florida's recent past, extensive coastal salt marshes produced enormous *Aedes* spp. broods, making coastal human habitation virtually impossible. Several source reduction efforts described in this chapter have greatly reduced saltmarsh mosquito production in salt marsh habitats through intensive management that relies upon artificial manipulation of the frequency and duration of inundation.

4.2.2 Freshwater Habitats

Environmental laws greatly restrict habitat manipulations in freshwater lakes, ponds, swamps and marshes, making permanent control there difficult. These areas can produce *Culex, Anopheles, Coquillettidia, Mansonia,* and *Culiseta* species. In these situations, other options are effective in controlling mosquitoes including periodic drainage, providing deepwater sanctuary for larvivorous fish, minimizing emergent and floating vegetation, and maintaining steep banks.

4.2.3 Temporarily Flooded Locations

Pastures and agricultural lands are enormous mosquito producers, frequently generating huge broods of *Aedes, Psorophora,* and *Culex*. Improved drainage is one effective tool for source reduction in these floodwater habitats. Another technique is the use of micro-jet irrigation for those agricultural areas that require artificial watering. For example, a water conservation requirement in some citrus groves has replaced flood-irrigation with micro-jet irrigation, resulting in almost a complete disappearance of *Ae. vexans* in some locales.

4.2.4 Containers

Containers such as flowerpots, cans, pet bowls, rain barrels and tires are excellent habitats for several *Aedes* and *Culex* species. Container-inhabiting mosquitoes of particular concern in Florida are *Ae. albopictus* and *Ae. aegypti.* In some parts of Florida *Ae. albopictus,* a species adapted to and closely identified with the human environment, has become a significant mosquito control problem. A container-inhabiting mosquito problem can be solved by properly disposing of the standing water in the containers, covering the containers, or tipping them over to ensure that they do not retain water. Due to recent threats of disease outbreaks, many Florida mosquito control agencies have established programs to address urban container-inhabiting mosquito problems through house-to-house surveillance and formalized education programs directed at elimination of container habitats.

4.3 SOURCE REDUCTION FOR SALTMARSH MOSQUITO CONTROL

4.3.1 Description of Florida's Saltmarsh Habitats

The first mosquito control programs in Florida were created in response to the need to control saltmarsh mosquitoes along areas of the central east coast with barrier islands and large and extensive high salt marshes. Along the central east coast of Florida's estuary, the Indian River Lagoon (IRL), mean daily tidal heights and ranges are small in comparison to other coastal areas of the continental United States which experience much larger seasonal tidal variations. Seasonal wind-generated water movements can be more important factors than lunar tides in determining maximum water levels. The difference between the large seasonal tides and relatively small daily tides results in a greater variability in inundation frequency between low and high marsh. Since low marsh is flooded by the year-round daily tidal changes and high marsh is flooded only by seasonal high tides, strong wind tides, or rainfall, a much greater proportion of high marsh compared to low marsh is created there. It is the high marsh that produces large numbers of saltmarsh mosquitoes and thus is the area targeted by most source reduction efforts.

Provost (1967) broadly classified Florida's salt marshes into three main vegetative types:

Grass Marshes*:* Grass marshes are typical of low marsh habitats and are dominated by *Spartina alterniflora* (cordgrass) or *Juncus roemerianus* (black rush). The relatively small high marsh portions of grass marshes usually are vegetated by *Distichlis spicata* or *Spartina patens,* which can be prolific producers of *Ae. sollicitans.* Areas of transition from low marsh to high marsh are usually narrow in grass marshes, as are the high marsh fringing areas themselves.

Scrub Marshes: Typical scrub marsh is dominated by *Batis maritima* (saltwort) and *Salicornia* spp. (glasswort). The scrub marsh can be variable in a cross-sectional area and usually lies behind a wave-action berm that limits inundation frequency. The berm is generally less than 100 feet in cross-section and consists of broken parts of sea shells, sand, mud, and plant materials trapped and retained along shorelines by fringing mangroves. Wave-action berms limit tidal inundation of the marsh to periods when water levels exceed mean high water (MHW) and thus contribute to the mosquito problems by reducing the periodic natural inundation to approximately six weeks per year.

High salt marsh, Pelican Island National Wildlife Refuge, Indian River County

Mangrove Swamps: Mangrove swamps are present in both low and high marsh forms. In the low marsh, mangrove swamps are dominated by *Rhizophora mangle* (red mangrove), while *Avicennia germinans* (black mangrove) and *Laguncularia racemosa* (white mangrove) dominate the high marsh. Red mangroves, with their extensive prop roots, protect the shoreline against erosion and typically lie at, or waterward, of the mean high water line (MHWL), except where management practices (*e.g.*, impoundments) have altered the wetland hydrology. Generally, a wave-action berm forms behind this mangrove fringe, effectively restricting tidal inundation frequency of the more inland sections of the swamp. Due to current rise in sea level and anthropogenic forces (ditching and artificial inundation activities), the tidal flooding frequency may be changing.

Low and high marsh vegetation types differ regionally. Grass marshes dominate in north Florida. Scrub and scrub marshes occur along the central east coast, roughly from St. Augustine to Indian River County and along the central west coast from Tampa Bay to

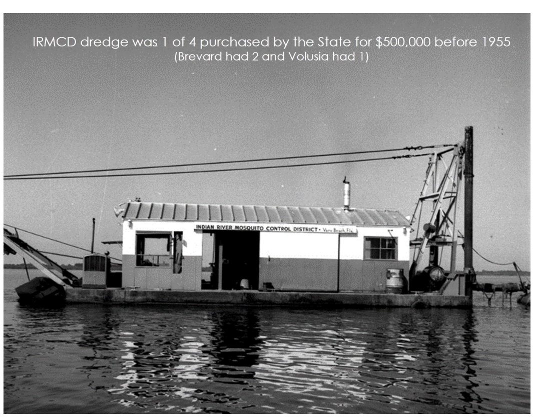
Naples. From Naples and St. Lucie County southward, mangrove swamps or mixed high mangrove scrub marsh dominate coastal wetlands. Both scrub marsh and high mangrove swamps produce saltmarsh mosquitoes, *Ae. taeniorhynchus,* and biting midges (*Culicoides* spp.), commonly known as no-see-ums, in vast numbers if uncontrolled.

4.4 HISTORICAL METHODS OF SOURCE REDUCTION IN SALTMARSH HABITATS

4.4.1 Ditching

Beginning in the late 1920s, ditching of high marshes – by hand using Work Projects Administration (WPA) workers or with explosives – was done to dewater the marsh within several days of rainfall events. This rapid dewatering prevented sufficient time for adult mosquito emergence by desiccating the stranded larvae. This technique was of limited success because ditches were not always dug where they were needed most and because many of these ditches were promptly obstructed, especially at the ditch-estuary interface. In addition, fish were generally not present where the larvae occurred in sufficient numbers to provide appreciable control. Furthermore, the ditch banks made perfect sites for biting midges to develop, exacerbating the biting insect problems for nearby communities.

4.4.2 Filling



During the 1950s and 1960s along the central east coast, placing earth or hydraulically dredged sediments to fill mosquito-producing areas was a common source reduction technique. However, it was generally too slow and expensive to be effective. Fissures and cracks developed in drying dredge/fill material, producing an abundance of saltmarsh mosquitoes. Environmental regulations have virtually eliminated the possibility of large-scale wetland filling because it eliminates this environmentally important habitat.

4.4.3 Impounding

Impoundment construction began in the mid-1950s and continued until the late 1960s. Impoundments consist of earthen dikes that isolate high salt marshes and mangrove swamps from the adjacent estuary. Impoundments are generally artificially flooded for mosquito control from April through September/October using water pumped from the adjacent estuary. Impounding and artificial flooding eliminates oviposition sites for saltmarsh mosquitoes and biting midges and is both effective and economical in reducing their populations, with limited need for additional chemical treatments. Pumping water out of a mosquito-producing dike marsh was initially attempted as a source reduction technique but was unsuccessful because it was impossible to completely dewater the area before mosquito emergence occurred.

There were environmental impacts resulting from isolating and flooding these impounded wetlands including interfering with the movement of water and organisms between the marsh and estuary, killing indigenous flora, and, in some instances, changing vegetation from high marsh to low marsh species. Since the early 1980s, these impacts received considerable scientific and regulatory attention resulting in management modifications designed to address both mosquito control needs and environmental benefits.

4.5 CURRENT SALTMARSH SOURCE REDUCTION TECHNIQUES

4.5.1 Environmental Considerations

Prior to the 1970s, when the majority of mosquito control ditching, filling, and impoundment construction was completed, mosquito control was usually the primary consideration when manipulating salt marshes. Little concern was given to environmental issues because the high salt marsh was not considered to be ecologically significant. Today, minimizing ecological impacts must be considered when designing a source reduction project and is of paramount importance in obtaining regulatory approval.

The importance of both mosquito control and natural resource implications of salt marsh manipulations is evidenced by the formation in 1986 of the Subcommittee on Managed Marshes (SOMM) in Chapter 388.46 Florida Statutes (F.S.). SOMM, a subcommittee of the Florida Coordinating Council on Mosquito Control (FCCMC), is an advisory group responsible for providing review and comment on saltmarsh management plans. SOMM, originally formed in 1983 and called the Technical Subcommittee on Mosquito

Impoundments, has developed guidelines for impoundment and mosquito control ditching management plans based on current research findings. These guidelines require that management plans be written with the mutual objectives of mosquito control, protection of fish and wildlife resources, and water quality enhancement. The most desirable management goals appear to be those that attempt to mimic natural marsh functions while providing for mosquito control. The goal of reducing insecticide use is a factor that weighs heavily in the overall management assessment equation.

4.5.2 Ditching

Over the past 40 years, rotary ditching has been implemented on both the east and west coasts of the U.S., as part of an Open Marsh Water Management (OMWM) system. While ditching has been utilized infrequently in recent years, it can be used in salt marsh or freshwater locations to control mosquitoes by:

1. Enhancing drainage, thus eliminating mosquito-producing sites

2. Allowing larvivorous fish access to mosquito habitats that can be enhanced through the creation of permanent water bodies which act as fish reservoirs

Rotary ditching involves the construction of shallow ditches, usually through grass or scrub marshes, typically four feet wide and two to three deep, using high-speed rotary equipment which broadcasts spoil evenly over the marsh surface. A ditching network frequently connects shallow ditches to permanent water habitats whether they are ponds or canals. Where it is impossible or impractical to connect to major waterways, a permanent pond is constructed deep enough to hold water throughout the year. These ponds harbor fish, and radial ditches connect the mosquito-producing locations to the pond. Research demonstrating some of the ecological effects of rotary ditching was conducted on Florida's west coast in Charlotte County and along the northern IRL in Brevard and Volusia Counties. A well-designed project avoids deposition of spoil that would alter the hydrography of the ditched wetland areas.

4.5.2.1 Benefits of a Properly Designed Rotary Ditching Plan

Rotary ditching is generally considered more environmentally acceptable than deep ditching (*e.g.,* dragline) because spoil material from these shallow ditches is evenly distributed in a very thin layer over the marsh surface – instead of accumulating on the marsh surface as banks of spoil. Consequently, the problem of invasion of exotic vegetation is eliminated. Impacts to grass or shrub vegetation are usually limited to the ditch area itself, as the low-ground pressure tractor will climb over the vegetation allowing it to spring back, causing little damage. Marsh ditching seems to affect the vegetation as a top-dressing of dirt might affect a lawn. Experience has repeatedly demonstrated that a properly designed rotary ditching system can decrease greatly the need for larvicide applications on the affected marsh. Rotary ditching can be cost effective and of lower management intensity when used in appropriate areas and where larval habitats are widely scattered.

4.5.2.2 Environmental Risks of Rotary Ditching

Rotary ditchers broadcast spoil indiscriminately and can throw debris great distances; Therefore, great care is necessary when working in congested areas. In loose soils, the size and shape of the finished ditch will not be maintained due to erosion from water movement through the ditch. The depth fixes the width of the ditch; Therefore, a shallow ditch is also narrow. Concerns have been raised about the possible marsh hydrological changes (*i.e.*, dewatering) that may occur from the installation of rotary ditches. This concern has been addressed typically through the installation of ditch sills, the tops of which are usually set at mean high water. The installation of sills can result in water being retained in the ditch and on the marsh surface; However, this result is not always the case, and some dewatering of the marsh may still occur. Though more frequent flooding of the marsh could conceivably alter soil salinities (by reducing hypersaline conditions), the possible impacts to the benthic invertebrate populations have not been thoroughly investigated. Soil salinity changes also may alter native plant communities, although the introduction of some non-native plants is restricted by marsh elevation. Rotary ditching projects in Volusia County have not experienced native vegetation changes post excavation. Grass marshes continue to thrive immediately adjacent to rotary ditches.

4.5.2.3 Rotary-Ditching Applications

Basic limitations on the use of rotary ditching revolve around the size of the ditch needed, soil types, access, adjacent terrain, and existing vegetation. Good marsh type candidates for rotary ditching include grass marshes, dredge spoils, temporary grassy ponds, scrub marsh, and savannas. Areas with sandy loose soil are not good ditching candidates. Ditch cleaning or new construction is possible in areas of limited woody vegetation, if planned carefully. Experience has shown that poorly engineered or poorly maintained ditches, especially ones that become disconnected from a permanent water body, can produce more mosquitoes than preconstruction conditions, as is true for any permanent control project.

Environmental regulatory agencies generally allow rotary ditching of impoundments because this practice usually reduces insecticide use and allows the impoundment to remain open to tidal exchange, resulting in the exchange of organisms for a longer period of the year. In some cases, it allows the impoundment to be opened permanently. Rotary ditching projects are usually undertaken by mosquito control offices and require permits from the Army Corps of Engineers (ACOE), the Florida Department of Environmental Protection (FDEP), and/or a local water management district (*e.g.*, St. Johns River Water Management District), along with local county approval.

4.5.3 Impoundments

Impounding has been used extensively along Florida's central east coast for mosquito and biting midge control. The principle is simple: Keeping a sheet of water across a saltmarsh substrate prevents *Aedes* spp. mosquitoes and biting midges from ovipositing (laying their eggs) on these otherwise attractive soils. On an impounded marsh, mosquito and biting midge control is effectively achieved with a minimum of insecticide use.

Bear Point Impoundment, St. Lucie County

4.5.3.1 Benefits of a Properly Designed Impoundment Management Plan

Based on research conducted during the 1980s and 1990s, Rotational Impoundment Management (RIM) is currently considered the most favorable and versatile management technique that provides the greatest public benefit. RIM accomplishes mosquito control while still allowing the marsh to function in a nearly natural condition for much of the year. RIM is implemented by installing culverts with water control structures through impoundment dikes to allow seasonal management via flooding and a connection between the marsh and estuary during the rest of the year. Pumps are installed to allow summer flooding of the marsh surface when it would normally be dry. Culverts serve as pathways for tidal exchange, rainfall runoff, nutrient exchange, and organism movement between the estuary and the wetland. Intensive sampling has shown that fish use these culverts as ingress and egress points to the impounded marsh and that these marshes now serve as nursery habitat for more than 100 species of juvenile fish and macrocrustaceans.

Culverts are strategically distributed around a dike at approximately 500-900-foot intervals, or one for every ten to fifteen acres of wetland area, in order to approximate natural tidal exchange rates and to meet water quality standards. Most favorable locations are generally sites where natural tidal creeks occurred or where flushing will be optimized or evenly distributed. Culvert invert elevations are generally set at -1.0 feet NGVD (National Geodetic Vertical Datum), so that they contain water at low tide in an estuarine system, where water levels typically reach 0.0 feet NGVD at low tide. At this elevation, maximum flow-rates can be achieved at MHW levels (culverts flowing at 67% full). Culverts are left open during the fall, winter, and most of the spring. In the late spring, the culverts are closed and remain closed through late summer/early fall (the most productive mosquito season), during which time the marsh is artificially flooded.

The water control structures attached to the culverts allow the marsh flooding height to be regulated to a minimum height necessary for mosquito control. Water levels exceeding control height automatically spill out into the estuary through overflow structures, thus preventing damage to marsh vegetation from excessive water levels or acidification processes. During the closure period (early spring through late summer/early fall), the impounded marsh is flooded by tide, rainfall trapping, and the pumping of water as needed from the adjacent estuary using either stationary electric or portable diesel-driven pumps. Pumping ceases, and the culverts are opened in late summer/early fall to allow the seasonally occurring fall high tides to flood the marsh. Marsh transient organisms enter and leave the marsh on these tidal events.

Modifications to these standard protocols include: Continuous summer pumping through open or partially open culverts or combinations of weirs and breaches incorporated into the summer water circulation programs. The latest efforts targeting marsh flooding with pumps or water circulators are designed to inundate the marsh floor and take advantage of natural tidal exchange, thus augmenting the natural tidal inundation frequency of a marsh during the low-water, mosquito-production period. Continuous pumping through open or partially-open culverts (employing flap-gates, bottom-water release gates, etc.) allows organism exchange between the estuary and the impoundment during the management season. This arrangement is especially beneficial to summer transient species such as tarpon. Excess pumping creates water turnover rates that improve water quality, enhance biodiversity, and protect the wetlands from acidification by preventing freshwater build-up. Further benefits of artificial pumping include protection of wetlands from exotic species invasion and enhancement of mangrove growth rates and productivity.

RIM achieves multipurpose management by allowing the impoundment to:

1) Control saltmarsh mosquito production with minimal insecticide use

2) Promote survival and re-vegetation of native plant species by maintaining open periods and sufficiently low water levels during the summer flooding period to protect plants with limited water level height tolerances

3) Help prevent exotic plant incursion into wetlands

4) Allow marine life to use the previously unavailable impounded high marsh

In order for a governmental mosquito control office or private developer to implement a RIM plan, it usually is reviewed and endorsed by the SOMM before being reviewed by the permitting agencies. The agencies involved in RIM permitting include the ACOE, the FDEP, the local water management district (*e.g.*, the St. Johns or South Florida Water Management District), and the local county government. When undertaken by a governmental mosquito control office, some streamlining of the permitting process for RIM projects has occurred under permitting changes adopted in 1995. Under Florida's Environmental Resource Permit (ERP), a Noticed General Permit is granted to mosquito control offices for the installation of culverts in impoundments for non-mitigation enhancement projects. While some review of the project is still necessary, this process speeds up the regulatory review.

4.5.3.2 Environmental Risks of Impounding

Before the 1970s, mosquito control considerations outweighed natural resource concerns in high marsh communities. This situation was due both to the urgent public health need to control tremendous saltmarsh mosquito broods and to the lack of understanding of the ecological significance of wetlands. In the 1950s and 1960s when impoundment construction occurred, little was known about the importance of high marshes and their role in estuarine productivity. Historically, black and white mangroves, *Batis,* and *Salicornia* dominated many high marshes that were impounded. These plants cannot sustain continual unregulated flood heights, where the succulent plants or black mangrove pneumatophores are completely inundated. During the early years of impounding, water levels were maintained at an elevation that killed virtually all the existing vegetation in some locations. This result left some impoundments barren of vegetation for many years, except where red mangroves intruded. Also, the perimeter dike virtually eliminated the natural movement of water and organisms between the marsh and adjacent estuary. Marsh transients, those organisms that use the high marsh during a portion of their life cycle [*e.g.*, *Elops saurus* (ladyfish), *Centropomus undecimalis* (snook), *Megalops atlanticus* (tarpon), *Mugil* spp. (mullet)], were excluded from the impounded marshes, primarily during the high fall and winter tidal period experienced on the central east coast of Florida.

4.5.4 Current Efforts in Saltmarsh Management

RIM management and rotary ditching as described above are marsh management techniques that are well accepted by both mosquito control agencies and those agencies responsible for protecting natural resources. Virtually all of the IRL marshes have been impacted in some way; Therefore, management diversity may be the best solution for the future. Toward that goal, SOMM participated in a project to develop regional management plans for IRL impoundments and marshes. This planning project regionalized the lagoon into ten management areas and assigned each marsh an optimal management scenario based on current best management information. In addition to RIM and OMWM utilizing rotary ditching, appropriate techniques include among others: Open marsh-lagoon connection, RIM modifications with near-continual pumping during the closed period for water quality improvement, RIM management with modifications to enhance wading bird feeding opportunities, waterfowl management, and stormwater retention. This planning document provides assistance to governmental agencies and private developers for specific marshes targeted for management. However, as always, recommendations made today can change tomorrow as further scientific information becomes available. (See Appendix I for a list and description of recognition of mosquito control professionals for their source reduction efforts that take into account environmental considerations.)

4.6 SOURCE REDUCTION IN FRESHWATER HABITATS

Source reduction for mosquito control in freshwater habitats typically involves constructing and maintaining channels (ditches) to reduce mosquito production in areas such as floodplains, swamps, and marshes. The principle that directs source reduction work entails manipulating water levels in low-lying areas to eliminate or reduce the need for insecticide applications.

Two different mosquito control strategies are considered when performing freshwater source reduction. One strategy involves reducing the amount of standing water or reducing the length of time that water can stand in low areas following significant rainfall events. This type of strategy involves constructing channels or ditches with control elevations low enough to allow for a certain amount of water to leave an area before immature mosquitoes can complete their life cycle.

Another strategy involves constructing a main central ditch with smaller lateral ditches at the lowest elevations of intermittently wet areas to serve as a larvivorous fish reservoir.

As rainfall increases, fish move outward to adjacent areas to prey on immature mosquitoes, and as water levels decrease, fish retreat to the ditches. Weirs are constructed in main ditches to decrease water flow, prevent depletion of the water table, and allow fish year-round refuge.

In Florida, most construction of source reduction projects occurred between the 1940s through the mid-1960s. Initially, these drainage projects were designed to reduce the production of *Anopheles* mosquitoes and lower the incidence of malaria. Later, drainage projects were constructed to help control other vector as well as nuisance species. Local mosquito control agencies wanting to construct drainage projects had to obtain approval from the state mosquito control office, originally located in the Florida State Board of Health (FSBH). Entomological data to support/justify the merit of projects along with design specifications had to be provided to obtain approval. Once projects were approved by the State, construction and maintenance activities were regulated by the State to ensure compliance with good mosquito control practices. In addition, for a period of more than twenty-five years, a specific type of financial aid – State II Aid – was provided to local mosquito control offices to supplement costs associated with constructing and maintaining source reduction projects.

Very few, if any, mosquito control offices are involved in construction of new drainage projects because of environmental restrictions associated with obtaining permits. However, several mosquito control offices are involved in maintenance work on existing drainage systems. This maintenance includes cutting, mowing, or the application of herbicides to overgrown, nuisance, and exotic vegetation and excavating built up spoil material. Florida law provides a permit exemption for mosquito control maintenance activities. This maintenance exemption allows mosquito control agencies to maintain the systems, provided that their sizes are not expanded beyond original design specifications. One important provision of the exemption states that up to 10,000 cubic yards of spoil material can be excavated from a project without a permit, provided that the material is deposited on a self-contained upland site.

Over the past several decades, urban development has occurred in areas of Florida where mosquito control drainage ditches were the primary drainage systems. If these systems are expanded to meet modern stormwater management specifications, mosquito control maintenance exemptions are no longer valid. In many cases, maintenance responsibility for mosquito control projects has been taken over by city and county public works departments and integrated into their comprehensive stormwater management programs.

4.7 STORMWATER MANAGEMENT

Florida largely depends on potable water pumped from aquifers supplied by rainfall. Much of Florida is flat with sandy soils resulting in a variety of percolation rates and water table depths. These characteristics make the management of stormwater and wastewater very important, and poor engineering and construction – or improper maintenance – can result in considerable mosquito problems.

From a legislative perspective, very little has been done to prevent the production of disease vector or nuisance mosquitoes from either stormwater or wastewater facilities. Wastewater facilities are regulated under FDEP. Stormwater is regulated by FDEP or the appropriate water management district, counties, and municipalities. In 1982, the Florida Department of Health and Rehabilitative Services (HRS), through the efforts of William Opp, and the Florida Department of Environmental Regulation (FDER) developed the original Florida criteria for considering mosquito problems resulting from stormwater facilities. The 72-hour recovery period associated with design criteria for retention and filter/under-drain systems was put into the rule at the suggestion of HRS solely to minimize mosquito production.

A few counties and municipalities have language prohibiting mosquito production in stormwater treatment facilities. This language was largely due to the efforts of William Opp of HRS in the late 1970s and early 1980s to develop guidelines for engineering mosquito-free facilities. Volusia County Mosquito Control built upon this work in the early 1990s by developing their own local Best Management Practices (BMP) for Mosquito Control in Stormwater Management Facilities (see Appendix II).

FDEP has delegated authority from the U.S. Environmental Protection Agency (EPA) to regulate municipal stormwater through the Municipal Separate Storm Sewer Systems (MS4s) permit and industrial stormwater through the Stormwater Association with Industrial Activity permit. These permits represent an expansion of Florida's State/Water Management District stormwater program in that they address existing systems – not just new development. The impact on mosquito control is relatively minor, since these permits do not typically require the installation of structural controls such as retention or detention ponds for compliance.

Research into mosquito problems associated with stormwater and wastewater facilities has been limited. Dr. George O'Meara of the University of Florida, Institute of Food and Agricultural Science, Florida Medical Entomology Laboratory (FMEL) has been the primary scientist to study this issue in the 1980s.

There are a wide range of mosquitoes produced in these facilities including floodwater *Aedes* and *Psorophora* spp. in intermittently wet facilities, *Culex* and *Anopheles* species associated with permanent or semi-permanent wet facilities, and *Mansonia* and *Coquillettidia* species associated with floating or emergent vegetation. The *Aedes, Psorophora, Mansonia,* and *Coquillettidia* species are the most pestiferous to humans. Mosquito control efforts in infested areas include larviciding, vegetation management, herbicide applications, barrier treatments, Ultra Low Volume (ULV) adulticiding, stocking with larvivorous fish, and the installation of reservoirs for larvivorous fish.

Engineering design can eliminate mosquito production from stormwater and wastewater facilities but not easily. Permanent water ponds can be kept free of nuisance and exotic vegetation with water quality sufficient to support mosquito-eating fish. Dry facilities can be designed to dry down in three days to prevent floodwater mosquito production, but some standing water beyond the three-day period may occur due to intermittent rainfall common to Florida in the summer.

4.8 WASTEWATER MANAGEMENT

In many parts of Florida, clean freshwater for domestic, agricultural, or industrial uses is becoming a critical resource. Wastewater recycling and reuse help to conserve and replenish freshwater supplies. Floridians produce approximately 100 gallons of wastewater per capita each day from domestic sources alone. Concern for water quality conditions in lakes, rivers, and marine areas has resulted in the enactment of state laws that greatly limit future disposal of wastewater into these aquatic systems. To adjust to these changing conditions, many communities must implement wastewater reuse and recycling programs. Mosquito problems are frequently associated with some conventional wastewater treatment techniques, and the expanded use of wastewater recycling and reuse may inadvertently create even more mosquito habitats.

4.8.1 Domestic Wastewater

4.8.1.1 Septic Systems

In 2016, approximately 30% of Florida's households used on-site treatment systems such as septic tanks and associated drain fields. With proper soil porosity, sufficient lateral fields, and low human congestion, these systems are safe and efficient. The wastewater in a properly located and maintained septic tank system will percolate into the subsoil without causing a surface water accumulation that may induce mosquito production. Yet, when these systems are placed in locations with inappropriate soil conditions, wastewater will flow laterally, often into nearby swales and ditches, thus providing habitats for *Culex* spp. oviposition.

4.8.1.2 Package Plants

Some central wastewater facilities in Florida are relatively small, treating less than 100,000 gallons of wastewater daily. In some instances, a small system known as a package plant can be used by private companies when establishing new subdivisions and related developments. However, this arrangement has become much less common as regulations require such developments to use large treatment facilities. Some of these remaining package plants provide inadequate wastewater treatment because they are poorly maintained or operated beyond their capacity. Generally, package plants discharge treated wastewater into small holding ponds. When these ponds receive poorly treated wastewater, mosquitoes may become abundant, especially when the ponds are invaded by aquatic plants. If aerators, pumps, and related components of package plants are not functioning properly, then mosquito production may not be confined to just the holding ponds.

4.8.1.3 Large Treatment Facilities
Large treatment facilities have large holding ponds that are less likely to be invaded by mosquitoes than the smaller ponds associated with package plants. Often, major mosquito problems associated with large municipal and county wastewater treatment facilities are confined to the advanced treatment phase of the overall process. Techniques used to improve water quality conditions beyond the levels obtained in the secondary treatment process include spray irrigation, rapid-dry ponds, aquatic plant/wastewater systems, and the use of natural or modified wetlands.

4.8.1.4 Spray-Irrigation Systems
Secondarily treated wastewater is used to irrigate golf courses, road medians, pastures, sod fields, citrus groves, and other types of crops. During the rainy season, it is not uncommon for spray fields to become waterlogged, particularly those in low-lying areas with high water tables or in poorly drained soils. Under these conditions, the continued application of spray irrigation will result in the accumulation of surface water, thus providing aquatic habitats for a variety of mosquito species.

4.8.1.5 Rapid-Dry Ponds versus Holding Ponds
Rapid-dry ponds are classified as dry-retention systems. In these systems, water flows into the pond and then percolates into the soil. By contrast, holding ponds are primarily flow-through systems. Typically, water enters and leaves the holding pond in some type of pipe. Soil percolation is an optional feature in holding ponds. Due to the regular inflow of wastewater, holding ponds are normally full and thus represent a type of wet-detention system. Rapid-dry ponds that fail to dry fast enough produce mosquito problems similar to those found in areas where surface water has accumulated from excessive spray irrigation.

4.8.1.6 Wastewater/Aquatic Plant Systems
At some wastewater treatment facility ponds in Florida, certain species of aquatic plants (*e.g.*, water hyacinths) have been added for nutrient removal and biomass production. Mosquito problems result in this type of system if the inflow is inadequately treated. Effective nutrient removal requires periodic harvesting of a portion of the aquatic plants.

4.8.1.7 Wetlands
Subject to regulatory permitting, secondarily treated wastewater can be pumped into wetland areas. Earthen dikes often are used to increase the water retention capacity of wetlands that are receiving treated wastewater. The responses of mosquito populations to wastewater inundations vary depending upon the type of wetland.

For example, coastal salt marshes and mangrove swamps are noted for producing large broods of pestiferous mosquitoes. Highly effective mosquito control has been achieved by surrounding these brackish wetlands with dikes and then flooding the enclosed area. Usually brackish or freshwater is used, but impoundments at a few locations have been operated effectively with secondarily treated wastewater as the influent.

The various types of freshwater wetlands in their natural condition provide suitable aquatic habitats for a variety of mosquito species. Adding treated wastewater to these aquatic systems rarely reduces mosquito production and often changes the relative abundance of different plant species and the associated mosquito species.

4.8.2 Agricultural and Industrial Wastewater

Many commercial operations have on-site treatment facilities for decreasing nutrient loads in wastewater and generally use techniques similar to those applied to domestic wastewater. The quantity of wastewater produced at some commercial locations, such as those processing certain crops, may be highly variable during the year. Therefore, the amount of surface water in the holding ponds or spray fields used in the wastewater treatment may fluctuate considerably, thereby contributing to the production of certain species of floodwater mosquitoes. Wastewater from feed lots and dairy barns often is placed in holding or settling ponds without any prior treatment. Several mosquito species of the genus *Culex* can become extremely abundant in these ponds, especially in the absence of aquatic plant control.

4.8.3 Major Pest and Disease-Vectoring Mosquitoes

4.8.3.1 *Culex*

Throughout much of southeastern United States, the dominant species of mosquito in wastewater ponds and lagoons is usually *Culex quinquefasciatus*. Major exceptions to this pattern occur in both central and south Florida where *Cx. nigripalpus* is often seasonally more abundant than *Cx. quinquefasciatus*. Especially in the southern half of peninsular Florida, *Cx. nigripalpus* is usually the dominant wastewater *Culex* in the summer and fall, whereas *Cx. quinquefasciatus* is more common in the winter and spring. Human activities are responsible for establishing the vast majority of the aquatic habitats used by *Cx. quinquefasciatus*, the southern house mosquito. A much wider range of larval habitats, including both artificial and natural aquatic systems, is used by *Cx. nigripalpus*. In large wastewater ponds, immature *Cx. quinquefasciatus* are generally most abundant near the inflow area where the nutrient loads are typically highest. By contrast, immature *Cx. nigripalpus* are more evenly distributed in wastewater ponds. In recent years *Culex coronator* has spread throughout Florida where it has the potential to serve as a vector of West Nile virus and possibly other pathogens.

Culex salinarius, another common mosquito in wastewater, is similar to *Cx. nigripalpus* in its range of larval habitats, but its seasonal pattern of abundance is similar to *Cx. quinquefasciatus*. *Culex salinarius* inhabit not only semi-permanent ponds but also more ephemeral habitats, such as temporary pools in spray irrigation fields. Occasionally, immature *Culex restuans* may become common in a wastewater system. Fortunately, *Cx. restuans* populations are inactive during much of the year in most of peninsular Florida. *Culex salinarius* is the most pestiferous wastewater *Culex* because it feeds mainly on mammals, while females of the other three species are either generalists or primarily avian feeders. *Culex nigripalpus* is the species of greatest interest because it is the dominant *Culex* in Florida during the summer and fall, occurs in wastewater systems varying widely in nutrient loads, and is the primary vector of St. Louis encephalitis virus (SLEV) and West Nile virus (WNV).

4.8.3.2 *Aedes* and *Psorophora*

Unlike *Culex*, whose eggs hatch within a few days after being laid in rafts on the water surface, *Aedes* and *Psorophora* species lay their eggs individually on moist substrate with hatching occurring only after the eggs have been flooded. Consequently, *Aedes* and *Psorophora* are seldom found in wastewater systems where there is little or no variation in surface water levels. However, poorly designed, improperly operated, or inadequately maintained systems often lead to conditions that are ideal for an invasion by floodwater mosquitoes. Poorly drained spray-irrigation fields often become water logged especially during the rainy season. Under these conditions, many broods of *Ae. vexans* and *Ps. columbiae* can be produced in a single season. Land application of wastewater may increase the salt content of the soils and cause inland sites to become suitable for saltmarsh mosquitoes. *Aedes sollicitans* has become a major pest species at some wastewater disposal or recycling sites.

4.8.3.3 *Mansonia* and *Coquillettidia*

Immature *Mansonia dyari*, *Mansonia titillans*, and *Coquillettidia perturbans* do not breathe at the water surface. They obtain oxygen from the root hairs of various species of aquatic plants and may stay attached to the plants for extended periods. Immature *Ma. dyari* are found almost exclusively in association with water lettuce, *Pistia stratiotes*, whereas *Ma. titillans* use several species of aquatic plants, notably water hyacinth (*Eichornia crassipes*) and water lettuce. Rooted and floating cattails (*Typha* spp.), especially floating mats, are the principal host plants for *Cq. perturbans*.

Of these three mosquito species dependent upon aquatic plants, *Cq. perturbans* is the most aggressive biter. It is an opportunistic blood feeder, occasionally taking multiple blood meals. These behavioral traits enhance the mosquito's potential for vectoring certain viruses. *Mansonia dyari* are less likely to feed on humans than are *Ma. titillans*. Nevertheless, at locations where *Ma. dyari* is extremely abundant, this species may be an important component in the enzootic cycle of St. Louis encephalitis (SLE) transmission.

4.8.4 Mosquito Control

The best approach to managing mosquitoes in wastewater systems is initial avoidance by incorporating features into the design and operation of wastewater treatment systems that will either preclude or greatly limit mosquito production. Special attention should be directed to the items listed in the remainder of Section 4.8.4.

4.8.4.1 Operating Capacity

Many systems provide inadequate wastewater treatment because the amount of inflow regularly exceeds treatment capacity. Treatment facilities must be designed and constructed to handle current and future demands that are based on realistic projections. Moreover, facilities need to be properly maintained to prevent any loss in operating capacity. Although wastewater treatment is expensive, cutting costs by overloading treatment facilities is counterproductive in the long term.

4.8.4.2 Water Quality

Wastewater should receive at least a good secondary treatment and preferably some advanced treatment before it is placed in detention/retention areas. Land applications, such as irrigation projects, should be used to complement rather than substitute for good secondary treatments. Poor water quality is a major factor contributing to *Culex* mosquito problems. Improved secondary and advanced treatments decrease the likelihood of *Cx. quinquefasciatus* and *Cx. nigripalpus* oviposition, make habitats more suitable for fish and other mosquito predators, and increase the effectiveness of various mosquito larvicides.

4.8.4.3 Wet-Detention Ponds

In wet-detention ponds, large ponds are much more desirable than small ones (*i.e.*, those with < 0.1 acre of surface area). In fact, small ponds and various types of wastewater holding tanks may require surface agitation from a sprinkler or an aerator to deter invasion by *Culex* mosquitoes. Pond banks should be relatively steep with a minimum water depth of at least two feet. Methods for preventing seepage should be incorporated into the design and construction of holding ponds. Water levels in wet-detention ponds should be kept constant. If ponds must be drained for maintenance, they should be equipped for rapid and complete drainage. These drainage/refill episodes should be infrequent. Debris and excessive vegetation should be removed from the banks and shoreline. The surface of wet-detention ponds should be kept free of floating and immersed aquatic plants. The deliberate introduction of aquatic plants, such as water hyacinths for biomass production or water quality improvement, should be limited to ponds receiving good secondarily or advanced treated wastewater. When plants are used for nutrient removal, they must be protected from insects, pathogens, and cold weather, otherwise, the dead plants will release nutrients back into the wet-detention pond and increase the likelihood of mosquito production. Plant harvesting schedules must be adjusted for variation in seasonal growth patterns. Failure to harvest the plants on time also increases the chances for a mosquito outbreak.

4.8.4.4 Dry-Retention Areas: Rapid-Dry Ponds and Spray-Irrigation Fields

In theory, the wastewater applied to rapid-dry ponds and fields should rapidly percolate into the soil so that surface water is present for brief periods (less than a few days). In practice, standing water is often present for longer periods. Even if 90 to 95% of the wastewater rapidly enters the soil, the amount of surface water remaining can cause major mosquito problems. Dry-retention areas must be restricted to sites with soil and water table conditions that will allow for the rapid absorption of all wastewater. The rate of application needs to be adjusted for seasonal patterns in rainfall and water table and for long-term changes in the soil's water holding capacity.

Depressions, potholes, and related irregularities should be removed from dry-pond bottoms and spray-irrigation fields. Grass-covered systems should be mowed without creating tire ruts, and the cuttings should be removed. Even when rapid-dry ponds and spray-irrigation fields operate satisfactorily, seepage to adjacent lowlands may create or aggravate mosquito problems. Therefore, the design of dry-retention areas should include provisions for adequate drainage in neighboring areas.

4.8.4.5 Wetlands

Environmental mandates and budget constraints may greatly limit the use of aquatic plant management or mosquito larvicides in freshwater wetlands that receive wastewater. Baseline information on mosquito production should be obtained following wastewater input. Access trails should be made available so that all major sections of the wetlands can be monitored periodically for mosquitoes. If plans call for deliberately adding aquatic plants to a wetlands/wastewater system, avoid using plant species that provide especially favorable microhabitats for mosquitoes. Flow rates and nutrient loading should not exceed the carrying capacity of the area. Wetlands receiving wastewater should be located away from residential and commercial areas. Future development should be limited to maintain buffer zones.

4.8.4.6 Larvicides

Several different types of larvicides are available for controlling mosquitoes. Generally, these larvicides are least effective in wastewater systems. The flow-through nature of many wastewater treatment, reuse, and recycling operations rapidly diminishes the effectiveness of many larvicides. Bacteria and other components of wastewater quickly break down or inactivate some larvicides. Increasing the dosage rate and the number of applications or using slow-release formulations may be required to achieve adequate control. At sites where mosquito outbreaks are large and frequent, larvicides may provide only temporary control and may not be cost-effective. Larvicide operations must be supported with a quality inspection program. Potential mosquito production sites must be identified and frequently inspected. Larvicide applications should be integrated with other mosquito abatement measures such as aquatic plant management and water quality improvement. A goal when using larvicides is that their use does not interfere with the level of mosquito control already provided by natural predators and parasites.

4.8.5 Recommendations

Ideally, all agencies involved in regulating stormwater and wastewater facilities should add language striving to minimize, and, where possible, eliminate mosquito production. In addition, a method for resolving problems in maintaining compliance with this goal is desirable, but this result may be difficult to achieve due to the large number of facilities. Partnerships between state and local government agencies (in particular local field inspectors) could be beneficial in helping to meet compliance requirements. Research is needed to establish testing/monitoring techniques and thresholds to allow applicants, operators, and independent inspectors to determine compliance with mosquito reduction goals.

All agencies involved in regulating stormwater and wastewater facilities should recognize that some wetland plantings, while providing habitat for fish and wildlife as well as other ecological functions, can create larval mosquito habitat. This problem should be taken into account in engineering a system design with vegetation that does not contribute to mosquito problems.

There should be state recognized and published BMPs for mosquito control in stormwater and wastewater management facilities. These BMPs would provide an educational tool guiding designers, builders, and operators. Volusia County Mosquito Control has such a policy. See Appendix II.

Mandatory mosquito biology and control training should be part of all stormwater and wastewater certification programs.

Finally, mosquito problems in stormwater and wastewater facilities are easy to prevent and sometimes easy to fix. The approaches are non-chemical and environmentally sensitive, and they have the potential to reduce mosquito populations in all areas.

4.9 AQUATIC PLANT MANAGEMENT AND THE EFFECTS ON MOSQUITO POPULATIONS

This section describes the practices used to control mosquitoes and aquatic plants associated with freshwater environments only. Saltmarsh environments are discussed in other sections of this chapter.

Certain mosquito species use various aquatic plants as a primary habitat for egg deposition and larval development. Because aquatic plants can, at times, produce heavily vegetated stands, the use of conventional mosquito management techniques, such as biological and chemical control, may be ineffective. Therefore, removal of the habitat may be the only means of reducing these mosquito populations to a desired level.

Aquatic plant management in Florida can have a positive effect on the control of mosquito populations. A primary goal in reducing mosquitoes that use aquatic plants is to eradicate or, at the very least, manage the aquatic plant communities at the lowest feasible level.

4.9.1 Aquatic Plants

The three most important aquatic plants that provide mosquito habitat in Florida are water lettuce, water hyacinth, and cattails. The following descriptions are reprinted from the *Aquatic Plant Identification Deck* by Victor Ramey.

Water lettuce (*Pistia stratiodes*) is a floating plant. Experts disagree as to whether water lettuce is native or has been introduced. Water lettuce occurs in lakes, rivers, and canals, occasionally forming large dense mats. As its name implies, water lettuce resembles a floating head of lettuce. The very thick leaves are light dull green, hairy, and ridged. There are no leaf stalks. Water lettuce roots are light-colored and feathery. Its flowers are inconspicuous.

Water hyacinth (*Eichornia crassipes*) is a floating plant. This exotic nuisance plant grows in all types of freshwater. Water hyacinths vary in size from a few inches to more than three feet tall. They have showy lavender flowers. Water hyacinth leaves are rounded and leathery, attached to spongy and sometimes inflated stalks. The plant has dark feathery roots.

Cattails (*Typha* spp.) are among the most common of all aquatic plants. They can reach eight or more feet tall and grow prolifically from thick underground rhizomes. Cattails often dominate large areas, especially where water levels fluctuate. Cattails get their name from their cylindrical flower spikes that can be more than one foot long. The flower spikes are densely packed with tiny flowers. Cattail leaves are strap-like, stiff, and rounded on the back. The leaves are sheathed together at their bases and appear to be flattened from the side. Leaves are straight in the bottom half but twist and spiral in the top half.

4.9.2 Mosquitoes

The three most important mosquito species that use aquatic plants in Florida are *Ma. dyari, Ma. titillans,* and *Cq. perturbans*. The following descriptions are taken from the Florida Mosquito Control Handbook.

Mansonia dyari is found in permanent lakes and ponds. This species is most closely associated with water lettuce but also occurs on water hyacinth, pickerel weed (*Pontederia* spp.), and arrowhead (*Sagittaria* spp.). The egg masses are attached to water lettuce leaves and, after hatching, the larvae and pupae attach permanently to the roots, getting their oxygen from the plant tissues. The females will bite humans but seldom become pests. In Panama, this species is a major vector of St. Louis encephalitis (SLE), but its relationship to the SLE virus in Florida is unknown.

Mansonia titillans is also found in permanent lakes and ponds. This species is most closely associated with water hyacinth but also occurs on water lettuce, pickerel weed, and arrowhead. This tropical species is found only in the southern half of the state. The egg masses are laid on the underside of floating leaves, and the larvae and pupae attach to and derive their oxygen from the roots. It can be a pest to humans near its larval habitats. In South America, this species is a major vector of Venezuelan equine encephalitis.

Coquillettidia perturbans is found in permanent lakes and ponds with cattails, sedges, maidencane (and other *Panicum* grasses), and arrowhead. This large black and white mosquito is a severe pest in inland Florida. The immature stages are found in established permanent freshwater marshes containing emergent vegetation where there is a layer of detritus on the marsh bottom. The eggs are laid in a raft on the water surface and the immature forms attach to the roots of the emergent plants. This aggressive mosquito is active for short periods at dusk and commonly flies three to five miles from its aquatic habitat, often much further. Females bite both humans and birds. This species is an important vector of eastern equine encephalitis (EEE) to humans throughout the eastern U.S. wherever it is associated with *Culiseta melanura*.

4.9.3 Surveillance

If adult *Mansonia* are discovered through routine surveillance, a thorough survey of the immediate area should be conducted to locate freshwater sources containing water hyacinths and/or water lettuce. If a suspected freshwater source is found, a larval survey should be conducted. If disturbed, the larvae attached to plant roots will immediately release and fall to the bottom of the water. As a result, a mosquito dipper is an inappropriate sampling tool. A good method for collecting *Mansonia* larvae is to place a shallow pan under the floating aquatic vegetation. Care must be taken not to disturb the aquatic plants or surrounding area. Once the pan is in place, it and the aquatic plant must be lifted slowly out of the water. Clean water may need to be added to the pan to accurately view and count any mosquito larvae. This method requires a great deal of patience and practice.

Cq. perturbans is also difficult to survey. They can fly several miles from their larval habitat, requiring a more widespread survey of freshwater sources containing cattails. Since the larvae are found in the detritus, a benthic sampling method is necessary, where a portion of the plant roots and surrounding detritus is collected.

4.9.4 Mosquito Control Measures

The use of biological control methods, such as mosquito fish, is usually not effective for mosquitoes associated with aquatic plants. The aquatic vegetation is too dense for predators to gain access to the mosquito larvae.

For *Mansonia* and *Coquillettidia*, chemical control methods, such as the larvicide *Bacillus thuringiensis israelensis* (*Bti*), may be effective if the product is applied directly to the areas containing mosquito larvae. This application may be difficult and labor intensive if the aquatic vegetation is dense.

In general, conventional mosquito control methods are not effective tools in reducing mosquitoes associated with aquatic plants.

4.9.5 Aquatic Plant Management Measures

Eradication or maintenance level control of aquatic plants is the best method of mosquito control for *Mansonia* and *Coquillettidia* species. There are three basic types of aquatic plant management:

Chemical control involves the use of aquatic herbicides to eradicate or manage the aquatic vegetation. Depending on the amount and accessibility of the vegetation, a backpack, truck-mounted, boat-mounted, or aircraft-mounted sprayer can be used. The aquatic herbicides used are specific for the aquatic plants. Diquat is used to control water lettuce, a 2,4-D amine is used for water hyacinths, and glyphosate primarily is used for cattails. Chemical control can be cost effective if the aquatic plants are managed at a maintenance level.

Biological control involves the use of insects or pathogens to eradicate or manage the aquatic plants. At present, there is no effective biological control for cattails. The water lettuce weevil and water hyacinth beetle have been used with limited success. There have been a few successful large-scale biological applications to date.

Mechanical control is a method in which equipment or tools are used to physically remove the aquatic vegetation. Examples include aquatic harvesters, bucket cranes, underwater weed trimmers, and machetes. Mechanical control is limited to areas that are easily accessible to the equipment. Also, mechanical control can be labor intensive and extremely expensive.

4.10 WASTE TIRE PROGRAM IN FLORIDA

4.10.1 Tires as Mosquito Producers

Tires have provided favored mosquito habitats since the first discarded tire filled with water. Waste tires have been legally and illegally accumulating in Florida for the past several decades. The legal accumulations usually take the shape of a somewhat organized pile containing up to several million tires. Illegally dumped tires may be scattered about from single tires to piles containing 40,000 to 50,000 tires. Unfortunately, most of the problem tires are not in large piles but are rather scattered about, making removal difficult and, at best, labor intensive.

The design of tires makes them ideal sites for producing several species of mosquitoes, and some of these mosquitoes are important disease vectors. The 20-80 rule probably applies to waste tires. Of the mosquito problems associated with waste tires, it is safe to say that 20% of the tires are responsible for 80% of the problem.

Until the mid-1980s, waste tires were considered more of a nuisance and environmental threat than the possible foci of mosquito-borne disease epidemics. This situation changed in 1985 when a substantial population of *Ae. albopictus* was discovered in Houston, Texas. It is probable that this population arrived from Japan as eggs deposited inside used tires. In 1986, this species was found in an illegal tire pile in Jacksonville. It was found in 62 counties in 1991 and, by 1994, was established in every county in Florida.

The potential importance of *Ae. albopictus* and waste tires became apparent in June 1991 when adult specimens collected from a large tire pile in Polk County tested positive for the eastern equine encephalitis virus (EEEV). This discovery called attention to a problem of enormous magnitude. Discarded automobile and truck tires are the preferred habitat of *Ae. albopictus*. Chemical treatment of tire piles to control either larval or adult stages is much more difficult than most routine applications and may not be fully effective. Shredding tires – or otherwise rendering them incapable of holding water and supporting mosquito production – is preferable to attempting chemical control. However, large piles, such as the one in Polk County, may contain an estimated 4.5 million tires, and it may take two or more years to complete the shredding and cleanup process.

4.10.2 Waste Tire Disposal Regulations

In an effort to promote recycling, slow the growth of landfills, and reduce pollution, a comprehensive solid-waste bill was enacted in 1988. This legislation empowered the FDEP to regulate the storage, transportation, processing, and disposal of waste tires. Under this bill, no one is allowed to have more than 1,500 tires except at a solid-waste management facility or a waste tire processing facility. Transporters are required to register each truck used to haul tires with the FDEP, dump only at approved locations, and maintain records for three years of where tires were obtained and finally deposited. Processors with fixed-site facilities are allowed to have more than 1,500 waste tires in storage but must comply with storage standards set by the Waste Tire Rule, Chapter 62-711 of the Florida Administrative Code. Landfills are allowed to collect all tires brought in but must have the tires on hand processed every 90 days. Landfills are allowed to bury tires that have been cut into eighths or smaller pieces. Most landfills have the tires shredded to a four-square-inch size that they can use as daily landfill cover. The FDEP also is involved in eliminating the state's large, illegal tire sites. If a site owner is unable or unwilling to abate the site, the FDEP can gain possession of the site through the court, process and remove the tires, and seek recovery of costs.

The legislation also established a waste tire fee of $1 collected on each new tire sold at retail. The waste tire fee generates more than $21 million annually, which goes into the Solid Waste Management Trust Fund. Up to 11% of the waste tire fees are allocated to local mosquito control agencies. In Fiscal Year 2016-2017, the Florida Department of Agriculture and Consumer Services (FDACS) received and administered approximately $2.6 million from this program.

Since the enactment of the Solid Waste Act of 1988, FDEP has cleaned-up more than 22 waste tire sites that contained more than five million tires. This effort reduces the waste tire problem to a more manageable level but does not alleviate the mosquito problems caused by the many thousands of tires scattered throughout the state that have been illegally discarded to avoid dumping fees. This situation is where mosquito control programs can make a real difference. During the first two years that mosquito control agencies participated in the waste tire program, they were responsible for the collection and removal of approximately 730,000 discarded tires.

The removal of waste tires can help reduce populations of *Ae. albopictus* and the threat of dengue, chikungunya and Zika. However, as tires disappear from the environment, the mosquitoes that found them an attractive habitat will quickly adapt to almost any type of water-holding container. In the final analysis, premise sanitation is the key to controlling container-producing mosquito problems. Mosquito control workers have daily contact with the public and are uniquely suited to the task of informing citizens about eliminating mosquito habitats around their residences.

4.11 BROMELIADS

Exotic (non-native) bromeliads are popular landscape plants to due their colorful leaves and flowers and their ability to survive and thrive with little if any care. Immatures of *Wyeomyia mitchellii* and *Wy. vanduzeei* are generally the most common mosquitoes found in native and exotic tank bromeliads in Florida. However, in parts of Miami-Dade County another mosquito species, *Culex biscaynensis,* is more common than either *Wy.* species in exotic bromeliads. These bromeliad specialists are not major pest or disease vectors, but they do play an important role in limiting the production *Ae. aegypti* and *Ae. albopictus* from these plants. Consequently, water trapped in the leaf axils of bromeliads only occasionally provides a suitable habitat for the production of substantial numbers of these *Aedes* mosquitoes. To lessen the potential for *Aedes* production from bromeliads avoid watering them with lawn sprinklers and prevent grass clipping from falling into the plants. Some species of bromeliads, especially those with a nested inflorescence, are more likely to harbor *Aedes* mosquitoes than plants with a large stalked inflorescence. Treating the plants with a mosquito larvicide may exacerbate problems with *Aedes* mosquitoes once such treatments are discontinued because these mosquitoes can reinvade the plants much more quickly than the *Wyeomyia* sp. and other bromeliad specialists. Authors of 2018 study by the University of Miami and Miami-Dade Mosquito Control Division (Wilke, *et al.* 2018) recommend that during emergency situations (*Aedes aegypti*-transmitted disease outbreaks) control of larvae in bromeliads, including plant removal, should be considered. Their recommendations were based on finding *Ae. aegypti* to be the most dominant species in bromeliads sampled in Miami-Dade County.

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Chapter 5

LARVICIDES AND LARVICIDING

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Summary

This chapter focuses on larviciding products and techniques used to control mosquitoes while in the larvae and pupae stage. Larval mosquito control in Florida has continued to advance with education and in technology over the past century. Integrated Pest Management (IPM), as defined by the Environmental Protection Agency (EPA), is a smart, sensible, and sustainable solution to pest management. Integrated Mosquito Management (IMM) is a derivative of this principle and has been widely adopted by mosquito control districts throughout the state of Florida. Larviciding is a vital component of IMM providing prescription applications to target mosquitoes in their most concentrated and vulnerable stage of life. Products and techniques currently used in Florida are discussed.

5.1 INTRODUCTION

Larviciding is a general term for killing insects in the larval stage by applying insecticides, collectively called larvicides, to suppress an insect population to an acceptable level. Although a general term, larviciding is most commonly used when referencing the treatment of mosquito larvae. Similar to larviciding, pupaciding is a general term for killing insects while in the pre-emergent pupal stage. Pupal control is commonly conducted by mosquito control technicians while targeting larvae in the field and for the clarity of this chapter, pupal control will be considered synonymous to larval control.

Larval Source Management (LSM) involves both the modification of water habitats, often referred to as Source Reduction (see Chapter 4), and the direct application of larvicides to control mosquito production. Depending on temperature and available resources, most mosquito species spend three to five days of their life cycle in the larval stage when they are highly susceptible to both predation (see Chapter 7) and control efforts. They often are concentrated within defined water bodies with limited mobility and ability to disperse, making them highly accessible. Well planned larviciding can efficiently reduce the number of adult mosquitoes that emerge, saving labor costs, reducing chemical use, interrupting arbovirus cycles, reducing public nuisance, and, ultimately, the laying of eggs which leads to more mosquitoes.

Larval control is a vital component of a proper Integrated Mosquito Management (IMM) program**.** Effective IMM involves understanding the local mosquito ecology and patterns of arbovirus transmission and then selecting the appropriate mosquito control techniques. The most common components of IMM include Environmental Management, Source Reduction (Chapter 4), Larviciding, and Adulticiding (Chapter 6). Other mosquito control principles include Biocontrol (Chapter 7), as well as additional methods not discussed here such as herbiciding and hand removal of aquatic plants. These methods may be used to control immature mosquitoes indirectly, usually when there is an obligatory association between the larvae and specific host plants. In Florida, mosquitoes in the genus *Mansonia* and *Coquillettidia* utilize oxygen in the roots of specific aquatic plants for respiration.

Mosquito species can vary in larval density. Common examples of highly concentrated broods include immature *Aedes taeniorhynchus* and *Ae. sollicitans* in saltmarsh pools, *Psorophora columbiae* in flooded pastures, and *Culex nigripalpus* or *Cx. quinquefasciatus* in wastewater treatment sites. In these situations, most Florida mosquito control programs larvicide as a management practice because it both minimizes the area in which control procedures must be applied and reduces the need for adult control. At these times, larviciding has a high impact on local population numbers with minimal application efforts. With other species, larviciding may be less rewarding because small numbers of larvae are widely and unevenly distributed. Examples include *Culiseta melanura* in bay tree swamps, *Mansonia* species and *Cq. perturbans* in large freshwater marshes with patchy host plant distribution, and *Anopheles quadrimaculatus* in large, overgrown grassy retention ponds.

Planning a LSM strategy is crucial to a highly effective control program. The first step begins with larval surveillance. Once surveys have been conducted, it is then important to map out and prioritize potential larval habitats in the surrounding area. Treatment thresholds, often based on the number of larvae encountered at a site, should be established to justify larviciding, and action plans appropriate for the sites should be developed.

It is important to select the appropriate larvicide and formulation based on product performance, target site, access to larval source, and stage of larval instar. It is critical to have a thorough knowledge of the biology of the targeted species to determine the appropriate larvicide, the timing of the application, and the amount of product to be applied. For example, *Ae. taeniorhynchus* tend to "ball up" when feeding as third instars (Nayar 1985). The larvae are unevenly distributed and the density where they do occur is much higher than at other times in their development when they tend to be more evenly dispersed in salt marsh pools. This situation may call for an application rate higher than what is normally used but never exceeding the maximum allowed on the label. Larvicides may be chosen which exhibit a selective mode of action and have a minimal residual activity, or which are not selective and exhibit long-term control. Many larvicides can be applied from either the ground by truck, boat, and hand-held devices or by air with fixed wing and rotary wing aircraft; However, some products are not suitable for aerial application. Follow-up efficacy checks are important to ensure a successful larviciding program, and rotation of products should be incorporated into any IPM program.

There is no perfect larvicide for every situation, and each larvicide has its strengths and weaknesses. Larvicides may be grouped into two broad categories: Biorational pesticides and conventional, broad-spectrum pesticides. The latter will be discussed in sections 5.2.3 thru 5.2.4.2.

The term “biorational” gained popularity in the climate of environmental awareness and public concern (Williamson 1999). It refers to pesticides of natural origin that have limited or no adverse effects on the environment or beneficial organisms. For a synthetically produced pesticide to be classified as a biorational, it must be structurally identical to a naturally occurring compound. Biorational pesticides are comprised of two major categories: Microbial agents (*e.g.*, bacteria) and biochemical agents (*e.g.*, pheromones, hormones, growth regulators, and enzymes).

Schuster and Stansly (2006) defined a biorational pesticide as any type of insecticide active against pest populations but relatively innocuous to non-target organisms and, therefore, non-disruptive to biological control. An insecticide can be "innocuous" by having low or no direct toxicity on non-target organisms or by having a short field residual, thereby minimizing exposure of natural enemies to the insecticide. By this definition, all larvicides registered for use in Florida, when applied according to label instructions, might be considered biorational. There is no legally clear, absolute definition of a biorational pesticide (Williamson 1999). The U.S. Environmental Protection Agency (EPA) considers biorational pesticides to have different modes of action than traditional pesticides (http://ipmworld.umn.edu/chapters/ware.htm), with greater selectivity and considerably lower risks to humans, wildlife, and the environment. The terms “biorational” and “biopesticide” overlap but are not identical.

Organic certification for food has been a growing trend in the United States. In 1997, the Organic Materials Review Institute (OMRI) was founded to evaluate materials used in organic agriculture. The OMRI evaluated materials also include pesticides that may be used near organic gardens that are sometimes interspersed within neighborhoods where mosquito control operations occur. A limited number of products have been developed to meet the OMRI requirements and offer mosquito control an opportunity to treat for mosquitoes without compromising the public’s organic certification of a food source. (https://www.omri.org)

5.1.1 History

Stories of prodigious numbers of mosquitoes occupy a special place in Florida's history (Patterson 2004) beginning with 16th century explorers. An 1888 yellow fever epidemic in Jacksonville set in motion the formation of the Florida State Board of Health (FSBH) in 1889. The Florida Anti-Mosquito Association was founded in 1922 and is now known as the Florida Mosquito Control Association (FMCA). The first mosquito control legislation was passed, and the Indian River Mosquito Control District was established in 1925 (Anonymous 1948, Patterson 2004).

Larviciding became prominent when implemented as an area-wide malaria control procedure in the early 1900s, but by then it had been used as a control technique for over a century in Florida (Floore 2006). From the earliest days, two types of larval control were employed: Larviciding as a temporary control method and ditching as a permanent control method (see Chapter 4 on Source Reduction). Larviciding using waste oil or diesel oil products was implemented to control mosquitoes in the early 1800s (Howard 1910). Paris green dust, an arsenical insecticide, was developed as a larvicide in 1865 and, along with undiluted diesel oil, was used through the 1960s (Anonymous 1970). In 1958, the FSBH developed its own Paris-green granular formulation as a general purpose larvicide (Mulrennan 1958). The FSBH went on to develop its own "Florida Mosquito Larvicide" in the 1960s which contained 99% mineral oil (unpublished 24-C label 1967).

After 1945, dichloro-diphenyl-trichloroethane (DDT), a chlorinated hydrocarbon compound, was used as both an adulticide and a larvicide in Florida (Anonymous 1970, Patterson 2004). Mosquitoes became resistant to DDT, and its use was discontinued in the late 1950s. As resistance to DDT increased, malathion, an organophosphate (OP) compound, was used increasingly to control both larval and adult mosquitoes. Soon, resistance to malathion was observed in saltmarsh mosquitoes (Rathburn and Boike 1967). The FSBH then implemented a policy limiting the use of malathion to adulticiding in areas where OP larvicides were not used. Resistance (see Chapter 10) has been a concern in the past (Boike and Rathburn 1968) and continues to challenge Florida mosquito control agencies (http://www.floridamosquito.info/insecticide-susceptibility-testing-results/). Rogers and Rathburn (1964) summarized early agency attitudes toward larviciding: "Although larviciding alone is not regarded as a practical procedure for mosquito control in Florida … the great value of larvicides is fully appreciated." Attitudes have changed, and by 2006 most mosquito control agencies in Florida had incorporated larviciding as one of their mosquito management practices.

During the twenty years that have elapsed since the first edition of this document, a number of larviciding formulations are no longer registered and likely will never again be available as tools for mosquito control agencies. These products include pyrethrum, temephos, Bonide Mosquito Larvicide (oil), and BVA Chrysalin (oil). Laginex AS (active ingredient *Lagenidium giganteum*) has not been enthusiastically accepted in Florida or elsewhere in the United States. Some agencies may list predatory minnows which they purchase for larval control as line items in their larvicide budgets, but these fish are considered biocontrol agents. Industry consolidation has placed the stewardship of the remaining larvicides into the hands of fewer manufacturers. Mosquito control professionals must be diligent with applications and guard against the loss of the remaining control agents.

5.1.2 Regulation

The regulation of larvicides and larviciding is provided for by a set of federal and state acts, statutes, and rules. Oversight includes both regulation of the pesticides themselves and regulation of pesticide applications. The principal controlling law is the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Chapter 487 Florida Statutes (F.S.), known as “The Florida Pesticide Law”, Chapter 388 F.S. known as “The Mosquito Control Act”, and associated Rules outlined in Chapters 5E-2 and 5E-13 of the Florida Administrative Code constitute the State’s authority. The Florida Department of Agriculture and Consumer Services (FDACS), Agricultural Environmental Services (AES), is tasked with ensuring compliance and regulates and licenses the pest control industry and mosquito control programs. The FDACS, AES administers various state and federal regulatory programs concerning environmental and consumer protection issues. These responsibilities include state mosquito control program coordination, agricultural pesticide registration, testing, and regulation, pest control regulation, and feed, seed, and fertilizer production inspection and testing.

The AES, Bureau of Scientific Evaluation and Technical Assistance (BSETA), Pesticide Registration Review Section (PRRS) registers federally approved pesticides that are distributed, sold, or offered for sale in Florida (https://www.freshfromflorida.com/Business-Services/Pesticide-Licensing/Pesticide-Product-Registration). Pesticides not requiring federal approval that are exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA section 25(b) or minimum risk) also must be registered in Florida to assure adherence with State law. Emergency exemptions from federal registration also are reviewed and processed by the PRRS and submitted to the Environmental Protection Agency (EPA) for action. Special registration actions for new active ingredients, special local needs, significant new uses, and experimental use permits are processed through the PRRS. To accomplish their mission, members of the PRRS consult with specialists within FDACS and other state and federal agencies, commissions, and councils.

Most special registration actions are reviewed by the Pesticide Registration Evaluation Committee (PREC). PREC was established by FDACS in 1984 as a means to implement pesticide review responsibilities and obtain input from involved FDACS staff and affected state agencies. PREC members include scientists from the FDACS, Florida Department of Environmental Protection, Florida Department of Health and Florida Fish and Wildlife Conservation Commission.

The Scientific Evaluation Section (SES) of the FDACS, AES, BSETA, includes scientists with expertise in geology, soil science, hydrology, mammalian and ecological toxicology, chemistry, and chemical fate modeling. The SES provides technical support and has five core functions/programs:

1. Pesticide Registration Evaluation Committee Reviews
2. Endangered Species Protection Program
3. Ground Water Protection Program
4. Surface Water Protection Program
5. Termiticide Efficacy Review

The SES functions and interacts with other stakeholders to ensure the safety of the State of Florida. The Entomology and Pest Control Section (EPCS) of the FDACS, AES, BSETA, includes scientists with expertise in mosquito control, invasive termite species, and bedbugs. The EPCS provides technical assistance and conducts outreach activities for the public and mosquito control and pest control communities to raise awareness on insect pests of public health, nuisance, and structural importance.

Chapter 388 F.S. provides the authority for mosquito control activities. The statute includes a provision that public lands may be designated as environmentally sensitive and biologically highly productive, thereby requiring special arthropod control plans for mosquito control activities on those "designated" lands. Many state and federal land management authorities [*e.g.*, Florida Department of Environmental Protection (FDEP), Florida Division of Forestry (FDOF), Florida Fish and Wildlife Conservation Commission (FFWCC), U.S. Fish and Wildlife Service (USFWS)], and regional water management districts designate their conservation lands similarly and have corresponding control plans in place.

The National Pollutant Discharge Elimination System (NPDES) was created in 1972 to protect the nation's water system from point source discharges from potential pollutant sources. The NPDES Storm Water Program in Tallahassee is responsible for the development, administration, and compliance of rules and policy to minimize and prevent pollutants in storm water discharges. Recently, Mosquito Control Districts have been considered as potential operators of these sources and rapidly have become required to follow NPDES requirements.

The control plans are initially proposed by the mosquito control agency for individual parcels and negotiated with the public land manager until mutually agreed upon. Either party may propose further amendments. There is no overarching agreement that certain control chemicals are approved for all such public lands. For example, in 1987, the Florida Park Service and various mosquito control agencies adopted control plans for many state parks (personal communication, Dana C. Bryan, Environmental Policy Coordinator, Office of the Director, Florida Park Service, December 2006). At that time, products containing *Bacillus thuringiensis israelensis* (*Bti*) and methoprene were widely approved for use. *Bacillus sphaericus* (*Bs*) had not yet been developed commercially and hence was not included in arthropod control plans. Many subsequent plans include *Bs* in addition to *Bti* and methoprene. See Chapters 9 and 13 for additional discussions of mosquito control agency interactions with other government entities.

5.2 Larvicides Available

Mosquito larvicides registered for use in Florida are discussed below within the following classification system:

- insect growth regulators (IGRS)
- microbial larvicides
- bacterial metabolites
- organophosphates (OPs)
- surface oils and films

Insecticide labels usually bear a precautionary signal word. The necessity for a signal word on labels (http://www.epa.gov/oppfead1/labeling/lrm/chap-07.htm) and which word is assigned is dependent upon the results of six separate acute toxicity studies which are performed with each product formulation.

There are a variety of products and formulations within each larvicide classification. Specific formulations are different from manufacturer to manufacturer. Application rates and suggested treatment sites may differ as well. Individual product labels and safety data sheets (SDS), usually downloadable from manufacturers' web sites, should be consulted for specific information, habitat dependent application rates, and restrictions, if any. FDACS should be consulted to ensure that a specific product is labeled for use in Florida.

5.2.1 Insect Growth Regulators (IGR)

The initial identification of a natural juvenile hormone (JH I) in insects occurred in 1967 and was followed rapidly by the discovery of JH II and JH III (Henrick 2007). JH is involved in the regulation of physiological processes in insects including mating and metamorphosis. Research was initiated in 1968 to determine if insect pests could be selectively controlled – without environmental concerns – by developing synthetic mimics of the natural JH. Since JH does not occur in vertebrates, it was expected that selective insecticides could be developed. Sacher (1971) reported on a group of chemicals that mimic juvenile hormone activity. These chemicals appeared to block naturally occurring ecdysone from initiating molting processes and inducing metamorphosis in mosquito larvae. Staal (1975) discussed several methoprene analogs that interfere with normal insect growth and maturation. Abnormal larval growth patterns plus malformed or smaller than normal forms were observed. The first IGR, which contained several methoprene isomers, was registered in 1975 (Henrick 2007). Methoprene, pyriproxyfen, and diflubenzuron products currently are the only IGRS registered for use in Florida.

5.2.1.1 Methoprene

Methoprene (Isopropyl (2E, 4E, 7S)-11-methoxy-3,7,11 -trimethyl-2,4-dodecadienoate) is a terpenoid compound. Technical methoprene is an amber or pale-yellow liquid with a faint fruity odor, which is slightly soluble in water and is miscible in organic solvents. Methoprene is a synthetic mimic and a true analog of naturally occurring JH found in mosquitoes and in other insects.

JH is found throughout the larval stages of a mosquito, but it is most prevalent during the early instars. As mosquito larvae mature, the level of naturally occurring JH steadily declines until just prior to the 4th instar molt, when larvae develop into pupae. This time is a sensitive period when all the physical features of the adult begin to form. Methoprene is absorbed through the insect's outer "skin" or cuticle and may be incidentally ingested or enter the body through other routes. The level of applied methoprene (parts per billion) in the larvae's water environment must be higher than the level of juvenile hormone circulating in the larvae's body in order for the disruption of endocrine processes to occur. Therefore, the application of methoprene larvicides is most efficacious during late fourth instar. Treated larvae reach the pupal stage and then cannot emerge to become adults. Since pupae do not eat, they eventually deplete body stores of essential nutrients and starve to death. Incomplete adult emergence is an indicator of methoprene efficacy.

Methoprene is listed by the EPA as a biopesticide. Methoprene based larvicides are General Use Pesticides (GUPs). Methoprene-based larvicides have undergone extensive studies both prior to and after registration to determine risk to humans and non-target organisms. When used according to label directions, methoprene is considered extraordinarily safe for humans and almost all non-target organisms. Methoprene does not produce the nondiscriminatory, rapid toxic effects often associated with central nervous system toxicants. The lethal effects of methoprene are based on the disruption of the insect's endocrine system mediated developmental processes, such as metamorphosis and embryogenesis. Consequently, control of mosquito larvae is relatively slow.

Methoprene is effective in a wide variety of both fresh and saltwater habitats. It is relatively selective for target species, and lingering mosquito pupae serve as a food for fish and other predators. The IGR is particularly effective against *Aedes* larvae. Methoprene does not bioaccumulate; It degrades into simpler compounds. Since ultraviolet light deactivates methoprene, many formulations incorporate activated charcoal or other dark inert substances to prolong product life. Early methoprene manufacturing products included two mirror-image molecules called r- and s-isomers. The racemic isomer (r-methoprene) is not active on mosquitoes. Improved manufacturing techniques allow current formulations to contain only active s-methoprene isomers. Methoprene labels bear the "CAUTION" signal word. Methoprene is available in solid (granular, pellet, and briquette) formulations.

5.2.1.2 Pyriproxyfen

Pyriproxyfen (4-Phenoxyphenyl (R/S)-2-(2-pyridyloxy)propyl ether) is a pyridine-based chemical. Like other insect growth regulators, pyriproxyfen mimics natural insect hormones and prevents larvae from maturing into adults. Pyriproxyfen has low acute toxicity to mammals (Anonymous 2000) and is lethal to mosquitoes at extremely low concentrations (Lloyd *et al*. 2017). Pyriproxyfen has potential for autodissemination of the product by mosquitoes to oviposition sources (Itoh 1994, Devine *et al*. 2009). Pyriproxyfen has low solubility in water but degrades fairly quickly due to aquatic photodegradation. In soils it is broken down via action of microorganisms (Sullivan and Goh 2008). Pyriproxyfen is available as liquid or dust formulation. Pyriproxyfen labels bear the "CAUTION" signal word.

5.2.1.3 Diflubenzuron

Diflubenzuron (*N*-[(4-Chlorophenyl)carbamoyl]-2,6-difluorobenzamide) unlike other IGRS is a chitin synthesis inhibitor and has much broader non-target impacts than methoprene, especially on marine and freshwater arthropods such as shrimp and crabs (Weis *et al.* 1987, Soltani *et al*. 2009). Diflubenzuron also can cause damage to some plants, particularly poinsettia, hibiscus, and some begonias (OHP, Inc. 2012). Therefore, although Dimilin® 25W has a special local need registration in Florida, its use is strictly confined to particular larval habitats and thus it is not widely used. Diflubenzuron degrades rapidly in soil but degrades more slowly in water (Ivie *et al*. 1980, Nimmo *et al*. 1984). Diflubenzuron labels bear the "CAUTION" signal word.

5.2.2 Microbial Larvicides

Microbial larvicides are formulated to deliver a natural toxin to the intended target organisms. Bacteria are single-celled parasitic or saprophytic microorganisms that exhibit both plant and animal properties and range from harmless and beneficial to intensely virulent and lethal. *Bacillus thuringiensis* (*Bt*) is the most widely used agricultural microbial pesticide in the world, and the majority of microbial pesticides registered with the EPA are based on *Bt*. The *Bt* serovar *kurstaki* (*Btk*) is the most commonly registered microbial pesticide, and this variety has activity against Lepidoptera (butterflies and moths) larvae. It was originally isolated from natural Lepidopteran die-offs in Germany and Japan. *Bt* products have been available since the 1950s. In the 1960s and 1970s, the World Health Organization (WHO) encouraged and subsidized scientific discovery and utilization of naturally occurring microbes. As a result of those early studies and a whole body of subsequent work, two lines of mosquito control products have been developed: Crystalline toxins of two closely related gram-positive, aerobic bacteria – *Bacillus thuringiensis israelensis* (*Bti*) and *Bs*. Mosquito control agents based on *Bt* are the second most widely registered group of microbial pesticides. Highly successful *Bti* products have expanded the role of microbial agents into the public health arena (de Barjac 1990). Reviews of microbial agents may be found in Lacey (1985), Lacey (2007), and Singer (1985).

5.2.2.1 *Bacillus thuringiensis israelensis*

Bacillus thuringiensis is a bacterium which occurs naturally in soils and aquatic environments globally. In 1976, Goldberg and Margalit (1977) isolated *Bti* from *Culex pipiens* collected in an Israeli riverbed. In 1977, de Barjac designated this *Bt* strain as H-14, noting that it is toxic to mosquito and black fly larvae. Over the last three decades, a number of other strains have been investigated, some with desired larvicidal effects. The active ingredients in *Bti* formulations are delta-endotoxin (d-endotoxin) crystals separated from bacteria near the end of manufacturing processes. These toxic crystals are incorporated into various products which allow their release into water so that they may be ingested by mosquito larvae. The d-endotoxin crystals are activated by the alkaline environment and enzymes of the mosquito midgut. The alkaline gut environment allows hydrolysis of the crystal's protein coating and the release of pro-toxins. Gut enzymes then activate the pro-toxins and facilitate their binding to the gut epithelium of the mosquito larva. Cells rupture and are destroyed at the binding sites, leading to a loss of body fluids which results in death. This rapid action typically controls larvae in four to twenty-four hours.

Bacillus thuringiensis israelensis is listed by the EPA as a biopesticide. *Bti* based larvicides have undergone extensive risk studies both prior to and after registration. *Bti* products are GUPs and are safe for non-target organisms in the environment. The crystalline d-endotoxins are not activated in the acidic guts of humans or other animals or in the alkaline guts of animals which do not contain the enzymes necessary for activation and binding of released pro-toxins. This specificity accounts for the highly selective nature of *Bti* larvicides which is limited to Dipterans, notably mosquitoes, black flies, and some midges. *Bti* controls all larval instars provided they are still feeding. It is effective on most mosquito species in a very wide variety of habitats, so *Bti* formulations are thus ideally suited for IPM.

Bti product labels show the potency of the product as the number of International Toxic Units (ITU) available. This value is more meaningful than the weight percent of the active ingredients, as it characterizes the formulation's effectiveness. ITU values are determined by a standardized laboratory bioassay which uses fourth instar *Culex quinquefasciatus*. Prepared volumes of toxins are applied to living mosquito larvae and the resulting mortality data provide a numerical measure of activity. *Bti* labels bear the "CAUTION" signal word.

Bacillus thuringiensis israelensis is available in liquid (aqueous suspension) and solid (granular, tablet, pellet, water dispersible granule, and briquette) formulations.

5.2.2.2 *Bacillus sphaericus*

In 2007 *Bacillus sphaericus* (*Bs*) was transferred to the genus *Lysinibacillus*, thus the taxonomically correct name for this bacterium is *Lysinibacillus sphaericus* (Ahmed *et al.* 2007). More recent work indicates that what was thought to be a single species with numerous toxic and nontoxic strains may in fact be a complex of species (Gómez-Garzón *et al.* 2016). Although the correct name is slowly appearing in entomological literature, most mosquito control personnel are familiar with the old generic placement, and that name will be retained in this edition of this document.

Bacillus sphaericus is a naturally occurring spore-forming bacterium found throughout the world in soil and aquatic environments. Kellen and Myers (1964) isolated *Bs* from *Culiseta incidens* larvae in California. Early studies were conducted on *Bs* strains isolated by the Pasteur Institute, while the commercial products discussed below are based on strain 2362 isolated in Nigeria. Lacey (2007) reported that serovarieties with the most pronounced larvicidal activity are 1593 and 2362. Some strains produce a protein d-endotoxin at the time of sporulation which is toxic to many species of mosquito larvae upon ingestion.

Bacillus sphaericus acts in a manner similar to *Bti*, except it has been shown to recycle in intact *Culex* cadavers, thus maintaining some residual activity (Becker *et al*. 1995). Once larvae ingest these *Bs* d-endotoxins, they are partially digested (their protein envelope is dissolved) in the alkaline gut, enabling the release of pro-toxins. These pro-toxins in turn are activated by enzymes and attach to the gut wall where they begin to disrupt, paralyze, and rupture the gut.

The activity of *Bs* d-endotoxins differs from that of *Bti* in several important ways. *Bs* toxins are attached to a living bacterial spore while the *Bti* toxins are not. The toxins of *Bs* and *Bti* bind to chemically different receptor cell sites. They are not related immunologically and are thought to have completely different molecular modes of action. Operationally, the most important differences between the toxins are speed of action and persistence in the larval habitat. *Bs* toxins are much slower acting than *Bti* toxins and can be more persistent. *Bs* has a slower settling rate, and the spores can invade the body cavity of the larvae where they have the capability to germinate, grow, and produce toxins. This process is known as recycling.

Bs is listed by the EPA as a biopesticide. *Bs* based larvicides are GUPs, which have undergone extensive risk studies both prior to and after registration. The crystalline d-endotoxins are not activated in the acidic guts of humans or other animals or in the alkaline guts of animals which do not contain the enzymes necessary to activate the pro-toxins. This specificity accounts for the highly selective nature of *Bs* larvicides; They do not target as wide a range of mosquito species as do *Bti* products. Formulations containing *Bs.* are most active against *Culex* and *Anopheles* larvae and less active against some *Aedes* larvae. Formulation effectiveness depends on the mosquito species and environmental conditions including water quality. In general, the best immediate results with *Bs* are obtained when applications are made to larvae in the 1st to 3rd instars. Larval mortality may be observed as soon as a few hours after ingestion, but typically it takes as long as two to three days depending upon dosage and ambient temperature. Adequate recycling of *Bs* for sustained control is dependent on the presence of dead mosquito larvae.

Bs International Toxic Units (Bs ITU) values are determined by a standardized laboratory bioassay similar to that developed for *Bti* H-14. The bioassay uses 3rd and 4th instar *Cx. quinquefasciatus*. The signal word "CAUTION" appears on *Bs* product labels. In Florida *B. sphaericus* is available in granules, water-dispersible granules, and a water-soluble formulation.

5.2.2.3 Spinosad

Spinosad is a bacterial metabolite larvicide product derived via fermentation from a naturally occurring soil bacterium, *Saccharopolyspora spinose*, discovered in an abandoned rum still (Mertz and Yao 1990). This product is in a new class of insect-control products known as naturalytes/acitnomycetes (Sanon *et al*. 2010).

Actinomycetes are gram-positive, filamentous bacteria, many of which are common in soil. The actinomycete *Saccharopolyspora spinosa* secretes a variety of metabolites when grown in the laboratory. These naturally-produced polysaccharides, or complex sugars, are called spinosyns. In 1989 the most active polysaccharides from *S. spinosa* contained two insecticidal factors, spinosyn A and D, in a ratio of about 17:3 (Kollman undated). Spinosad is slower to penetrate the insect cuticle than pyrethroid insecticides, but once it enters the body it is metabolized very slowly (Sparks *et al*. 2001). The mode of action of spinosad is apparently unique; The chemical does not interact with any known insecticidal target sites within the insect (Orr *et al*. 2009).

Dow AgroSciences named this insecticidal mixture of spinosyns A and D as spinosad (Mertz & Yao, 1990; Dow Agrosciences 2001). Spinosad was first registered in the U.S. for agricultural use in 1997. It has highly favorable mammalian and environmental toxicity profiles. Spinosad is registered under the U.S. EPA Reduced Risk Pesticide Registration Program and received the U.S. EPA Presidential Green Chemistry Award in 1999.

Spinosyn A and spinosyn D are photolabile and have half-lives on soil of 17 and 7 days, after which degradation slows, giving estimated half-lives of over 100 days. Spinosyn A dissipates rapidly in soil – almost 99% is gone within 8 days. Spinosyn A and D degrade rapidly in water due to photolysis and adsorption to sediments. Long term ingestion of residues is unlikely to present public health concern, and short-term consumption unlikely to pose risk to consumers (Anonymous 2002). Spinosyns A and D have very low vapor pressures, making them essentially non-volatile. Aqueous photolysis of A and D is rapid, with a half-life of less than one day at pH 7 (WHO 2007).

Spinosad is active by contact or ingestion, but ingestion is the primary route of entry for mosquito larvae. Spinosad acts through a unique insecticidal mode of action targeting the nicotinic acetylcholine receptor (nAchR) that contain the Dα6 subunit. Although neonicotinoids also target insect nAchRs, target-site cross-resistance has not been observed between spinosad and neonicotinoids (Geng, Watson, and Sparks, 2013). More recent studies with a variety of pyrethroid and organophosphate insecticide-resistant insects also found no cross-resistance to spinosad (Geng *et al*. 2013).

Because mosquito larvae must ingest spinosad, full cumulative mortality is typically observed by 48 to 72 hours after application. Efficacy of spinosad can be influenced by larval age and environmental parameters, including water temperature, pH, and amount of organic debris. Liquid and granular formulations of spinosad have been developed for both single-brood and floodwater mosquitoes, including common nuisance species in Florida. Slow-release formulations have been developed for multiple-brood and permanent water-breeding mosquitoes, such as *Culex* and *Anopheles* species.

Spinosad is not acutely toxic to terrestrial birds, wildlife, or to fish and most aquatic invertebrates. Field studies have found that, once spray residues of spinosad have been allowed to dry for up to 3 hours, spinosad is not acutely toxic to foraging honeybees. Spinosad presents a relatively low risk to beneficial and non-target insects compared to broad-spectrum insecticides. Extensive field experience indicates that spinosad has a generally limited and transitory effect on beneficial insects and fits well into Integrated Pest Management (IPM) programs. After years of use in crop markets and, more recently, in many mosquito larval habitats, spinosad is widely recognized as compatible with integrated pest management and environmental stewardship objectives. Spinosad is available in liquid (emulsifiable concentrate) and solid (granule and tablet) formulations.

5.2.3 Organophosphates

The term organophosphate (OP) refers to all pesticides containing phosphorus. OPs were discovered in Germany during a search for a substitute for nicotine, which was heavily used as an insecticide but was in short supply. The insecticidal qualities were first observed there during World War II (http://ipmworld.umn.edu/chapters/ware.htm). OPs have been used for mosquito control since the early 1950s. OPs work after entry into and distribution through the body of a target organism by modifying the normal functions of some nerve cells by inhibiting the activity of cholinesterase enzymes at the neuromuscular junction. This action results in the accumulation of acetylcholine, thereby interfering with neuromuscular transmission. In insects, OPs produce a loss of coordination leading to paralysis and ultimately death.

5.2.3.1 Temephos

Temephos (O, O’-(thiodi-4, 1-phenylene) O,O,O’,O’-tetramethyl phosphorothiolate) is an OP compound. During the 1960s, temephos was studied extensively as a replacement for the persistent organochlorine DDT in malaria control programs. It was registered as a mosquito larvicide in 1965. A review of Florida pesticide use records indicates that temephos has been utilized in the state since 1969.

Temephos is labeled for use in many habitats including tidal marshes, woodland pools, polluted water, tires, and as a pre-hatch treatment. Temephos often is recommended as a rotation larvicide where it is used in place of the microbial or IGR larvicide in an IPM program. Temephos is a GUP with a low toxicity when used according to the label with little or no detrimental effects on non-target organisms. Temephos is one of the least toxic OPs to mammals

On February 25, 2011, EPA issued a cancellation order affecting all uses of temephos. As of December 31, 2015, the pesticide manufacturer can no longer sell any temephos products, although many districts still maintain the legal use of any temephos that was purchased prior to 2015 and can purchase any temephos that remain in distribution. Product labels bear either the signal word "WARNING" or the signal word "CAUTION."

5.2.4 Surface Oils and Films

Surface oils and films used as larvicides include oils and ethoxylated isostearyl alcohols. As previously noted, surface oils, such as waste motor oil and diesel, were the first larvicides used for mosquito control in Florida. Howard (1931) considered low grade kerosene or fuel oil more satisfactory than other larvicide methods. The State of Florida developed its own "Florida Mosquito Larvicide" oil, also called the "Florida Formula", in the 1960s, but by the 1980s, crude formulations such as these were losing status in Florida. Studies reported potential replacement products such as Arosurf, a thin layer alcohol-based surface film (Mulrennan 1982), and highly refined petroleum oils (Mulrennan 1983). New oil formulations replaced the "Florida Formula" by the mid-1980s (Mulrennan 1986). The new thin layer surface films and highly refined oils are virtually colorless and odorless (Floore *et al.* 1998), and they exhibit the same larval and pupal control properties as the waste oils they replaced.

5.2.4.1 Larviciding Oils

The larviciding oils are probably the least studied of the mosquito larvicides, despite their long period of use for mosquito control. Specific control mechanisms are difficult to pinpoint but likely include poisoning of the larvae (pers. comm., E. J. Beidler, Indian River MCD). Oils also can suffocate – but only at the very highest dosage rates. Inert ingredients include emulsifiers which help them spread over the water's surface and kill larvae and pupae when inhaled into the tracheae along with air. With low dosages (*e.g.*, 1 gallon per acre), oils can work very slowly, taking four to seven days to provide control. Higher dosage rates (3-5 gallons per acre) usually are used to decrease the control time. Surface oils also are considered one of the most effective tools for pupal control and can control newly emerged adults that are resting on the water surface when drying their wings.

Larviciding oils are GUPs that are non-selective, and mosquito control efficacy is limited to those species which breathe air at the water surface. They have a low toxicity when used according to the label with minimal detrimental effects on non-target organisms. An "oil slick" can be viewed on the water surface. Both their odor and appearance may be objectionable, precluding widespread use in some areas. Larviciding oil labels bear the "CAUTION" signal word.

5.2.4.2 Monomolecular Surface Films

Monomolecular films (MMFs) are biodegradable, ethoxylated alcohol surfactants, made from renewable plant oils. MMFs are lighter than water and do not mix particularly well with it. As their name implies, MMFs produce an extremely thin film on the water's surface. They were originally developed by the U.S. Navy during World War II to help remove oil slicks. MMFs have been widely used in the cosmetics industry for over 50 years as a component of skin care products. Monomolecular films were investigated as mosquito larvicides and pupacides beginning in the early 1980s. Nayar and Ali (2003) have reviewed MMFs and their mosquito control uses.

Monomolecular surface films do not kill by toxic action but exert a physio-chemical impact on mosquito populations. When applied, they spontaneously and rapidly spread over the surface of the water to form an ultra-thin film that is about one molecule in thickness. They act by significantly reducing the surface tension of the water and wetting mosquito structures, which leads to drowning (Garrett and White 1977). Mosquito adults, eggs, larvae, and pupae utilize the surface tension of water in various aspects of their life cycle. With the surface tension reduction, mosquito larvae, pupae, and emerging adults cannot properly orient at the air-water interface and eventually will drown. Adults of both sexes that utilize the water surface for normal resting, and adult females who use the surface for oviposition also may drown. Eggs and egg rafts of certain species may not float normally or may sink and become unviable (Nayar and Ali 2003).

Monomolecular surface films can affect species that depend on the air-water interface. They may be used safely in potable waters, waters bearing fish and other aquatic organisms, and in runoff waters that enter fish-bearing waters. Monomolecular film labels bear the "CAUTION" signal word.

5.2.5 On-site Formulations and Combined Larvicides

Mixing materials "on-site" to formulate products has historically been popular with mosquito control operations in Florida. Applying liquid larvicides to granular carriers has been the most widely used type of home-made formulation. One early product involved applying Paris-green liquid to light-weight silica particles (pers. comm., E. J. Beidler 1996). Another notable practice involves combining two mosquito larvicides into a single-end product to take advantage of the properties of each component ingredient. Industry partners recognized the desire for a larvicide with multiple-faceted approach and now offer products that are pre-mixed and optimally formulated.

Methoprene sand granules are on-site granular formulations that are produced by combining liquid methoprene with washed sand. Thirty years ago, this process was developed at the Indian River Mosquito Control District in Vero Beach, and the formulation was named "Altosand" because Altosid Liquid Larvicide was used as the active material (pers. comm., E. J. Beidler 1996). Altosand was developed primarily to control mosquitoes in densely canopied mangrove swamps and coastal salt marshes where it is often necessary to penetrate dense canopies. Methoprene sand granules, prepared on-site, are used in Florida.

***Bti* sand granules** were not available as commercial formulations until the latter part of 1996. However, technical *Bti* powder and labeling has been available since the mid-1980s to allow end-users to make their own "on-site" *Bti* sand granules. Sand formulations require coating the particles with oil (GB-1111 or BVA 2) and then applying dry *Bti* powder which will stick to the oil.

Duplex is the name that has been attached to the end-user formulation which is made by combining *Bti* liquid and liquid methoprene. This mixture was developed principally to control larvae such as *Culex* spp. where many different instars may be present. The rationale for this mixture is that lethal *Bti* doses are somewhat proportional to a mosquito larva's body size and therefore less *Bti* is required for control of early instars. The opposite is true for methoprene which is most effective after 4th instars have stopped eating and the amount of methoprene required for control is the least. Combining *Bti* with methoprene theoretically allows operations to use less of each product than if using only one product.

Spinosad sand granules are registered in two formulations of Spinosad on granules: A corn cob-based single-brood formulation and a sand-based 30-day formulation. However, recognizing there are select habitats where a heavy single-brood granule is a best-fit formulation, an emulsifiable concentrate (liquid) was custom-blended onto a 20-45 mesh washed-sand and introduced in March 2015. Since then it has served primarily coastal mosquito control districts that have a need to treat marshland areas. A "mix and use" protocol is recommended vs. "mix and store" to ensure consistent performance.

Monomolecular films used with other larvicides have been investigated. Levy *et al.* (1982, 1984) reported significantly improved efficacy of several larvicides when formulated with ethoxylated alcohol surfactants. The authors indicated that "the use of mixtures of Agnique MMF or Agnique MMF mosquito larvicides and pupacides with other mosquito biolarvicides, IGRS, and/or central nervous system inhibitors has been shown to enhance the translocation of the bioactive agents over the surface of the water and provide improved joint-action mosquito-controlling efficacy." The dual-action larvicide formulations also are expected to be a good tool for use in resistance management programs. The use of a variety of ethoxylated alcohol surfactants that are approved by the EPA for use as inert materials in pesticide formulations is being evaluated on an operational basis as adjuvants for a variety of conventional mosquito larvicides. Lee County Mosquito Control District (LCMCD) has been using a mixture of a MMF and temephos for many years as a joint-action larvicide that rapidly spreads over the water surface (pers. comm., W. Gale and R. Levy 2006).

Pasco Mosquito Control District employed a MMF-temephos combination for several years with low doses of the monomolecular film used as a spreader and temephos as the intended active ingredient. However, a laboratory study with this mix introduced into long gutters populated with live larvae showed that temephos by itself spread nearly as well as when mixed with a MMF and that there was a slight tendency for reduced mortality when the two were combined (Wassmer, unpublished data). Consequently, the mixture was abandoned in favor of a temephos and water only mix. The results suggest a need for further study.

5.3 REPORTING ORGANIZATIONS AND RECENT LARVICIDE USE

Pesticide usage reports for 2013 and 2014 are available for downloading at https://www.freshfromflorida.com/Business-Services/Mosquito-Control/Mosquito-Control-Reports. Many special taxing districts, municipalities, developments, golf courses, and individuals throughout Florida also conduct mosquito control operations but do not report activities to FDACS.

5.4 EQUIPMENT AVAILABLE

Florida mosquito control operations employ a variety of larviciding equipment for both aerial and ground applications, as necessitated by the wide range of larval mosquito habitats, target species, site access, and budgetary constraints. Each operation typically will use more than one type of application equipment. There are advantages and disadvantages to each application system used and to the aerial and ground treatments themselves.

5.4.1 Ground Application Equipment

Almost all Florida mosquito control agencies use some type of four-wheel drive equipment as a primary larvicide vehicle. In most cases an open-bed pickup is equipped with a chemical-container tank, a high-pressure, low-volume electric or gas pump, and a spray nozzle. A switch and an extension hose allow the driver to operate the equipment and apply the larvicide from inside the truck's cab. Some agencies have the sprayer mounted on the front bumper of the truck and install a mechanical control that allows the driver to direct the spray while remaining in the cab. With the use and reduced cost of T.V. monitors, some districts have developed rear mounted spray systems that can be controlled from inside the cab. Roadside ditches, swales, retention ponds, treatment ponds, and other similar bodies of water can be treated with these setups. High velocity turbine spray machines have been utilized to target cryptic mosquito larva and can be mounted in the truck bed or on a trailer pulled by a truck.

Mosquito control agencies also use all-terrain-vehicles (ATVs), which allow operators to reach larval habitats that are inaccessible by truck. These units can carry a reasonable payload allowing operators to treat many remote sites consecutively without having to return to replenish pesticides. As with a truck, a chemical container is mounted on the ATV, a 12-volt electric pump supplies a high-pressure low-volume flow, and a hose and spray tip allow for manual application by an unaccompanied operator while steering the ATV with the other hand. Boom actuated granular spreaders can be mounted on the rear rack of an ATV when liquid larvicide is not necessary. ATVs are ideal for treating areas such as agricultural fields, pastures, salt marsh areas, and other off-road sites. Training in ATV safety and handling should be provided to employees operating these machines.

Ultra Low Volume (ULV) machines also can be mounted in the bed of the truck or on the back of an ATV to apply larvicides. These setups require the installation of a gas engine and compressor plus a metering system to accurately control output (see Chapter 6 for a detailed description of ULV systems). ULV applications of liquid larvicides from the ground were introduced in the late 1980s and early 1990s. ULV larviciding allows the product to drift into inaccessible areas. A more common use of ULV equipment involves diverting air from the compressor to propel granules and briquets into the target habitat via special granule nozzles or pneumatic guns.

Additional equipment used in ground applications includes dippers, horn seeders, hand-held sprayers, and backpack blowers and sprayers. Dippers and horn-seeders may be used to broadcast small amounts of granular or pelletized larvicides in spots that require minimal treatment. Hand-held sprayers are standard one- or two-gallon garden style pump-up sprayers used to treat small isolated areas with liquid larvicide formulations. Backpack sprayers usually have a gas-powered blower with a chemical tank and calibrated proportioning slot. Generally, pellet or small granular material is applied with a gas-powered backpack sprayer. They are extremely useful for treating tire piles. Pump-up backpack sprayers are sometimes used for dispensing liquid larvicides.

5.4.1.1 Advantages of Ground Application

There are several advantages to using ground application equipment when on foot or from vehicles. Ground larviciding allows more accurate pesticide applications to the intended treatment area and consequently to only those micro-habitats where larvae are present and is especially beneficial in urban areas. Ground larviciding applications are less affected by weather conditions than are aerial applications and are less susceptible to drift and product deposition outside the intended treatment area. This feature reduces the likelihood of unnecessary pesticide load on the environment and the financial cost of wasted pesticide. Also, initial and maintenance costs of ground equipment are generally less than those for aerial equipment.

5.4.1.2 Disadvantages of Ground Application

With ground application, there is greater risk of chemical exposure to applicators than aerial larviciding. Ground applications rely on human estimates of both the size of treatment areas and of equipment output during pesticide applications. Calibration of the applicators to the equipment can be difficult since an applicator's pace can vary, especially in areas with uneven terrain. It is difficult to provide even coverage with manually-operated ground equipment, and the possibility of under-applying or over-applying a larvicide is problematic. Ground larviciding is impractical for large, inaccessible, or densely wooded areas.

5.4.2 Aerial Application Equipment

Many of Florida's organized mosquito control operations have adopted aerial larviciding as a control strategy on otherwise large, unmanageable larval mosquito habitats. Agencies may not actually own the aerial equipment, as agricultural flying services can be contracted to apply larvicides as needed. Outsourcing the usually seasonal activity of aerial larviciding eliminates the need for and expense of an aircraft purchase, aircraft maintenance costs, and the expenses associated with having a pilot and perhaps an aircraft mechanic on staff.

Aerial larviciding is accomplished via fixed wing or rotary aircraft. Both types of aircraft can apply both solid and liquid larvicide formulations. A variety of hoppers, nozzles, and metering systems can be adapted to the aircraft, depending upon the desired equipment configuration and its size. The decision on whether to use liquid or granular applications depends on the target habitat and prevailing meteorological conditions.

Granular formulations provided by manufacturers incorporate a paper product, sand, gelatinous material, or corncob particles as the carrier for the active ingredient. Granules also may be prilled (pelletized) and contain little if any carrier. One prilling process is similar to that of making large snowballs, where the active ingredients are continuously packed onto a small seeded core as the ball of material is slowly rolled in a rotating tray. The tilt of the tray and the rotational speed help determine the resulting product size, as larger balls of material roll off the edge. In some instances, agencies can formulate their own granular materials (*e.g.*, sand mixes). Most granular formulations are applied at rates ranging from six to twenty pounds of product per acre.

5.4.2.1 Selecting Larvicide Formulations for Aerial Applications

Deciding which larvicide formulation to apply is critical for successful control efforts. There is considerable debate about which formulations are best for each mosquito control program. Debates often focus on habitat differences and which product type (liquid or granule) will best reach the target habitat or combination of habitats to be treated. The relative efficacy of pesticide types, their initial cost, the costs of any mixing, and the costs of loading and ferrying the pesticides to the application sites also need to be considered.

With liquid applications, there is debate over the ideal droplet size and carrier. Wind, temperature, evaporation, and droplet movement have major impacts on the success or failure of ULV applications. Using large droplets eliminates some of the drift problems of ULV. Low volume or ULV applications of undiluted liquid products (no water added) maximize acreage per load, thereby reducing overall costs. Diluting liquid products increases the costs of loading and ferrying and greatly reduces the payload. However, dilution may allow the application of more droplets within an application site, which in some circumstances may lead to a better presentation of the toxicant to the mosquito larvae and thus better control.

Liquid larvicides can deposit and stick on foliage, reducing the amount available for larval control. Using small droplets or ULV may reduce the loss due to canopy impaction, but the amount of material reaching the target under these conditions is not well documented. Some organizations attempt to minimize losses by using "raindrop" nozzles which produce extremely large droplets. These large droplets are thought to "punch" their way through the canopy. This type of application may render overall efficacy unacceptable for some target areas with specific canopy types and density. Despite these shortcomings, ease of product handling and relatively lower product costs combine to make liquid larviciding a viable operational option.

Dry pesticides formulations such as powders utilize bulky and/or heavy carriers to prevent them from drifting away from target application sites. Granule products, in contrast to liquid formulations, usually have less drift and are less apt to stick to foliage, allowing somewhat better penetration. Granules are not as easy to handle as their liquid counterparts because of their bulk (*e.g*., corncob formulations) or their weight (*e.g.*, sand formulations). Initial costs (especially the costs of premixed formulations) tend to be higher than the initial costs of closely related liquid formulations. Aircraft load weight restrictions limit the amount of granules per load and thus the number of acres that may be treated as compared to diluted liquid formulations. In addition, pilots and their mechanics are extremely cautious about applying formulations containing sand or other hard carriers with turbine driven aircraft.

Over the past two decades since the first printing of this document, the Florida Mosquito Control Association (FMCA) has held annual aerial application workshops, called the Aerial Short Courses, at the Lee County Mosquito Control District (LCMCD) in Ft. Myers, Florida. The courses have included expert presentations on relevant pesticide application topics, field demonstrations, and actual on-site application.

5.4.2.2 Measuring and Perfecting Aerial Larvicide Applications

When attempting to control larvae and/or pupae of many Florida mosquito species, complete coverage of the larval mosquito habitat is critical. Missing just a tiny fraction of the target area can result in the emergence of huge numbers of biting adults. A pilot must be completely familiar with the application equipment and know what kind of swath width to apply for each product under different environmental conditions. A pilot must know the mosquito-producing habitats and know when to apply "heavy" in order for enough pesticide to reach the water's surface to establish control. While many pilots claim that they can fly accurate swaths based on their skill alone, some type of guidance and offset system is necessary when performing aerial larviciding over large areas.

Deposit variability can be minimized only through rigorous calibration programs and optimum flight-path positioning. Using flaggers is a simple alternative to the use of GPS guidance if the influence of wind has been considered in advance. One or two flaggers use a flag or other signaling device on each end of the treatment area and pace off a measured distance for each swath. The pilot is guided by the flaggers, who then pace off the next swath, and so on. While not practical for all areas, when used it greatly increases the accuracy of the treatment coverage.

With today's electronic environment, a ground-to-air radio also may be employed where a field technician on the ground guides the pilot by pointing out landmarks that are easily seen from the air. This arrangement works especially well at small sites and where there is dense canopy since it is often impractical to flag these areas. Another method is to show the pilot a recent aerial photo of the target site during the period when the aircraft is being loaded and explain which spots are to be treated. In contrast, contour flying of large areas while applying pesticides in multiple swaths requires special GPS equipment because no one can recall all of the necessary landmarks to maintain proper lane spacing.

With improved GPS equipment, new computer-guidance programs for aircraft are now available. These new systems can accurately track the mission parameters (*e.g.*, treatment area, coordinates of treatment area, swath width, etc.) and provide the pilot with almost instant necessary course corrections. In addition to improving treatment accuracy, these systems log flight information which may be downloaded and used to produce a map or a visual display, providing mosquito control operations with accurate records of treatments.

Spray system calibration is necessary to ensure that pesticides are being applied according to label requirements. For liquid formulations, a spray calibration confirms that the droplet size distribution is appropriate. For both liquid and granular larvicides, swath characterizations and trial applications highlight the need for modifications that should provide the best chance for uniform deposit at labeled rates. In an effort to assist in spray calibration efforts, two free companion software programs – Grainalysis and Stainalysis – have generously been made available by REMSpC Spray Consulting at http://www.remspc.com/.

Grainalysis: REMSpC Granular Deposit and Larval Mortality Analysis Tool
The Grainalysis program can be used to calculate the deposit characteristics of granular swath-characterization trials from input data including product weight, number of granules, and measured larval mortality at each sampler. Output, available in tabular or graphic form, is displayed relative to the aircraft flight line and includes deposit (kilograms per hectare or pounds per acre), cumulative deposit fraction, number of

granules per unit area (square feet or square meters), number of granules per gram weight of product, and mortality. Swath analyses of deposit and granular uniformity also are available.

Stainalysis: REMSpC Stain Analysis Tool
The Stainalysis program can be used to analyze drop characteristics on Kromekote cards that have been scanned using any flatbed scanner (256 color depth). BMP, GIF and TIFF file formats are supported. Notch filtering allows for stain discrimination by color. Correcting for spread factor, an output file for each card includes: Spray documentation, digitization documentation, drop density, deposit volume (ounces per acres or liters per hectare), volume median diameter, and the contribution of individual drop sizes to volume fraction, number fraction, and cumulative volume fraction.

5.4.2.3 Advantages of Aerial Larvicide Applications

The number of programs utilizing aerial larviciding has been increasing in recent years suggesting that there are advantages to larviciding by air. Aerial larviciding poses a lower risk of chemical exposure to applicators than ground larviciding. Aerial applications can be more economical for large sites, especially when larvae are distributed throughout the area. Utilizing aircraft is often the only way to treat remote sites and those sites inaccessible by ground equipment. Calibration is simplified by the fact that target areas are often mapped, and the larvicide to be applied is usually measured or weighed when loading.

5.4.2.4 Disadvantages of Aerial Larvicide Applications

If the costs of the aircraft and aircraft maintenance are included, it is generally more expensive to aerially larvicide than to perform ground applications. To ensure accuracy in hitting the target, either additional labor for flagging or an expensive electronic guidance system is needed. As with all aerial applications, treatment windows can be narrow due to adverse weather conditions. Aerial applications also require special licenses, staff training, and additional liability insurance.

5.5 CHOOSING WHEN TO LARVICIDE

Historically, mosquito control agencies have adopted the general view that larviciding is typically not as effective or as economical as permanent source reduction but is usually more effective than adulticiding. However, this view was derived long ago when wetlands were not considered to be as important as they are today. Many of the compounds used were different as were costs in terms of money, manpower, and equipment. It was easy to assume that it was "cheaper in the long run" to move soil and change the hydrology of an area than to apply pesticides. With federal, state, and local government agencies strongly advocating that wetlands not be drained, the engineers who ran control operations had only to decide if it was "cheaper" to chemically control larvae or adults, and larval control through water manipulation won out.

The enlightened view of modern mosquito control professionals includes a strong commitment to minimizing environmental impacts. They recognize that undisturbed wetlands should remain pristine and that any disturbance will have long-term effects on non-target species of plants and animals. Source reduction in these areas should be avoided. One debate is over how to simultaneously manage mosquitoes in wetlands and at the same time maximize the wetlands' value to ecosystems. Our modern approach to mosquito control is reflected by the FMCA's commitment, along with the American Mosquito Control Association (AMCA), as a Partner in the EPA's Pesticide Environmental Stewardship Program (PESP) since the late 1990s (http://www.epa.gov/pesp/).

A successful IPM program relies on a variety of control methods and often on a combination of management techniques. As a practical matter, a director will view an agency's entire area of responsibility before making an informed decision on whether or not to employ source reduction techniques, larvicides, or adulticides to control mosquito populations. The director must carefully weigh potential risks and benefits associated with each method in an integrated program and then utilize the method that is most appropriate.

5.6 MANAGING LARVICIDE RESISTANCE

Selecting the proper class of larvicide and the formulation are both important in larval resistance management (see Chapter 10). The FDACS, AES discourages control agencies from using the same (or any) OP compound to larvicide when it or another OP is used to adulticide because this practice may lead to resistance.

Resistance also may arise by applying sublethal dosages. Many people feel that the EPA erred when it began allowing the market (cost) to dictate what the low dosage would be, despite the recommendations on the product label. Insects with inherent tolerances for weakly applied pesticides may survive to produce tolerant offspring. Soon, an entire population of tolerant mosquitoes may arise. Beyond recommended use periods, slow-release formulations may cause resistance if larvae are exposed to sublethal doses of the active ingredients. Agencies that use slow-release formulations should be aware of this possibility and monitor treatment sites.

Dame *et al*. (1998) reported resistance to methoprene in an island population of *Ae. taeniorhynchus* in Lee County after control problems were noted in areas treated with extended life (briquet) formulations. However, the problem appeared to be local. The Florida Keys had been using methoprene briquets since the early 1980s. Floore *et al*. (2002) reported no methoprene resistance in Florida Keys' *Ae. taeniorhynchus* populations at sites also controlled with slow-release formulations when control levels were compared to those for a susceptible colony.

The loss of any mosquito larvicide because of resistance would have a tremendous impact on Florida mosquito control operations. Proper product rotation – along with susceptibility monitoring – are the keys to ensuring that the pesticides currently available to mosquito control professionals remain effective for continued use.

5.7 UNDERSTANDING LARVICIDE NON-TARGET EFFECTS

Currently used mosquito larvicides, when applied properly, are efficacious and environmentally safe. Typically, there is less concern for the drift of mosquito larvicides than for the drift of adulticides, primarily due to the droplet size. Larvicides are typically dispensed aerially through spray systems producing larger droplets (300 - 400 microns) for canopy penetration, while adulticides are applied as smaller droplets (15 - 60 microns) for space spraying. Mosquito larvicides usually are applied directly into natural and artificial aquatic habitats as liquid or solid formulations, and aerial drift is negligible. Drift into water can result from tidal flushing or rainwater runoff. Under these conditions, dilution greatly reduces post-application pesticide concentration and consequently reduces exposure to non-target organisms.

It is possible to reduce non-target exposure to larvicides by using novel application techniques and new product formulations. Larviciding with machines that produce fine airborne particles, such as *Bti* applied with rotary atomizers or turbines, spreads the larvicides so that the concentration of active ingredients at any one point is minimized. In addition, these techniques may have the added benefit of allowing control agents to drift to inaccessible containers and remote aquatic habitats. Using different granular carriers, these new formulations provide better canopy penetration and larval control, while reducing the acute exposure rate for non-target organisms.

A variety of aquatic habitats and communities, ranging from small domestic containers to larger agricultural and marshland areas, are treated with larvicides. Natural fauna inhabiting these sites may include amphibians, fish, and invertebrates, particularly insects and crustaceans. Frequently, the aquatic habitats targeted for larviciding are temporary or semi-permanent. Permanent aquatic sources usually contain natural mosquito predators such as fish and do not require further treatment, unless littoral vegetation is so dense that it prevents natural predation. Temporary sites such as tidal marshes, flooded agricultural areas, and woodland depressions produce prolific numbers of floodwater mosquitoes. These sites are generally very low in species diversity due to the time needed for most species to locate and colonize them (Ward and Busch 1976, Pierce *et al.* 1991). While floodwater mosquitoes develop during the first week post-inundation, it may take several weeks for the first macro invertebrate predators to become established. Finally, many non-target species exploiting temporary aquatic habitats are capable of recovering from localized population declines via recolonization from proximal areas. Currently used larvicides, applied properly, have no known phytotoxic effects.

The use of any pesticide always involves a tradeoff between desired effects (effective control) and undesired side effects. No known larvicides are exempt from this conundrum. Even the seemingly innocuous use of predatory fish may result in an unwanted or unknown impact on an aquatic community, however temporary. More effective methodologies are needed to apply larvicides that will minimize undesirable impacts. As a group, mosquito control agencies constantly seek new and better application techniques. Mosquito control professionals are committed to the development and evaluation of new materials, as shown by the activities of numerous university, industry and mosquito control scientists around the state.

5.8 REFERENCES AND GENERAL READING

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Chapter 6

ADULTICIDES AND ADULTICIDING

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Summary

Chemical treatment for adult mosquitoes – adulticiding – is the most visible form of mosquito control. In Florida, ground and aerial applications for one or more of the state's more than 80 mosquito species may occur year round, although are most commonly used during the wet summer months of May thru October. These applications may be for pestiferous mosquitoes or for mosquitoes that vector disease. The adulticide treatments typically are space sprays of cold aerosols at Ultra Low Volume.

Adulticides used in Florida include malathion, naled, chlorpyrifos, permethrin, resmethrin, sumithrin, etofenprox, deltamethrin, and other products. The decision about which material to use is based on several factors including the efficacy as determined by scientifically conducted field trials, mosquito species susceptibility, safety, and cost. The insecticide choice is made by each mosquito control agency and varies throughout the state due to differing mosquito species and application requirements. Applications are made to coincide with mosquito flight activity so that the insecticide droplets contact the target insects and to avoid the flight activity of non-target insects such as bees and butterflies.

Training and certification are an integral part of adulticiding operations. The Florida Department of Agriculture and Consumer Services oversees the certification of public health pesticide applicators and routinely inspects mosquito control operations. This inspection checks surveillance records to verify the need for chemical applications and reviews application methods and amounts.

6.1 INTRODUCTION

Pest management techniques are many and varied: Mechanical, cultural, biological, and chemical. Treatment of adult mosquitoes – adulticiding – is achieved entirely via pesticide applications targeted to adult mosquitoes. The process of adulticiding is a step wise process that is included in an Integrated Pest Management (IPM) approach to mosquito control. Information on the biology of the pest organism is required, and thresholds must be determined before treatments begin. Once the thresholds have been met, the target is defined as flying insects, a barrier (vegetation), and/or a solid surface. Then, the appropriate equipment and chemical must be chosen, and the application must be made in a timely fashion. The chemical dose and type has a significant effect on the outcome of an application. The chemical must reach the adult mosquito through the most appropriate use of available methods.

The majority of adulticiding in Florida is conducted using the "cold fogging" technique, commonly (but erroneously) referred to as ULV (Ultra Low Volume) spraying. Space sprays are applied with specialized spray equipment mounted in aircraft, on the back of trucks or all-terrain vehicles, carried by personnel on their back, or even carried by hand. With space sprays, aerosols are released to drift through a target zone. Chemical concentrates most often are used and, even if diluted, volumes of material used remain low, thus "ULV". The aerosol persists in the air column for an appreciable length of time at suitable droplet densities to contact the flying mosquito and is only effective while the droplets remain airborne. Hence, a space spray is short-lived and is not intended nor is expected to have any residual effect.

Where a more long-term effect is required, residual spraying is employed. In this case, the mosquito is required to land on a surface deposit of the insecticide to pick up a toxic dose. Residual sprays often are referred to as barrier or surface treatments. A barrier treatment is applied to prevent adult mosquitoes from moving into an area such as a stadium, park, or resident's yard and often is applied with a modified vehicle mounted hydraulic sprayer. Interest in this technique is continuing to develop in Florida. A surface treatment is used to kill and/or exclude adults from a harborage area or resting site often around the home. Because the areas treated are generally small, handheld devices such as a backpack mist blower or a compression sprayer are employed. In Florida, surface sprays are used primarily in urban pest management scenarios but are becoming more commonly utilized by mosquito control agencies.

Adulticides are broad-spectrum pesticides that have the potential to impact non-target organisms. Space spraying relies on the prevailing meteorology to carry the pesticide as small droplets (aerosols) to and through the target area, which increases the probability of off-target drift. To minimize the potential for environmental impact, the applicator needs to understand the methods and equipment used and the potential risks involved.

This chapter discusses current and historical adulticiding and best management practices used by mosquito control programs in Florida. For a discussion on the risks and benefits of adulticiding, see Chapter 9, Mosquito Control Benefits and Risks.

6.1.1 Surveillance and Thresholds

Accurate detection and assessment of the current mosquito population is essential and is achieved through regular monitoring and surveillance programs. Mosquito numbers and distribution patterns are assessed, and this surveillance data is used to determine the area(s) to be treated. Surveillance methods to gather this data vary among mosquito control programs and are discussed in the Chapter 3, Mosquito Surveillance and Environmental Monitoring. Adulticiding is commonly utilized when larvicide and cultural control methods are not practical due to concerns about sensitive habitats, when these methods have failed, or adult thresholds have been exceeded.

When chemicals must be used, IPM strategies aim to maximize on-target deposition and minimize off-target deposition. Adult mosquito control via aerosol application is extremely complex, because it attempts to control numerous species over vast areas and changing habitats in a three-dimensional space. Thresholds, which are the keystone of most agricultural IPM programs, are difficult to establish in mosquito control. To treat or not to treat is typically a response to a nuisance level or an individual perception of the problem rather than a precisely quantifiable number of mosquitoes. Locally developed thresholds can change with time and location as rural areas are urbanized and the human population's tolerance to biting changes. When there are issues of public health, typical locally developed thresholds can be superseded by criteria described in approved emergency response plans. Section 5E-13.036 of the Florida Administrative Code ("Demonstrable Increase or Other Indicator of Arthropod Population Level") defines what minimum criteria must be used to justify adulticide applications. These include a quantifiable increase detected by landing rate or trap counts, "25 mosquitoes per trap night" or citizens service requests where the visual presence of mosquitoes has been confirmed. While most programs conduct routine surveillance in the form of trapping and/or biting counts, there is no one standard trapping method or sampling density. For instance, one program uses pure suction traps with no attraction (no light or CO_2), and they would consider catches above 100 mosquitoes per trap night to be very high, whereas other programs that do use light and CO_2 as attractants for their traps routinely catch mosquito numbers into the 1000s or tens of 1000s per trap night.

Section 5E-13.036 (3) requires that programs keep all surveillance and adulticiding records for a minimum of 3 years, thus allowing the calculation of baseline population levels for each trap/biting count location. And Section 5E-13.036 (2) requires the use of this baseline population level such that aerial adulticiding over beaches and bayshores may only be conducted when there is a demonstrable three-fold increase over a base population.

Setting a realistic trigger or action threshold for management decisions is specific to each mosquito control program and must be in compliance with Section 5E-13.036 of the Florida Administrative Code. Once all the criteria have been met to treat an area, the appropriate application may be initiated.

6.1.2 Timing

Timing is essential for space sprays to target actively flying mosquitoes. The timing needs to be precise because different species are active at different times. In general, most mosquito species targeted by space sprays fly in the crepuscular hours, and, hence, most adulticide applications occur in the crepuscular hours. Problems may arise with timing applications because:

1) the meteorology is inappropriate for good downwind dispersal

2) continuous late nights and overtime can cause personnel management problems

3) ground spray missions are typically not conducted when people are on the streets

Some targeted species are not active during the crepuscular hours. The Anopheline malaria vector, *Anopheles quadrimaculatus* in Florida, exhibit a nocturnal activity pattern. They are most active in the middle of the night when their blood hosts (humans and other mammals) are sleeping and usually exhibit no daytime activity. Florida's common domestic mosquitoes, *Aedes aegypti* and *Ae. albopictus*, are day-biters or diurnal. They tend to have peaks of activity around the hours of sunrise and sunset with less activity during the heat of the day and little to no activity at night unless disturbed by the presence of humans or other host animals. Their activity coincides with the times of highest vehicular traffic in their urban environments and poor meteorology (unstable atmospheric conditions), making aerial or truck adulticiding at this peak time somewhat impractical. However, in response to the recent concerns posed by dengue, chikungunya and Zika viruses, several programs have conducted operational evaluations of aerial adulticiding with the organophosphates naled and malathion during the post-sunset hours and have reported excellent efficacy (Lee County MCD and Manatee County MCD). Localized spot treatments with handheld equipment still remain the most common adulticiding method for these species at this time.

Meteorological parameters also influence mosquito activity and timing of the application. Some general trends are:

- Increased humidity = increased activity
- Increased temperature = increased activity (to a limit above which activity decreases)
- Increased wind = decreased activity
- Lunar illumination = increased activity and an extended activity period

The activity of some mosquito species is more affected by meteorological parameters than the activity of other species. The principal vector of St. Louis encephalitis in Florida, *Culex nigripalpus*, is a prime example; It is very sensitive to meteorological changes.

Timing of residual spraying is not nearly as critical as the timing of space spraying. Residual spraying targets the mosquito in harborage at rest on vegetation or other surfaces. An effective residual spray uniformly coats a target surface with an insecticide that will last an appreciable length of time. Applications should be conducted when conditions are conducive to provide the best coverage. Timing is not critical in relation to mosquito behavior. Instead, applications must be made to achieve the best deposit. Winds should be low or favorable to the direction of the target related to the sprayer. Conditions should be dry since though most compounds are considered rain-fast, they need time to dry.

6.1.3 Choosing the Chemical

Once the application type has been determined, the chemical to be applied and the dose rate must be selected. This decision is dictated in part by the size of the application area. For example, large area spraying with some compounds can be cost prohibitive. The habitat can have some influence. For example, the use of some chemicals may have to be restricted around waterways. The species that is being targeted also may affect the choice of compound. The comparative efficacy of one compound over another is disputable, but one thing that is known is the effect that mosquito species, habitat preference, and behavior has on ease of control. For example, *Psorophora columbiae* in open field is a species that is generally considered easy to knock down, so reduced doses may be applied. On the other hand, *Cx. nigripalpus* is a cryptic species that often is not active unless meteorological conditions are just right. Maximum label rates and perfect timing may be required to get enough of the spray cloud into the wooded areas to achieve significant control of *Cx. nigripalpus*.

Of increasing importance in the chemical selection is the issue of resistance, covered in more detail in Chapter 10: Insect Resistence Management. With the recent incursions of dengue, chikungunya and Zika viruses into Florida, the need to effectively control *Ae. aegypti* has become paramount. However, resistance to synthetic pyrethroids has been found to be common and widespread in this species in Florida, resulting in the increasing aerial use of the organophosphates malathion and naled in urban environments.

6.2 ADULTICIDES USED IN FLORIDA

Pesticides kill or alter an organism by disrupting some vital physiological function. The method by which this occurs is called the pesticide's mode of action. The most typical mode of action involves disruption of the insect's nervous system. One variation is insect growth regulators which mimic insect hormones and disrupt the insect's development. Also, soaps and oils affect the exoskeleton of the insect causing the insect to suffocate or desiccate. The mode of action of mosquito adulticides, however, is only through disruption of neuronal activity. General descriptions of the pesticide classes are provided here along with specific information on the individual compounds used in Florida.

Descriptions of individual compounds include the mode of action, general uses, and non-target toxicity data. The toxicity data is presented as an LC_{50}, the lethal concentration that will kill 50 percent of the target population. The LC_{50} is the most universal measure and allows for comparisons on relative non-target mortality between chemicals. Where possible, toxicity data was accumulated using the Re-registration Eligibility Decisions (RED) of the U.S. Environmental Protection Agency (EPA). RED data, however, are not available for all the compounds used in mosquito control. When data was not available from the RED, The Pesticide Manual (2000), a world compendium of pesticide data, was consulted, and information from this source is marked with an asterisk (*). Further comment on risk assessment, pesticide fate, and the re-registration process is outside the scope of this document.

The Florida Department of Agriculture and Consumer Service (FDACS) tracks and oversees pesticide usage by mosquito control agencies in Florida. Pesticide usage reports (in PDF format) for 2013 and 2014 are available for download at http://www.freshfromflorida.com/Business-Services/Mosquito-Control/Mosquito-Control-Reports.

6.2.1 Organophosphates - General Description

Organophosphates (OP) generally are acutely toxic and work by inhibiting important enzymes of the nervous system that play a vital role in the transmission of nerve impulses. Nerve impulses usually travel along neurons (nerve cells) by way of electrical signals. However, at the junction between two neurons (a synapse) and between a neuron and a muscle (neuromuscular junction), the impulse is transmitted in the form of a chemical substance (neurotransmitter). The neurotransmitter operating in the autonomic nervous system, neuromuscular junctions, and parts of the central nervous system is acetylcholine. In basic terms, acetylcholine fires the nerve impulse. Acetylcholine is broken down and inactivated in milliseconds by the enzyme cholinesterase. With exposure to OPs, cholinesterase is inhibited, and a build-up of acetylcholine occurs. If acetylcholine is not broken down, the nerve impulse does not stop, ultimately causing paralysis of the insect and eventually death. The organophosphates used in Florida include malathion, naled, and rarely, chlorpyrifos.

6.2.1.1 Malathion

Malathion is used for both ground and aerial adulticide applications. In FY 2013-14, malathion treatments constituted 5.0% of all the acreage sprayed by ground adulticiding but only 0.4% of the acreage treated by aerial application.

Mode of Action**:** Malathion is a non-systemic contact and stomach poison with respiratory action. Malathion is used to control Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera in a wide range of crops. It also is used extensively to control major arthropod disease vectors (Culicidae) in public health programs, ecto-parasites of animals, and for the protection of stored grain products. One disadvantage is that it has been used for a long time resulting in many cases of localized resistance. The International Program on Chemical Safety (IPCS) utilized by WHO lists Malathion as “Class III”, only “slightly hazardous”, and Malathion is one of only three actives available for vector control in the US that is on the WHO list of insecticides recommended for space spraying.

Malathion Toxicology: Class III	
Mammals	Acute oral LD_{50} for rats 390 mg/kg
Birds	LC_{50} (8d) for a ring necked pheasant 2369 mg/kg
Fish	LC_{50} (69h) for a Bluegill sunfish 30 ppb
Invertebrates	*Daphnia magna* (48hr) 1.0 ppb
*Bees	LD_{50} (topical 0.71 µg/bee)

6.2.1.2 Naled

Naled is the primary chemical used in aerial adulticiding in Florida. In FY 2013-14 naled applications constituted 84.9% of the total area sprayed by aircraft. Although labeled for ground adulticiding, no naled formulations were used for this purpose during this period.

Mode of Action: Naled is a non-systemic contact and stomach poison with some respiratory action. Naled is used to control spider mites, aphids, and other insects on many crops. It also is used in animal houses and in public health for control of insects such as flies, ants, fleas, cockroaches, and extensively for the control of mosquitoes. Naled breaks down rapidly in the environment. This product, however, is highly corrosive and therefore requires special consideration in handling and equipment design.

Naled Toxicology: Class I	
Mammals	Acute oral LD_{50} for rats 92 - 371 mg/kg
Birds	Canada goose LC_{50} 36.9 mg/kg
Fish	LC_{50} (24hr) for: Bluegill sunfish 2.2 ppb Lake trout 87 ppb Fathead minnow 3.3 ppb
Invertebrates	*Daphnia magna* 0.3 ppb
Bees	0.48 µg ai/bee

6.2.1.3 Chlorpyrifos

Only one county reported the use of chlorpyrifos to FDACS in FY 2013-14. They applied a 24.6% formulation to 1805 acres, only 0.01% of the total area adulticided by ground equipment in Florida.

Mode of action: Chlorpyrifos is a non-systemic contact and stomach poison with respiratory action. Chlorpyrifos is used to control Coleoptera, Diptera, Homoptera, and Lepidoptera in soil and on foliage. Although FDACS reports indicate that it is rarely used in mosquito control in Florida, several programs are evaluating it for use against pyrethroid resistant *Aedes aegypti* populations.

Chlorpyrifos Toxicology: Class II	
Mammals	Acute LD_{50} for Rats 97 mg/kg
Birds	Mallard duck LC_{50} 136 ppb
Fish	LC_{50} for: Bluegill sunfish 1.8 ppb Fathead minnow 0.57 ppb Atlantic silverside 0.28 ppb
Invertebrates	*Daphnia* LC_{50} 0.1 ppb
*Bees	Toxic to bees 0.07 µg/ bee

6.2.2 Pyrethroids – General Description

Pyrethroids are synthetic chemicals whose structures mimic the natural insecticide pyrethrum. Pyrethrins are found in the flower heads of some plants belonging to the family Asteracae (*e.g.,* chrysanthemums). These insecticides have the ability to knock down insects quickly. Pyrethrums can be degraded very easily by ultraviolet light which oxidizes the compounds. In general, this phenomenon leads to lower environmental risk. Pyrethroids can pose significant hazards to aquatic organisms, and the potential for build up within sediment is a concern. Pyrethroids are highly toxic to insect pests at very low rates (often one order of magnitude less than OPs). Synthetic pyrethroids have been chemically altered to make them more stable and safer to mammals. Pyrethroids are axonic poisons. They poison the nerve fiber by binding to a protein in nerves called the voltage-gated sodium channel. Normally, this protein opens causing stimulation of the nerve and closes to terminate the nerve signal. Pyrethroids bind to this gate and prevent it from closing normally which results in continuous nerve stimulation. Control of the nervous system is lost, producing uncoordinated movement and ultimately mortality.

6.2.2.1 Pyrethrum (Natural pyrethrins)

Pyrethrum is rarely used in Florida, only being reported to FDACS by two counties as a ground adulticide treating 16 acres in FY 2013-14.

Mode of Action: Pyrethrum binds to sodium channels prolonging their opening and thereby causes paralysis with death occurring later. It has a non-systemic contact action and some acaricidal activity. Pyrethrum is used to control a wide range of insects and mites in public health and agriculture. It normally is combined with synergists that inhibit detoxification by the insect. A benefit to its use is that it is considered to be a naturally occurring compound and therefore more environmentally acceptable to the general public. It also breaks down rapidly in sunlight, so it has few negative residual effects.

Pyrethrum Toxicology: Class III	
Mammals	Acute oral LD_{50} for rats 700 mg/kg for 57% ai

Pyrethrum Toxicology: Class III	
Birds	Oral LD_{50} Mallard duck 5,620 mg/kg
Fish	Toxic to fish LC_{50} (96h) for: Rainbow trout 5.1 µg/l Sheepshead minnow 16 µg/l
Invertebrates	*Daphnia magna* LC_{50} 11.6 µg/l Mysid shrimp LC_{50} 1.4 µg/l
*Bees	Toxic to bees but exhibits repellant effect LD_{50} (oral) 0.02 µg/bee (contact) 0.13-0.29 µg/bee

6.2.2.2 Resmethrin

Resmethrin is no longer being supported by the registrant, Bayer. However existing stocks can still be used, although no Florida programs reported using it in FY 2013-14.

Mode of Action: Resmethrin is a non-systemic insecticide with contact action and is a potent contact insecticide effective against a wide range of insects. It often used in combination with more persistent insecticides. Benefits include rapid mosquito knockdown properties and a low mammalian toxicity. It is photo-labile so does not persist. The disadvantages are that it is highly toxic to aquatic organisms and relatively expensive.

Resmethrin Toxicology: Class III	
Mammals	Acute oral LD_{50} for rats >4639 mg/kg
Birds	LD_{50} for bobwhite quail >5000 ppb
Fish	LC_{50} (96h) for: Rainbow trout LC_{50} 0.28 ppb Sheepshead minnow 11 ppb
Invertebrates	*Daphnia magna* 3.10 ppb Pink shrimp 1.3 ppb
Bees	Toxic to bees LD_{50} 0.063 µg/bee (contact)

6.2.2.3 Lambda-cyhalothrin

Lambda-cyhalothrin is not listed in the FDACS reporting for 2013-14, but some programs now use it for barrier treatments.

Mode of Action: Lambda-cyhalothrin is a non-systemic insecticide with contact and stomach action and repellant properties. Lambda-cyhalothrin provides rapid knockdown and has a long residual activity. It is used to control a wide range of insect pests in agriculture and public health. Benefits of this barrier product include its relatively safety margin to mammals and a long residual activity.

Lambda-cyhalothrin Toxicology: Class II	
*Mammals	Acute oral LD_{50} for rats 79 mg/kg
*Birds	Acute oral LD_{50} for Mallard ducks >3950 mg/kg

Lambda-cyhalothrin Toxicology: Class II	
*Fish	LC_{50} (96h) for: Bluegill sunfish 0.21 µg/l Rainbow trout 0.36 µg/l
*Invertebrates	*Daphnia* EC_{50} (48h) 0.36 µg/l
*Bees	LD_{50} (oral) 0.038 µg/bee (contact) 0.909 µg/bee

6.2.2.4 Cyfluthrin

Cyfluthrin is not listed in the FDACS reporting for 2013-14, but some programs now use it for barrier treatments.

Mode of Action: Cyfluthrin is a non-systemic insecticide with contact and stomach action that acts on the nervous system. It has a rapid knockdown and long residual activity. Cyfluthrin is effective against many pests in crops and also is used against migratory locusts and grasshoppers. It can be used against Blattidae, Culicidae, and Muscidae in public health situations.

Cyfluthrin Toxicology: Class II	
*Mammals	Acute oral LD_{50} for rats 500 mg/kg
*Birds	Acute oral LD_{50} for bobwhite quail >2000 mg/kg
*Fish	LC_{50} (96h) for: Golden orfe 3.2 µg/l Rainbow trout 0.6 µg/l Bluegill sunfish 1.5 µg/l
*Bees	Toxic to bees

6.2.2.5 Bifenthrin

Bifenthrin is listed in the FDACS reporting for 2013-14 as a minor use (barrier treatments) by 12 programs covering 8,513 acres, just 0.04% of the total ground-based adulticiding. However, this amount is virtually 100% of all the acreage reported for a barrier treatment only product.

Mode of Action: Bifenthrin is a pesticide with non-systemic contact and stomach action. Bifenthrin is effective against a broad range of foliar pests and is a preferred residual; However, it is not compatible with alkaline materials.

Bifenthrin Toxicology: Class II	
*Mammals	Acute oral LD_{50} for rats 54.5 mg/kg
*Birds	Acute oral LD_{50} for: Bobtail 18 mg/kg Mallard ducks 2150 mg/kg
*Fish	LC_{50} (96h) for: Bluegill sunfish 0.35 µg/l Rainbow trout 0.15 µg/l
*Invertebrates	*Daphnia* LC_{50} (48h) 0.16 µg/l
*Bees	LD_{50} (oral) 0.1 µg/bee (contact) 0.01462 µg/bee

6.2.2.6 D-phenothrin

D-phenothrin (sumithrin) is labeled for both ground and aerial use in Florida either as a stand-alone product (Anvil®) synergized with piperonyl butoxide or in combination with another pyrethroid, Prallethrin, which is added for its "excitation" properties (Duet®). In FY 2013-14 Anvil was used to treat 0.8% of the total acreage for ground adulticiding and 9.9% of the total acreage for aerial adulticiding. Duet was used to treat 1.3% of the total acreage for ground adulticiding and 1.4% of the total acreage for aerial adulticiding.

Mode of Action: D-phenothrin is a non-systemic pesticide with contact and stomach action. D-phenothrin provides rapid knockdown. It is used to control injurious and nuisance insects of public health importance and to protect stored grain.

D-phenothrin Toxicology: Class III	
*Mammals	Acute oral LD_{50} rats 5000 mg/kg
*Birds	Acute oral LD_{50} bobtail quail >2500 mg/kg
*Fish	LC_{50} for: Rainbow trout 2.7 µg/l Bluegill sunfish 16 µg/l
*Invertebrates	*Daphnia* EC_{50} (48hr) 0.0043 mg/l

6.2.2.7 Etofenprox

Etofenprox is labeled for both ground and aerial adulticiding in Florida. The total acreage treated by ground adulticiding with Etofenprox in FY 2013-14 and reported to FDACS was 274, 850 acres, 1.4% of the total for Florida. For aerial adulticiding, there were 153,003 acres treated, representing 2.2% of the total aerial acreage.

Mode of Action: Etofenprox is a so-called pyrethroid ether pesticide, whereas most synthetic pyrethroids are pyrethroid esters. However, the mode of action of both types is basically the same, being non-systemic contact and stomach action. Like most of the pyrethroids, Etofenprox provides a rapid knockdown of targeted pest species.

Etofenprox Toxicology: Class III (EU Regulatory Information)	
*Mammals	Acute oral LD_{50} for rats >2000 mg/kg
*Birds	Acute oral LD_{50} for: Mallard ducks >2000 mg/kg
*Fish	LC_{50} (96h) for: Rainbow trout 2.7 µg/l
*Invertebrates	*Daphnia* LC_{50} (48h) 1.2 µg/l
*Bees	LD_{50} (oral) 0.27 µg/bee LD_{50} (contact) >0.13 µg/bee

6.3 METEOROLOGY

Increased understanding of meteorology and the integration of detailed meteorological data into routine operations are some of the major developments in recent years. Clearly, the meteorology at time of application must be considered and, where practical, the application should wait for conditions conducive to successful dispersal of the spray.

Many new methods for measurement and logging of meteorology at the time of application are available to mosquito control programs. Networks of local meteorological stations can be accessed for free through websites such as http://weather.weatherbug.com or http://www.weatherunderground.com. Detailed information, therefore, on weather is available to all programs with a minimal fiscal outlay. At the other end of the spectrum, highly sensitive anemometers are available for installation at the office, on a local tower, or even on the application equipment. An anemometer installed on the application equipment is best since it provides detailed information at the time and the location of the application. Many aircraft are now equipped with such an onboard meteorological device, the AIMMS-20 or AIMMS-30, manufactured by Aventech. A single location meteorological source may not be appropriate for a coastal county because one part of the county may be experiencing the effects of coastal meteorology, while at the same time inland areas may be on the other side of a "sea-breeze front" and experiencing totally different weather.

Every effort should be made by programs to equip and educate themselves about the effects of meteorology on adulticiding. Given the droplet size spectrum that is applied for space sprays, meteorology is the primary parameter controlling droplet dispersal.

6.4 DROPLET SIZE

Although meteorology is considered to be the primary concern for space sprays as the cloud produced is highly subject to meteorological change, droplet size is still extremely important. Unlike atmospheric conditions, droplet size is controllable. Certain droplet sizes will be more likely to provide effective control, and other sized droplets will be lost either downwind or to the ground. It is therefore particularly important for operators to be familiar with the appropriate droplet size distribution for the application at hand. The descriptive statistics of adulticide plumes important to mosquito control relate a particular droplet size being produced to the volume proportion (volume percentage) of the cloud composed of drops equal to or smaller than the droplet size of interest.

With the implementation of new adulticide label requirements in 2006, the spray plume statistics have been changed. A spray plume descriptive statistic was needed which, like the volume median diameter (VMD), relates plume volume and drop size but that could be applied to an infinite number of volume percentages or drop sizes. The Diameter Volume (Dv) is such a statistic. The diameter volume is a decimal value between 0 and 1 which relates the volume proportion of the spray cloud to the drop diameter at which the cloud is made of drops equal or smaller to this drop diameter and whose cumulative volume equals the proportion of interest. The diameter volume for the size drop at which 50% of the spray volume is composed of drops equal to in diameter and smaller, written as Dv 0.5, represents the same value as VMD or mass median diameter (MMD). The diameter volume can provide the size drop at which 10% of the spray volume is composed of this drop diameter and smaller [Dv 0.1] as well as the 90% value [Dv 0.9]. The combination of Dv 0.1, Dv 0.5 and Dv 0.9 provides an understanding of the whole droplet size distribution that a particular spray system is producing.

The adulticide label requirements (PR 2005-1) implemented in 2006 place two requirements on the applicator:

1) Use spray nozzles for which there exists the capability to maintain the spray cloud within the Dv 0.5 and Dv 0.9 requirement of the label. For recently approved labels, typical upper limits are as follows:

   Ground Adulticiding: Dv 0.5 < 30 microns and Dv 0.9 < 50 microns
   Aerial Adulticiding: Dv 0.5< 60 microns and Dv 0.9 < 100-115 microns

2) Annually confirm that the pressure at the nozzle and nozzle flow rate(s) are properly calibrated.

During the implementation of PR 2005-1 it was recognized that the standard waved (or spinning) slide method developed by Yeomans in 1949 was still reasonably accurate for droplet calibration of truck-based cold aerosol adulticiding systems (typical Dv 0.5 of 10-20 microns) but gave erroneously small indications of droplet size for aerial adulticiding systems (typical "true" Dv 0.5 of 30-100 microns, but "measured" as 20-40 microns). The label now instructs the end-user to check with the equipment or chemical manufacturer to ensure that the systems they are using are capable of meeting the labeled droplet size requirements.

6.5 GROUND ADULTICIDING

6.5.1 Barrier Treatments

Barrier treatments work through the application of insecticides to foliage where adult mosquitoes may rest. The insecticide needs to be applied at a concentration where a mosquito landing upon the treated vegetation will pick up enough of the active ingredient through contact to cause mortality. Typically, these types of treatments are used in very limited areas to protect the public during nighttime outdoor events such as weddings, parties, and sporting events. Barrier treatments can provide control for days or even weeks depending on the insecticide formulation.

The insecticide can be applied to the foliage by one of three methods:

1. **Drenching Sprays**
   Drenching sprays are applied as a very dilute aqueous formulation typically using a vehicle mounted larvicide type system (tank, pump, hose, and spray-gun) capable of producing very coarse "raindrop" droplets of 500 to 1000+ microns in diameter. Foliage is treated with a spray-wand or spray-gun at the end of the hose to the point of runoff ("dripping"). Typically, it would take 50 gallons or more of formulation to treat an acre.

2. **Mist Sprays**
   Mist sprays are typically applied as an aqueous insecticide formulation (sometimes oil-based) as a mist type spray with droplets of 100 to 150 microns in diameter using an air blast type sprayer with either a backpack or vehicle/trailer mounted equipment such as a "Buffalo Mist Turbine." Insecticides are less heavily diluted for mist sprays than for drenching sprays. The smaller droplets in the high energy air blast (100+ miles per hour) readily impinge upon the vegetation and the surfaces that they contact.

3. **Electrostatic Sprays**
   Electrostatic sprays are applied as electrically charged insecticides that more effectively "sticks" to the barrier to which it is applied. Although not widely used for mosquito control in Florida, this method has promise due to the low volumes needed for effective coverage and the minimal waste/contamination to the ground.

The amount of barrier treatment sprays used in Florida mosquito control programs is insignificant when compared to the amount of ground adulticiding, but barrier treatments are gaining in popularity and are a significant portion of the mosquito control services offered by commercial pest control companies to property owners.

6.5.2 Space spray

Space spray ground adulticiding is the most commonly used method of controlling mosquitoes in Florida today and often is perceived by the general public as the only method in use. In 2014, mosquito control agencies reported adulticiding 19,618,312 acres by ground. This amount is 74 percent of the adulticiding acreage reported. Aerial applications accounted for 7,002,164 acres.

Ground adulticiding consists of dispersing an insecticide as a space spray of fine aerosol droplets ("spray cloud") into the air column, which then moves through the habitat where adult mosquitoes are flying. In order to be effective, the drops must contact an actively flying adult mosquito. Once the spray has deposited on the ground or has been intercepted by very fine elements of foliage (hairs and tiny leaflets), it is no longer effective because the concentration is far too small to act as contact or residual treatment. Very small droplets are necessary so that they: 1) remain airborne for a significant period of time in order to increase the probability of encountering an actively flying mosquito, 2) are large enough to have a high probability of impinging when encountering a mosquito, and 3) are not so large that they deposit out close to the vehicle in concentrations that may be harmful to non-target organisms.

Understandably, very small (aerosol) droplets rely on prevailing meteorological conditions to move to and through the target zone. As such, meteorology is one of the primary factors governing the effectiveness of a spray operation. Applications conducted in low wind and very stable inversion conditions may keep the spray cloud in the target zone for a significant period of time, but such applications may fail to penetrate densely vegetated habitats or move the spray cloud through areas with wider than normal street separations. Applications conducted in higher more turbulent winds ("neutral" atmospheric conditions) may be more effective at forcing the spray into densely vegetated habitats but also may move the spray cloud too quickly through open areas and may not target the mosquitoes in these areas since their flight activity behavior may be inhibited by the high winds. The operator/manager needs to understand these meteorological issues and the particular mosquito species/habitat that they are targeting.

Historically, two techniques of mosquito control insecticidal space spraying have been utilized: Thermal aerosol and ULV cold aerosol. Truck mounted thermal aerosol equipment has been phased out, and only a few programs now use handheld thermal aerosol "foggers" for treating very small areas. Thermal fogging is covered in this section for historical purposes.

6.5.2.1 Thermal Fog

Thermal foggers were developed largely from smoke generators built principally for concealing military maneuvers. The first units were built by a Navy contractor, Todd Shipyards Corporation. The insecticide is mixed into a fog-oil, usually with #2 Diesel or a light petroleum distillate, which is injected into a heated, often double walled nozzle. The mixture is vaporized by the heat, which may be in excess of 1000º F. A source of forced air drives this vapor out of the nozzle where the outside cooler air condenses it into a visible fog with droplets ranging from 0.5 - 1.5 microns.

If the insecticide flow does not overwhelm the vaporization capacity (sufficient BTUs/gallon/hour) of the machinery, all of the droplets will be in this near sub-micron range and often are referred to as a dry fog. If the insecticide flow is increased or the heat reduced, some of the material will not be completely vaporized, and larger droplets will be produced. The insecticide's contact time with the high temperature is so short that little if any degradation takes place.

6.5.2.2 Ultra Low Volume

Cold aerosol generators or cold foggers were developed to eliminate the need for the great quantities of petroleum oil diluents necessary for thermal fogging. These units originally were constructed by mounting a modified vortical nozzle on a thermal fogger's forced air blower. Most of the nozzles owe a great deal of homage to a design patented by the U.S. Army. The insecticide is applied as a technical material or at moderately high concentrations, as is common with the pyrethroids, which translates to very small quantities per acre (typically less than one fluid ounce) and hence, is often erroneously referred to as ULV (Ultra Low Volume, which is more correctly used to describe the application rate rather than the application method). The optimum size droplet for ground application with cold aerosols has been determined to be in the range of five to twenty-five microns (Haile *et al*. 1982). Much of the work developing and improving our understanding of this method was conducted from the mid-1960s through the early 1980s by researchers at the United States Department of Agriculture, Agricultural Research Station laboratory in Gainesville, most notably under the leadership of Dr. Gary Mount (Mount *et al*. 1998).

The sprayers in use today utilize several techniques to meet these requirements. Air blast sprayers are almost universal. They use either high volume/low pressure vortical nozzles or high pressure air shear nozzles to break the liquid into very small droplets. Rotary atomizers, ultrasonic nozzles, and electrostatic nozzles are other rarely used forms of atomization equipment. Centrifugal energy nozzles – rotary atomizers – form droplets when the liquid is thrown from the surface of a high speed spinning porous sleeve or disc. Ultrasonic equipment vibrates and throws the droplets off. Electrostatic systems repel the droplets.

6.5.2.3 Risks and Benefits of Thermal Fogging and ULV

A benefit of thermal fogging is its ability to atomize more insecticide with much less energy (BTUs) input than air blast ULV delivery techniques. Thermal fogging produces a uniform droplet spectrum of very small droplets if a dry fog is maintained. The small droplets do not settle quickly and may penetrate foliage better than the larger cold aerosol droplets. Also, the cloud is very visible, allowing the applicator to observe its movement through the area, which is particularly useful when wind indications are non-existent.

Dense enveloping fog creates a traffic hazard. Additional concerns include the amount of non-insecticidal petroleum distillates, which function only as a carrier, and their possible damaging side effects on the environment. Thermal fogs are considerably more expensive when the cost of the petroleum oil is considered. Thermal aerosols often are utilized in third world countries because the population can easily see that something is being done.

ULV cold aerosols do not require large amounts of diluents for application, making them cheaper and placing a lower petroleum product load on the environment. The spray plume is nearly invisible and does not create a traffic problem due to reduced visibility and may not be perceived as an undesirable function. The machinery to generate cold aerosols can be much simpler in design and operation than thermal foggers but requires sophisticated nozzles and, with pneumatic equipment, a great deal of energy input (horsepower) to atomize even a small flow of insecticide. A typical energy requirement would be 0.5 horsepower per ounce per minute of formulation to be atomized.

Risks associated with ULV cold aerosols include the problems related to applying any technical pesticide undiluted. The material is being handled and transported in a concentrated form. The droplet spectrum is rather wide (1 µm - 40 µm), can be difficult to change, and may settle into non-target areas more readily than a dry thermal aerosol.

Any discussion of risk versus benefits needs to note that this "space spray" form of control has been in extensive use for more than fifty years. There have not been any glaring adverse impacts attributed to ground adulticiding when done properly. Population growth along the coastal areas of Florida and the state's appeal as a tourist destination attest to the benefits of this technique and mosquito control in general.

Although ground adulticiding is the most widely used mosquito control technique in terms of acreage treated, the limitations of this method bear noting. Vehicle mounted equipment can only be effectively utilized where there is a good street network. As roads become more widely spaced in suburban and rural areas, the coverage afforded by the wind-driven spray cloud becomes diluted to the point of being ineffective. Similarly, only those properties on the downwind side of the street are treated by the spray. Dense vegetation and high building densities also may reduce the effective movement of the spray.

6.5.3 Equipment

Ground adulticiding equipment is normally mounted on some type of vehicle, but smaller units are available that can be carried by hand or on a person's back. Pickup trucks are the most common motorized vehicle for conveyance. All-terrain vehicles and golf carts are occasionally utilized for ground adulticiding with various equipment configurations.

Of the 50 organized mosquito control agencies in Florida reporting to FDACS in 2006, all but one agency listed ground adulticiding machines in their inventory of equipment. A total of 352 vehicle mounted ULV adulticiding machines were reported with programs listing as few as one to as many as 27.

Cold aerosol generators (ULV) are available in a broad range of sizes and configurations. The largest units offered by most manufacturers are often termed "heavy-duty" units and are sold as being the most applicable for community/county sized operations. This "heavy duty" label is more tied to larger flow capabilities than to the durability of the equipment. Large area operations once utilized the largest equipment available because their choice of insecticide often included malathion (over 45% usage in 1995), which required the highest flow rates (up to 8.6 ounces per minute at 20 miles per hour) and is considered to be the most difficult mosquito adulticide to atomize to label specifications. However, by 2014 malathion comprised only 5% of the total usage (in acres sprayed) for ground adulticiding. The majority (>90%) of ground adulticiding in 2014 was being conducted with various formulations of permethrin that are significantly easier to atomize to correct droplet sizes.

Most manufacturers offer a "heavy-duty" machine, typically utilizing a large twin cylinder gasoline engine (16-18 horse power) driving a rotary lobed blower. The nozzles on these machines may differ, but they all resemble the old vortical nozzle patented by the U.S. Army. The Clarke Pro-Mist Dura ULV Sprayer differs; It is an electric driven rotary atomizer type machine that operates off of the vehicle's electrical system. Only a few of these machines are used in Florida.

The insecticide metering equipment available on these machines ranges from a simple glass flow meter and a pressurized tank (only found on very old machines) to an electric pump on fixed flow machines to computer-controlled, speed correlated, event recording, and programmable flow management systems. The fixed flow units are designed to be operated with the vehicle traveling at a constant speed. Most of these utilize 12-volt laboratory type pumps that are quite accurate.

Variable flow metering systems regulate insecticide flow relative to the distance the vehicle travels and are therefore forgiving of speed irregularities. The majority of all truck-mounted cold aerosol generators used in Florida are equipped with speed control variable flow metering systems. Vehicle monitoring systems record vehicle speed and insecticide pump operations over time. This information may be incorporated with the flow control systems to provide complete spray operations management systems.

Historically, many programs constructed their own ULV adulticiding machines from off-the-shelf components. Some of these machines were built from new pieces, but other machines were fabricated from scavenged equipment that began life elsewhere. They may have been locally made for economic reasons or to customize a certain function for a particular operational need. Many, if not most, of these "home-built" machines are being replaced by commercially manufactured units incorporating the many improvements and new technologies available today.

Several manufacturers now produce a mid-range machine in the eight to twelve horsepower (or equivalent) class, as well as a few even smaller (<six horsepower) machines. These units are more compact, lighter, and typically use less fuel than their larger relatives. The atomization capabilities of the machines in this class are normally sufficient for many of the pesticides now being used (lighter, lower flow-rate pyrethroid formulations), particularly at the ten miles per hour rates. All of the flow systems available for the larger units may be fitted to this class machine as well. These smaller machines are suitable for use on golf carts or all-terrain vehicles.

Several handheld, 2-cycle engine driven, ULV sprayers are available for spot treatments. Several units are configured as backpack sprayers with the engine/blower mounted on a pack frame connected to a remote nozzle with a hose. These units utilize an orifice to control flow and either an aspirating or a gravity feed to supply the insecticide. With the high cost of these relatively small, simple machines, several programs have now taken to manufacturing their own handheld equipment.

6.5.4 Training and Maintenance

Operators of adulticiding equipment must be trained not only in the proper use and maintenance of the pesticide equipment but also in the proper application of the insecticide that they are using. Pesticide labels specify application details including acceptable droplet spectrum, flow rates, application rates, areas to avoid, and target insects. The law requires that any operator be certified in the Public Health category through FDACS or be supervised by a licensed person. A certified applicator may supervise up to ten operators. Some programs have all their personnel certified including office staff.

The Florida Mosquito Control Association (FMCA) Dodd Short Courses regularly offer programs designed to educate operators, mechanics, and supervisors in the proper techniques of calibration, maintenance, operation, and scheduling of spray activities. FDACS encourages agencies to budget course fees and travel monies to attend the courses every year.

6.6 AERIAL APPLICATIONS

In 2014, there were twenty-one operational mosquito control programs in Florida that conducted aerial adulticiding. Fifteen programs own/lease their own aircraft, and six programs contract with one of several private aerial applicators. They have chosen aerial application as a very effective means of controlling adult mosquitoes particularly in inaccessible areas. Some of the agencies base almost all of their operations on this form of application. Normally, adulticiding would not be the primary operational response. Where aerial adulticiding is the primary response, it is because:

- permits to construct new source reduction projects are essentially unobtainable
- larviciding is most effective when a high percentage of the mosquito production sites are regularly treated which may be difficult and expensive
- aerial applications may be the only reliable means of getting effective control if the areas lack a network of roads

Aerial adulticiding may be the only means of covering a very large area quickly during severe nuisance mosquito outbreaks (particularly after natural disasters such as hurricanes) or vector- borne disease epidemics. One of the advantages of aerial application of organophosphates (naled and malathion) is that the pesticide labels permit as much as five times the amount of toxicant to be applied by air as by ground. An example is the Dibrom (naled) label with a ground maximum rate of 0.198 ounce per acre but an aerial maximum of one ounce per acre (over 5 times the ground rate). This opportunity for aerial operations biases it heavily toward better levels of control. However, this advantage does not apply to the synthetic pyrethroids (permethrin, resmethrin, and D-phenothrin), as the per acre rates for these products are the same for both ground and aerial applications.

Aerial applications are expensive due to the pesticide costs per acre, the high cost of owning and maintaining (or leasing) aircraft, and the inherent increased salary for professional pilots. Low level flying, most often conducted during the night hours to coincide with peak mosquito activity, is a dangerous activity requiring a high degree of skill and professionalism, particularly considering the many obstructions such as towers, high rise buildings, and construction cranes. Flying also is very dependent on good weather conditions. Due to the commitments for any spray mission, decisions are given much thought and are commonly scheduled when adult mosquito population levels are at their peak.

Three aerial adulticiding techniques have been used in Florida: low volume spraying, thermal fogging, and ULV aerosols.

Low volume (about a quart per acre) sprays were commonly applied with the pesticide diluted in light petroleum oils and applied as a rather wet spray with a large droplet size. Their effectiveness as a localized residual treatment was negated by problems of spotting car paint or anything else left outside. The size of the droplets reduced drift, thus limiting swath widths, and was not ideal for impinging on mosquitoes. This technique which would be the aerial equivalent of ground applied barrier treatments, has not been used for some time and is not permissible with the small droplet sizes required by today's label language.

Thermal aerosol applications used the exhaust heat of the aircraft's engines (including the helicopter's turbine) to atomize a very dilute mixture of petroleum oil and insecticide. These applications were popular with pilots who easily could see where the spray plume was drifting. It also was an efficient means of producing a very small droplet and tight spectrum. The small droplets would remain airborne much longer than larger droplets and at very high densities, thus increasing the probability of impact with a flying mosquito. However, the large quantities of fog oil required larger heavy lift aircraft and even then limited the area that could be covered economically to about one-tenth that of the area covered by ULV applications. The insecticide mix needed to be completely atomized, or larger oil droplets could potentially put a sheen on the water beneath the flight path. The amount of petroleum oil dispensed as a carrier may have created environmentally undesirable effects. Although still included on some product labels, aerial thermal aerosol applications are rarely if ever used today.

The primary aerial adulticiding technique in use today applies the insecticide in a technical concentrate or in a very high concentration formulation as an ULV cold aerosol (Mount 1996). Lighter aircraft, including helicopters, can be utilized because the insecticide load is a fraction of the weight of other techniques. As with ground adulticiding with a cold aerosol generator, the intent of this method of aerial adulticiding is to produce a spray cloud of very fine droplets (aerosol) that moves through the target zone and kills any mosquitoes upon which the droplets impinge. Aerial adulticiding applies the spray well above the target zone, unlike ground adulticiding which sprays within the target zone. Aerial adulticiding relies on a number of different phases of particle (=droplet) movement to bring the spray cloud down into the target zone (within 50 feet of the ground). These phases are:

1. **Aircraft Vortices**
   Fairly soon after being emitted at the nozzle, spray drops are caught up or "entrained" within the wingtip or rotor wash vortices. This energetic turbulence produced by the aircraft sinks toward the ground taking the spray cloud with it before dissipating. The descent distances and life of the vortices before decay vary between aircraft and atmospheric conditions (stable, neutral or unstable) but typically drop 30-50 feet from the aircraft and last several minutes. In some aircraft under very stable atmospheric conditions, vortex descent distances can exceed 100 feet and last more than five minutes.

2. **Atmospheric Dispersion**
   Once released from the dissipated vortices, the spray cloud is now subject to general atmospheric turbulence, which dilutes the spray cloud through vertical spread as well as horizontal (wind) movement. The degree of atmospheric turbulence (vertical spread) is related to the stability of the atmosphere. In a highly stable atmosphere under inversion conditions typical of low wind nights, the vertical spread is minimal, resulting in the spray cloud hanging together as a fairly concentrated plume. In neutral to slightly unstable conditions typical of windy overcast nights without inversion conditions, the vertical spread is significant, bringing the spray cloud down towards the ground much faster and also diluting the spray cloud concentration.

3. **Droplet Sedimentation**
   Spray droplets also are subject to the forces of gravity, sinking toward the ground with a sedimentation velocity related to their size and density. For example, a 10 μm diameter drop would take 2.8 hours to fall 100 feet, a 20 μm drop would take 42 minutes, and a 50 μm drop would take only 6.7 minutes. Under stable nighttime conditions with little vertical movement in the atmosphere (typical night spray conditions in Florida), the sedimentation velocity may play a significant role in the droplets movement towards the target zone (near the ground).

The flight parameters for aerial adulticiding differ by program and by technique. Some mosquito control programs fly during the hours of daylight so their applications begin either at morning's first light or before sunset and work into twilight. At these times, the pilots should be able to see towers and other obstructions, as well as keep track of the spray plume. This timing makes it safer for the aircraft to be flown at less than 200 feet altitude which may make it easier to hit the target area. Although potentially safer and more comfortable for the pilot, this period may not coincide with the times of peak mosquito activity, thereby reducing the effectiveness of the spray.

Other operations fly in the dark of the night, typically after twilight or early in the morning before dawn. The aircraft typically are flown between 200 and 300 feet altitude, which is not ideal for accurate targeting of small spray blocks but is more appropriate for the treatment of larger (10,000+ acre) areas. A few programs utilize night vision goggles, allowing for safer low altitude (100-200 feet) applications during nighttime hours. Most mosquito flight activity is crepuscular, so these flights catch the adults at their peak activity. Bees are not active prior to full daylight so should not be at risk of serious impact from the insecticide application.

Application altitude not only has an impact on the accurate targeting of small spray blocks but also affects the insecticide deposition levels more than might be expected. Lower application altitudes result in higher deposition levels with the potential for non-target impacts. This phenomenon has been recognized by the EPA which now requires minimum application altitude statements on all new labels – 75 feet for helicopters and 100 feet for fixed wing aircraft.

Swath widths also vary from operation to operation but normally are set somewhere between 500-1,500 feet. Swaths are flown as close to perpendicular with the wind as is possible, working into the wind and commonly forming a long, tight S pattern. Many factors affect the spray drift offset, the horizontal distance traveled between spray being released at the aircraft and reaching the target zone closer to the ground. Pilots rely to a degree on experience for determining this offset, although there are now Global Positioning System (GPS) guidance systems with built in computer spray modeling which provide reasonable estimates for accurate placement of spray clouds. Spray drift offset distances can vary from less than 1,000 feet (low level applications in light winds) to greater than 7,000 feet (high altitude and/or moderate winds).

The relative importance of the three phases of droplet movement – Aircraft Vortices, Atmospheric Dispersion, and Droplet Sedimentation – depends upon the type of operational application. Low level (<100 foot altitude) treatments conducted during the dawn hours to target narrow mosquito harborage habitats (such as mangrove forest shorelines) rely almost exclusively on the aircraft vortices to move the spray into the target area. While this method has the ability to target a small area, it also runs the risk of creating a potentially damaging deposit peak close to the flight line. High altitude (300 foot altitude) nighttime applications rely on a combination of all three phases but more significantly on atmospheric dispersion and sedimentation. High altitude sprays have a higher probability of spray drift outside the intended target zone, but the spray deposit concentration is likely to be below a level that could be considered biologically significant.

It may be for this particular reason that droplet sizes that are effective for ground adulticiding (Dv 0.5 of 12-20 microns with droplet sizes of 5-25 microns) do not appear to be most effective for aerial adulticiding (according to anecdotal reports from several programs utilizing small droplet high pressure spray systems in aircraft). Although not conclusively proven at this time (there are several research projects being conducted to answer the question), it may be that the most effective droplet sizes for aerial adulticiding are in the range of 15-50 microns with spray cloud Dv 0.5 of 25-40 microns depending on operational parameters, target species, and target habitat. Label language sets the upper limit for droplet size, described by the terms Dv 0.5 and Dv 0.9. Since the introduction of PR Notice 2005-1, all aerial adulticiding labels contain standard language setting the upper droplet size limits at a Dv0.5<60 microns and a Dv0.9<100-115 microns.

6.6.1 Equipment

The only aerial thermal fogging equipment still in existence in Florida is at the Lee County Mosquito Control District, and these systems are rarely, if ever, used. The spray apparatus consists of a series of large nozzles arranged in a radial pattern directing the insecticide/oil mix into the engine's hot exhaust. The tanks are quite large, 800 gallons for a C-47 and 300 gallons for a Huey helicopter.

ULV systems are as diverse as the aircraft on which they are mounted. Many fixed wing twin engine aircraft and helicopters are equipped with external belly tanks suspended under the fuselage, cabin, or in pods under the wings (BN Islander). Other programs install their insecticide tanks within the aircraft's passenger compartment. Some of the tanks are commercial fiberglass units, but most tanks are custom fabrications of stainless steel, polypropylene, or fiberglass. One operation utilizes the aircraft's own auxiliary fuel tanks which were separated from the fuel system and now carry an oil/pyrethroid mix. Some units are equipped with in-flight (or return flight) tank flushing capability. Tank capacity ranges from 350 gallons for a C-47 to 20+ gallons for a Hughes 269A helicopter.

Much of the following information describing “typical” aerial application equipment comes from observations of the diverse systems at the FMCA Aerial Short-Course programs held annually in Florida over the past 20-25 years.

A wide variety of aircraft are utilized by mosquito control programs including both helicopters and fixed wing aircraft. Most aircraft are owned by the local mosquito control agencies, while some aircraft are contracted. One program contracts for pilots but owns the aircraft. Although many mosquito control agencies developed aerial application programs by acquiring inexpensive government/military surplus aircraft, the majority of the agencies now are purchasing and using newer (or brand new) civil aircraft.

6.6.1.1 Fixed Wing Aircraft

Fixed wing, multi-engine aircraft account for most of the acreage aerially adulticided in Florida. They have a reasonable payload and are moderately fast, economical to operate, and practical to maintain. Aircraft use and selection is changing in Florida. Older military cargo planes (C-45 and C-47) which carried the heavy payloads required for the dilute formulations used in thermal aerosol applications are being replaced by smaller, newer, and more practical aircraft capable of treating the same or greater acreage using adulticide concentrates applied as ULV aerosols. In the mid-1990s there were four programs that used DC-3/C-47’s, but currently there is only one program, Lee County

MCD, and their aircraft have been modified with turbine engines. A common replacement for these larger old aircraft is the Beechcraft KingAir, a mid-sized turboprop common in commercial aviation as an inexpensive short-haul executive transport.

Light general aviation twins (including Cessna 336, Piper Aztec, and Britten Norman Islander) may be smaller but still have payloads suitable for ULV spraying. They can be economical to purchase and operate, simple to maintain, nimble to fly, and somewhat less conspicuous when spraying. The fuel consumption of a smaller light twin engine plane may only be 30 gallons per hour, but the useful payload is limited to about 1,000 pounds.

6.6.1.2 Helicopters

Helicopters are seeing wider use for adulticiding activities. Many programs that operate helicopters for larviciding change the spray equipment and use the helicopters to adulticide. Additionally, programs utilize helicopters for adulticiding smaller areas with difficult obstructions or meandering shapes and also operate fixed wing aircraft for larger spray blocks. Helicopters are capable of much tighter turns, are more maneuverable, and can be serviced at field sites thus reducing ferry times. However, their operating costs are far more expensive than fixed wing aircraft. They may be considered safer, but autorotations during an engine failure at low level may be beyond recovery. No twin engine helicopters are used in mosquito control because the increased acquisition and maintenance costs far exceed the added benefits. Air speeds are somewhere between 60 knots for piston engine ships and 100 knots for the faster light turbines.

6.6.1.3 Inventory of Aerial Adulticiding Aircraft in Florida

Organized mosquito control programs have reported ownership of the following aircraft ownership to FDACS:

16	**Fixed Wing Aircraft**
2	Douglas DC-3/C-47 (Twin Turbine Conversion)
1	Beech King Air C90 (Twin Turbine)
1	Beech King Air A90
4	Beech King Air 200
2	Piper Aztec (Twin Piston)
2	Britten Norman Islander BN2T (Twin Turbine)
1	Britten Norman Islander BN2 (Twin Piston)
3	Shorts SkyVan (Twin Turbine)

33 Helicopters	
2	Bell UH-1B (Single Turbine)
6	Airbus H125 (previously named the AS350 B3e, Single Turbine)
12	Hughes/MD 500 C,D,E&N (Single Turbine)
7	Bell 206 (Single Turbine)
2	Bell 407 (Single Turbine)
2	Hughes 269 A (Single Piston)
2	Eurocopter Astar B3 (Single Turbine)

6.6.2 Training and Requirements

Pilots operating aircraft spraying for mosquitoes must hold a Public Health Aerial Applicator's certification issued by FDACS. The Aerial Training Committee of the FMCA educates personnel involved with aerial operations about new developments, demonstrates calibration procedures, and brings experts from related fields to special work sessions. In the wake of 9/11 and the potential use of agricultural spray aircraft by terrorists, all spray aircraft must be registered with FDACS each year and must be well secured at their home location. Any sale also must be reported to FDACS.

All aircraft applying insecticides are operated under the Federal Aviation Administration (FAA) FAR Part 137 (Agricultural Aircraft Operations). However, government owned aircraft may be operated as public aircraft, a category originally intended to allow government entities to use surplus non-certificated military aircraft. Public aircraft are exempt from a number of the provisions of FAR Part 137 including the need for a Part 137 Certificate and the need for an Airworthiness Certificate. It should be noted that most, if not all, aircraft flown by mosquito control programs are maintained according to the FAA's strict maintenance requirements as if they were civil aircraft rather than the more lax requirements of public aircraft. Private aerial applicators cannot operate as public aircraft and must conform to the full provisions of FAR Part 137. These aircraft have a certificate of airworthiness and are maintained, modified, and flown in strict conformance to the FAA's regulations governing civil aircraft.

Part 137.51(4)(iii) states: "No person may operate an aircraft over a congested area during the actual dispensing operation, including the approaches and departures for that operation, unless it is operated in a pattern and at such an altitude that the aircraft can land, in an emergency, without endangering person or property on the surface." This statement might be interpreted to mean that any aircraft other than a helicopter must be multi-engine (in case one fails) if spraying over congested areas.

6.7 TECHNOLOGICAL IMPROVEMENTS, GUIDANCE SYSTEMS, AND DOCUMENTATION

Ground adulticiding by truck mounted equipment always has had a simple form of geographical reference since the vehicles were driven on street networks. Drivers could follow paper maps with delineated spray zones for guidance. Aircraft, however, do not follow streets and must rely on other forms of reference for flight line guidance. In the early days "flagmen" with flashing beacons on vehicles were used to mark the beginning or ending of a spray run. This arrangement required streets at the edges of the spray block, the ability of the driver to move to the correct marking position, and a lack of potentially conflicting flashing beacons (*e.g*., emergency vehicles). Some aerial programs simply relied on the pilots ability to read a map, recognize spray block boundaries by visual cues, and estimate the correct spray line separation (be it 500 feet, 1,000 feet, or some other distance).

Based on radio signals triangulated between towers, LOng RAnge Navigation (LORAN) systems were used from the 1970s through the 1990s but suffered from signal interference particularly around dawn and dusk (meteorological effects). However, they were useful for flying large rectangular blocks with accurate swath separation since many incorporated a "grid" program developed for search and rescue flights. The pilot simply flew a known baseline (spray block downwind edge), entered the desired swath width, and the instrument gave guidance to succeeding flight lines.

In the early 1990s GPS became available and affordable. Navigation was based on signals from geostationary satellites and did not suffer from the same interference as LORAN. Even basic units are accurate to within 30 feet, providing the federal government does not re-introduce an error, "Selective Availability", during times of heightened national security. Systems are designed specifically for aerial application (including agriculture, forestry, and mosquito control) with the "grid" spraying program, flight recording, and the ability to upload irregular shaped spray blocks, "no spray" zones, and obstructions (towers) onto a moving map screen for pilot viewing and guidance. They are usually equipped with a light bar mounted in the pilot's field of view that indicates how far off track the aircraft might be so that the pilot does not have to look down at a map screen.

All of these improvements in technology allowed a pilot to fly a very accurate flight path within a defined spray block, but mosquito control programs still relied on a "best guess" as to where to fly (what offset distance) to ensure that the spray effectively covers the target zone. The U.S. government embarked on a program to develop computer models that could predict spray drop movement after leaving an aircraft. These models incorporated all the complex interactions between aircraft type, size, weight, nozzle position, formulation, droplet size, spray altitude, meteorological conditions (wind, temperature, humidity, atmospheric turbulence), and other parameters to give a reasonable estimate of spray deposit and drift. The original program was known as FSCBG (Forest Service, Cramer, Barry, Grim) and incorporated a "modeling engine" known as AGDisp (Agricultural Dispersal). The Spray Drift Task Force, a consortium of pesticide manufacturers and regulatory agencies, spent millions of dollars validating the AGDisp model and released a regulatory version known as AGDrift. This model is used by the EPA for pesticide application risk assessments. The AGDisp and AGDrift models are relatively simple and can be run on a desktop computer to view the effects of changing operational parameters (including droplet size, wind speed, spray altitude) on the eventual drift and deposit of the spray and allow the user to estimate the correct offset distances to use under a given set of conditions (Latham 2004).

In recent years, GPS guidance system manufacturers have taken this work a step further by incorporating the AGDisp modeling algorithms into the onboard computer processing unit to create, in essence, an "intelligent" unit that takes real-time information and calculates offset distances and correct flight paths for the pilot. The pilot simply flies the headings indicated by the unit on his light bar. The unit also takes into account "no spray zone" avoidance using the same process. The meteorology necessary to run the model in real time can be obtained from one of a number of sources: A fixed weather balloon (Kitoon), a tower based system that broadcasts the data to the GPS via a spread spectrum modem, or a meteorological probe mounted on the aircraft that calculates the meteorological conditions (wind speed, direction, temperature, and humidity) at the aircraft. Alternatively, wind information from another source can be manually entered by the pilot into the system. Further improvements include flow control valves that can automatically turn the spray on and off based on the aircraft's position within the spray block or regulate the flow based on aircraft speed or special remote sensing in the system.

Improvements also have occurred in technology for truck-mounted ground adulticiding equipment. Different forms of speed regulated flow control are common in many systems. Automated GPS systems with fully functional mapping and guidance systems are available. These units can be preprogrammed to turn the spray on or off at different locations, enabling automatic avoidance of no spray zones and ensuring that double spraying of streets (particularly cul-de-sacs) does not occur. These improvements have reduced the probability of human error in misapplications of pesticides, although it should be noted that failures and errors can occur in even the most sophisticated technologies.

FDACS reporting system requires records on the total acres sprayed and total chemical used for any application of pesticides. However, most operations keep more detailed records of their adulticiding missions, especially with aircraft/vehicle GPS recording devices that provide accurate information on location, time, and spray status (on or off), usually at one second intervals. Many units also record flow rate and meteorological information. Additionally, programs should include relevant adult mosquito sampling information (*e.g.*, landing rates and trap counts, preferably both pre- and post-spray) or at least a reference to where this information is retained. See Rule 5E-13.06(5) of the Florida Administrative Code.

6.8 DRIFT AND DEPOSITION MANAGEMENT

An effective insecticide application in mosquito control provides maximal target control and minimal non-target mortality. Two avenues are available for non-target impacts in mosquito adulticiding: 1) drift of the insecticide outside of the intended spray zone and 2) deposition to the ground.

Defined as the movement of spray material outside of the target area, drift is a negative term when used in pesticide application technology. However, many mosquito control professionals use the term 'drift' incorrectly to describe the general movement of mosquito control adulticide sprays, putting a negative slant on the correct use of these operational applications. Drift from an agricultural application is a negative term and is not relevant to mosquito control spray applications. While a mosquito control aerosol cloud is inside the intended application zone, the spray is actively controlling mosquitoes. Mosquito control aerosols should only be termed "drift" when they move into a no spray zone with potentially negative consequences.

Ground truck spraying applies a smaller volume of pesticides than aerial spraying, and the droplet size distributions are much smaller, reducing concerns for non-target communities. However, if the conditions are not consistent for the projected downwind dispersal, reduced concern may be misplaced.

Both drift and deposition effects must be considered and taken into account in aerial applications. Drift into a no spray zone can occur when conditions are beyond normal limits or incorrect operational parameters are used. Over the past decade, the use of GPS to precisely locate operations in relation to no spray areas has improved application efficiency. Moreover, computer models (AGDisp) integrated with real time meteorological data are available to calculate downwind dispersal of the spray. These models are accepted by the EPA and are being continually improved upon for mosquito control. The calculation and estimation of long range movement can be provided by models, and these models are being validated by dedicated research projects (Dukes *et al*. 2003 and 2004).

Deposition is the other avenue for non-target impacts; Minimization of deposition is far more critical than off-target drift. If off-target drift does occur, the spray cloud usually is so diluted the probability of impact with a non-target is low. Deposition, however, can occur in peaks close to the flight line, particularly with large droplets and low spray altitudes. This peak deposition has the potential to be problematic for non-target organisms.

Deposition can be controlled by droplet size. The larger droplets deposit quickly rather than move downwind. Mosquito control obtains no benefit from these larger droplets as deposition is waste. It is therefore within everyone's interest to minimize this depositing fraction of the spray. The EPA has refined its stance on this issue and created an extra required measure of droplet size distribution on the labels (PR 2005-1).

Historically, labels only required that the spray be below a maximum Dv 0.5, but now labels require that the spray must be both below a maximum Dv 0.5 value and a maximum Dv 0.9 value. The addition of the Dv 0.9 value now means that the upper end of the spray distribution can be limited so that spray systems producing the larger depositing drops that could impact non-targets are excluded. This change is a profound step forward for mosquito control; Adherence to this rule leads to more effective mosquito control and minimizes non-target impacts.

Deposition also is affected by altitude. Aircraft have wake effects and create vortices which entrain the spray, bringing it down in a concentrated plume from flight altitude. Although vortices descent distances and life are limited, if the flight altitude is low enough, the spray cloud can be placed close to the ground as a concentrated plume, resulting in high deposition peaks that could be potentially damaging to non-target organisms. Downwind movement and deposition of the spray can be calculated and viewed using AGDisp, the primary spray-fate model for long distance movement of aerially applied pesticides. This model has been adopted by the EPA and is used as a regulatory tool with other eco-toxicological models such as PRISM EXAMS to conduct risk assessments during the registration and re-registration of mosquito control pesticides. These tools can improve the efficacy of our operations and further minimize the potential for unintended non-target impacts.

This chapter describes the current technology used in mosquito adulticiding. In the last three decades, Florida mosquito control professionals have focused their attention on technological developments that locate, measure, and record mosquito control activities. With the re-registration of mosquito control products, the mosquito control industry is in a state of flux. However, the close relationship between the operators and researchers within this industry provides a unique ability to stay at the forefront of all changes and challenges. In this highly sensitive discipline there is no room for "Status Quo." Mosquito control techniques need to be the best available. As new technologies and changes in understanding arise, mosquito control managers need to incorporate them into operations in order to truly be integrated pest managers.

6.9 REFERENCES AND GENERAL READING

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Chapter 7

BIOLOGICAL AND ALTERNATIVE CONTROL

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Summary

Biological control is a significant component of most integrated mosquito management programs in Florida. Currently, the most widely used biological control agents are the bacteria Bacillus thuringiensis *subsp.* israelensis *and* Lysinibacillus sphaericus *(previously known as* Bacillus sphaericus*). Spinosad, a fermentation product from the Actionmycete bacterium* Saccharopolyspora spinosa*, also has become part of mosquito control over the past decade. Predatory fish, primarily* Gambusia *species, also are often used in manmade habitats. Recent technologies, including* Wolbachia *based biocontrol strategies and the release of genetically engineered mosquitoes carrying lethal genes, hold promise and are being field tested to combat domestic mosquito vectors. Continuous support from federal, state, and local agencies for biological and alternative control research is necessary to ensure the continued effectiveness of mosquito control efforts. Also see Chapter 5: Larvicides and Larviciding, 5.2.2 Microbial Larvicides.*

7.1 INTRODUCTION

The use of living organisms (predators, parasites and pathogens) or their byproducts to suppress populations of arthropod pests and disease vectors, such as mosquitoes, is known as biological control, or biocontrol (Eilenberg *et al*. 2001). Biologically based pest management includes the use of biorational insecticides (bioinsecticides) or environmentally benign toxins that do not persist in the environment produced by bacteria such as *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and *Lysinibacillus sphaericus* (*Ls*) to control the pests/vectors (Eilenberg *et al*. 2001). Biological control can involve four strategies: Classical, inoculative, inundative, and conservation biological control (Eilenberg *et al*. 2001, Hajek 2004). The overall premise is simple: Organisms that attack pests are grown in a laboratory or brought from the pest's native origin and then released into the environment to control target pest species. Releases can be inoculative, where the biocontrol agent is released in low numbers and allowed to multiply naturally, or inundative, where massive amounts of the control agent are released to control the pests usually with no expectation that the biocontrol organism will reproduce and persist naturally. Additionally, biocontrol efforts also can involve enhancement of existing naturally occurring enemies of the pests in areas where control is desired. While the vast majority of successful biocontrol programs are in agricultural systems in relatively more predictable habitats, very few biocontrol agents, with the exception of *Bti* and *Ls*, have been implemented in large-scale mosquito vector control programs (Thomas 2017, van Lenteren *et al*. 2017). One of the challenges of using biological control agents for vector control has been lack of efficient delivery mechanisms for operational purposes and scale (National Academies of Sciences and Medicine 2016, Thomas 2017). In addition, there are historical instances where some biocontrol agents did not work as intended and resulted in unintended environmental consequences (Howarth 1991).

Biocontrol is popular because of its potential to be host-specific with little or no effect to non-target organisms. In Florida there has been a re-emergence of *Aedes aegypti* and diseases it can vector; This insect has been difficult to control and in certain areas has developed resistance to the very limited adulticides available. This situation has created a potential market for alternative control strategies. A detailed discussion of the intricacies of biocontrol/alternative control strategies or of the many environmental and biotic factors that influence their effectiveness is beyond the scope of this white paper. Comprehensive reviews of biological and alternative control strategies to control mosquito vectors worldwide can be found here (Bukhari *et al*. 2013, Alphey 2014, Benelli *et al*. 2016, Huang *et al*. 2017, Thomas 2017, van Lenteren *et al*. 2017). The objective of this chapter is to update knowledge on the biocontrol and alternative control strategies that are currently used or have potential to be used in the near future by Florida mosquito control programs.

7.2 USE OF BIOLOGICAL CONTROL AGENTS

7.2.1 Microbial Control Agents

Bacillus thuringiensis* subsp. *israelensis

The mosquitocidal bacterium *Bacillus thuringiensis* subsp. *israelensis* is the most extensively used bacterial-based biological control agent in Florida and worldwide. These soil-dwelling bacteria produce mosquitocidal crystal toxins during sporulation. Mosquito larvae ingest the toxins during filter-feeding, and the toxins are activated by the mosquitoes' high gut pH and midgut proteases (Gill *et al*. 1992). The activated toxins kill the mosquitoes by degrading the internal tissues (Bravo *et al*. 2007, Ben-Dov 2014). Despite a long period (~ 4 decades), usage of *Bti* to control pestiferous nematoceran dipterans, including mosquitoes, blackflies, and midges, no widespread resistance and non-target impacts directly attributable to *Bti* have been reported to date when used at recommended label rates (Lacey 2007, Ben-Dov 2014, Lacey *et al*. 2015, Duguma *et al*. 2017b, Lawler 2017).

Lysinibacillus sphaericus (formerly known as *Bacillus sphaericus*)

Lysinibacillus sphaericus is another frequently applied microbial control agent in Florida. It has a relatively narrow host range compared to *Bti* and is effective against *Culex*, *Anopheles,* and some *Aedes* mosquito species (Berry 2012, Lacey *et al*. 2015). For example, it is not recommended for the control of *Ae. aegypti* (Berry 2012). High levels of resistance to *Ls* in the field were reported in mosquito populations in different countries (Rao *et al*. 1995, Silvafilha *et al*. 1995, Yuan *et al*. 2000, Nielsen-Leroux *et al*. 2002, Su and Mulla 2004, Lacey *et al*. 2015). These findings strongly suggest a need for novel mosquitocidal bacteria with complex toxins having different modes of actions. One way to overcome this problem is to make recombinant bacteria with improved efficacy. A good example of this approach is the construction of *Bti* producing *Ls* toxins with higher potency than the wild-type *Bti* (Park *et al*. 2005, Wirth *et al*. 2010). Alternatively, research is needed to isolate novel and more potent strains of *Bacillus* or other bacteria.

Spinosad

Spinosad is a mixture of compounds naturally derived from the soil Actinomycete bacterium *Saccharopolyspora spinosa* during fermentation (Duke *et al*. 2010). It consists of two major larvicidal toxins Spinosyn A and D, with the former being the dominant component of the mosquito larvicidal formulation. Spinosad is absorbed by mosquito larvae via contact and ingestion and induces nervous system paralysis and eventually death (Duke *et al*. 2010, Hertlein *et al*. 2010). While Spinosad has been widely used for control of agricultural crop pests for over three decades, it has only been used to control mosquitoes of public health concern and other nuisance pests in the past decade (Hertlein *et al*. 2010). Spinosad received the U.S. EPA Presidential Green Chemistry Award for its favorable environmental profiles (Hertlein *et al*. 2010). Spinosad products have been shown to be effective against a broad range of mosquito species including *Aedes, Culex* and *Anopheles* species (Bond *et al*. 2004, Jiang and Mulla 2009, Allen *et al*. 2010, Marina *et al*. 2011, Lawler and Dritz 2013, Marina *et al*. 2014, Su *et al*. 2014, Dias *et al*. 2017). An outdoor mesocosm experiment using the slow-release Natular G30 formulation at a recommended label rate showed a control of freshwater Florida mosquitoes for more than a month (D. Duguma, unpublished data). Spinosad has become an integral component of larval control in Florida due to its efficacy on a wide range of larval mosquito species.

Wolbachia-based Control Strategies

Wolbachia-based biological control strategies are one of the most promising mosquito vector controls that have been in accelerated development over the past 5-10 years. Although the idea of using *Wolbachia* as a biological control agent goes about six decades back when it was first used to suppress *Culex quinquefasciatus* (then known as *Cx. fatigans*) populations in Myanmar (then Burma) during a multi-week trial (Laven 1967), significant research advances have been made over the past 5-10 years to understand the underlying mechanisms and to develop *Wolbachia*-based biological control strategies to curb the major mosquito-borne diseases, including dengue, Chikungunya, Zika, malaria, etc. (Hoffmann *et al*. 2015, Lambrechts *et al*. 2015). *Wolbachia pipientis* often referred to as just "*Wolbachia*" is a maternally inherited, intracellular, obligate bacterial endosymbiont, first discovered by Hertig and Wolbach from *Culex pipiens* mosquitoes in 1924 (Werren 1997). It is one of the most well studied and naturally widespread (~ 65%) endosymbionts in arthropod and nematode species, including in several mosquitoes, fruit flies, wasps, moths, seed shrimp, copepods, springtails, filarial nematodes, etc. (Hilgenboecker *et al*. 2008, Werren *et al*. 2008, Wiwatanaratanabutr 2013, Chrostek *et al*. 2014). However, this bacterium is absent from some medically important mosquito vectors including *Aedes aegypti*. In mosquitoes, this bacterium causes a phenomenon called cytoplasmic incompatibility, which results in early developmental arrest of embryos when a *Wolbachia*-infected male mates with an uninfected female or a female that is infected with a strain of *Wolbachia* different from that of her mate (Zabalou *et al*. 2004, Bourtzis 2008). Some *Wolbachia* types also can cause nonlethal traits including shortening the life span, reduced fitness, and exhibit antiviral/pathogen properties (Hedges *et al*. 2008, Hoffmann *et al*. 2015). *Wolbachia*-induced traits are being harnessed by researchers to impact disease vector mosquito populations and to curb the burden of mosquito-borne diseases.

Several strains of *Wolbachia* have been described from different hosts including from fruit flies (*Drosophila melanogaster* and *D. simulans*) and *Ae. albopictus*. The most common strains studied and are being considered for biological control of mosquitoes and mosquito-borne-pathogens include: wMel, wMelPop-CLA, wMel, wAlbB, wAlbA, wRi, wMelPop, and wPip (Hoffmann *et al*. 2015). Numerous studies have demonstrated impacts of *Wolbachia*-induced traits on mosquito vectors and their pathogens including interference with pathogen transmission and cytoplasmic incompatibility when introduced into a new host such as *Ae. aegypti* (Hoffmann *et al*. 2015).

Three strategies using *Wolbachia* as part of mosquito and mosquito-borne disease control strategies have been proposed (Fig. 7-1) and currently are in various stages of efficacy trails (Hoffmann *et al*. 2015). These include: 1) population suppression via release of *Wolbachia*-infected males, 2) population replacement with *Wolbachia*-infected mosquitoes refractory to arboviruses and other mosquito-borne pathogens, and 3) release of *Wolbachia* strains that produce deleterious fitness effects on vectors. Population suppression strategy rely on the introduction of (only) non-biting males, whereas population replacement strategy relies on introduction of female mosquitoes that can bite and blood feed (female release is required because *Wolbachia* is maternally inherited). Population suppression is achieved by inundative release of *Wolbachia*-infected male mosquitoes into naive mosquito populations, which is analogous to the classical sterile insect technique (SIT) (see SIT section below), whereas the population replacement strategy is more of an inoculative biological control and is considered to be self-sustaining. The former strategy is led by researchers at the University of Kentucky and MosquitoMate, Inc. (http://mosquitomate.com), whereas the latter program is led by group of researchers in the Eliminate Dengue Program in Australia (http://www.eliminatedengue.com).

Successful experimental releases of mosquitoes infected with various strains of *Wolbachia* have been carried out in recent years including in the United States, Australia, Vietnam, Indonesia, China, and Brazil (Hoffmann *et al*. 2011, O'Connor *et al*. 2012, Frentiu *et al*. 2014, Nguyen *et al*. 2015, Schmidt *et al*. 2017). While results of experimental trials thus far have been exciting and promising, epidemiological data, which is the key measurement for the success of vector control strategies and will be a challenging task to measure, is still needed (Lambrechts *et al*. 2015, Thomas 2017).

An experimental release trial of wAlbB strain-infected male *Ae. aegypti* mosquitoes is currently underway in Stock Island, Florida, and Clovis, California to suppress *Ae. aegypti* mosquito populations (A. Leal and S. Dobson, pers. comm.). This particular bacterial strain was originally isolated from *Aedes albopictus* and transinfected into *Aedes aegypti* for biological control of *Ae. aegypti* mosquito populations (Xi *et al*. 2005). This bacterium causes a complete embryonic death (*i.e.*, prevents laid eggs from hatching) when a wAlbB strain-infected male *Ae. aegypti* mosquito mates with a naive (uninfected) female *Ae. aegypti*. MosquitoMate Inc. (http://mosquitomate.com), a start up company founded by researchers at the University of Kentucky (Waltz 2016), has registered this bacterium in the category of mosquito biopesticides for experimental trials by the U. S. Environmental Protection Agency (https://www.epa.gov/pesticides/epa-grants-extension-experimental-use-permit-wolbachia-mosquito). In addition, a permit has been obtained from the Florida Department of Agriculture and Consumer services for experimental trials in Florida (A. Leal, pers. comm.).

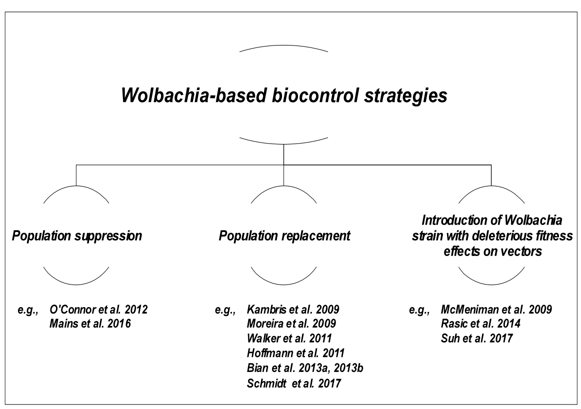

Fig. 7-1. Diagram of three major *Wolbachia*-based control strategies to suppress mosquito borne diseases (adapted from Hoffmann *et al*. 2015). These strategies are not necessarily mutually exclusive.

Protozoa
Despite the ubiquitous presence of protists in mosquito larval habitats, only a few studies explored protozoa as possible biological control agents (Washburn 1995). Some studies suggested their potential utility as delivery vehicles of other microbial control agents such as *Bti* (Manasherob *et al*. 1994). A recent study at the Florida Medical Entomology Laboratory showed a biomass reduction of mosquitoes when exposed to naturally occurring free living protists (*e.g.*, *Paramecium* sp.), suggesting potential negative impacts on mosquito life history traits (Duguma *et al*. 2017a). Research is needed on how this group might impact mosquito populations or mosquito control agents in the field including the potential for using these ubiquitous microorganisms for delivering more effective control agents.

Fungi
There is a renewed interest in using entemopathogenic fungi for use in suppressing mosquitoes and mosquito-borne diseases worldwide. A number of fungal pathogens, including *Beauveria* species (*B. bassiana* and *B. brogniarii*), *Metarhizium anisopalae*, *Coleomycets* spp*., Isaria fumosorosea*, *Anncaliia algerae* (formerly known as *Nosema algerae*)*, Lecancillum* spp., etc. have been known to kill mosquitoes (Scholte *et al*. 2004, Kanzok and Jacobs-Lorena 2006, de Faria and Wraight 2007, Bukhari *et al*. 2013, Huang *et al*. 2017). Although the majority of the fungal pathogens are used to control other agricultural pests and are commercially available, some, including *Beauveria bassiana* and *M. anisopalae* have been used for domestic mosquito control (de Faria and Wraight 2007, Scholte *et al*. 2007). A recent study demonstrated a potential for use of *B. bassiana* for large-scale suppression of malaria mosquito vectors (Heinig *et al*. 2015). In addition, recent studies showed that some transgenic fungal species (*e.g.*, *Metarhizium anisople*) can have potential impact on pathogens such as malaria parasites (Fang *et al*. 2011). Research is needed including on delivery mechanisms of some of the effective fungal biocontrol agents to incorporate fungi into the integrated mosquito control programs in Florida.

Nematodes
Parasitic nematodes, such as *Romanomermis* spp. (Mermithidae), have been considered for mosquito biocontrol in Florida and elsewhere (Platzer 1981, Giblin 1987, Bukhari *et al*. 2013, Pérez-Pacheco *et al*. 2015). Although earlier releases of mermitid nematodes (*e.g.*, *Romanomermis culicivorax*) have shown good results in controlling mosquitoes, the discovery of *Bti,* which required less technical difficulty in mass production and distribution, dampened commercial success of other microbial-based products, including nematodes for mosquito control (Peters 2013). *Romanomermis culcivorax* is a mosquito specific pathogen native to Florida and Louisiana (Giblin 1987). Nematodes are considered easy to rear for use in biocontrol (Lacey *et al*. 2015).

Viruses
There are some viruses (*e.g.*, nucleopolyhedrovirus isolated from *Culex nigripalpus*) that have been reported to kill mosquito larvae or can be used as delivery mechanisms for genes that could adversely impact mosquitoes or their pathogens (Andreadis *et al*. 2003,

Bukhari *et al*. 2013). However, currently there is no virus-based product used for mosquito control in Florida or elsewhere.

7.2.2 Invertebrate Arthropod Mosquito Predators

Toxorhynchites

Species of predaceous mosquitoes in the genus *Toxorhynchites* have been studied in a variety of urban areas for control of domestic and tree-hole mosquitoes such as the Asian tiger mosquito, *Ae. albopictus*. However, their introduction into urban container habitats has proven unsuccessful (Schreiber and Jones 1994). In certain containers, *Toxorhynchites* may consume a large number of prey mosquito larvae, such as *Ae. aegypti* and *Ae. albopictus.* All larval instars of *Toxorhynchites* spp. are known to be predators (Collins and Blackwell 2000). However, this predator does not disperse well enough to impact the vast number of natural and artificial containers used by these mosquitoes (Collins and Blackwell 2000). Additionally, their development time is 2-3 times that of their prey making it difficult for them to keep up with the other more rapidly developing mosquito species (Service 1983). Lee County Mosquito Control District uses *Toxorhynchites r. rutilus* to control bromeliad-inhabiting mosquitoes on a limited time basis (W. Gale, pers. comm.). To our knowledge, there is no other mass production and release of these mosquitoes for operational mosquito control in Florida.

Copepoda

Another group of biocontrol agents with promise for mosquito control is comprised of predaceous copepods particularly those in the genus *Mesocylops*. In Florida, season-long (Schreiber and Jones 1994, Soumare and Cilek 2011) and multi-year (Rey *et al*. 2004) control was achieved in trials in tire habitats. Copepods are easy to rear and to deliver to target sites in the field (Hallmon *et al*. 1993), and they perform well when used with certain narrow spectrum insecticides (Tietze *et al*. 1994). Copepods were used in integrated mosquito-borne disease prevention campaigns and contributed to dengue decline in Vietnam (Kay *et al*. 2002, Nam *et al*. 2005). Copepods consume a broad variety of prey and can survive in the field even when mosquitoes are absent or less abundant. More research is needed on the operational use of copepods in natural field conditions.

Other invertebrate predators

Experimental studies have shown impacts on mosquito populations by other invertebrate predators of mosquitoes such as dragonfly and damselfly naiads (Odonata), backswimmers (Notonectidae), beetle larvae (Dytiscidae and Hydrophilidae), etc. in certain habitats (Quiroz-Martínez and Rodríguez-Castro 2007). However, currently there is no small or large-scale development program for the application of these organisms for biological control purposes.

7.2.3 Vertebrate Mosquito Predators

Mosquitofish

Mosquitofish (*Gambusia* spp.) are widely used for biological control of mosquitoes in Florida and elsewhere. *Gambusia holbrooki*, commonly known as the eastern mosquitofish, is native to all regions of Florida, while *Gambusia affinis* (western mosquitofish) is limited to the northwest panhandle region of the state. Both species are considered invasive in other areas of the country and the world. These fish feed on mosquito larvae and can be placed in a variety of permanent and semi-permanent aquatic habitats, where *Culex* and *Anopheles* are the primary mosquito residents and where emergent vegetation is not too dense. Mosquitofish are commonly used in manmade habitats such as in abandoned swimming pools. However, in habitats such as salt marshes, fish alone cannot provide effective control of the sudden explosion of larvae produced following rainfall or rising tides. This situation is particularly true during the first hatch of the season, where the mosquito populations numerically exceed what the fish can consume during the brief immature mosquito developmental period. However, in many situations, effective control can be achieved if the larvivorous fish are afforded access to the mosquito-producing areas.

It is a common misconception that when mosquito control programs encourage residents to eliminate standing water, this request includes swales, lakes, ponds, canals and other large permanent bodies of water where *Gambusia* naturally occurs. For the most part *Gambusia* control the mosquitoes in these large bodies of water except for those mosquitoes associated with certain types of aquatic vegetation. “Eliminate standing water” as intended by mosquito control agencies really means to empty standing water in manmade containers.

Differences of opinion exist regarding the utility and actual control benefits derived from use of *Gambusia* to control mosquitoes in certain situations, and actual results vary from excellent to no control at all. There is also a growing concern over introducing nonnative fish species. such as *Gambusia affinis* into new habitats where other native fish species assemblages have been threatened (Azevedo-Santos *et al*. 2017, Huang *et al*. 2017). Care must be taken in placement of these species in areas where endemic fish species are sensitive to further environmental perturbation. Additionally, investigation of alternative endemic fish species in these areas of concern as potential biological control agents deserves greater attention.

Frogs/tadpoles

Tadpoles in Florida are known to be herbivores/detritivores and therefore are not predators of mosquito larvae (J. Wrublik, pers. comm, McGarrity and Johnson 2010). A similar observation was reported for tadpole species in Thailand (Weterings 2015). However, some laboratory studies indicated that tadpoles prey on mosquito eggs and also deter mosquitoes from laying eggs (Bowatte *et al*. 2013). Frogs also may consume some adult mosquitoes, but not enough to impact mosquito populations (Schaefer and Liebertz 2001). Because existing scientific evidence does not support that tadpoles significantly impact mosquito populations, they are not being used in Florida mosquito control programs.

Birds and bats

Very little published scientific information exists on the use of birds and bats for actual mosquito control because mosquitoes are not primary food sources for these predators (Kunz and Whitaker Jr. 1983, Whitaker Jr. and Clem 1992, Carter *et al*. 2003, Whitaker 2004, Boyles *et al*. 2013). In addition, although both of these kinds of predators eat adult mosquitoes they do not do so in sufficient amounts to impact the mosquito populations. For example, a study that investigated the role of birds (*e.g.*, purple martin) for mosquito control concluded that they do not have a significant impact on mosquito populations (Kale 1968). Additionally, because mosquito flight behavior is crepuscular, they are not active during the feeding periods of most birds. However, one study reported a 32% reduction of *Culex* spp. in the presence of the northern long-eared bats (*Myotis septentrionalis*) in an enclosure experiment (Reiskind and Wund 2009). While it is a common belief by the public, there is no scientific evidence that increasing the population of bats in an area will reduce the numbers of mosquitoes enough to affect human biting rates.

In an early laboratory experiment in a room filled with only mosquitoes less than 10 percent of the bats hunted mosquitoes (Griffin *et al*. 1960). A bat's diet in a room filled only with mosquitoes provides no information about what bats eat in the wild. Evidence from analyzing bat stomach contents or fecal pellets has shown that mosquitoes comprise a small percentage of their natural diet (*e.g.*, Whitaker and Lawhead 1992, Agosta 2002), and thus bats are not currently deployed as part of mosquito control operation in Florida. In addition, bats are known to carry a greater proportion of viruses that potentially can be transmitted to humans than other mammals (Olival *et al*. 2017).

7.3 ALTERNATIVE CONTROL TECHNIQUES

The term "alternative control techniques" in essence is a catch all for biotechnological developments, including Sterile Insect Technique (SIT), removal-trapping techniques, repellents, mechanical traps, bug zappers, and socio-cultural changes. A thumbnail sketch of some of these alternative control techniques, their merits, and their disadvantages follows.

7.3.1 Sterile Insect Technique (SIT)

Sterile Insect Technique, first invented by USDA entomologist Edward Knipling, is a method of species-specific pest control in which sterile insects are mass-produced and released into the environment to interfere with the reproductive success of the target insect pest population (Knipling 1955). This result was achieved first by the use of gamma radiation to sterilize male insects (Krafsur 1998). Building upon the classical SIT method, other novel ways of generating sterile insects, including chemical sterilization, classical genetic methods, biological sterilization, and by manipulation or alteration of the genome of the target population also have been achieved.

Classical SIT using irradiation

The successful sterile-male eradication programs of the screwworm fly in North America and Central America, Mediterranean fruit fly, and tsetse fly, have proven the ability of SIT to control pest insects over large areas including entire continents (Krafsur 1998). Sterile-male release works optimally in situations where target population density is low and dispersed and mating frequencies are limited such as in screwworm flies and Mediterranean fruit fly populations. Various SIT efforts targeted mosquitoes but with mixed successes (Benedict and Robinson 2003). For example, despite millions of sterile *Ae. aegypti* males released in Pensacola (Florida) in 1960's, no population level suppression was observed (Morlan *et al*. 1962, Weidhaas 1973, Benedict and Robinson 2003). Implementation of a sterile-male technology for controlling mosquitoes has a number of technical and economic obstacles including the ability to mass rear insects cost effectively, the efficiency of the sterilization technique, and fitness effects of irradiation. Certain species of mosquitoes present challenges when using SIT; Consider the number of sterile males that would have to be synchronously raised to compete with the single emergence from a 500 acre salt marsh, where billions of adult *Ae. taeniorhynchus* emerge in a 24-hour period. In addition, the classical size-based mechanical separation of immature males from females as has been used for screwworm flies may be difficult and expensive when attempting to release large numbers of sterile male mosquitoes into the environment. However, there are certain species of mosquitoes that are well suited to SIT techniques, such as *Ae. aegypti*, *Ae. albopictus*, several species of *Anopheles* and *Culex* species. These species occur in relatively low density, generally the females only mate once, and can be mass reared relatively cheaply. The problem in the past has been that sterilization by irradiation has caused too much damage to the mosquitoes, effecting their fitness and subsequent competitiveness in the field. But there have been several advances in recent years that have brought the SIT technique back as a viable option for controlling mosquitoes. The following subtopics discuss some of those advances.

Lee County Mosquito Control District in collaboration with the International Atomic Energy Agency is currently developing a SIT program to release sterile male *Ae. aegypti* mosquitoes as part of their integrated management of *Aedes* mosquito populations and *Aedes*-vectored disease control program (W. Gale, pers. comm.).

Oxitec Self-limiting Insect Control

Oxitec's self-limiting approach is an innovative method of controlling insect populations using genetic engineering (Phuc *et al*. 2007, Alphey *et al*. 2013). Oxitec's male insects are engineered to contain a self-limiting gene that causes their offspring to die before they reach adulthood, but these insects can live and reproduce normally when they are reared in the lab and fed a diet containing an antidote (tetracycline). They also contain a heritable, fluorescent marker, which has several unique benefits include: An easy way to distinguish Oxitec insects from native pest insects, a tool for quality control in mass production, and a method to monitor progress and efficiency of control in the field. The released insects do not perpetuate in the environment, as there is no or insufficient antidote present to rescue the offspring (Curtis *et al*. 2015), and the released adult males die within a few days (Lacroix *et al*. 2012). With repeated releases of sufficient numbers of these self-limiting males, there is a reduction in the wild populations in a similar manner as the classical SIT control strategy (see SIT above). This approach is designed to overcome many problems the classical SIT methods including poor male competition against wild males for mating and has a potential to suppress populations of economically and medically pestiferous insects including disease-vectoring mosquitoes (Alphey *et al*. 2010).

Oxitec has developed genetically engineered male Friendly™ *Aedes*, which is the self-limiting strain of the *Ae. aegypti* mosquito (also known as OX513A) for use in vector control. *Ae. aegypti* is considered the primary vector for many human diseases including Zika, dengue, chikungunya and yellow fever. Oxitec has developed an innovative approach to mosquito control by reducing the populations of *Ae. aegypti* mosquitoes in an area (with repeated release of male OX513A *Ae. aegypti*) and/or preventing recurrence of the population once control in the area has been achieved. Oxitec has used recombinant DNA (rDNA) technology to insert a "self-limiting" gene and a marker gene into the genome of *Ae. aegypti*. The self-limiting gene is present in OX513A males as a homozygous dominant trait (two copies). When male OX513A *Ae. aegypti* are released into the environment and mate with wild *Ae. aegypti* females, their offspring inherit a single copy (are hemizygous) of the self-limiting trait. Over 95% of the resulting offspring die before reaching adulthood (Phuc *et al*. 2007), and the local *Ae. aegypti* mosquito population declines within four to eight months, depending on several factors including initial *Ae. aegypti* populations, temperature, and numbers of OX513A released. In five separate suppression projects (trials), regular releases of OX513A *Ae. aegypti* resulted in substantial reductions in wild *Ae. aegypti* populations of over 90% (Harris *et al*. 2012, Carvalho *et al*. 2015, Gorman *et al*. 2016). Population reduction was measured by comparing the number of mosquitoes collected from ovitraps (abundance data) from treated areas where releases of OX513A males occurred compared to untreated areas. These projects were conducted in Brazil (3 different sites), the Cayman Islands, and Panama.

Public support for the Oxitec self-limiting approach has been strong. In Brazil, a project using Oxitec's Friendly™ *Aedes* mosquitoes was started in the city of Piracicaba in April 2015. An opinion poll of 1,200 city residents conducted in November 2016 showed that 92.8% of the residents supported the project. In Monroe County, FL, more than 40,000 residents voted in the world's first ever referendum on a genetically engineered organism (to our knowledge). The results showed, on average, that 58% of residents were in favor of using the Oxitec approach to control mosquitoes. In addition, 31 of the 33 precincts in Monroe County voted in favor of conducting a trial there. These results reflect other surveys about the Oxitec approach in the U.S. (Cobb 2013, Ernst *et al*. 2015).

Regulatory agencies around the world have evaluated the Oxitec Friendly™ *Aedes* mosquitoes for open release. A 2017 report by Dutch National Institute for Public Health and the Environment (RIVM) GMO (Genetically Modified Organisms) Office concluded that potential release of these mosquitoes poses negligible risks to human health and the environment. In addition, the World Health Organization (WHO) has posted the conclusions and recommendations of the Vector Control Advisory Group (VCAG) meeting from March 14-15, 2016 (http://www.who.int/neglected_diseases/news/mosquito_vector_control_response/en/), regarding vector control tools for use in response to the recent Zika virus outbreak. After their review of several new tools with the potential to reduce vector populations, the VCAG issued a positive recommendation in support of Oxitec's self-limiting mosquito (OX513A). Open releases have been conducted in Brazil, the Cayman Islands, Panama, and Malaysia (Harris *et al*. 2012, Lacroix *et al*. 2012, Carvalho *et al*. 2015, Gorman *et al*. 2016). In 2016, the United States Food and Drug Administration (FDA), with a team consisting of experts from the Center for Veterinary Medicine (CVM), the Environmental Protection Agency (EPA), and the Centers for Disease Control and Prevention (CDC), issued a finding of no significant impact for the Oxitec OX513A mosquito; It concluded that there was no significant risk to human health, animal health or the environment (FONSI 2016). Independent review has also concluded there is negligible risk (Prabhakargouda 2010). Open release trials of the Oxitec Friendly™ *Aedes* mosquito since 2009 have demonstrated the efficacy of the Oxitec self-limiting approach (Harris *et al*. 2011). The technology has been deemed safe in several countries and is now being deployed on a city-wide scale in Brazil. Upon regulatory approval, it is anticipated that the Oxitec technology will be deployed in the U.S. as a new tool for mosquito control teams to use to combat *Ae. aegypti*.

The Florida Keys Mosquito Control District (FKMCD) in Monroe County is working with Oxitec to implement a pilot study using OX513A Friendly™ *Aedes* mosquitoes to control *Ae. aegypti*. The FKMCD board has signed an investigation agreement to proceed with the pilot study after regulatory approval has been given. A mass rearing facility has been built, and the production of male OX513A Friendly™ *Ae. aegypti* will begin when regulatory approval is received.

RNA interference

The RNA interference (RNAi) is a naturally occurring gene silencing pathway used to degrade unwanted (foreign) gene products (Sen and Blau 2006). This naturally occurring species-specific RNAi pathway can be exploited to suppress pathogens or generate desirable mosquito phenotypes by introducing a complementary double stranded RNA (dsRNA) and has a potential to be used for mosquito control in the future (Airs and Bartholomay 2017). For example, a study has shown that feeding of dsRNA associated with testis of *Aedes* mosquito larvae resulted in sterile males, which is thought to be another way of producing males for SIT program (See SIT above) program (Whyard *et al*. 2015). While the prospect and research is advancing to utilize this technology for pest control, currently there is no RNAi technology approved by EPA for use in mosquito control in Florida or elsewhere.

7.3.2 Trapping and Removal Techniques

The premise underlying removal trapping is that a trap attracts, captures, and removes a significant portion of the biting mosquito populations (Kline 2006). Subsequent mosquito populations are smaller and, therefore, require fewer chemical applications for control. Some of these studies use commercially available attractant baited traps, and others are using baited targets. Up to now, these studies have largely been conducted on a limited spatial and temporal scale. So far, the technology has worked best on isolated islands where one species was clearly dominant compared to the mainland residential areas where many different important nuisance species of different genera were present. Different attractant combinations, delivery systems, and trap types may be required to attract and effectively capture different species. Parks, resorts, golf courses, and other recreation areas may be good candidates to evaluate this technology. With the development of sufficiently effective traps and a diversity of effective attractant combinations for different mosquito species, trapping systems could be used as behavioral control measures and added to the growing list of biologically-based technologies for mosquito control.

7.3.3 Trapping Devices and Bug Zappers

There are several devices, including electronic devices to attract and kill flying mosquitoes or to repel them by sound (acoustic), available on the market, but most of these devices are not supported by scientific research. A FMEL factsheet that discusses the advantages and disadvantages of various mosquito control devices is available at http://solutionsforyourlife.ufl.edu/hot_topics/environment/mosquitoes.shtml. Bite Back!® is an example of a simple passive mechanical trap that uses the mosquito larvae's need to come to the water surface to breathe air to trap them. While it may remove some mosquitoes from an area, the device may be more useful as a surveillance tool than a control strategy. In2Care® is another ovitrap strategy complemented with *Beauveria bassiana* and pyriproxyfen thought to reduce populations of domestic mosquitoes (Snetselaar *et al*. 2014). The CDC autocidal gravid trap also has been shown to be effective against container mosquitoes (Barrera *et al*. 2014). Acoustic devices such as Larvasonic SD-Mini Acoustic Larvicide® that uses sound to disrupt the internal structure of mosquito larvae over short distances have been shown to be effective in killing mosquito larvae (Britch *et al*. 2016). Novel trapping technologies such as one currently developed by Microsoft (https://www.microsoft.com) to trap target species of interest are currently in the testing phase and are thought to facilitate surveillance of arboviruses. To our knowledge, no large scale use of these traps or acoustic devices for mosquito control is currently used operationally in Florida.

7.3.4 Socio-cultural Changes

This category of alternative controls includes human avoidance of mosquitoes. Two lifestyle changes that have greatly reduced mosquito contact with human beings are air conditioning and television viewing. Additional cultural changes that can be employed include wearing protective clothing and the curtailment of outdoor activities when the potential for mosquito attack is greatest. Mosquito control can influence the public perception of mosquito problems through educational programs in schools, through the media, and by cooperation with government agencies at the local, state, and federal level. Public education and awareness as part of vector-borne disease control strategies needs greater attention and resources (Omodior *et al*. 2017). With continuing urbanization, residents seem to be becoming even less tolerant of insect pests. However, with the recent publicity of mosquito-borne diseases such as Zika, West Nile and other encephalitides, public receptiveness to lifestyle changes for protection against mosquito bites may be increasing.

7.4 CONCLUSIONS

Biological control is an integral component of mosquito control in Florida. The dominant biological (biorational) control agents that are currently components of the integrated mosquito control programs in Florida include the mosquitocidal bacteria *Bacillus thuringiensis* subsp. *israelensis* and *Lysinibacillus sphaericus*, spinosad (a fermentation product from the Actionmycete bacterium *Saccharopolyspora spinosa*), and mosquitofish *Gambusia* spp. Although there is a desire to integrate other previously known as well as novel biocontrol tactics particularly against domestic mosquito vectors, many unanswered questions, including lack of suitable delivery mechanisms, public acceptance, etc., prevent immediate implementation of more than a few of these methods. Novel technologies such as *Wolbachia*-based biological control and release of transgenic sterile (Oxitec strain) mosquitoes hold great promise and are either in experimental trial or awaiting regulatory approval for field trials in Florida. While biocontrol agents are generally considered safe and effective, careful selection and introduction of the use of biocontrol agents is also critical to avoid unintended environmental consequences (*e.g.*,Walsh *et al*. 2016). While some of the promising alternative technologies are being field-tested and further developed for operational mosquito control usage, mosquito control programs and researchers need to continuously monitor the efficacy of existing products, may need to phase-out some products that are found to be ineffective, and use improved technologies to enhance the efficacy of existing biological control products (Ritchie and Johnson 2017).

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Chapter 8

DISEASE SURVEILLANCE, OUTBREAKS, AND CONTROL IN FLORIDA

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Summary

Historically, Florida has suffered from repeated large epidemics of serious mosquito-borne disease, including yellow fever, malaria, dengue, and several arboviral encephalitides. Many of these diseases remain a serious threat to Florida residents. Florida's proximity to areas in the Caribbean Basin and northern South America where many of these diseases are endemic contributes to concerns about the potential for their resurgence in the state. Florida is also susceptible to invasion by other emerging vector-borne pathogens from around the globe. The introduction of West Nile virus (WNV) into Florida in 2001 resulted in an additional arbovirus that has become endemic and creates the potential of epidemic transmission throughout the state. Recently, dengue, chikungunya, and Zika virus transmission has been reported in the southern half of Florida. Though poorly documented, the economic costs associated with mosquito-borne disease transmission to humans, domestic animals, and wildlife are probably growing rather than subsiding as development of the state progresses.

Surveillance and control of mosquito-borne disease were once largely coordinated and financed by the state of Florida. In recent decades, however, state involvement has declined. Active disease surveillance and control is now predominantly a local initiative supported by local revenues. Approximately half of Florida's 67 counties conduct active surveillance for mosquito-borne disease. It is generally accepted that Florida mosquito control agencies are maintained by local taxpayers only where there is demand for continuous relief from biting "nuisance" mosquitoes – with concern for mosquito-borne disease being only a secondary consideration. The Division of Environmental Health, Florida

Department of Health, monitors human cases of mosquito-borne and other reportable human diseases, while the Florida Department of Agriculture and Consumer Services independently monitors and reports equine cases of West Nile and eastern equine encephalitis. Active sentinel chicken surveillance for the transmission of West Nile, St. Louis encephalitis, eastern equine encephalitis, and Highlands J viruses is conducted in Florida on the local level and results are analyzed and reported by the Florida Department of Health as weekly arbovirus summary reports. In addition to sentinel chicken surveillance data, the reports include horse, mosquito pool, wild bird, and human arboviral surveillance data.

Local arbovirus surveillance programs in Florida generally include: 1) monitoring virus exposure in sentinel chickens through the weekly testing of blood samples for arbovirus-specific antibodies and 2) monitoring the abundance, spatial distribution, and age structure of known mosquito vector species. Wild avian mortality, especially that of corvids (blue jays and crows), and equine mortality also is used to monitor the spatial and temporal distribution of WNV. Climatic conditions (daily rainfall and temperature data) and mosquito infection rates are also monitored by some jurisdictions. Elevated rates of virus exposure in sentinel chickens can indicate an elevated risk of transmission to the human population. A cluster of positive horses also may indicate potential of eastern equine encephalitis virus (EEEV) or WNV transmission to humans living in close proximity to the positive horses. Properly interpreted, vector density, and vector population age structure data can be used to assess arboviral transmission risk. High vector mosquito populations may support extensive enzootic transmission of these viruses, and, under the proper environmental conditions, elevated vector populations may result in epidemic arboviral transmission to humans. Agency-level responses to the indicators of a potential vector-borne disease epidemic may include: 1) aggressive insecticide applications targeting the immature and adult stages of the vector mosquito and 2) public notification concerning the increased arboviral transmission risk to reinforce mosquito avoidance, the source reduction of larval habitats on private property, and the use of personal protection and behavior modification to reduce exposure to biting mosquitoes.

8.1 HISTORY OF MOSQUITO-BORNE DISEASE OUTBREAKS IN FLORIDA

The prevalence of freshwater and coastal wetlands in Florida and the subtropical climate of much of the state were formidable obstacles to its colonization by Europeans. These characteristics made the human inhabitants (and their domestic animals) particularly vulnerable to a variety of mosquito-transmitted pathogens and parasites. Most of these disease agents are endemic in Florida, and some of them have taken on a greater prominence as human development increasingly impinges on previously undeveloped and uninhabited regions. But because of its position at the crossroads of the Caribbean, Florida is also highly vulnerable to the introduction of new vector-borne pathogens from around the world as evidenced by the 2016 introduction and transmission of Zika virus in Dade, Broward, and Palm Beach Counties.

8.1.1 West Nile Virus

West Nile virus (WNV) is in the genus *Flavivirus* (family Flaviviridae) and is the etiological agent that causes West Nile fever. The virus was first detected in the Florida Panhandle in 2001, after introduction to the U.S. by way of New York the preceding year. The most likely mode of WNV transport into Florida was in migrating birds. The virus spread rapidly throughout Florida in 2001, 2002, and 2003 when there were 139 human cases reported along with 1,197 WNV-positive horses. Since 2004 WNV has settled into an endemic transmission pattern similar to that observed for St. Louis encephalitis virus (SLEV), with sporadic and relatively localized epizootic transmission. From 2004 through 2016 there has been an annual average of 16 human cases of WNV reported in Florida. Wild birds are important amplification hosts for WNV and the major mosquito vectors include *Culex nigripalpus* throughout the state and possibly *Cx. quinquefasciatus* in the Florida Panhandle. Compared with other parts of the continental United States where Flavivirus epidemics have been reported in the past and epidemics of WNV have been reported since 1999, Florida has been spared from a major human epidemic caused by WNV. The reasons for the absence of WNV epidemic transmission in Florida are unclear but may involve the distribution, abundance, susceptibility to infection, and age structure of the avian amplification hosts and mosquito vectors of WNV.

8.1.2 St. Louis Encephalitis Virus

St. Louis encephalitis virus (SLEV) is a New World Flavivirus that causes St. Louis encephalitis. The virus is endemic in Florida and is normally associated with wild birds (where it causes no disease symptoms) and several species of mosquito, most notably *Culex nigripalpus*. In the second half of the 20th century, SLE became the predominant mosquito-borne disease of humans in Florida and was responsible for epidemics in the south and central parts of the state. Epidemics caused by SLEV occurred in 1959, 1961, 1962, 1977, and 1990. The 1990 epidemic was the largest (226 documented cases with 11 deaths) and most widespread (human cases were reported from 28 counties). The 1959-1962 outbreaks in the Tampa Bay area involved 315 clinical cases and 55 fatalities. The true extent of St. Louis encephalitis disease during epidemics is difficult to assess, since there are typically several hundred mild or asymptomatic non-clinical cases generated for every laboratory-diagnosed clinical case. The 1977 and 1990 epidemics resulted in considerable disruption of normal nighttime activities and negatively impacted tourism in the affected parts of the state. Economic loss to the state of Florida due to arboviral transmission has not been well-documented, but the 1990 St. Louis encephalitis epidemic alone is likely to have been responsible for millions of dollars of direct and indirect losses.

8.1.3 Eastern Equine Encephalitis Virus

Eastern equine encephalitis (EEE) disease is caused by the Alphavirus eastern equine encephalitis virus (EEEV). Infection with EEEV is frequently fatal in humans, equines, and exotic avian species (*e.g*., pheasants, emus, and ostriches). The veterinary importance of EEEV adds considerably to the economic impact of the virus in Florida. The virus is endemic in the eastern half of the U.S., while a closely related pathogen (Madariaga virus) circulates throughout Central and South America. Two transmission cycles of EEEV are reported in Florida. The enzootic transmission cycle involves transmission of EEEV between *Culiseta melanura* and wild birds in and around freshwater swamps. Periodically, EEEV breaks out of the enzootic transmission cycles and is transmitted to horses and humans in areas surrounding the freshwater swamps. Humans and horses are regarded as biological dead-ends (*i.e.*, mosquitoes feeding on infected humans and horses do not acquire enough virus to become infective) despite the severe neurological disease symptoms displayed by these hosts. A number of mosquito species are involved in the secondary transmission cycles of EEEV including members of the genera *Culex*, *Aedes*, *Mansonia*, and *Coquillettidia*. Human epidemics caused by EEEV have never been reported in Florida, and human cases of EEE are rare (1.33 cases per year from 1955-2016) and widely dispersed. For example, five human cases were reported in 2005 from five Florida Counties (Gadsden, Leon, Pasco, Polk, and Suwannee). However, environmental conditions, especially heavy winter rainfall, often predispose large regions to springtime EEEV enzootic transmission. Human infections caused by EEEV often occur around areas where horse cases are reported. Despite the commercial availability of an effective horse vaccine against EEEV, the annual mortality rate in Florida horses remains high. Between 2001 and 2016 an average of 62 horses per year died of EEE infection in Florida. The annual cost of veterinary care for the prevention and treatment of EEEV infection in horses was estimated to exceed $1,000,000 per year in a study conducted in 1982 and 1983 (Wilson *et al*. 1986).

8.1.4 Dengue Viruses

Dengue is a disease with symptoms ranging from simple flu-like illness and body aches to severe hemorrhagic symptoms, shock, encephalitis, or death. Dengue disease is caused by any of five closely related yet distinct dengue virus (DENV) serotypes (DEN-1, -2, -3, -4, or -5) belonging to the virus family Flaviviridae. Epidemics of this disease had a major impact during the early development of the state. Dengue was first recognized in Florida in 1850 and was a persistent threat in Florida in following years. A 1934 dengue epidemic of more than 15,000 human cases was reported throughout peninsular Florida. Humans serve as the amplification host in all of the DENV transmission cycles where there is mosquito-to-human-to-mosquito transmission. *Ae. aegypti* and *Ae. albopictus* are the principal mosquito vectors of DENV in most parts of the world where dengue transmission occurs. These mosquito vectors are not native to

Florida but were introduced into the state and are now firmly established. Dengue has become an increasingly serious threat throughout the Caribbean and Central and South America in the past 30 years, and all DENV serotypes have been reported in the region. In 2009, an outbreak of 22 locally-acquired cases of DENV was reported in Old Town, Key West. Prior to this outbreak, the last locally-acquired cases of DENV were reported in Florida in the late 1940s. The origin of the dengue virus of the 2009 outbreak is believed to be Venezuela, and the virus was likely brought to Key West in an infected traveler. Between 2009 and 2016 a total of 130 locally-acquired cases of DENV infection have been reported in 8 Florida Counties (Broward, Dade, Hillsborough, Martin, Monroe, Osceola, Palm Beach, and Seminole). The widely dispersed distribution of locally-acquired dengue cases suggests that vector populations (*Ae. aegypti* and *Ae. albopictus*) are prevalent throughout south Florida. Although endemic dengue transmission is currently relatively rare in Florida, imported cases are frequently reported, and the potential for infected tourists to interact with competent vector mosquitoes is extremely high, resulting in likelihood of future local transmission events.

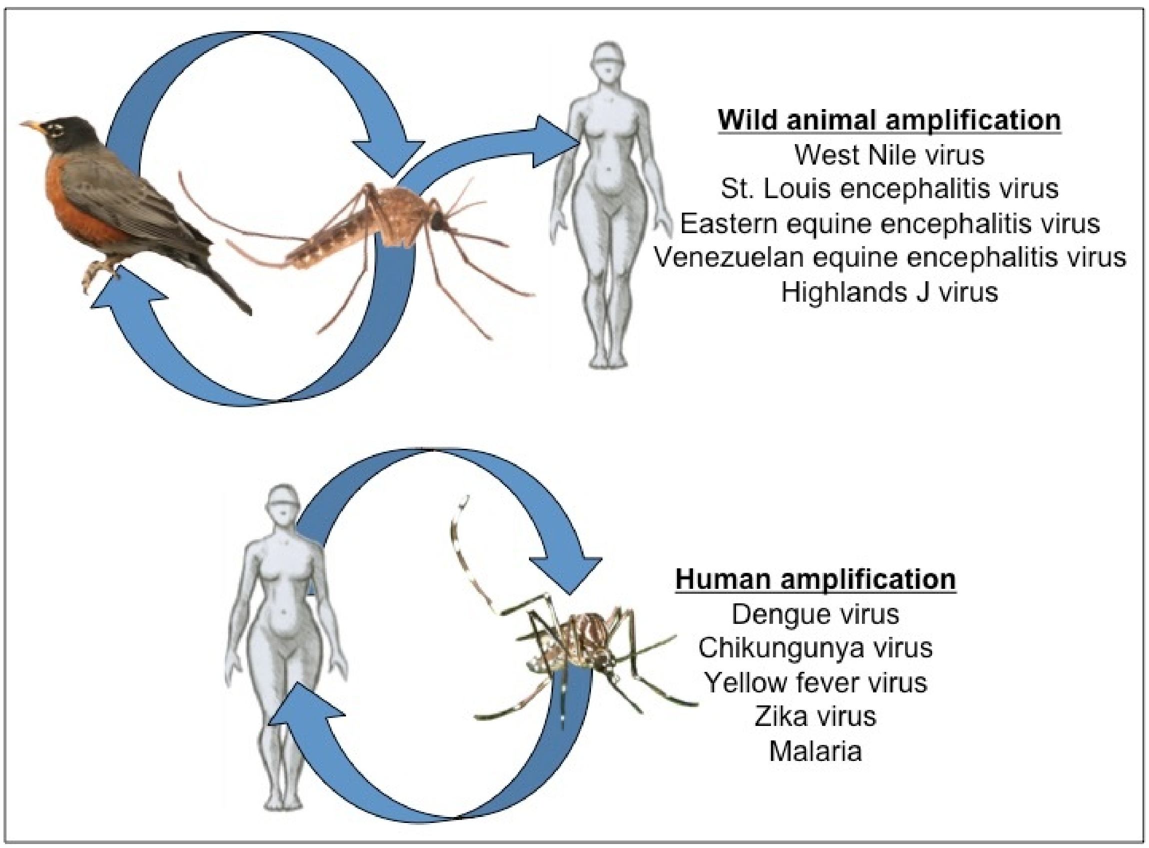

Figure 1. Vector-borne disease cycles involving wild animal amplification hosts (top) and human amplification hosts (bottom) (Diagram designed by N. Burkett-Cadena).

8.1.5 Chikungunya Virus

Chikungunya virus (CHIKV) is in the family Togaviridae and the genus *Alphavirus* (related to EEEV). The word chikungunya is from the Makonde language of southeast Tanzania and northern Mozambique and means “that which bends up.” Symptoms of chikungunya are similar to those of dengue including an elevated sustained fever, rash, neurological involvement, and severe joint pain. Chikungunya is primarily an Old World disease, originating in East Africa around 1700 and spreading throughout Africa and Central Asia. A widespread outbreak of CHIKV was reported in the islands of the Indian Ocean in 2006 and from there the virus was transported in infected travelers into the New World. Transmission of CHIKV is very similar to that of DENV including two essential factors: 1) humans serve as the primary amplification host and 2) important vectors include *Ae. aegypti* and *Ae. albopictus*. Prior to 2014, all of the CHIKV cases reported in Florida were imported from other parts of the world. In June, July, and August of 2014, 11 locally-acquired cases of chikungunya were reported from four Florida Counties (Broward (1 case), Palm Beach (5), and St. Lucie (3)). As with DENV, CHIKV transmission in Florida highlights two important points, 1) CHIKV is transported into Florida in infected travelers and, 2) populations of susceptible mosquito vectors are present throughout the state and are capable of initiating locally-acquired transmission events.

8.1.6 Zika Virus

Zika virus (ZIKV) is in the family Flaviviridae and the genus *Flavivirus* (related to DENV, SLEV, WNV, and Yellow Fever Virus) and was first isolated in Uganda in 1947. The word Zika originates from the Zika Forest where the virus was discovered and means “overgrown.” In adults, symptoms of Zika infection are similar to those caused by infection with chikungunya and dengue viruses and include an elevated sustained fever, rash, conjunctivitis, neurological involvement, and severe joint pain. Zika virus spread from its origins in East Africa to West Africa in the 1960s and then east through Central Asia in the 1970s. In 2007, Zika virus was reported to have spread eastward through Micronesia and then into French Polynesia in 2013. The virus appeared in Brazil in 2014 and spread throughout the Caribbean Basin. Zika virus was first detected in Florida in 2016. The transmission of ZIKV is similar to that of DENV and includes two essential factors: 1) humans serve as the primary amplification host and 2) *Aedes aegypti* is the primary epidemic vector. There is some evidence that *Ae. albopictus* also may be a competent vector of ZIKV, however, this mosquito does not appear to have been involved in the transmission of ZIKV in the New World in 2014-2016. A total of 1,124 imported cases of Zika were reported in 38 Florida counties in 2016. A total of 285 locally-acquired cases of Zika were reported in four South Florida counties 2016, most of which occurred in Miami-Dade County. As with DENV and CHIKV locally-acquired transmission in Florida indicates two important points, 1) ZIKV is transported into Florida in infected travelers and, 2) populations of susceptible *Ae. aegypti* are present in the state, especially in south Florida counties, and are capable of initiating locally-acquired transmission events.

8.1.7 Yellow Fever Virus

Yellow Fever virus (YFV) is in the family Flaviviridae, genus *Flavivirus*. The virus was perhaps the most feared mosquito-borne disease in colonial Florida and was first reported in Pensacola in 1764 and in 1874 where it was responsible for the deaths of 354 of the city's 1,400 residents. Similarly, 1,500 of 1,600 residents of Fernandina, Florida were infected with YFV in 1877. Local transmission of YFV has not been reported in Florida since 1905. The virus is transmitted in two distinct, yet related cycles. The urban YFV transmission cycle involves humans as the amplification host and is transmitted between humans by *Ae. aegypti* mosquitoes. Laboratory studies have shown that *Ae. albopictus* is a potential YFV vector as well. The jungle YFV cycle is maintained in non-human primates by a variety of mosquito vectors, including species of *Aedes*, *Haemagogus* and *Sabethes*. The YFV has not been eradicated from South America where it currently circulates in a jungle transmission cycle that occasionally results in sporadic human cases in Bolivia, Brazil, Columbia, Ecuador, Peru, and Venezuela. Yellow fever also occurs in much of Africa. In 2016, a yellow fever outbreak was reported in Angola, with more than 5,000 cases and 600 deaths, and in the Democratic Republic of the Congo, with more than 5,000 suspected cases and 200 deaths. Clearly, in spite of a highly effective vaccine against YFV, outbreaks continue to be reported around the world. Travelers to jungle areas of South America or Africa are strongly advised to vaccinate against YFV.

8.1.8 Venezuelan Equine Encephalitis Virus and Everglades Virus

Venezuelan equine encephalitis (VEE) is caused by a number of closely related, but antigenically distinct, Alphavirus subtypes in the Venezuelan equine encephalitis virus (VEEV) complex. One VEEV subtype, Everglades virus (EVEV), has been reported to occur throughout Florida, although all reported human cases have been from the southernmost portion of the peninsula. The virus was discovered in the 1960s in the Florida Everglades, where humans inhabiting the area demonstrated evidence of frequent exposure. The EVEV is maintained in cotton mice, cotton rats, raccoons, and opossums and is transmitted by the bite of infected *Culex (Melanoconion) cedecei* mosquitoes. The EVEV virus is apparently not a source of disease in horses, but human illness due to infection with this virus has been documented in at least two Florida residents. Other variants of VEEV are responsible for periodic equine epidemics in northern South America and Central America. A 1995 VEE epidemic in South America reportedly involved at least 13,000 human cases. In Colombia, transmission involved *Ae. taeniorhynchus* and *Psorophora columbiae* mosquito vectors.

8.1.9 Malaria

Human malaria is caused by infection with one of four protozoan parasites (*Plasmodium* species) that have a complex life cycle requiring *Anopheles* mosquitoes as intermediate hosts. Nearly all of the fifteen species of *Anopheles* mosquitoes found in Florida are capable of transmitting *Plasmodium* parasites. Historically, malaria was a major impediment to the economic development of the state. Between 1917 and 1930, 33 of Florida's 67 counties had annual malaria death rates of >100 deaths per 100,000 residents. During the 1930s and 1940s, mosquito control efforts contributed to a large reduction in the human malaria cases in Florida. Despite concerted efforts of U.S. government and the Bill and Melinda Gates Foundation to combat this disease, malaria is a growing international problem. Annually, there are 400-500 million cases of malaria, with at least 2 million fatalities. Despite a substantial decline in locally-acquired malaria transmission in the U.S., dozens of imported malaria cases are reported annually in Florida residents returning from international travel. These imported cases could serve to reestablish local malaria transmission in Florida if native *Anopheles* mosquitoes feed upon infected travelers. In fact, a camper in the Florida Panhandle (Gulf County) acquired malaria from local mosquitoes in 1990, as did two individuals in Palm Beach County in 1996 and eight individuals in the same area of Palm Beach County in 2003, highlighting the potential for renewed endemic malaria transmission in the State.

8.1.10 Dog Heartworm

Dog heartworm is a chronic disease of dogs and sometimes cats that is due to a mosquito-transmitted filarial worm (*Dirofilaria immitis*). Male and female adult worms reside in the pulmonary artery of dogs where they mate and female worms release large numbers of microscopic, embryonic worms (microfilariae) into the host's bloodstream. Microfilariae are ingested by susceptible blood-feeding mosquitoes and develop into infective L3 larvae which eventually infect the mosquito salivary glands. The L3 larvae escape through the mosquito's proboscis during blood feeding on a new mammalian host (ideally, another dog). The L3 larvae molt to the L4 stage in the new host's subcutaneous tissue at the site of the initial mosquito bite. The L4 larva molts to become an immature adult and migrates to the pulmonary artery where it attaches for life and becomes a mature adult worm. Untreated, heartworm is often a fatal disease in dogs. Human exposure to infective heartworm larvae is probably common, since many of Florida's common mosquito species are potential vectors of dog heartworm. Although heartworms fail to fully complete development in humans and cause no real disease, the invading worms are sometimes detected in lung X-rays, where they are easily confused with cancerous lesions. Prevention and management of heartworm in dogs is best accomplished by use of available pharmaceuticals which kill the infective larvae that are introduced by infected mosquitoes.

8.1.11 Mosquito Annoyance, Discomfort, and Allergic Reactions

Although mosquito control programs cannot directly address the needs of those effected, a few minor public health consequences caused by mosquitoes should be mentioned. An undocumented but presumably small proportion of the human population experiences true allergic reactions to mosquito bites. Undoubtedly, a larger number of people (especially children) suffer from self-induced injury related to excessive scratching of fresh mosquito bites. This scratching response can cause considerable alarm in parents of infants and does produce some risk of secondary bacterial infection. Another documented though rare phenomenon is the syndrome described as delusional parasitosis (DP), also known as Ekbom's syndrome. Sufferers of DP experience an uncontrollable, irrational fear of insects resulting from their belief that their bodies are infested (despite evidence to the contrary). Sufferers may induce numerous secondary skin lesions by cutting and scratching imaginary bites, and this behavior reinforces their conviction of a personal insect infestation.

8.2 ECONOMIC COST OF MOSQUITO-BORNE DISEASE SURVEILLANCE, PREVENTION, AND CONTROL

There has never been a careful analysis of the state-wide costs of surveillance, prevention, and control of mosquito-borne diseases in Florida. Such an analysis should include costs that accrue during normal arboviral transmission years as well as during epidemic years. Costs incurred by mosquito control programs, public health agencies, and businesses – as well as the cumulative medical and other costs incurred by individual citizens – should be part of the analysis. The tourism industry and school administrators bemoaned loss of revenue during the 1990 SLE epidemic in Florida when cancellation of school-sponsored outdoor athletic events (especially high school football games) was frequently cited as an example of an adverse economic impact of SLEV transmission. During non-epidemic years, disease-related responses by mosquito control programs and public health agencies do not constitute a major part of their budgets, but the collective, state-wide expenditure of arboviral surveillance would probably still be substantial. Mosquito-borne diseases in Florida produce a significant cost to the state, even when not at the forefront of public attention.

Costs associated with a single human case of eastern equine encephalitis, St. Louis encephalitis, and West Nile fever and encephalitis can be enormous and drain personal and public funds alike. Non-fatal human cases resulting from infection by one of these viruses can result in residual neurological damage, and full recovery to a productive life often is not possible. A 1995 cost analysis of EEE survivors in Massachusetts (Villari *et al.*) revealed that life-time medical care and support for an individual surviving severe infection was approximately three million US dollars per case. This cost exceeded the economic burden imposed by other major infections (including AIDS). Even mild cases of EEE that did not cause chronic residual neurological damage resulted in an economic burden of $21,000 per case. The Massachusetts study concluded that the costs of a single severe case of EEE far exceeded direct costs of statewide aerial adulticiding during periods of high epidemic risk. There is also a growing body of evidence that West Nile survivors frequently require costly long-term medical care. A 2006 study by Carson and colleagues of 49 patients surviving West Nile fever or West Nile neuroinvasive disease concluded that half of the infected individuals had lingering health issues thirteen months after diagnosis, including fatigue, memory problems, depression, and tremors.

8.3 MOSQUITO-BORNE DISEASE SURVEILLANCE IN FLORIDA

Vector-borne disease systems offer special challenges to those responsible for the prediction, prevention, and control of disease outbreaks. With the exception of vaccines manufactured for YFV and Japanese encephalitis virus, commercially produced human vaccines are not available for other mosquito-borne viral pathogens. Most of the vector-borne disease systems that are endemic to Florida have complex transmission cycles that involve multiple vertebrate and invertebrate host species. The abundance, reproductive success, and age structure of host populations is influenced by rainfall and temperature patterns, factors that are also inherently unpredictable. Consequently, outbreaks of mosquito-borne disease cannot be reliably predicted except in a general way or, at best, only over a short period of time.

Dengue provides a dramatic illustration of the difficulty in predicting impending epidemic activity. Dengue outbreaks are notoriously difficult to predict, despite the fact that humans are the only vertebrate host involved. This situation is especially true in Puerto Rico, which experiences endemic dengue activity annually with periodic epidemic outbreaks, despite the presence of the Centers for Disease Control and Prevention's prestigious dengue laboratory in San Juan. Outbreaks and epidemics of zoonotic diseases caused by WNV, SLEV, and EEEV that involve disease cycles in other vertebrate species (wild avian species for all three of these viruses) are even more difficult to predict.

Unlike many human diseases, mosquito-borne diseases are, for the most part, not transmitted from person-to-person. Notable exceptions include WNV that can be transmitted via blood transfusion and organ transplant, and ZIKV that can be transmitted sexually. For most mosquito-borne viruses the main mode of transmission is through the bite of an infective vector. Medical and public health workers that are more accustomed to dealing with the transmission patterns of conventional infectious diseases do not always appreciate this fundamental distinction. Public health agencies are understandably reluctant to issue *Mosquito-borne Illness Alerts* until at least one human case is confirmed. However, it has been repeatedly established that emergency measures to reduce arboviral encephalitis transmission are largely ineffective if delayed until a human case of locally-acquired infection is confirmed. In the case of the 1990 epidemic caused by SLEV in Indian River County, Florida, most of the St. Louis Encephalitis cases in that county were already contracted by the time the index case (the first regional case) was confirmed. Timely surveillance data and a quick vector control response to sentinel, animal disease, and vector surveillance data are clearly essential to mitigate the threat of a widespread arboviral disease outbreak.

8.4 ARBOVIRAL DISEASE SURVEILLANCE IN FLORIDA

Surveillance and control of mosquito-borne disease in Florida was initially the responsibility of the State government. In fact, epidemics caused by YFV were the stimulus behind the creation of the Florida State Board of Health (FSBH) in 1889. In its early years, FSBH was heavily involved with issues related to YFV, DENV, and malaria, while in the early 1960s emphasis switched to the emerging problem of SLEV. This focus prompted the development of the Epidemiology Research Center in Tampa to provide laboratory diagnostic services and conduct research related to Florida vector-borne diseases. Following the 1977 central Florida SLE epidemic (110 cases in 20 counties), the Florida Sentinel Chicken Arboviral Surveillance Program (FSCASP) was established by FSBH. This program provided laboratory services and financial assistance to local agencies to monitor transmission of SLEV and EEEV by screening blood samples from sentinel chickens maintained in the field for antibody production to these viruses. In 2001 the program was expanded to include the monitoring of sentinel chickens for antibody production against WNV and Highlands J virus (HJV). This support provided important data concerning the seasonality of arboviral transmission in and around participating counties. The FSBH was merged into the Department of Health and Rehabilitative Services (FDHRS) in 1976. In 1997, the FDHRS was reorganized and all health functions were transferred to the Florida Department of Health (FDOH).

Annual participation in the FSCASP has varied from county to county since its inception in 1978. Eleven Florida counties (Charlotte, Duval, Indian River, Lee, Leon, Manatee, Martin, Orange, Palm Beach, Pinellas, and Volusia) have monitored sentinel chickens for all 40 years of the program's existence. Although the State continues to provide serologic testing of sentinel blood samples, state funding for other costs of the program such as flock establishment and maintenance disappeared in the 1980s. It can be argued that the sentinel chicken program is no longer fundamentally a state surveillance program, as participation is entirely dependent on the interest and resources of the local agency. Current participants are typically mosquito control programs, although a few county health departments (CHDs – part of the FDOH structure) are directly involved. Participants submit sentinel chicken serum samples to the FDOH Bureau of Laboratories in Tampa, where hemagglutination-inhibition (HI), IgM ELISA, plaque reduction neutralization tests (PRNT), polymerase chain reaction (PCR), and virus isolation tests are performed to detect evidence of exposure (antibodies or antigens to) or infection with (virus nucleic acids or infectious virus) SLEV, WNV, EEEV, and HJV. Each week, participating agencies receive electronic copies of the test results for their serological and whole blood submissions of that week. Combined results from all participating counties also are provided when requested. These combined results are also distributed to each mosquito control program director and to all CHDs in the state. The FDOH reports the weekly serologic data to the local agency but does not recommend any specific courses of action relative to vector control. Active surveillance for mosquito-borne disease is non-existent in most Florida counties.

While the FDOH Division of Environmental Health conducts surveillance for arboviral encephalitis in humans, the FDOH CHD Director is responsible for issuing local *Mosquito-borne Illness Advisories* and *Alerts* for WN, SLE, or EEE. The local mosquito control agency should be consulted prior to the issuance of a *Mosquito-borne Illness Advisory* or *Alert* since in addition to being a "first responder" the mosquito control agency is usually the collector of all local surveillance data. Keeping this line of communication open necessitates a close collaboration between the local mosquito control programs and the local Public Health Unit. Unfortunately, this communication does not always exist. In some counties there is a long-standing communication problem that undermines the local ability to respond to increased epidemic risk.

Supplemental arboviral surveillance data are provided by the FDACS Bureau of Diagnostic Laboratories in Kissimmee, which tests veterinarian-submitted blood and serum samples from horses and other domestic animals for antibodies to WNV, EEEV, VEEV, and other agents. These data are frequently of lesser public health value because the number of submissions is generally too low to adequately sample any particular locality and data are generally reported long after infection or onset dates. Lee County Mosquito Control District performs its own testing on sentinel chicken sera collected by its program, although paired samples also are sent to the FDOH Bureau of Laboratories in Tampa. Other counties and districts of the state also collect serological samples from wild birds to monitor arbovirus activity.

The FDOH also monitors Florida Fish and Wildlife Conservation Commission wild bird mortality data as a passive indicator of WNV transmission. For mosquito-borne diseases other than WN, SLE, HJ, and EEE, surveillance is largely restricted to the monitoring and reporting of human cases by Florida public health agencies. In 2016 FDACS began testing selected mosquito pools for arboviral agents including EEEV, WNV, Zika virus, and dengue viruses.

8.5 GENERAL APPROACHES TO ARBOVIRAL SURVEILLANCE IN FLORIDA

One of the principal goals of most arbovirus surveillance programs is to anticipate circumstances conducive to the appearance of disease in humans before this occurs (or at least before many cases of disease are suspected or diagnosed). From this perspective, it is possible to group all surveillance methods or approaches into two broad categories: **Predictive factors** and **direct transmission monitors**. Ideally, an integrated surveillance program (Day 2001, Day and Lewis 1992) will incorporate surveillance components from each category. Integrated programs provide more complete data to guide control decisions than do single factor surveillance programs such as those that only monitor vector abundance.

Predictive factors are not directly linked to virus transmission *per se* but rather have an indirect correlation or relationship to transmission. Direct monitors of arboviral transmission, such as sentinel chicken seroconversion rates, are more likely to influence decision-making by public health agencies, since they provide data that suggest imminent viral transmission risk. Predictive factors that may be monitored as part of an integrated arboviral surveillance program include the following:

- Population density and distribution of known and suspected vector species

- The age structure and population dynamics of known and suspected vector species

- Real-time, recent and long-term local rainfall and temperature data (These factors not only affect vector numbers but also can influence vector behavior, vector population age structure, and the reproduction, migration, and dispersal of vertebrate amplification hosts of the virus.)

- Abundance, immunological status, and age structure of known and suspected vertebrate amplification host populations

- Prevalence of infected mosquitoes in field samples (Minimum Field Infection Rates (MFIR) in mosquitoes)

- Temporal and spatial distribution of recent viral transmission events

Testing the immunological status of wild vertebrates, such as birds, may provide a direct measure of virus transmission under four conditions:

1) Serological tests are diagnostic of a recent infection (presence of IgM antibody).
2) Positive antibody samples come from a nestling or hatching-year bird or juvenile animal.
3) A series of negative serum samples precede a positive sample.
4) Live virus is isolated from a blood sample.

Without meeting one (preferably more than one) of these conditions, it is difficult to determine where or when an antibody-positive wild animal was bitten by a virus-infected mosquito. Consequently, because of the mobility of wild animals, it is not typically possible to determine the temporal or spatial infection parameters of an antibody-positive wild animal unless one or more of the conditions listed above are met.

Mosquito infection rate data do not necessarily predict present or future arboviral transmission risk. Even assuming that an adequate number of mosquitoes are sampled and screened (which is rarely achieved), the detection of virus in a mosquito pool (typically 50 females from the same species from a specific locality) cannot distinguish between mosquitoes capable of transmission and those that do not have a disseminated infection and are therefore cannot transmit. These “non-transmitters” could include individuals that are incompletely susceptible (perhaps with infection limited to the midgut), as well as potentially competent individuals who were infected too recently to have developed salivary gland infections. Climatic factors such as extreme temperature and drought affect mosquito longevity and may prevent newly infected individuals from surviving to an age when transmission will occur. Unfortunately, mosquito infection rates calculated by assay of pooled mosquitoes are usually assumed to be far more precise than can be justified. Seemingly large differences in calculated infection rates are generally statistically identical, since such group-testing inherently creates extreme confidence limits that can only be overcome by testing much larger mosquito samples than those generally processed. The extreme abundance and mobility of *Cx. nigripalpus* in Florida also has implications for the use of mosquito infection rates as a primary surveillance tool for WNV and SLEV. The SLEV infection rates rarely exceeded 1:1000 in *Cx. nigripalpus* during the 1990 epidemic in Indian River County (Shroyer 1991) and mosquito infection rates for these viruses during non-epidemic years are much lower. During low transmission periods exceedingly large numbers of mosquitoes need to be collected, pooled, and assayed in order to achieve the statistical probability of a single virus-positive pool. The method of mosquito sampling can have a strong impact on the perceived infection rate in the vector population. Traps that attract host-seeking females, such as carbon dioxide-baited light traps, collect a relatively large number of recently emerged, unfed females that have never taken a blood meal. These virus-negative females may cause an underestimation of the natural MFIR in the vector population. On the converse, traps that selectively sample egg-laden (gravid) females, such as CDC gravid trap, often yield much higher MFIR in mosquitoes, as these traps target females that have previously taken blood from a potentially infective host. These trap-related biases in MFIR should be considered when interpreting surveillance data to inform transmission risk.

Direct monitors of arboviral transmission include:

- Regular serological testing of sentinel vertebrates maintained in habitats appropriate for sampling the target vector mosquito species

- Isolation of live virus from wild or sentinel vertebrates

- Monitoring human cases of arboviral disease (*i.e.*, human sentinels)

The last option is not acceptable as a primary arbovirus surveillance tool, yet it is often the default method employed because resources (or interests) will not support a more comprehensive surveillance program. Clinical symptoms of most arboviral diseases are non-specific and, unless alerted to the possibility, local physicians often fail to recognize the initial human cases in an outbreak. As noted previously, retrospective analysis of arbovirus epidemics provides examples in which a substantial proportion of all human cases were actually infected before the index case (first case) was identified.

When the goal of arbovirus surveillance is to provide an early warning during times of elevated risk for disease transmission to humans, the integrated arboviral surveillance system will yield one of three possible outcomes:

1) Ideally, the surveillance system will detect a period of risk in advance of the appearance of human disease. This outcome not only provides an opportunity to initiate preventive and emergency control procedures but also provides time to alert the medical

community about potential human cases that might not otherwise be recognized as mosquito-borne disease.

2) Surveillance systems may fail to predict increased transmission risk. Due to inaccuracies in data sampling or interpretation a surveillance system may fail to indicate an imminent risk of human infection, when in fact an outbreak is about to occur. In this false-negative scenario, the responsible agencies are caught off guard by the virus. The appearance that virus activity is at a low level can sometimes foster a false sense of security.

3) Surveillance data may instead suggest the imminent occurrence of a disease outbreak, when in fact one will not materialize. This false-positive outcome sometimes provokes charges that the responsible agency is "crying wolf" and that surveillance activities are a waste of time.

In addition to the obvious potential of endangering public health, either type of failure can seriously jeopardize local support for surveillance programs and personnel. Responsible public health agencies should endeavor to err on the conservative side by issuing public alerts when field data indicate that increased arboviral virus transmission to humans will possibly occur.

In their efforts to establish the best arbovirus surveillance program possible, agencies sometimes fail to recognize that there is no single, universally applicable, superior arboviral surveillance method. Integrated strategies of arbovirus surveillance for prediction of disease outbreaks are the least likely to fail, and different agencies concerned with the same virus may be completely justified in following different strategies in their surveillance programs.

Heavily-populated urban regions generally will require different surveillance strategies than predominantly rural ones. Rural-oriented programs often contend with extensive natural or agricultural wetlands, as well as a smaller human population (*i.e.*, smaller tax base to provide operating funds). Even when concerned with the same disease, different local agencies must deal with differing terrain, mosquito habitats, human population patterns, financial constraints, and logistical limitations on their ability to effectively control vector mosquitoes. It would be inappropriate and dangerous to expect each local jurisdiction in Florida to design its surveillance program along identical lines.

No matter what specific surveillance methods are employed, a local arbovirus surveillance program needs several years of operation to accumulate a baseline surveillance data set that is useful for assessing epidemic risk in that geographic area. Surveillance programs can succeed in the long run only to the extent that they are able to clearly define realistic surveillance goals that are appropriate for the available control and response resources.

The organizational structure of the local mosquito control office and its relationship to the local County Health Department (CHD, which has responsibility for issuing a *Mosquito-borne Illness Advisory* or *Alert*) are also important considerations. Where active arboviral surveillance is conducted locally by an independent mosquito control district, it is especially important to have pre-established lines of communication with the CHD. Otherwise, it likely will prove impossible to effectively motivate the CHD to issue a medical alert when surveillance data clearly warrant one.

8.6 AN OVERVIEW OF CURRENT ARBOVIRAL SURVEILLANCE METHODS IN FLORIDA

As noted above, surveillance of human cases of mosquito-borne disease in Florida is done by the state FDOH Division of Environmental Health in Tallahassee and is dependent upon data provided by individual physicians and CHDs. Surveillance of equine cases of EEE and WN is conducted by the State Veterinarian's Office and the FDACS Bureau of Diagnostics Laboratories in Kissimmee. These data are made available to local mosquito control programs on a weekly basis. However, serologic testing for arboviruses is done for patients and animals with generalized symptoms consistent with mild forms of WN, SLE or EEE only infrequently, unless there has already been a recent medical alert notifying local physicians of the possibility of local arboviral transmission. This situation highlights why human and animal case surveillance usually provides data too late to allow implementation of a meaningful vector control intervention.

Aside from human and equine case surveillance and reporting, other forms of arboviral surveillance currently used in Florida include:

- Monitoring transmission of WNV, SLEV, HJV, and EEEV to sentinel chickens and occasionally other birds such as pheasants and quail. In 1990, data from sentinel chicken flocks maintained throughout south Florida detected the imminent arrival of Florida's most extensive SLE epidemic *before* the appearance of the human index case.

- Monitoring an aspect of vector population density, usually by selectively trapping some fraction of the population that is actively flying at night. In many cases, this type of surveillance is performed primarily to assess the need for insecticide applications to reduce the annoyance of biting mosquitoes.

- Monitoring qualitative indices of selected vector populations to track (in real-time) the physiological status, blood-feeding activity, oviposition, and physiological age of local vector mosquitoes

- Monitoring and testing dead wild birds for WNV

- Serologic sampling of wild vertebrates for evidence of recent and past arboviral infection

- Laboratory analysis of field-collected mosquito pools for arboviruses.

Sentinel chicken surveillance, vector and mosquito surveillance, and the collection of wild bird mortality data are commonly used by Florida agencies. The physiological monitoring of vector mosquitoes, serological sampling of wild vertebrates, and mosquito pooling are done either experimentally or in limited areas, usually with active participation by university entomologists and vector specialists.

Innovative molecular technologies are being applied to many diagnostic problems in medicine and have application to the highly specialized needs of those monitoring vector-borne diseases. Polymerase chain reaction (PCR)-based diagnostic tests, in particular, are attractive candidates for increasing the speed and, in some cases the accuracy, of detecting the presence of an arbovirus. PCR technology has been field-tested for all of the major North American arboviruses and has proven exceptionally efficient for detecting viral RNA in vertebrate tissues (particularly blood and serum) and pooled mosquitoes. Analytical problems associated with false-positive tests that rely upon the detection of relatively small sections of total viral genome, rather than the replication of live virus, remain an important hurdle that needs to be overcome before PCR will become a complete surveillance tool.

There is an unfortunate tendency to view the welcome development of molecular tools as a “magic bullet” that will eliminate the need for other forms of arboviral surveillance, particularly the monitoring of virus transmission to sentinel animals. Yet, no matter how useful, these molecular techniques merely represent advances in virological methods and do not replace or eliminate the need for monitoring virus transmission to wild and sentinel animals. The molecular monitoring of arboviral infection rates in field-collected mosquito pools represents a fundamentally different part of the virus transmission cycle than does the monitoring of actual virus transmission to sentinel chickens. Pool screening of field-collected vectors using PCR or other advanced molecular methods should not be used in isolation but rather together with other surveillance techniques to augment information in an integrated approach to arbovirus surveillance (Day and Lewis 1992).

8.7 CONTROL OF ARBOVIRAL DISEASE EPIDEMICS

When an epidemic of mosquito-borne disease is imminent or in progress, the primary means of vector control and disease mitigation are: 1) aggressive insecticidal treatment of suspect vector populations including aerial applications and 2) notification and education of the medical community and the general public. Notification of the medical community enhances the likelihood of proper clinical diagnosis of suspected cases and makes it possible to follow the course of the epidemic. This information can be useful in determining when the risk of further transmission has declined and when emergency surveillance and control measures can be terminated. While notification of the public of an impending epidemic can generate anxiety if treated sensationally by news media, surveillance personnel have an obligation to accurately share information regarding the epidemic health risk with Florida residents and visitors. The overriding benefit of public notification is that citizens can be encouraged to take personal protective measures, such as reducing exposure to mosquito bites by use of repellents, by wearing protective clothing, or by modifying outdoor activity. The benefit of public notification is probably most pronounced in situations where insecticidal control of the vector mosquito is difficult and/or ineffectual (*e.g.*, control of SLEV or WNV vectors). It is believed that aggressive public notification prior to the 1990 SLE epidemic in Indian River County substantially reduced the number of human cases (Day and Shaman 2011).

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Chapter 9

MOSQUITO CONTROL BENEFITS AND RISKS

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Summary

In Florida, both mosquito control and the protection of environmentally sensitive habitats are legislatively mandated. Clearly, modern mosquito control poses some environmental risks, yet it just as obviously provides benefits. Public health protection, improved human comfort from mosquito annoyance, and economic gains are the most obvious benefits. Impacts on fish, wildlife, and non-target arthropods are some of the risks. There is also a growing concern about the risks of human exposure to pesticides in general. These potential impacts to both natural communities and to humans need to be sufficiently understood to help risk/benefit analysis that can result in informed decision making.

Modern mosquito control consists of an integrated pest management program, utilizing adulticiding, larviciding, and source reduction as appropriate and incorporating a public education component. Mosquito control agencies and environmental land management agencies are required to work together, carefully weighing the risks and benefits in each situation, to resolve any controversial issues that arise.

9.1 INTRODUCTION

The use of various chemicals to attempt to control pests of humans, crops, and animals has been documented since ancient times. Homer described how Odysseus fumigated a house with burning sulfur to control pests (Ware 1994). The Chinese used arsenic sulfide to kill insects (Pimentel and Lehman 1993). The use and success of chemicals drastically changed with the development of synthetic pesticides a little over fifty years ago. The Swiss chemist Paul Müller discovered the insecticidal properties of the organochlorine pesticide dichloro-diphenyl-trichloroethane (DDT), and the United States Department of Agriculture (USDA) laboratory in Orlando developed it for field use by the armed services. An arsenic compound (Paris green) was used in Florida for larval control during the 1960s.

These and many other synthetic pesticides were developed by scientists for the control of insects and other pests in many situations in both public health and agriculture. In the early years their effectiveness, just like that of antibiotics, was so dramatic that their development was considered miraculous. As a result, these chemicals were widely and often indiscriminately applied. While some people questioned such a widespread use of pesticides, many more people praised it. At that time, research had not yet documented the environmental, ecological, or human hazards of these materials. What people did know throughout the world was that chemical control of mosquitoes and other pests significantly reduced human illness and death and greatly improved human comfort.

The risks involved with pesticide use were not widely questioned until the early 1960s when Rachel Carson published *Silent Spring* (Carson 1962). Although some considered the science controversial (*e.g.*, Edwards 2002), this publication increased public awareness of issues such as:

1) Acute and chronic pesticide impacts to humans, wildlife, and other non-target species
2) The persistence of certain pesticides in the environment
3) The transport of pesticides outside target areas, which can cause unintended environmental damage

Mosquito control contributes to some of these environmental problems, but compared to agricultural methods and materials, mosquito control pesticides are applied at lower dosages and in smaller amounts (Lyon and Steele 1998). In Florida, agriculture and lawn care are believed to represent much greater potential impacts to the aquatic environment than does mosquito control (Hushon 2006).

Mosquito control pesticides are regulated federally by the U.S. Environmental Protection Agency (EPA), which is responsible for authorizing labels for allowable chemicals. The legal authority for mosquito control in Florida is Chapter 388 Florida Statues (F.S.). Mosquito control is regulated by the Florida Department of Agriculture and Consumer Services (FDACS), which designates which chemicals are permitted for use. FDACS also oversees Florida mosquito control operations by making certain that they comply with Florida Statutes and any appropriate rules.

The United States Census Bureau estimates that from 1900 to 2010 Florida's population increased by almost 3,500%. There is little doubt that implementation of both physical and chemical mosquito control techniques aided in the development and utilization of areas that previously were not considered acceptable for human habitation. Certainly, Florida's explosive growth in coastal areas after World War II was due in large part to the use of synthetic pesticides and physical methods to control mosquitoes.

Controversy often accompanies mosquito control, because the chemicals frequently are applied in developed areas, and some people are concerned with their own exposure. Treatment also occurs in natural areas, including protected public lands, and some people are concerned about effects on wildlife. It is vitally important that the risks and benefits of mosquito control practices are analyzed scientifically so that the control decisions can be made with a good understanding of their effects.

Prior to the early 1980s, mosquito control practices were questioned only when obvious, adverse effects on wildlife were observed (*e.g.,* Patterson 2004). We now better appreciate the complex interrelationships of organisms within an ecosystem. For instance, the food of many marine organisms consists of small arthropods or organisms that are similar in size to mosquito larvae, and such organisms differ greatly in their susceptibility to pesticides (Curtis and Profeta 1993). Some organisms may be more sensitive to pesticides than mosquitoes. Impacting any portion of this food web may affect other parts or even the entire web. The current lack of knowledge concerning the biology of many non-target species and their community functions further complicates the problem of risk/benefit analysis.

Mosquito control practices usually focus on the monitoring of mosquito populations with little or no routine monitoring of non-target species. The difficulty and cost of monitoring non-target effects in the natural environment has impeded this type of work. Ideally, long-term goals for non-target assessments are:

1. Identify key non-target indicator species to monitor on a routine basis
2. Establish insecticide impact thresholds for these indicator species
3. Develop standardized methodologies for monitoring post-application insecticide residues

Moreover, mosquito control programs must place more emphasis on non-chemical techniques to control mosquitoes in order to reduce non-target impacts.

9.2 INTEGRATED PEST MANAGEMENT (IPM)

It is important that mosquito control agencies maintain a broad selection of tools, both chemical and non-chemical, to use in managing mosquito populations in Florida. It is also important that the potential impacts to both natural communities and to humans are understood sufficiently to help in risk/benefit analysis that can result in informed decision-making. Mosquito-borne diseases have had a great impact on the settlement and development of Florida (Hribar 2013). For the most part, since 1949, mosquito agency activities have been directed primarily towards nuisance mosquitoes, those which are of economic importance but do not transmit diseases to humans. However, recent outbreaks of chikungunya, dengue, and Zika viruses in Florida are reminders that mosquito-borne diseases are on the rise worldwide, and several diseases are threats to public health and animal health in Florida. These diseases include St. Louis encephalitis, eastern equine encephalitis, Highlands J encephalitis, West Nile encephalitis, California group encephalitis (*i.e.,* Keystone and Trivitattus), dengue, chikungunya, and Zika. With Americans making millions of visits per year to developing countries the risk of imported viral diseases is significant (Mirzaian *et al.* 2010).

The most effective and environmentally sound pest control programs are based on a combination of methods including source reduction, chemical control, and biological control (Rose 2001). Using a combination of these techniques is termed Integrated Pest Management (IPM). IPM has been developed to encourage a balanced usage of cultural and insecticidal methodologies and habitat manipulations in order to minimize adverse environmental impacts. To effectively use IPM, it is necessary to have a thorough understanding of the basic biology of the pest species and the many factors that influence their density. Because of rapid mosquito population reduction and economic considerations, many mosquito control programs use chemical applications as their primary control method. A program that relies solely on chemical control is not an IPM program. While most components of an IPM program have some level of environmental risk, the overall risks are likely to be less than a program that relies solely on chemical control, which might cause undesirable non-target mortality and contribute to chemical resistance in mosquitoes.

9.3 MOSQUITO CONTROL INSECTICIDES: PAST AND PRESENT

The synthetic pesticides used for mosquito control over the years have varied greatly in structure, toxicity, persistence, and environmental impact. These chemicals include the following:

Organochlorine pesticides are no longer used for mosquito control in Florida, although methoxychlor was labeled for use until its cancellation in 2003 (Edwards 2004). Some organochlorines that were formerly used included DDT, BHC, chlordane, heptachlor, aldrin, and dieldrin. Organochlorines are relatively non-soluble in water and very persistent in soils. Also, organochlorines are lipophilic, *i.e.*, they bioaccumulate in fat and other lipids. Largely, it was these lipophilic properties that resulted in organochlorines no longer being labeled for use in the U.S. These bans are still actively criticized by some (*e.g.*, Tren and Bate 2000, Bailey 2002, Edwards 2004). In spite of cancellation of all uses of these chemicals in the U.S. by the EPA between 1973 and 1988 (Ware 1994), many soils and rivers are still contaminated with residues of the most persistent of these compounds (*i.e.*, DDT, endrin, dieldrin) (White and Krynitsky 1986), and they continue to be detected in wildlife (Clark *et al.* 1995, Sparling *et al.* 2001). Although the total concentration of DDT residues in the U.S. appears to be declining (Nowell *et al.* 1999), they still pose risks to wildlife. In late 1998 and early 1999 hundreds of water birds were killed from eating contaminated fish at the North Shore of Lake Apopka Restoration Area linked to organochlorine pesticide residues, including DDT and DDE, in farm fields (USFWS 2004). Millions of public dollars were subsequently spent to mitigate and sequester the contamination. The Lake Apopka disaster was a classic case of bioaccumulation of organochlorine residues that had lain dormant in soil until restoration activities created conditions for their release. Organochlorines continue to be used for agricultural and mosquito control in developing countries.

Organophosphates (OP). Although OPs are generally less persistent than organochlorines, some have higher acute toxicities for mammals and other organisms (Pimentel and Lehman 1993). Currently recommended OP compounds are the adulticides malathion (Fyfanon®) and naled (Dibrom®). These compounds have relatively low mammalian toxicity and most usually break down rapidly; However, some intermediate breakdown products are also toxic. Accidental discharge of organophosphorus insecticides into aquatic environments has caused fish kills, and some of the OP compounds are toxic to microcrustaceans such as *Daphnia* spp. (WHO 1986a). Fenthion (Baytex®) is no longer used for mosquito control in the U.S. The registration of Abate® (temephos) for mosquito control was voluntarily cancelled by the manufacturer; Stock currently possessed by districts may be used, but no new product will be manufactured or sold (Keigwin 2011).

Pyrethroids. Pyrethroid insecticides are based on the chemical structure of a group of naturally occurring compounds, pyrethrums, derived from a flower native to Africa. Products extracted from these flowers have been used for thousands of years and are still used today but are extremely expensive. Artificially created pyrethroids used today in Florida for mosquito control are bifenthrin, resmethrin, permethrin, and sumithrin. Pyrethroids are more persistent than natural pyrethrums and in a few cases are more persistent than OPs, although resmethrin degrades rapidly in the environment (WHO 1989). Pyrethroids are broad-spectrum toxicants that are very toxic to fish, aquatic organisms, and most other cold-blooded animals. Due to their high and broad range of toxicity to insects, they may affect beneficial species, thereby lessening natural controls and, for some pests, may actually increase the need for further chemical control (Edwards 1993). However, to date, a need for increased chemical control, because of pyrethroid use for mosquito control, has not been demonstrated. Pyrethroids exhibit low toxicity to birds and mammals (EPA 2002). The registration of Scourge® (resmethrin) for mosquito control was voluntarily cancelled by the manufacturer; Existing stock may be sold and used, but no new product will be manufactured (Goodis 2013).

Carbamates. Methyl carbamates are related chemically to physostigmine, a naturally-occurring alkaloid isolated from the calabar bean (WHO 1986b). No carbamates are currently used for mosquito control in Florida, although propoxur has been used. Carbamates are broad-spectrum, tend to be more persistent than organophosphates in soil, and thus have the potential for considerable environmental impact (Edwards 1993). However, data exist that suggest carbamates are liable to degradation by soil microorganisms (WHO 1986b). Propoxur is considered to be moderately toxic to mammals (WHO 1986b).

Insect Growth Regulators (IGR). IGRS interfere with insect development typically resulting in larval or pupal mortality. For more than thirty years, the insect growth regulator methoprene, Altosid®, has been a widely used mosquito larvicide in Florida and elsewhere in the world. Methoprene is specific to immature insect larvae, especially dipterans, which include mosquitoes. Methoprene has extremely low mammalian toxicity. Diflubenzuron (Dimilin®), a chitin inhibitor, has much broader non-target impacts than methoprene, especially on marine and freshwater arthropods such as shrimp and crabs. Therefore, Dimilin is severely restricted to certain sites and is not widely used.

Biologicals. *Bacillus thuringiensis israelensis* (*Bti*) and *B. sphaericus* (*Bs*) are both bacterial larvicides (acting as stomach poisons) that are quite specific to mosquito larvae and a few other aquatic dipterans. *Bti* is used worldwide. *Bs* is more recently labeled and is only effective in freshwater habitats. *Bs* has a narrower host range than does *Bti* (Bauman *et al.* 1991). *Bs* can be used in water of much lower quality than can *Bti* and can actually improve water quality by suppressing algal growth (Silapanunatakul *et al.* 1983, Su and Mulla 1999). Both are non-toxic to mammals and exhibit few or no non-target effects (WHO 1999, Ware 1994, Boisvert and Boisvert 2000). Spinosad is an insecticide derived from fermentation metabolites of the actinomycete bacterium *Saccharopolyspora spinosa.* Spinosad is a stomach and contact poison for insects; Its toxicity is species-dependent, ranging from very highly toxic to essentially nontoxic. Spinosad is very slightly toxic to mammals, essentially nontoxic to birds, and slightly to moderately toxic to fish and aquatic invertebrates (EPA 1999).

Surface films. Petroleum distillates (*i.e*., oils) are used as pupacides to suffocate mosquitoes prior to adult emergence. These oils can be toxic to predatory Hemiptera and Coleoptera, as well as sheepshead minnows but are not toxic to rotifers and some protozoa (Mulla and Darwazeh 1981; Tietze *et al*. 1993, 1995). Monomolecular films, alcohol ethoxylated surfactants, are used as larvicides and pupacides. They disrupt surface tension and cause larvae and pupae to drown. Monomolecular films currently are being evaluated in Florida regarding their toxicity to non-target insects in salt water marsh habitat. Monomolecular films are not as efficacious when exposed to high winds (Nayar and Ali 2003).

9.4 BENEFITS OF MOSQUITO CONTROL

Broadly speaking, the benefits of mosquito control can be divided into three classes: Nuisance benefits, economic benefits, and public health benefits. Nuisance benefits include relief to people around homes or in parks and recreational areas. Nuisance benefits can even be said to extend to pets and to wildlife. Economic benefits include increased real estate values, enhanced tourism and related business interests, or increased livestock or poultry production. Public health benefits include the reduction of infectious disease agents.

9.4.1 Nuisance Benefits

A benefit of mosquito control that has contributed greatly to Florida's growth is the tremendous progress made in controlling pestiferous mosquito species, especially those that are found in coastal marshes. Although many of these pest mosquitoes do not present a threat of disease transmission to humans, they significantly effect human comfort. Prior to the advent of organized mosquito control in Florida, mosquito numbers were such that residents could not go outdoors after dark, and many coastal towns closed down for the summer season (Harden 1981). The influx of an estimated 700-800 people moving to Florida daily and the fact that much new development occurs near mosquito-producing habitats put increasing pressure on mosquito control agencies to maintain effective control programs.

The nuisance factor to pets also may be considered important to many people. Video evidence exists that mosquitoes are severe pests of purple martin nestlings (Hill 1994). The introduction of novel viral pathogens into naïve populations may have impacts (Farajollahi *et al*. 2004, Miller *et al*. 2005). For example, during the 2001 West Nile virus outbreak in Florida, 1,106 dead birds reported to the Florida Department of Health were found to be infected with West Nile virus. The affected birds comprised 10 orders and 25 families (Blackmore *et al*. 2003).

9.4.2 Economic Benefits

Florida's economy benefits from tourism (106.6 million visitors in 2015) which depends on the beaches, fishing, golfing, amusement parks, and the outdoors in general. Tourism resulted in $108.8 billion in taxable sales in 2015 and supported over one million jobs for Floridians (Visit Florida 2017). Most of these visitors have little tolerance for mosquitoes, and it seems reasonable that mosquito control helps many visitors enjoy their stay and, therefore, helps the Florida economy. Perhaps the most striking illustration of the economic benefits of controlling mosquitoes is the classic graph by Dr. John A. Mulrennan, Sr. showing that for the period 1950-1967, the decline in average light trap catch for the female saltmarsh mosquito (*Aedes taeniorhynchus)* correlated with increasing tourist expenditures (Breeland and Mulrennan 1983). The dramatic decrease in saltmarsh mosquitoes during this period, in large measure due to impoundment, ditching, and filling of salt marshes, facilitated the development of large areas of coastal Florida and a general increase in tourism (Gaiser 1980, Harden 1981, Thomas 1981).

Economic impacts of mosquito-borne diseases have not been well documented in the past, but recent research suggests that any type of vector-borne epidemic will have local and statewide, direct and indirect economic impacts that may be in the multi-millions of dollars. For example, the 1990 SLE epidemic not only caused considerable illness (223 confirmed cases with 11 deaths), but Florida saw a 15% decrease in tourism-related revenues in the last quarter of the year (Mulrennan 1991). The 2002 West Nile virus (WN) epidemic was estimated to have cost over $20 million in Louisiana alone (Zohrabian *et al.* 2004). Mosquito control possibly decreases these impacts by reducing the chances for outbreaks and by helping to control them when they occur (*e.g.,* Ruiz *et al*. 2004).

Another economic benefit of mosquito control is increased worker productivity. In outdoor work areas, such as crop fields, marinas, orchards, sawmills, and the construction trades, productivity of work crews can fall to near zero in the presence of large numbers of mosquitoes (Williams 1986).

A wide cross-section of domestic animals also benefit from mosquito control. Data on loss of meat or dairy production due to mosquito attack are difficult to come by, although older literature reported losses in milk production of up to 40% and losses in beef cattle weight gain (reviewed in Steelman 1976). Research conducted in Louisiana showed that a combination of mosquito control and improved diet resulted in significant increases in weight gain by beef cattle (Steelman *et al.* 1972, 1973). The suffocation of cattle by hordes of mosquitoes prior to modern mosquito control was documented in news reports and has occurred in recent times as well (Addison and Ritchie 1993). One source estimated an economic loss of $61 million dollars in one year due to mosquitoes (Hamer 1985, cited in Frank *et al.* 1997).

Birds and other wildlife may serve as reservoirs for mosquito-borne diseases that can impact animals of economic importance (USDA 2005). Horses in Florida are at risk from infection by Eastern equine encephalitis virus and West Nile virus and potentially from Venezuelan equine encephalitis virus (Lord and Rutledge-Connelly 2006). During the 2001 West Nile virus outbreak in Florida, 492 horses were confirmed to have had acute West Nile encephalitis (Blackmore *et al*. 2003).

9.4.3 Public Health Benefits

Another important benefit of mosquito control is the targeting of mosquitoes that transmit diseases. Mosquito control is an important and basic public health service (ASTHO 2003). Since 1978, some public health departments and mosquito control agencies throughout the state have participated in a surveillance program using sentinel chickens to closely monitor for St. Louis encephalitis (SLE) and eastern equine encephalitis (EEE) viruses. Arbovirus outbreaks, like the 1990 SLE epidemic (223 confirmed cases with 11 deaths) (Mulrennan 1991) and the 2002 West Nile virus (WN) epidemic in the United States (4,156 reported cases with 284 fatalities) (O'Leary *et al*. 2004) typically result in increased and targeted mosquito control to stem the outbreaks. On a personal level, a survivor of La Crosse encephalitis or EEE infection may need lifetime medical support costing into the millions of dollars (Villari *et al.* 1995, Utz *et al*. 2003). Long-term sequelae of West Nile virus infection include abnormalities of motor skills, attention, and executive functions, all of which may negatively impact quality of life and productivity (Carson *et al.* 2006).

There are also social justice benefits to mosquito control. At least three different studies (Kutz *et al.* 2003, Ruiz *et al.* 2004, Rios *et al.* 2006) have suggested that the burden of mosquito-borne viral diseases falls more heavily upon lower-income residents and minority group communities.

9.5 COSTS OF MOSQUITO CONTROL

9.5.1 Human Health Concerns

A consideration associated with the overall use of pesticides, of which mosquito control is a part, is the potential human health risk of pesticide exposure. In the last several years, more evidence has been evaluated concerning the impact on humans from a half-century of exposure to synthetic chemicals and other environmental contaminants. Human health problems associated with the effects of severe, acute exposure to organophosphate pesticides include irreversible neurological defects, memory loss, mood changes, infertility, and disorientation (Savage *et al.* 1988). Guillette (1998) documented decreases in stamina, gross and fine eye-hand coordination, 30-minute memory, and ability to draw in children exposed to pesticides in the Yaqui Valley, Mexico. However, health effects are generally attributed to exposure to agricultural applications to food – not to mosquito control applications. No clear evidence exists for adverse effects on human health from long-term exposure to organophosphate insecticides at levels that do not affect acetylcholinesterase levels (WHO 1986a). Attempts to quantify human exposure to adulticides used in ULV mosquito control operations found no significant increase in levels of naled or permethrin in humans after wide-area spray missions in Mississippi, North Carolina, and Virginia (Currier *et al.* 2005). Duprey *et al*. (2008) determined that aerial application of naled did not result in increased levels of naled in humans when applications were conducted according to label directions. In fact, recent research suggests that human health risks from mosquito control pesticides are low and that risks from mosquito-borne diseases greatly exceed risks from pesticides to human health (Peterson *et al.* 2006, Schleier *et al*. 2009, Macedo *et al*. 2010).

Idiopathic Environmental Intolerance (IEI) ("idiopathic" meaning of "unknown origin") is the name currently applied to a phenomenon formerly known as Multiple Chemical Sensitivity (ACOEM 1999). The newer name does not assume a chemical, biochemical, or immunologic cause for the patient's symptoms and was adopted because there is no medical consensus as to its diagnostic criteria, etiology, or therapy (AAAAI 1999, Poonai *et al*. 2001). Symptoms are said to be caused by exposure to a wide range of human-made chemicals at doses far below those known to cause toxic effects to humans (ACOEM 1999, Bailer *et al*. 2005). Symptoms may include weakness, dizziness, headaches, heat intolerance, difficult in concentrating, depressed mood, and memory loss (Pirages and Richard 1999).

IEI is said to be "the only ailment in existence in which the patient defines both the cause and the manifestations of his own condition" (Gots 1995). Many IEI patients self-report allergies to chemicals, but IgE levels have been shown to not support an allergic cause (Bailer *et al*. 2005). Another study found that IEI patients showed no difference from control subjects in responses to solvents or placebos (Bornschein *et al*. 2008). Other researchers suggest that IEI patients have an exaggerated response due to hypersensitivity to odors (van Thriel *et al.* 2008). Currently, IEI is not recognized as an organic disease by the American Academy of Allergy and Immunology, the American Medical

Association, the California Medical Association, the American College of Physicians, nor the International Society of Regulatory Toxicology and Pharmacology (Gots 1995).

This notwithstanding, medical research continues to investigate the causes of the phenomenon. Preliminary data indicate that IEI and panic disorder are related and may have a common neurogenetic origin (Poonai *et al*. 2000, Binkley *et al*. 2001). Other data indicated that IEI patients may have variant genes that code for altered drug-metabolizing enzymes (McKeown-Eyssen *et al.* 2004, Schnakenberg *et al.* 2007). Still other researchers report that IEI appears to be a variant of somatoform disorders, in which psychiatric disorders cause unexplained physical symptoms (*e.g*., Bailer *et al*. 2005).

Regardless of the cause of their symptoms, IEI patients can suffer severe disruption of work and daily life (Magill and Suruda 1998). IEI is given credence in regulatory actions, tort liability, and workers compensation claims (Gots 1995). In Florida, private pest control operators are legally required to notify registered persons prior to chemical applications (Chapter 482 F.S.). In addition, FDACS maintains a list of persons who claim to be pesticide-sensitive, requiring a physician's certification of a health concern, and typically mosquito control offices avoid spraying their residences or notify them prior to spray operations.

9.5.2 Chemical Trespass

The concept of chemical trespass (*i.e.*, applying chemicals to an individual or their property against their wishes) extends back to old Florida statutes. However, statutory law (Chapter 388 F.S.) now permits the application of mosquito control chemicals in the public domain. The potential for conflict is obvious, and this conflict has been the basis for some claims in the past (*e.g.*, by beekeepers).

Adulticide drift, in particular, invites claims of chemical trespass. Mosquito adulticides are not labeled for application to wetlands, and most environmentally sensitive publicly owned upland is also off-limits. Because any wind will create drift, mosquito control operators face the difficult task of both hitting their targets and avoiding the adjacent non-target areas. Adulticides have been shown to drift three miles and in some extreme instances up to five miles (Dukes *et al.* 2004). One study in the Florida Keys found that aerial thermal fog drifted 750 meters (½ mile) into protected no-spray zones which harbored endangered vertebrate and plant species, though no harm was demonstrated (Hennessey and Habeck 1991, Hennessey *et al*. 1992). Such data may appear to suggest the need for larger buffer areas and/or careful attention to meteorological conditions to fully protect no-spray zones. With the general replacement in Florida of aerial thermal fogging by aerial ULV treatments, some of these concerns may be allayed.

Tietze *et al*. (1992) and Tietze and Shaffer (1997) documented microscopic damage to automotive paint finishes due to the application of malathion and naled.

9.5.3 Potential Problems of Chronic Chemical Exposure

Problems resulting from chronic exposure to chemicals are a general public health issue, because everyone is exposed daily to chemical and pesticide residues in food, water, and air. In regard to chronic exposure to chemicals, animal endocrine and immune system dysfunction studies have provided evidence that synthetic pesticides and industrial chemicals in very low quantities, after repeated exposures, may affect these functions (Pimentel and Lehman 1993). Such chronic exposure has been associated both with decreases in human sperm counts and sperm abnormalities. Swan *et al.* (2003) and Swan (2006) examined effects of pesticides on quality of human semen in the United States. These studies revealed that among men living in agricultural areas exposure to atrazine, alachlor, and diazinon appeared to decrease sperm concentration and motility, whereas exposure to malathion and DEET did not. A documented problem in Lake Apopka believed to be caused by chronic exposure to chemicals, included small genitalia size and sperm abnormalities in male alligators (Guillette, *et al*. 1994, Colburn *et al*. 1996). While mosquito control chemicals are not implicated in these instances, they are a part of the total insecticide use picture. It should be noted that organophosphates, such as malathion, have been used routinely for over 40 years in some Florida communities without any documented chronic effects. This lack of documentation should not be misunderstood to be proof of absence of risk, however (Thier 2001). This lack of data may be a detriment to public relations. For example, Petty *et al.* (1959) observed the development of two extreme points of view regarding the use of organophosphate pesticides in Louisiana. On the one hand were people who were too casual in mixing and applying pesticides. On the other were people so frightened by any use of pesticides that they created “localized hysteria”.

There do not appear to be significant ill effects to humans attributable to long-term, low-level exposure to organophosphate pesticides (WHO 1989, Steenland 1996, Leon-S. *et al*. 1996, others reviewed by Eskenazi *et al*. 1999). Insecticides used for mosquito control in Florida have been evaluated for this use by the EPA. They pose minimal risk to human health and the environment when used according to label directions. The EPA estimates that the exposure and risks to adults and children posed by ULV aerial and ground applications of malathion and naled range from 100 to 10,000 times below the quantity of pesticide that might present a health concern (IDPH undated). Lal *et al.* (2004) examined blood cholinesterase levels of applicators and residents of villages involved in a kala-azar control program in India. These researchers found that blood cholinesterase levels of applicators and villagers declined immediately after treatment of homes with 5% malathion suspension but still were within the normal range of blood cholinesterase levels. One week after application the applicators’ blood cholinesterase levels were still depressed but remained within normal limits. After one year of exposure, the villagers’ blood cholinesterase levels had returned to pretreatment levels (Lal *et al*. 2004). Few data concerning inhalation toxicity of malathion to humans are available, but Culver *et al*. (1956) and Golz (1959) found no significant health effects beyond nasal irritation.

Beyond the risks to humans and wildlife from pesticide exposure, application procedures may cause problems by promoting pesticide resistance, resulting in the need for increasing doses or new chemicals. In some locations, the widespread use of pesticides by agriculture, homeowners, and mosquito control may have contributed to resistance (Boike *et al*. 1989). In some geographically distinct areas (*i.e.*, island situations), spraying has helped lead to mosquito resistance to certain chemicals (Reimer *et al*. 2005). Mosquito populations subject to chemical control operations may be especially vulnerable to development of resistance due to widespread applications of a single pesticide coupled with the short generation time with abundant progeny of the mosquito life cycle (Hemingway and Ranson 2000).

Since it is currently impossible to predict the long-term consequences of human exposure to synthetic compounds, including mosquito control agents, a prudent strategy is for society to reduce all unnecessary chemical applications. For mosquito control, strides have been made in this direction by regulations that allow adulticide applications only after adequate surveillance verifies a nuisance level. Mosquito control and all other industries applying chemicals should use alternative procedures that reduce the need for chemical applications whenever possible. Such actions may result in decreased environmental risks.

9.5.4 Environmental Costs of Adulticiding

In recent years, some politicians, private interest groups, and the general public have become increasingly vocal in their concerns about potential human and environmental hazards associated with the use of chemicals to control mosquitoes especially aerially applied adulticides (Gratz and Jany 1994). This concern has generated greater accountability by mosquito control operations when applying insecticides, and some tighter environmental restrictions have been implemented at the federal and state levels. Hopefully in the future, more effective alternative strategies such as biological control agents and non-chemical larvicides will be available for mosquito control. Realistically, however, chemical companies see the mosquito control market as being relatively small and usually not providing adequate economic incentive to allocate the tremendous costs (easily tens of millions of dollars) necessary to develop and receive a label for a new and safer product (Rose 2001).

9.5.4.1 Impacts on Insects

Insects are among the most abundant and diverse organisms on Earth. Most insects cause no harm to humans and are beneficial, even necessary in the case of pollinators, to the continued well-being and functioning of the biosphere. The chemicals used to control mosquitoes are likely to have sublethal and lethal effects on other kinds of insects, but so far research has focused on just a few non-target species such as honeybees and butterflies. The types of insects most likely to be affected by adulticides are small, night flying, and mosquito-like. Larvicides, including growth regulators and Bti, are also likely to effect other aquatic insects, especially the larvae of small flies.

Honeybees have been shown to be very sensitive to organophosphates in laboratory studies, and extensive kills from mosquito control have been documented in the field. Acute problems usually include immediate bee kills, but sublethal amounts of organophosphates also can cause a general decline in hive vigor and/or a loss of feeding ability (Atkins 1975). Bee exposure to ground adulticiding is usually minimal because treatment is almost always conducted after the evening or before the morning crepuscular periods. However, under certain conditions, mosquito adulticiding sometimes occurs while bees are foraging and therefore can be an increased threat.

Mosquito control agencies operate over a wide area, are very visible, and often investigated when there is a reported bee kill. In addition mosquito control applications are specifically sought out by EPA and State bee kill investigation guidelines (EPA 2013). The incidence of conflicts between beekeepers and mosquito control in Florida peaked in the 1980s and has declined in recent years. Some Florida mosquito control programs now notify beekeepers in advance of spray operations to give the beekeepers the option of covering or moving hives. The impact to honeybees within target areas can be minimized if insecticide deposition on the ground is reduced to below the effect threshold (Zhong *et al*. 2003, 2004). Improvements in mosquito control equipment also have led to reductions of honeybee mortality (Zhong *et al*. 2004). In the 1980s, the State of Florida distributed to beekeepers a state map depicting where most aerial operations occurred (Sanford 1998). Currently, mosquito control and beekeepers maintain communication about timing of insecticidal treatments (Ellis and Hayes 2014). It is important to note that for the most part beekeepers use the same two classes of pesticide inside their hives to control deadly Varroa mites that mosquito control entities use outside the hives to control adult mosquitoes: Pyrethroids and organophosphates. The most prevalent in hive pesticides found in the pollen are miticides beekeepers apply directly to hives to control Varroa mites (Rennich *et al*. undated).

Other pollinators less well known than the honeybee may be impacted by adulticiding. Perhaps 65% of flowering plants depend upon insect pollination with many plant species relying upon a specific insect species. With that in mind there are general concerns about declining pollinator populations that usually are focused on habitat loss as well as insecticide applications, especially on agricultural crops. However, butterfly gardeners and butterfly conservatories sometimes claim to be affected by mosquito control operations. Anecdotal accounts of butterflies and other insects found dead on ground following adulticiding are sometimes heard. It is difficult to confirm such claims (Schweitzer *et al*. 2011).

In Florida and particularly in the Florida Keys, there has been controversy regarding the impact of mosquito control on rare butterflies. Emmel (1991) reported that insect diversity was much lower in areas subjected to mosquito control operations (*i.e.,* Key Largo) compared to areas not exposed to mosquito control (*i.e.,* Elliott Key). Eliazar and Emmel (1991) and Salvato (2001) calculated LD_{50} values for some mosquito control adulticides applied to butterflies. However, laboratory analyses are not always reflective of events in the field (Clark 1991, Charbonneau *et al.* 1994, Blus and Henny 1997).

Salvato (2001) found more Bartram's scrub-hairstreaks along transects in sprayed areas on Big Pine Key but doubted his result. Walker (2001) also suggested that mosquito control was responsible for extirpating a wood cricket (*Gryllus cayensis*) from the Florida Keys, although he stated he had no proof this was the case.

Butterflies have definitely declined in southern Florida. At least two, the Zestos skipper (*Epargyreus zestos oberon*) and rockland Meske's skipper (*Hesperia meskei pinocayo*), are thought to be extinct (Schweitzer *et al*. 2011). In addition, the U.S. Fish & Wildlife Service has listed four endemic subspecies as Endangered: the Schaus' swallowtail (*Papilio aristodemus ponceanus*), Miami blue (*Cyclargus thomasi bethunebakeri*), Bartram's scrub-hairstreak (*Strymon acis bartrami*), and Florida leafwing (*Anaea troglydyta floridalis*). The leafwing disappeared from Big Pine Key following Hurricane Wilma in 2006 and is currently only known to occur in Everglades National Park. Up to 20 other kinds of butterflies in Florida may be imperiled. This situation is especially meaningful when considering that out of some 800 species and thousands of subspecies of butterflies found in the United States and Canada (Pelham 2008), only eight other subspecies, all western, are believed to be extinct (Opler 1976).

Among the many factors influencing butterfly populations, mosquito spraying must be considered along with fire, habitat destruction, drought, and other sources of pesticides including agriculture, golf courses, and consumer use (Schwarz *et al*. 1996, Carroll and Loye 2006, Hoang and Rand 2015a). Adult butterflies may be exposed via aerial spraying while in flight or when landing on treated vegetation; Treated vegetation may also be a source of exposure for caterpillars (Oberhauser *et al.* 2006; Hoang and Rand 2015a, b). Field experiments on Key Largo found higher mortality of laboratory reared Miami blue butterfly larvae closer to adulticide release areas than at control sites further away (Zhong *et al*. 2010) suggesting that adulticiding does affect butterfly populations. Hoang *et al.* (2011a, b) reported that butterflies may be more sensitive to mosquito control pesticides than are honeybees. Bargar (2012) conducted a risk assessment on adult butterflies exposed to naled and found that mortality was varied according to dose per unit body weight. He developed a risk model predicting greatest risk of adulticiding to small butterflies (Lycaenidae) and lowest risk to larger bodied Hesperiidae. Extensive field surveys throughout the Keys from 2006 to 2010 (Minno and Minno 2013) found high levels of butterfly species richness and abundance on some of the most highly developed and sprayed islands – Key West and Stock Island, while unsprayed conservation lands such as Everglades National Park and Biscayne National Park have lost butterfly species. Two relatively large hesperiid butterflies are likely extinct, while the small Cassius blue (*Leptotes cassius*) is still one of the most abundant and widely distributed species, commonly found in butterfly gardens in urban areas. Minno and Minno (2013) attributed the butterfly losses in southern Florida mostly to exotic predatory ants that became established during the 1970s but acknowledged that complex interactions between many factors have shaped the populations we see today.

The impact of adulticides on the nocturnal insect fauna, both flying and non-flying, has not been well documented. One study in California evaluated the effects of aerial application of pyrethrin, malathion, and permethrin on night flying non-target insects. A significant reduction in numbers of non-target insects was observed on the night of the insecticide treatments, but insect numbers had rebounded 24 hours later (Jensen *et al.* 1999). Peterson *et al.* (2016) discuss a number of other studies and their own work with lady beetles. Nontarget effects may vary depending on whether the organism in question is flying, settled on vegetation, and on or near the ground. Among their conclusions was that acute mortality of nontarget terrestrial insects exposed to mosquito control ULV applications is largely dependent on the insect's position relative to that of the aerosol cloud.

9.5.4.2 Impacts on Insectivores

Just as the impacts of mosquito adulticiding on non-target insects are not well quantified (Stevenson 1980), the ecological impact from the reduction of mosquitoes is also largely unknown. Nevertheless, it is commonly claimed that mosquitoes play an important role as a food source for larger organisms. Claims include that larvae are an important food for other aquatic organisms, that adults of many mosquito species have an important role in the pollination of plants, and that adults serve as important food sources for birds, bats, and other arthropods including dragonflies and spiders.

The evidence is lacking for commonly cited species such as purple martins (Kale 1968) and bats (Easterla and Whitaker 1972, Vestjens and Hall 1977, Sparks and Valdez 2003). Adults of most mosquito species are not active during the hours that most dragonflies are seeking prey (Pritchard 1964a, Walton 2003). Nevertheless, adult dragonflies will prey on adult mosquitoes when the two are present in the same habitat (Wright 1944a, 1944b; Pritchard 1964a). Analysis of gut contents has revealed that consumption of mosquitoes by dragonflies is greater in the early morning hours; Up to 19% of gut contents consisted of mosquitoes (Pritchard 1964a). The importance of mosquito larvae as food for fish, aquatic salamanders, and predatory aquatic insects seems better demonstrated (*e.g.*, Pritchard 1964b, Mathayan *et al.* 1980, Whiteman *et al.* 1996, Lundkvist *et al*. 2003). Boone and Bridges (2003) have pointed out that control measures that reduce population sizes of plankton and aquatic invertebrates can have adverse effects on amphibians due to reduction of available foods.

9.5.4.3 Fish

Impacts of mosquito adulticides on fish have received considerable attention. Fish may be killed in small streams or ponds where slow flow rates allow pesticide concentrations to increase in excess of toxic levels or where heavy rainfall within a large watershed area allowed high pulse loads to enter small aquatic habitats (Hinton 1998). Risk to fish is lower in swiftly flowing streams because pesticides are transported downstream and rapidly diluted (Neumann *et al*. 2002). Field studies have shown that operational mosquito control applications of pesticides can be of shorter duration and of lesser concentration that those used in worst-case scenarios for environmental risk assessments (Clark 1991). For example, in one field study, application of naled according to label directions did not impact fish (Bearden 1967). Temephos applied at label rates resulted in no adverse impact on bluegill (Sanders *et al*. 1981). Malathion ground ULV and thermal fog applications presented no acute toxicity to fish (Tagatz *et al*. 1974). Clark *et al*. (1989) and Coates *et al.* (1989) have reviewed the literature pertaining to toxicity of pesticides to aquatic organisms.

9.5.4.4 Aquatic Crustacea

Aquatic crustaceans – cladocerans, copepods, lobsters, and shrimp – can be impacted by mosquito control adulticides, probably due to their close phylogeny to insects (Clark 1991). Older studies documented effects of fenthion on ostracods and cladocera (Khudairi and Ruber 1974, Ruber 1963). Zulkosky *et al*. (2005) reported that resmethrin was more toxic to American lobsters (*Homarus americanus*) than was malathion during 96 hour tests. Operational application of naled according to label directions resulted in no significant mortality of shrimp or crabs (Bearden 1967). Aquatic habitats are avoided operationally to minimize such impacts.

9.5.5 Environmental Costs of Larviciding

Controlling a brood of larval mosquitoes while they are still concentrated in a pool of water is easier, more efficient, and less costly environmentally than controlling dispersed adults. Nevertheless, there still are costs, and they should be recognized and minimized to the extent practicable. Using biorational materials (*e.g., Bti*, methoprene) minimizes non-target effects because of the specificity of these materials. Nevertheless, research has shown there are short-term effects on non-target insect species when methoprene is used for mosquito larviciding (Hershey *et al.* 1998). That same study revealed that there was a delayed effect of 2-3 years between initiation of treatment with *Bti* and evidence of effects on the wetlands food web. Methoprene can affect copepods, crabs, and shrimp, although effects generally are seen at concentrations higher than those of operational rates (Miura and Takahashi 1973, McAlonan *et al.* 1976, Christiansen *et al.* 1977, Bircher and Ruber 1988). A review of 75 studies of non-target effects of *Bti*, concerning nearly 125 families, 300 genera, and 400 species is available (Boisvert and Boisvert 2000). Most research on the use of monomolecular films to control larvae or pupae has shown that there is little or no effect on non-target organisms (reviewed by Stark 2005). However, Takahashi *et al*. (1984) observed mortality of aquatic Hemiptera (Corixidae, Notonectidae), Coleoptera (Hydrophilidae), and clam shrimp (Limnadiidae) in field trials of Arosurf®. Regarding the loss of mosquitoes as important prey, in the case of methoprene, since mortality generally occurs during the pupal stage, larvae remain as a prey source. Nevertheless, the reduction of the huge biomass of saltmarsh mosquitoes (potentially many millions of larvae per acre) must be significant to some aquatic predators. Nielsen and Nielsen (1953), for example, described the voracious consumption of *Ae. taeniorhynchus* larvae by minnows and water beetle larvae. The loss may be mitigated by some species, however. Harrington and Harrington (1961, 1982) have shown that a few species of fish are capable of dietary shifts following impoundment when mosquito broods were lost as a food source.

9.5.6 Adulticiding versus Larviciding

Both larvicide and adulticide chemicals may impact non-target species, although it is widely accepted that larvicides have less environmental impact than adulticides. Larvicides can be quite target specific (*e.g., Bti*, methoprene) and are used in specific habitats and under certain conditions. Adulticides, on the other hand, are more broadly distributed by truck or aircraft, thus impacting both the target area and potentially other nearby areas through drift and run-off. Such movement is a problem when the insecticide enters wetlands or public lands where they are not allowed. All mosquito control programs should continue to concentrate their efforts on developing effective larval surveillance and control programs in order to effectively minimize the need for adulticiding.

All industries need to continually review and improve their operations. Mosquito control is no exception. When larval or adult control has not worked effectively, a thorough assessment should be conducted so the program can be improved. Larval control will usually allow some mosquitoes to emerge, mostly due to the inspection program's failure to identify a mosquito brood or to implement thorough treatment coverage. Likewise, adulticiding is by no means 100% effective. An education program to inform the public that at least some mosquitoes are to be expected in Florida is warranted.

9.6 SOURCE REDUCTION

Achieving permanent mosquito control by eliminating mosquito larval habitats is called source reduction. It ranges from efforts as simple as collecting discarded tires to long-term habitat altering measures. Several source reduction techniques for saltmarsh mosquito control are presently used. For more information about source reduction, see Chapter 4.

Ditching is a strategy whereby mosquito producing depressions of tidal water or rainfall are engineered to drain and larvivorous fish are allowed access. Ditching is most effective where daily tides flush the potential mosquito oviposition sites on the marsh. Ditching can increase tidal flushing of soils, increase oxygen availability to plants, reduce soil salinity, and contribute to increased primary productivity of salt marsh plants. It also can increase fish diversity within the marsh and can provide additional habitat for birds (Anonymous 1990, Resh and Balling 2003). The environmental costs of ditching include creation of permanent scars on the marsh and adverse effects on natural hydrology and biological productivity. Ditching historically has created berms which allow encroachment of woody, often exotic, vegetation. While ditching can be effective for mosquito control, it also can create larval habitat for biting midges (*Culicoides* spp.), insects which are difficult to control and frequently are perceived as being much more annoying than mosquitoes.

Impounding became popular along the Indian River Lagoon in the 1950s and 1960s when earthen dikes were built around approximately 42,000 acres of high salt marsh to allow for their seasonal flooding. This technique became the most effective and economically feasible approach to saltmarsh mosquito control on Florida's central east coast. Although early impounding efforts greatly decreased the need for adulticiding and virtually eliminated the need to larvicide, the environmental consequences included high mortality of the native marsh vegetation and the isolation of thousands of acres of salt marsh. These habitats are critical for the development of many important marine species (*e.g.*, fish, crustaceans, mollusks), and their loss negatively affected the multibillion dollar commercial and recreational fishery. Despite these impacts, high saltmarsh impoundments have provided good feeding opportunities for ducks and wading birds (Provost 1959, 1969), although some use of these impoundments may be due to loss of habitat elsewhere (*e.g.*, loss in the Kissimmee River and St. Johns River flood plains due to human development and drainage).

Unintentional effects of source reduction practices have included: Changes in plant composition and abundance that affect their value as forage or shelter, changes in animal diversity and abundance which alter the food web, changes in competitive relationships between predators and prey, and increased susceptibility to disease and parasitism. An extreme example of unintentional pesticide impacts is that the use of some agricultural chemicals has altered entire ecosystems resulting in freshwater eutrophication.

Since the early 1980s scientific research has identified improved water management techniques that reintegrate impounded marshes with the estuary. This reconnection restores many natural marsh functions while still controlling mosquito populations with a minimum of pesticide use. There are two salt marsh management techniques which best accomplish these desirable goals, and they have been aggressively implemented by mosquito control agencies: Rotational Impoundment Management (RIM) and Open Marsh Water Management (OMWM), typically utilizing rotary ditching (Carlson 2006).

9.7 MOSQUITO CONTROL ON BIOLOGICALLY PRODUCTIVE STATE-OWNED LANDS

Florida public land management agencies generally believe that any external influence that potentially threatens the flora, fauna, or natural systems under their management must be considered with caution. For example, although pest control once was a priority in Florida’s parks (*e.g.,* Provost 1952), park managers now pursue an ecosystem management approach that considers the well-being of entire biological communities (*e.g.,* Stevenson 1991). Chapter 388.4111 F.S. mandates that public lands may be designated by their managers to be “environmentally sensitive and biologically highly productive”. Once declared, and where such lands have public health or nuisance levels of mosquitoes, their mosquito control activities are conducted according to a special “public lands arthropod control plan”. The plan is written by mutual agreement between the agency and the mosquito control program to authorize activities that are the minimum necessary and economically feasible to abate the health or nuisance problem and impose the least hazard to fish, wildlife, and other natural resources. Since adulticiding is not highly selective and non-target species can be adversely affected, state land managers generally believe adulticiding is contrary to the legislative mandate to protect environmentally sensitive and biologically productive state lands. Other control methods, ideally biological controls (*e.g.*, *Gambusia* spp. for larval control) or larviciding with *Bti* or methoprene, which are mostly target-specific, are usually acceptable to the agencies. Allowing these practices on most properties is viewed by the state as a reasonable compromise for adhering to the legislative mandates regarding public land protection and mosquito control.

9.8 MUTUAL ACCOMMODATION

The effects of pesticides on target and non-target organisms, wildlife, soil, and water can both benefit and negatively impact Florida's quality of life. Both mosquito control and the protection of environmentally sensitive habitats in Florida are legislatively mandated, needed, and important to the state. Indeed, they need not be mutually exclusive goals *(e.g.,* O'Bryan *et al.* 1990, Batzer and Resh 1992). Because the selection of chemicals available for both larviciding and adulticiding is becoming increasingly limited without many new products in development and because of the possibility of non-target insecticide effects, it is incumbent that mosquito control pesticides be applied wisely in integrated pest management programs. It is also important that new, more environmentally acceptable methods are developed, tested, and used as they become available, and that research continues to document non-target and human health effects of the pesticides used. The American Public Health Association has noted, "debates over the use of pesticides for public health vector control have sometimes divided the public health and environmental communities … at a time when maximizing public health and environmental protection requires close coordination and mutual trust between those communities" (APHA 2001). The mosquito control and resource management communities need to be aware of each other's concerns and be familiar with scientific literature pertaining to each other's field (Willott 2004, Rey *et al*. 2012). Continued dialogue between mosquito control and environmental resource agencies is necessary to make certain that mosquito control minimizes all its adverse environmental effects while protecting the public health and welfare.

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Chapter 10

INSECTICIDE RESISTANCE MANAGEMENT

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Summary

This chapter discusses development of resistance to insecticides and best management practices to maintain efficacy of chemical means for mosquito control. Topics covered are the: 1) history of insecticide resistance in Florida mosquitoes, 2) definition of genetic insecticide resistance, 3) resistance mechanisms, 4) methods to detect insecticide resistance in both larvae and adult mosquitoes, 5) current research, 6) strategies of resistance prevention and management, 7) resistance surveillance, 8) future research

10.1 INTRODUCTION

Development of resistance to insecticides is a potential threat to any long term mosquito control program. Like populations of all living organisms, mosquito populations are dynamic, responding to selective pressure. The challenge to mosquito control programs is to stay a few steps ahead of the target species' ability to defeat control efforts. Insecticide resistance can be prevented and mitigated in a number of ways including greater reliance on Integrated Pest Management (IPM), the development of better methods of resistance detection and monitoring, and improved management of insecticide resistant populations through better coordination among mosquito control programs, state agencies, university and government scientists, and insecticide manufacturers.

10.2 HISTORY OF INSECTICIDE RESISTANCE IN FLORIDA MOSQUITOES

Insecticide resistance developed shortly after the earliest attempts at large-scale chemical control in agriculture, and there is a long history on this subject. See Appendix III. In

1993, Dr. Tom Breaud published a review of scientific literature on insecticide resistance in Florida mosquitoes (Breaud 1993). This section is based on that report.

The rapid development of resistance to the organochlorine insecticide, dichloro-diphenyl-trichloroethane (DDT), shortly after its introduction during World War II, has been well documented. DDT was first used for mosquito control in Florida in 1943. During and immediately following World War II, Florida mosquito control programs relied almost exclusively on DDT for mosquito control. This over-dependency quickly led to resistance to DDT, and, subsequently, to dieldrin resistance. In 1947 the black saltmarsh mosquito, *Aedes taeniorhynchus,* began to show resistance to DDT (Brown 1986).

The response to this problem was to look for chemical alternatives to DDT. During the 1950s, Florida mosquito control programs utilized a different class of insecticides, the organophosphates (OP), which were shown to be more effective than DDT. By 1963, the OPs malathion and naled had replaced DDT as the adulticides of choice. A few years later, Gahan *et al*. (1966) reported poor results with aerially applied malathion for the control of *Ae. taeniorhynchus* in Lee County. In laboratory studies, Glancey *et al*. (1966) confirmed the development of resistance to malathion showing that it took ten times the baseline amount of malathion to achieve the median lethal concentration (LC_{50}) with susceptible mosquitoes and thirteen times the baseline to obtain the LC_{90}, the concentration at which 90% of a population dies. This account was the first published report of malathion resistance in Florida.

In the last four decades, there were few confirmed incidences of mosquito resistance to other OP adulticides (chlorpyrifos, fenthion, naled) or to carbamates in Florida, despite their extensive usage. Furthermore, resistance has not prevented the continued use of OP insecticides in Florida, where tolerance or resistance tends to be localized and not a general or widespread phenomena. This situation is probably a direct result of the adoption of Florida's policy in the late 1960s to restrict the use of a class of insecticides to either adulticiding or larviciding but not both.

The synthetic pyrethroid resmethrin, synergized with piperonyl butoxide (PBO), was introduced in the 1970s in part to control malathion-resistant mosquitoes, followed later by synergized permethrin and sumithrin (d-phenothrin) products. At present, pyrethroids are the chemical treatment of choice for ground adult mosquito control in Florida. To date (2017), few documented reports of pyrethroid resistance negatively impacting mosquito control in Florida have been published. However, permethrin resistance has been reported in *Culex* mosquitoes in California (McAbee *et al*. 2004) and in *Aedes* mosquitoes. Laboratory tests conducted at Auburn University, Alabama, have documented elevated LC_{50} values for mosquitoes from the Southeastern United States (Liu *et al.* 2004a,b, 2006). Eternal vigilance is in order.

Although over three decades of larviciding with temephos (Abate®) in Lee County and other Florida districts has not led to resistance to this OP insecticide, resistance to an insect growth regulator, methoprene, was documented in an isolated saltmarsh habitat of

Lee County following the long-term use (150-day) of briquets for control of *Ae. taeniorhynchus* (Dame *et al.* 1998).

10.3 DEFINITION OF RESISTANCE

Insecticide resistance is defined as the genetic response of a population of mosquitoes that enables some members of that population to survive exposure to a chemical that would prove lethal to a susceptible population (WHO 1992). This definition distinguishes insecticide resistance from treatment failures that may result from any number of other problems such as operator error, formulation error, equipment failure, etc. This definition is vitally important because it enables the establishment of standardized procedures for the early detection of insecticide resistance. Without standardized procedures, meaningful comparisons are not possible.

Insecticide resistance originates in the genetic variability of an insect population. Mutations give rise to some individuals with an enhanced ability to survive exposure to chemicals that would kill fully susceptible individuals.

Insecticide resistance is inherited. The basic genetic mechanisms are well understood. Genes are the units of inheritance. Alternative forms of genes are called alleles. The resistant allele may be either recessive (as in certain DDT-resistant mosquitoes) or dominant (as in organophosphate resistance). Some alleles are co-dominant, and the resistant-susceptible hybrids are intermediate in susceptibility (as in dieldrin resistance). Resistance increases in the population when susceptible alleles are selectively removed by insecticide treatments, leaving an increased proportion of resistant alleles. Proportionally more eggs of resistant mosquitoes hatch than those of susceptible females of the target population under selective pressure from chemical insecticides.

10.4 RESISTANCE MECHANISMS

Two major classes of insecticides are currently used to control adult mosquitoes in Florida:

- Organophosphates (malathion and naled)
- Pyrethrins/pyrethroids [deltamethrin, etofenprox, natural pyrethrin, permethrin, resmethrin, and sumithrin (d-phenothrin)]

Each class has a particular mode of action, so various mechanisms of resistance may operate. Specific types of resistance are: Behavioral, metabolic, target site insensitivity, and cross resistance.

10.4.1 Behavioral Resistance

Genetic variation in behavior may contribute to resistance by enabling the mosquito to avoid contact with the insecticide. When resting surfaces are treated with pesticide, some mosquitoes in the target population may never contact the treated area. This difference in exposure alters survival rates of the next mosquito generation and may increase the allele frequency of the genetic factors contributing to the avoidance behavior. Over time, fewer and fewer mosquitoes will be killed by the pesticide. This type of resistance is most common in the case of insecticide-treated surfaces.

10.4.2 Metabolic Resistance

Detoxifying enzymes present in mosquitoes, such as the oxidases and esterases, may inactivate an insecticide before it can kill the mosquito. Mixed function oxidases (MFOs), in general, deactivate pyrethroids. Esterases are responsible for detoxifying organophosphates, such as malathion.

Synergists like piperonyl butoxide (PBO) work by defeating the mosquito's detoxifying enzymes. PBO is not an insecticide at the dose it is applied, but together with the active ingredient (AI), reduces the mosquito's ability to detoxify the insecticide, thereby making the AI more effective. Synergists can be used experimentally to detect the mechanism of resistance. For example, if the synergist DEF (S,S,S,-tributyl phosphorotrithioate – a defoliant) or TPP (triphenyl phosphate) increases susceptibility, then esterases are the mechanism of resistance. If addition of PBO increases susceptibility, then MFOs are the mechanism of resistance.

10.4.3 Target Site Insensitivity

Organophosphate and carbamate insecticides work by inhibiting the enzyme acetylcholinesterase. Some mosquito species have developed insecticide resistance by structural modification of acetylcholinesterase so that it is less sensitive to the insecticide. In order for this type of insecticide to work properly, it must attach to the target molecule which is acetylcholinesterase. Genetic modification of the shape of the acetylcholinesterase molecule prevents proper attachment and results in resistance.

Pyrethroids work by interfering with the normal function of the nerve membrane. Because pyrethroids target the nervous system they possess rapid "knock-down" capability. In order to be effective, pyrethroids must bind with certain molecular structures on the nerve surface called sodium channels. Genetic variation may lead to altered molecular surface structures, or altered "target sites." Mosquitoes with these altered target sites may not be killed with pyrethroid insecticides. This type of altered target site resistance is also known as "knockdown resistance," or *kdr*, which reduces the effectiveness of natural pyrethrins and the synthetic pyrethroids.

10.4.4 Cross Resistance

Selection pressure on a mosquito population by one specific insecticide may result in resistance to other insecticides to which the population has not been exposed. This type of cross-resistance is most common among insecticides that belong to the same class. However, cross class resistance has been reported in, for example: 1) target-site DDT-pyrethroid resistance in the malaria vector, *Anopheles gambiae* and 2) carbamate-organophosphate cross resistance in Central American *Anopheles albimanus*. In both of these cases, a similar mode of action contributed to the cross resistance. DDT and pyrethroids target the sodium and potassium channels of the nerve membrane. Carbamates and organophosphates work by inhibiting the enzyme acetylcholinesterase.

10.5 DETECTION OF RESISTANCE

Insecticide resistance is often first observed in the field as a failure to control the target population with a dosage applied at the label rate. The next step is to rule out treatment failure due to operator error, equipment failure, unfavorable weather conditions, formulation error, failure to expose (hit) the target population, or some other non-genetic cause. To confirm genetic resistance, it is essential to test a sample of the target population by means of a standardized test in the laboratory. Methods for susceptibility-resistance tests have been standardized by the World Health Organization (WHO 1981) for both larval and adult mosquitoes. In addition, the Centers for Disease Control and Prevention (CDC) developed rapid diagnostic tests that can provide useful information to operational control programs (CDC 2002; 2016). Bioassays and biochemical tests are used to detect genetic insecticide resistance and to establish the median lethal concentration, or LC_{50} (the quantity of an insecticide per unit volume of solvent that kills 50% of the test sample).

10.5.1 Bioassay

A bioassay uses live mosquito larvae or adults to determine the response to known concentrations of an insecticide under controlled conditions. The bioassay incorporates sufficient replication to estimate experimental error accurately. Two bioassays used in Florida are the:

1. CDC bottle bioassay, which measures the response of adult mosquitoes over time to a single diagnostic dose

2. Standard beaker test, which measures the response of mosquito larvae to different concentrations of an insecticide

An advantage of the bottle bioassay is that it can be modified by adding synergists to inhibit the detoxification enzymes and expose the nature of the resistance.

Beaker tests were employed during the Florida Abate® (temephos) monitoring program of the 1980s conducted by Boike *et al*. (1982). These long-term studies made a significant contribution to insecticide resistance management by establishing a convention that facilitated comparison of data from different tests. Boike *et al*. (1982, 1985) defined the resistance ratio (R/R) as the median lethal concentration (LC_{50}) for the test strain divided by the LC_{50} of the susceptible strain. Resistance ratios are now usually calculated at the median lethal doses, LD_{50} and LD_{95}, of the test populations. Resistance ratios make it much easier to compare populations with respect to their insecticide susceptibility.

10.5.2 Biochemical tests

Biochemical assays can detect resistance mechanisms in single mosquitoes, enabling resistance monitoring when only a small sample size is available. For example, biochemical assays to detect target site resistance measure changes in the affinity of acetylcholinesterase (the target of organophosphates and carbamates) to its substrate resulting from the alteration of the amino acids responsible for insecticide binding at its site of action (Brogdon and McAllister 1998a). Acetylcholinesterase activity can be measured using acetylthiocholine iodide as a substrate and measuring the released thiol colorimetrically at a specific absorbance (Grafton-Cardwell *et al*. 2004). Changes in the affinity of acetylcholinesterase from the resistant strain compared to the susceptible strain indicate that resistance is due to modified acetylcholinesterase activity.

Resistance by detoxification of the insecticide includes measuring changes in protein levels or activity of enzymatic members of a large multigene family of esterases, oxidases, and glutathione-S-transferase. The biochemical analysis can be measured using a microtiter plate and a spectrophotometer.

The most common resistance mechanisms in insects are esterase detoxification enzymes that metabolize a broad spectrum of insecticides (Brogdon and McAllister 1998b). Reduction in insecticide susceptibility may be due to changing a single amino acid, resulting in the conversion of an esterase to an insecticide hydrolase. The modification may result, also, in the presence of multiple esterase genes that have been amplified to produce numerous copies in resistant insects. Increase in esterase activity can indicate resistance to organophosphates or cross resistance to OP, carbamates, and pyrethroids (Brogdon and McAllister 1998a; Vulule *et al*. 1999).

Detoxification of insecticides is also a function of cytochrome P450 oxidase, including monooxygenases or mixed function oxidases. Oxidases responsible for insecticide resistance result from increased concentration rather than gene amplification. High oxidase metabolic activity, for example, has been implicated in permethrin EC tolerance (Etang *et al*. 2004). Glutathione-S-transferase (GST) exists in insect genomes as multiple copies of one of the classes of glutathione-s-transferase. GST has been implicated in DDT resistance and exists as gene clusters scattered throughout the insect genome via recombination. In fact, multiple forms of GST in the same insect have been found and implicated in resistance (Ferrari 1996).

10.5.3 Gene expression (transcriptomics)

Changes in the amount of insecticide detoxification protein produced in resistant mosquitoes can be measured by quantifying gene expression levels (transcriptomes). Microarray, RNAseq analysis (global detection of changes in gene expression), and quantitative reverse transcription polymerase chain reaction (measurement of the expression level of specific genes) can be used to determine alterations in the expression of genes involved in chemical insecticide detoxification, such as genes encoding esterases, cytochrome P450 oxidases, etc. (Eans *et al*. 2009, Kasai *et al*. 2017). A study using RNAseq to compare whole transcriptome expression changes between a *Bacillus thuringiensis israelensis* (Bti)-resistant population of *Ae. aegypti* with susceptible mosquitoes revealed that Bti-resistance involved changes in the transcription level of enzymes involved in detoxification and chitin metabolism and Bti-receptors were differentially expressed between the resistant and susceptible larvae (Després *et al*. 2014). Bti-resistance resulted in two larval phenotypes, fast and slow growing, that differed in expression of the genes involved in immunity.

10.5.4 Genomics

Small alterations in the genomes of detoxification proteins with a role in mosquito insecticide resistance and susceptibility and between populations from different geographic areas can be detected using next generation sequencing techniques, *i.e.*, RNAseq or DNAseq (Bonizzoni *et al*. 2015, Faucon *et al*. 2015), and these genomic markers can be associated with the regulation of detoxification enzymes and the selection of particular variants in field mosquitoes, which will allow better tracking of metabolic resistance. For example, a study on pyrethroid resistance in *Ae. aegypti* from eight populations (resistant vs susceptible), analyzed by comparing RNAseq and DNAseq data, revealed novel genomic resistance markers associated with gene expression control mechanisms and modifications in detoxification protein structure. The investigators found gene expression polymorphism patterns were geographic region-specific but differed among continents confirming worldwide variability in resistance (Faucon *et al*. 2017).

10.6 CURRENT RESEARCH IN FLORIDA

Available information about insecticide resistance of Florida mosquito vectors is limited (Liu *et al.* 2004a, b, Shin and Smartt 2016, Connelly and Parker 2018). With the increase in resistance worldwide, additional research on insecticide resistance in Florida mosquitoes needs to be evaluated. This need is especially true for Florida as recent years has seen transmission of a number of mosquito-borne viruses, such as DENV, CHIKV, and ZIKV, by local *Aedes* species mosquitoes (Graham *et al.* 2011, Kendrick *et al*. 2014, Likos *et al*. 2016). Florida is also home to *Culex* species mosquitoes where *Culex nigripalpus and Cx. quinquefasciatus* are considered important West Nile virus vectors as field collected samples were found infected with WNV (Blackmore *et al*. 2003).

Field populations of *Cx. nigripalpus* mosquitoes collected in two Florida counties, Manatee and Indian River, were assessed for resistance to an organophosphate. Expression analysis of an esterase gene and results from insecticide susceptibility tests suggest that the level of esterase expression differed depending on the frequency of exposure to the organophosphate insecticide (Shin and Smartt 2016). Insecticide susceptibility tests showed that field populations with previous exposure to OP resulted in higher resistance and esterase gene expression results corroborated what was found with the bottle bioassay, reinforcing the idea that expression of this esterase gene might be used as a marker for increasing OP resistance. Future research should directly link expression of this esterase with OP resistance using gene expression silencing. RNAi molecules designed to esterase will be injected into OP resistant populations mentioned above and sensitivity to OP assessed.

In 2016, the University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory began a state-wide evaluation of the insecticide susceptibility status of *Ae. aegypti* and *Ae. albopictus* against organophosphates and pyrethroids, the two classes of insecticides approved for use in Florida for adult mosquitoes. In one year, 256 CDC Bottle Bioassays were conducted. A trend was observed for high levels of phenotypic resistance to pyrethroids in *Ae. aegypti*. The work was extended for 2017-2018. Updates can be reviewed quarterly at http://www.floridamosquito.info/insecticide-susceptibility-testing-results/.

The United States Department of Agriculture-Center for Medical, Agricultural and Veterinary Entomology (USDA-CMAVE) and Naval Entomology Center of Excellence (NECE) have continued to devote extensive resources to defining the spread and intensity of insecticide resistance in Aedes from the southern U.S. with a focus on Florida and Arizona. They provided no cost access to toxicological and genetic resistance testing for many local mosquito control programs and state public health programs to allow more effective operational decision making. They have examined over 45 strains of *Ae. aegypti* from Florida (including 14 from Miami-Dade County) and found widespread but varied intensity of pyrethroid resistance. Pyrethroid resistance of up to 60-fold is not uncommon; However, nearly all strains were relatively susceptible to available organophosphates. A rapid molecular testing program processed thousands of samples and confirmed the presence of varying levels of sodium channel mutations kdr linked to pyrethroid resistance. In collaboration with the CDC Southeast Vector Center, USDA-CMAVE has started to train personnel from well-equipped mosquito control programs in genetic testing methods. Collier Mosquito Control District was the first to be trained and successfully instituted an in-house testing program. USDA-CMAVE also has provided source material from Miami Beach for the current project to sequence the genome of a pyrethroid resistant American *Ae. aegypti* strain. In the future, the USDA-CMAVE will provide training and assistance to local mosquito control programs and continue collaborations with industry, academic labs, state governments, the military, and the CDC Southeast Vector Center. The laboratory is attempting to define an algorithm to rapidly quantitate pyrethroid resistance using genotypes from surveillance collections of *Ae.* aegypti without having to collect and colonize viable field strains. Collaborative studies to examine repellence and arboviral vector competence are planned using recently field collected strains. This laboratory also plans to implement a testing program to examine resistance in Culex species in Florida.

10.7 STRATEGIES OF RESISTANCE PREVENTION AND MANAGEMENT

This section is based on Yu (2015).

10.7.1 Reduce the frequency of resistant genes in a population

- Use the lowest dosage that gives control, sparing a portion of the susceptible target population. This practice does not seek complete control and conserves susceptible alleles in the mosquito population.
- Apply insecticide less frequently
- Use chemicals of short environmental persistence
- Rotate chemicals of different classes
- Use localized rather than area-wide applications
- Leave some mosquito generations untreated
- Preserve "refugia" of susceptible mosquitoes
- Do not treat adults and larvae with the same class of insecticide
- Consider acceptance of a higher pest population threshold before applying insecticide

10.7.2 Management by Multiple Attack:

- Use mixtures of chemicals of different classes
- Suppress detoxification mechanisms by use of synergists such as PBO
- Alternate or rotate use of insecticides of different classes for an entire season
- Use different classes of insecticides for adulticiding and larviciding

10.7.3 Management by Integrated Pest Management

Integrated Pest Management (IPM) is the prudent combination of control methods designed to minimize over-reliance on any single means of control. The effect of IPM is to minimize exposure of target species to a given class of pesticides.

For example, mosquitoes that show evidence of resistance to OP insecticides still show high levels of susceptibility to *Bti*. Take advantage of this differential susceptible by using *Bti* against the larvae where feasible, eliminating the use of OP larvicides, and reducing the use of OP adulticides. This approach illustrates another principle of effective resistance management: Different control strategies should be directed to the different life stages of the mosquito in order to minimize selective pressure.

Another effective strategy is to maintain "refugia" of susceptible mosquitoes. Refugia are areas in which the target population remains untreated. The fundamental concept here is that mosquitoes that are not exposed to pesticides will maintain high levels of susceptible alleles and low levels of resistant alleles. Susceptible mosquitoes usually have higher biotic potential, which enables them to be more successful and live longer than resistant mosquitoes. Thus, they can reduce the proportion of resistant alleles when they migrate from refugia and infiltrate resistant populations. State and federal lands, where pesticide use is prohibited, effectively function as refugia.

10.8 RESISTANCE SURVEILLANCE

The main defense against insecticide resistance is close surveillance of mosquito susceptibility to pesticides. Insecticide application methodology should include regular measurement of target insect susceptibility. Early diagnosis improves long-term prognosis. In addition to surveillance using the bioassays, new tools are being developed to enable quick evaluation of changes in insecticide susceptibility.

It is now possible to utilize deep genome sequencing technologies (high throughput) to obtain sequence information about common mutations responsible for insecticide resistance in field mosquitoes. Technology such as the MinION (Oxford Nanopore), a portable device that performs DNA/RNA sequencing, can detect the presence or absence of sequence variation in genes involved in insecticide detoxification, *i.e.* cytochrome P450 and voltage gated sodium channel genes. Whole genome sequencing was instrumental in the discovery of a genetic signature of pyrethroid-based interventions in an African population of *Anopheles funestus* following an increase in insecticide-based malaria control in Africa (Barnes *et al*. 2017). Also deep sequencing was used to elucidate gene amplifications and polymorphisms associated with resistance to the insecticide deltamethrin in *Ae. aegypti* (Faocon *et al*. 2017). Comparing *Ae. aegypti* from different origins and resistance levels, sequence analyses identified multiple genomic regions under selection, and several nonsynonymous nucleotide sequence variants were associated with deltamethrin resistance (Faocon *et al*. 2017). From these results, simple

PCR based assays can be developed to specifically target the most common polymorphisms associated with resistance to insecticides thereby facilitating surveillance.

10.9 FUTURE RESEARCH

Resistance to the commonly used chemical insecticides by mosquitoes is worldwide. New mosquito control technologies are needed to restrain the spread of new and re-emerging mosquito-borne diseases. This section presents two of the newest most promising strategies for mosquito control that warrant further research.

RNA interference (RNAi)-mediated gene expression silencing has been successfully used to characterize functions of numerous genes from different organisms (Zamore *et a*l. 2001). Employing this technique allows one to specifically decrease the expression of a gene to study the resultant phenotype. In an attempt to counteract chemical insecticide resistance, RNAi mediated gene expression knockdown has been used to reduce the expression of detoxifying genes of commonly used insecticides and has proven successful against pest insects, including the Colorado potato beetle and western corn rootworm and the yellow fever mosquito (Bona *et al*. 2016, Fishilevich *et al*. 2016, Clements *et al*. 2017). Double stranded RNA (RNAi) designed to a voltage gated sodium channel gene was able to rescue the pyrethroid susceptible phenotype in a population of resistant *Ae. aegypti* (Bona *et al*. 2016). Adults from the dsRNA exposed *Ae. aegypti* larvae were susceptible to a range of deltamethrin concentrations and silencing of the detoxification gene was stable though the adult stage suggesting further studies on the use of RNAi to control insecticide resistance should be performed.

Nanotechnology allowed the development of targeted drug delivery through using biocompatible nano-carriers such as nanoparticles. Nanoparticles are extensively used for drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering (Morones *et al*. 2005). In addition to uses in the medical field, silver nanoparticles were shown to be potent antimicrobial and antifungal agents and some synthesized silver nanoparticles were shown to kill mosquito larvae. Recent studies show that silver nanoparticles synthesized with various leaf extracts tested against *Anopheles stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* have larvicidal activity (Santhoshkumar *et al.* 2011, Muthukumaran *et al.* 2015). However these extracts were solubilized in non-aqueous solvents making their production costly and environmentally hazardous (Muthukumaran *et al.* 2015). Presently, it is possible to synthesize biodegradable plant based silver nanoparticles, and there are many plants shown to have some level of mosquitocidal activity (Benelli *et al.*). Oral exposure to *Acacia caesia* leaf extract fabricated silver nanoparticles (Ag NPs) revealed ovicidal, larvicidal, and adulticidal toxicity against *Anopheles subpictus, Ae. albopictus*, and *Cx. tritaeniorhynchus,* and the Acacia leaf extract Ag NP was less toxic to three aquatic mosquito predators than the mosquito larvae suggesting reduced non-target effects (Benelli *et al*.).

10.10 CONCLUSION

The application of chemical insecticides is the mainstay of mosquito abatement in Florida. It is essential that great care is taken to maintain efficacious, cost-effective, and safe control methods. Monitoring for insecticide resistance on a regular basis can provide early warning, so that corrective measures can be taken. Use of alternative classes of active ingredient, increased larviciding with *Bti* and other biorational techniques, source reduction, rotation of chemical classes, and mechanical control measures all contribute to sound management practices that will result in sustainable maintenance of successful mosquito control.

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Chapter 11

MOSQUITO CONTROL RESEARCH

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Summary

The goal of mosquito control is to limit the impact of nuisance and disease carrying mosquitoes on Florida residents and tourists, while simultaneously maintaining and, where possible, improving the environment. Mosquito control is too often in the middle of a conflict between citizens who may feel that mosquito control is insufficient and those people who believe mosquito control is harming the environment. To strike a balance, mosquito control programs need to be based on solid scientifically based research that provides safe, effective, economical, and environmentally sensitive mosquito control technologies. In the development of an effective mosquito control program, the most important concerns facing mosquito control today requiring research are:

- *surveillance*
- *mosquito biology*
- *wetlands ecology*
- *human-made mosquito problems*
- *disease detection and prevention*
- *repellents*
- *improving attractants and traps*
- *improving on existing chemical technology*
- *non-target organisms*
- *biocontrol including*
- *sterile insect technique*

The history, accomplishments, and needs of the principal university, government and private laboratories, and agencies involved in Florida's research effort are described. Without strong funding for mosquito

control research and extension, these laboratories will be forced to conduct research in other more readily funded areas. Already, mosquito control is losing some of its effective tools due to insecticide resistance, development and operational costs, or concern that some chemicals and/or techniques may be harmful to the environment. Without research laboratories searching for new innovative methods and technology and verifying their safe use, mosquito control will have increasing difficulties providing protection for Florida's citizens at the level expected today. The threat of emerging vector borne pathogens has effected and will continue to effect the well-being of Floridians and tourists. Without a strong mosquito control effort, Florida will be an uncomfortable and dangerous place to live and visit.

11.1 INTRODUCTION

• **Surveillance**: Good mosquito and mosquito-borne pathogen surveillance systems are the heart of an effective mosquito control program. Efficient and accurate methods to survey mosquito populations, to identify and predict mosquito outbreaks, and to detect and predict the occurrence of mosquito-borne pathogens are needed so that the most efficient and environmentally sound control methods can be implemented. Research is needed to improve current surveillance practices.

• **Mosquito Biology**: Mosquitoes have been around for millions of years and have mastered ways to survive – despite habitat manipulation and chemical control. New exotic species invade the state on a regular basis, bringing with them new threats. Research on the biology of many of the species has provided much of the information that led to today's control methods. These methods resulted from an understanding of mosquito biology, ecology, reproduction, and habits. The more we know about mosquitoes, the greater the potential for developing improved techniques for their control without harming the environment.

• **Wetlands Ecology**: Originally, mosquitoes inhabiting salt marshes along Florida's coast were the major source of complaints. The use of draining, impounding, and chemicals brought saltmarsh mosquitoes largely under control. Unfortunately, some of the past solutions caused some environmental problems. Research has demonstrated that mosquito control and wetlands preservation are not mutually exclusive goals. More research is needed to fine-tune and enhance mosquito control programs in wetlands.

• **Human-made Mosquito Problems**: Wastewater, rainwater, and artificial containers discarded by humans are now a major source of mosquito problems in Florida. Research is needed to provide creative solutions to mosquito problems created by humans. For example, research is urgently needed on the biology, surveillance and control of *Aedes aegypti*, the primary vector of Florida's recent dengue, chikungunya and Zika outbreaks. Additionally, research is also needed on the biology and control of *Aedes albopictus,* the

Asian tiger mosquito, and other invasive species introduced into the U.S. The Asian tiger mosquito is considered by most people to be among the more important pest species throughout much of Florida. It also is considered an important potential vector of pathogens that cause disease.

• **Disease Detection and Prevention**: While Florida has been relatively free for the last 40 or more years from the most infamous mosquito-transmitted diseases such as yellow fever, dengue, malaria, and filariasis, Florida still experiences periodic outbreaks of St. Louis encephalitis (SLE), eastern equine encephalitis (EEE), and West Nile encephalitis (WNE). West Nile virus (WNV) entered the U. S. in 1999, was detected in Florida in 2001, and has caused West Nile disease in 3-100 Floridians each year since. There is great risk of a substantial WNE epidemic in Florida with hundreds to thousands of human cases. More recently, Florida has been afflicted with the *Aedes aegypti* transmitted emerging mosquito borne dengue, chikungunya, and Zika viruses. The dengue outbreak in the Florida Keys infected hundreds of people over a two year period from 2009-2010, followed by several years of local transmission in other parts of southern Florida. Local transmission of chikungunya occurred in 2011, and a large Zika outbreak in Miami-Dade County resulted in over 285 confirmed cases of locally transmitted virus in 2016.
The entry of WNV, dengue, chikungunya and Zika viruses into Florida demonstrates the great risk from other emerging vector-borne pathogens, some of which are already circulating in South America, Central America, and the Caribbean region. These pathogens circulating in other parts of the America directly threaten Florida, which each year reports dengue, chikungunya, malaria and Zika infections in travelers to the state with more than 1,100 cases of travel related Zika infection in 2016. Expertise and research to identify the mosquito vectors, the pathogens they carry, and new methods to control them are essential to detect outbreaks early and implement control measures to interrupt transmission to prevent human disease.

• **Repellents**: Safe and effective personal protection is needed to ward off biting insects, especially if funds, time, or location do not allow for mosquito control activities. Recently developed repellents have improved user acceptability, although some products remain oily, have an offensive odor, or have other unpleasant properties, and some individuals are allergic to the ingredients. A number of plants, bug zappers, buzzers, etc., are advertised and sold as mosquito repellents and yard insect-control devices. To date, all of these devices have proven useless, however, new products offer potential to be used as spatial repellents. Research is needed to evaluate and develop new repellents and improved methods of personal protection especially those with enhanced spatial effects.

• **Attractants**: In contrast to repellents, mosquito attractants also exist. Research has shown that some gases (*e.g.*, CO_2 and octenol) attract large numbers of some mosquito and biting midge species to surveillance and /or control traps or into killing zones treated with an insecticide. Through research, we are able to develop more integrated methods of control with less reliance on chemical insecticides.

• **Improving Existing Chemical Technology**: New chemicals for mosquito control are expensive to develop. Research to develop new or different application techniques, formulations, and synergists can extend the effectiveness of existing chemicals for mosquito control by delaying resistance and/or improving performance. For example, recent discussions concerning the efficacy of microbial insecticides suggest that time of application and larval feeding habits may be important for treatment success. There are variations in application methods among mosquito control programs. It is not unusual for a chemical to work well for one program but not work for another program. Research to compare and demonstrate the best methods and techniques of application are needed. Work on development of novel molecular pesticides that target specific areas of the mosquito genome has the potential for producing new, effective, and safe public health pesticides.

• **Non-target Organisms**: The Florida Coordinating Council on Mosquito Control (FCCMC) has been concerned about the effects of insecticides such as temephos and permethrin on non-target organisms especially when applied on state lands. Further research is needed to better assess and mitigate the non-target impacts of mosquito control insecticides. There is also a need to weigh the benefits and limitations of deploying a particular chemical versus other control techniques (*e.g.*, ditching, impounding). The opposite situation, the effects of non-target fauna and flora upon the effectiveness of mosquito control interventions, is also in need of further research.

• **Biocontrol**: Although a number of mosquito predators, parasites, and pathogens have shown promise as biological control agents, few species other than the mosquitofish, *Gambusia* spp., have been effectively integrated into mosquito control due to efficacy, as well as logistical and economic problems. Research has shown that, with the exception of mosquito-eating fish, natural levels of predators, such as purple martins and dragonflies or pathogens and parasites, do not significantly reduce mosquito populations. Yet, with additional research, biological control may represent a target-specific alternative to chemical insecticides. Most recently the development of new products utilizing sterile insect techniques, a proven strategy for other insect pests, offer the prospect for an added tool to the mosquito control arsenal (see discussion below).

• **Sterile Insect Techniques**: Testing of new and old mosquito control techniques involved in sterile male release should be continued and expanded to determine their efficacy and the circumstances under which they are most effective. Included in this category are the use of transgenic mosquitoes, irradiated mosquitoes, and strain specific *Wolbachia* infected mosquitoes, and several others. Some of this work is already underway in Florida at U.S. Department of Agriculture, Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS CMAVE) and the University of Florida in collaboration with mosquito control districts and the Florida Department of Agriculture and Consumer Services (FDACS).

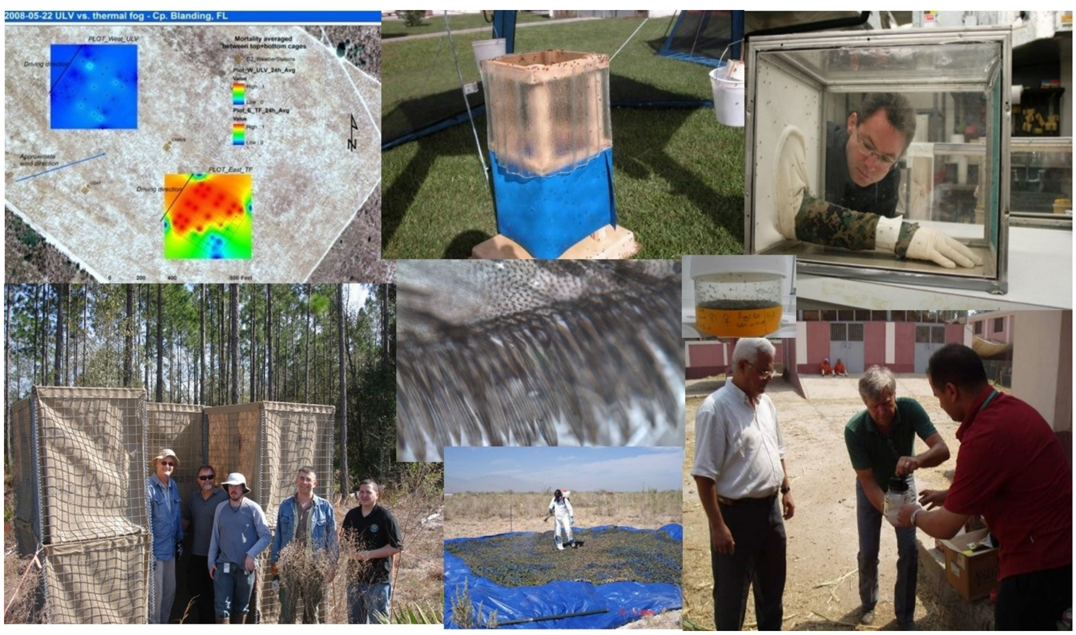
11.2 RESEARCH ORGANIZATIONS

Florida is fortunate to have a number of federal, state and local government agencies, as well as private organizations, that conduct or support mosquito biology and control related research.

11.2.1 Federal

11.2.1.1 U.S. Department of Agriculture, Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology

History: During World War II, the United States Department of Agriculture (USDA) cooperated with the United States Department of Defense (DoD) to establish a research laboratory in Orlando, Florida. The mission of the laboratory was to develop technologies for the protection of military personnel against insect vectors of disease. In 1951, the laboratory was named the Insects Affecting Man and Animals Research Laboratory. In 1961, the Secretaries of Defense and Agriculture signed a memorandum of understanding to continue the research program under USDA funding. In 1963, the laboratory moved into new federal facilities located adjacent to the campus of the University of Florida (UF) in Gainesville. The laboratory's name was changed to Medical and Veterinary Entomology Research Laboratory in 1990 and to the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in 1996. Annual base funding for the Center exceeds $12 million at present with in excess of $5 million in extramural funding and over 350,000 square feet of office and laboratory space.



Collage of CMAVE Activities

Mission: The mission of CMAVE is to conduct research on insects of agricultural, medical, and veterinary importance with the goal of achieving control of pest species through environmentally compatible approaches. CMAVE consists of four Research Units:

1. Insect Behavior and Biocontrol
2. Chemistry
3. Imported Fire Ant & Household Insects
4. Mosquito and Fly

The Mosquito and Fly Research Unit's mission is to develop novel technologies for detection and population monitoring, repellents for the protection of humans and animals from biting and filth breeding flies, and effective chemical, biological, and genetic control technologies, and integrated management strategies for insects and arthropods of medical and veterinary importance. For many years, the laboratory has had important national and international roles in studying and developing new and innovative methods for controlling blood-sucking insects and flies. This research has resulted in improved management technologies for insects that are pests or transmit disease agents (such as those that cause West Nile fever, dengue, chikungunya, Zika, Rift Valley fever, and malaria) to humans and animals worldwide.

The Unit's research mission includes the study and development of:

- New, more efficient insect trapping systems
- Biologically-based control technology;
- Host protection methods for use against insect pests of medical and veterinary importance

In this research, the Unit has works closely with mosquito control and public health specialists, researchers, and industry partners involved in control of these insect pests to the ultimate benefit of consumers and stakeholders in the U.S. and internationally.

The results of research undertaken at CMAVE have application to programs of animal and public health in international, national, state, and local agriculture and public health government agencies, private industry, and the general public. The medical and veterinary entomology staff of CMAVE consists of fourteen permanent scientists, six postdoctoral/visiting scientists, and approximately fifty technical/support personnel including numerous students and volunteers. The laboratory facility is modern and well equipped and comprises approximately 100,000 square feet of space with one of the largest mosquito and fly insectaries in the country.

Staff: Mosquito-related research at the laboratory is undertaken by seven permanent, full-time category I scientists, a full-time category III scientist, and four visiting scientists from the U.S. military including a uniformed medical entomologist from the U.S. Navy and one from the U.S. Army.

Budget: The allocation of base funding is defined by the number of permanent, full-time category I positions. At present, approximately $3,700,000 per year is committed to research involving mosquitoes. Base funds are received via the agriculture appropriation approved yearly by the U.S. Congress. Current total extramural funding in support of mosquito research is more than $2,000,000 from DoD and industry sources.

Major Contributions: CMAVE accomplishments derive from multi-disciplinary team research and a wide range of cooperative efforts. Scientists interact with colleagues and with animal/public health agencies and organizations worldwide. Cooperators include the DoD, the World Health Organization (WHO), the Food and Agriculture Organization, the International Atomic Energy Agency, the Animal and Plant Health Inspection Service, the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the National Aeronautics and Space Administration (NASA), the Department of Transportation (DOT), various universities, local and state mosquito control programs, sister Agriculture Research Service (ARS) laboratories, and industry. CMAVE has an outstanding record of chemical and biological control research accomplishments. Research accomplishments of the scientists concerned with mosquito research are documented in approximately 3,000 publications in scientific journals, conference proceedings, books, book chapters, and handbooks and via patents.

Major research accomplishments related to biodegradable pesticides and personal protection chemicals include the:

- Development of N,N-diethyl-meta-toluamide (DEET), the principal active ingredient in most insect repellents
- Development of the Ultra Low Volume (ULV) method of insecticide application for use in mosquito control
- Development of a clothing treatment for personal protection against biting arthropods
- First operational use of sterile insect technique to control mosquito vectors
- First development of molecular pesticides for mosquito control

Major research accomplishments related to the **Biologically Based Research Program** include developing new biologically based control strategies for mosquitoes, house flies, and stable flies. Development of new biological pesticides and/or control strategies for vector and pest flies becomes increasingly important as human populations grow and new and exotic disease agents appear. Alternative control methods also are needed to combat high levels of insecticide resistance in flies that affect animal production and well-being. Such strategies can help prevent contamination of the environment with chemical pesticides that threaten humans and contribute to a decline in biodiversity. Examples of new technologies under development by unit scientists are:

- Discovery of a new baculovirus to combat mosquitoes inhabiting agricultural wastewater
- Establishment of a mosquito pesticide resistance testing laboratory using classical bottle bioassays and molecular techniques
- Development of a new protozoan parasite method for control of *Aedes aegypti,* the yellow fever mosquito
- Development of a new method using a nematode parasite for mosquito control
- Discovery of a new parasitic wasp for control of house flies and stable flies
- Improvement of the quality of commercially produced wasps for fly control
- Development of new traps to prevent fly immigration into neighborhoods around farms
- Discovery of chemicals that cloak humans (make them "invisible") from mosquito detection
- Development and evaluation of all permethrin-treated military uniforms including quality control for all first article assessments
- Discovery of new potential natural products as active ingredients for vector control
- Pioneered the discovery of RNAi technology and continue to develop products that impact mosquito vectors and potentially could be used for operational control
- Development of the use of irradiated mosquitoes for use in sterile insect technique
- Discovery of potentially new personal and spatial repellents

Major research accomplishments of the **Surveillance and Ecology of Mosquito, Biting and Filth Breeding Insects Program** are directed at meeting public/animal health and military needs for low-cost, attractant-based detection systems that determine the presence and abundance of nuisance flies and vectors and developing faster, less expensive, more specific, and more sensitive methods to detect vectors that may be carrying endemic, exotic animal, or human pathogens. There is a critical need to develop a Geographic Information System (GIS)-based system that integrates these detection methods with knowledge of the target insect's biology and environmental factors for accurate disease risk assessment and vector disease forecasting. Examples of new technologies under development by unit scientists are:

- Discovery of new attractants for house flies, *Ae. aegypti,* and mosquitoes that transmit arboviruses and malaria

- Discovery and isolation of environmental factors that attract or repel mosquitoes during oviposition

- Elucidation of dispersal patterns, breeding habits, and host attractions of horn flies, house flies, stable flies, and mosquitoes

- Development of marking systems to study dispersal and survival patterns of mosquitoes

- Development of a new generation of CO_2 and/or heat producing mosquito traps for improved surveillance and population management of selected mosquito species

- Identification of octenol as an important mosquito attractant and work with private industry to develop readily available lures for mosquito surveillance programs

- Understanding the role of species biology and population genetics in the transmission of arboviruses

- Development of species-specific traps that are light weight, inexpensive, low maintenance, and which are surrogates for individual human or livestock bait

- Identification and synthesis of host specific and oviposition attractants and adaptation for use in traps or bait stations

- Design and testing of a model using GIS technology and remote sensing to predict the ideal placement of traps for vector and fly surveillance and to assess the risk of disease transmission

- Development of first technology to predict vector-borne disease, including application to mitigate Rift Valley fever outbreaks in Africa and the Middle East Rift Valley fever risk monitoring and dengue and chikungunya outbreaks in Africa and Asia
  http://www.ars.usda.gov/Business/Docs.htm?docid=23464

- Investigation of the neural and sensory ultrastructure of ticks and Diptera

- Development of measurements of electrophysiological activation for use in selecting vector attractants, repellents, and new generation pesticides

The **Deployed War-Fighter Protection (DWFP) Program** is a DoD-sponsored research program administered by the Armed Forces Pest Management Board (AFPMB) that was stood up in 2004. It is tasked with the development and testing of management tools for pest and vector species that transmit diseases to deployed war-fighters. Due to a shrinking list of safe, cost-effective pesticides for control of disease vectors, new and improved materials and methods for pesticide delivery are needed by the Armed Forces to prevent diseases that threaten the deployed troops. Research at CMAVE focuses on the discovery, evaluation, development, and optimization of: 1) new pesticides effective against mosquitoes and flies, 2) new personal protection products effective in preventing mosquito and fly bites, and 3) new application and personal protection methodologies and strategies.

Recent accomplishments include:

- Development of new pesticide application technologies for use by military and civilian vector control programs [Mobile Pesticide App (in development) http://www.ars.usda.gov/Business/docs.htm?docid=24908]

- Re-evaluation of new and old chemistries for their effect on mosquitoes

- Development of new pesticides using molecular biology techniques to target physiological process in the insects

- Development of spatial repellent delivery field kits to be used by war-fighters

- Testing of spatial repellents for use in military tents, blast-wall protective structures known as HESCO barriers, and other military barricades with various types of military camouflage netting

- Development of a new generation of barrier-spray strategies for use by deployed troops

- Selection of more efficient fly traps for use in arid conditions where U.S. troops are deployed

Future research is being directed at the following:

- Discovery and development of new biological, behavioral, physiological, genetic, and chemical regulating mechanisms that can be used for mosquito, sand fly, and higher diptera (fly) control

- Validation of recently discovered biological, chemical, and genetic mosquito control technologies in large-scale, area-wide management programs targeted at natural populations of mosquitoes

- Scientific, economic, and sociologically sound analyses of the costs of mosquito control in relation to benefits accruing to the public (improved quality of life) and animal/public health (disease vector abatement) worldwide

11.2.1.2 U.S. Navy Entomology Center of Excellence

History: Following World War II, only a handful of entomologists remained on active duty in the Navy. A majority of the Navy Epidemiology Units which proved so successful during the war were disbanded. One that remained, however, was the Malariology and Pest Control Unit at Naval Air Station, Banana River, Florida. The unit was moved to Naval Air Station, Jacksonville, Florida in 1947. In 1949, it was commissioned the Malaria and Mosquito Control Unit No. 1 with an entomologist as the Officer in Charge. In 1952, the Unit was renamed Preventive Medicine Unit No. 1 (PMU-1), and, in 1957, PMU-1 became the Disease Vector Control Center (DVCC) with an expanded mission and area of operation which included approximately one-half of the world. In 1971, DVCC JAX was re-designated the Navy Disease Vector Ecology and Control Center (DVECC). Finally, in 2006, DVECC JAX was re-named the Navy Entomology Center for Excellence (NECE) to reflect an expanded role involving active collaboration with federal, state, and local agencies, academia, and world-recognized specialists and organizations to develop state-of-the-art disease vector control tactics, techniques, and procedures, protecting both military and civilian personnel around the globe.

Mission: NECE continues to help ensure the readiness of our military forces and support Geographic Combatant Commander-led global health initiatives. To accomplish this mission, NECE provides direct entomological support to operational units, conducts training on a variety of preventive medicine skills including disease vector surveillance and control with an emphasis on integrated vector management, engages in research, development, testing, and evaluation of insecticides, application equipment, and novel technologies to meet any existing or emerging vector borne disease threat, and works with military and civilian personnel from partner nations around the world to enhance their capability and capacity in reducing the impact of disease on their populations, economies, and health security.

11.2.2 State

11.2.2.1 Florida Department of Health, Tampa Branch Laboratory, Virology Section

The Florida Department of Health, Tampa Branch Laboratory, Virology Section tests the sentinel chicken blood from various counties for St. Louis encephalitis, eastern equine encephalitis, and West Nile encephalitis virus antibodies collected on a weekly or biweekly schedule. Results are sent to all mosquito control programs by the FDACS. This Laboratory also collaborates with University of South Florida, the Florida Medical Entomology Laboratory, and on arboviral research projects.

11.2.2.2 Florida Institute of Technology

Scientists and graduate students at the Florida Institute of Technology (FIT), a private university in Melbourne, have conducted research on the ecosystem effects of mosquito control source reduction projects (*e.g.*, impoundment management and rotary ditching). FIT is now called Florida Tech.

11.2.2.3 Florida Medical Entomology Laboratory, University of Florida, Institute of Food and Agricultural Sciences

The Florida Medical Entomology Laboratory (FMEL) is one of the world's largest research institutions devoted to the understanding and control of medically important biting insects. Modern laboratory and support facilities and easy access to natural habitats offer an environment conducive to scientific investigation.

FMEL is a Research and Education Center of the University of Florida, Institute of Food and Agricultural Sciences (IFAS). The FMEL is located about three miles south of Vero Beach along the Indian River Lagoon (IRL) on Florida's subtropical east coast. The laboratory was established in 1956 and consists of a group of buildings among 38 acres of an oak-palm forest, a scrub oak-pine forest, mangals, and an extensive salt marsh. The convergence of these habitats provides an exceptional outdoor classroom setting which affords students the opportunity to experience the contrasts and similarities of all these habitats. The facilities include modern laboratories, offices, a library, a dormitory, and classrooms. Other facilities include a biological safety level III laboratory for handling arboviruses, an insectary for holding exotic mosquitoes, a metal and woodworking shop, biochemistry and molecular biology laboratories, a graphics laboratory, and an animal facility for housing experimental animals. A screened pavilion sits in the midst of a coastal oak hammock for use as an outdoor mosquito cage and also is available to accommodate up to 150 guests and students for lectures. Field classroom and wet lab facilities also are available nestled among mangroves on the FMEL campus.

Mission: In 1979 the Florida Legislature passed House Bill 684. This bill placed the FMEL under the UF/IFAS. The Legislature, through House Bill 684, recognized the need for greatly expanded research on the biology and control of mosquitoes, especially about the effects of insect-borne diseases on the citizens of Florida and its tourist industry. House Bill 684 mandated that the FMEL:

- Conduct basic and applied research in the biology and control of biting insects and other arthropods which are important transmitters of disease or pest annoyances, giving special attention to the needs of Florida's mosquito control organizations (districts, counties, and municipalities).

- Be a center to train students and personnel in the entomological aspects of public health, veterinary science, sanitation, mosquito control, drainage and irrigation design, wetlands management, and other areas of service requiring knowledge of medical entomology.

- Extend research and training to international programs.

FMEL scientists conduct laboratory and field work on both vectors and pathogens and this allows them to ask unique questions about the interactions between hosts, pathogens, vectors, and the environment.

Over 1,500 peer reviewed scientific publications have been published by FMEL faculty and staff in about 100 national and international journals. The FMEL staff of about fifty people includes 13 faculty holding doctoral degrees with a tenure home in the Department of Entomology and Nematology of the University of Florida. The faculty include five full professors, three associate professors, two assistant professors, and three research assistant professors. The staff includes several visiting scientists, affiliate faculty, postdoctoral fellows, and graduate students earning M.S. and Ph.D. degrees at the UF. Faculty and staff represent an array of expertise contributing to multi-disciplinary projects that remain the hallmark of the FMEL and why the Laboratory provides unparalleled opportunities for studying vectors and vector-borne diseases.

The FMEL has a proven track record of research on all aspects of the biology of mosquitoes and of the arboviruses that they transmit. Prior to the recent threats to Floridians by emergent arboviruses such as dengue, Zika, and chikungunya, FMEL scientists contributed important research about the life cycle of St. Louis, eastern equine, and West Nile encephalitis viruses in Florida. Research includes the relative importance of abiotic factors (*e.g.*, weather and tides) and biological factors (*e.g.*, predation and competition) in determining the distribution and abundance of pests and vectors. The mating, feeding, and egg-laying behaviors of many mosquito and biting insect species have been published in numerous scientific reports. This information has allowed mosquito control to take advantage of the specific features of the biology of important species to control them more effectively. Furthermore, the impact of mosquitoes as vectors depends strongly on what species of animals they bite, plus when and where the blood feeding occurs. Research in the 1960s and 1970s at FMEL provided the first comprehensive studies on the host-feeding patterns of (nearly) all the region's mosquito species. Because this research extended across habitats and seasons, investigators were able to detect seasonal host shifts by vector mosquito species, which facilitates the maintenance of some arboviruses, such as SLE, in their enzootic cycles. Important offshoots of this research include recognition and first descriptions of host defensive behaviors, which reduced mosquito feeding rates on some local birds, especially salt marsh species.

FMEL researchers developed a sensitive, simple, and fast method that can be used by mosquito control programs to detect EEEV and SLEV in mosquitoes. Scientists verified that sentinel chickens provide early warning of SLEV and WNV transmission to humans. FMEL evaluated the importance of exotic avian species (*i.e*., emus, rheas, peafowl, and parrots) as possible arboviral amplification hosts and evaluated how meteorological factors and biological (*e.g.*, larval competition) factors influence arboviral transmission in Florida. Important discoveries were made on the role of mosquitoes as vectors of filaria, arboviruses, dog heartworm, turkey malaria, turkey pox, and other vector-borne diseases in Florida.

FMEL scientists demonstrated field transmission of WNV by *Cx. nigripalpus,* providing evidence of the ability of this species to vector WNV under natural conditions and developed on-line risk maps for mosquito control programs to use in making decisions on control operations based on arbovirus activity and environmental conditions in their areas. Additionally, the natural reductions (biological control) of mosquitoes by *Toxorhynchites*, *Corethrella*, copepods, tadpoles, fish, and other organisms and their importance to mosquito control has been demonstrated in several studies. Traditional insecticide evaluations also are performed at FMEL. These efforts assist Florida mosquito control programs in evaluating and improving biting fly control.

Current wetlands management practices pertaining to mosquito control are in large part based on collaborative research done at the FMEL. Research on all aspects of the Florida coastal environment and on the effects of mosquitoes control practices was conducted at FMEL and led to development of management strategies such as Rotational Impoundment Management (RIM), that allowed mosquito control agencies in the state to continue to use ecologically sound source reduction techniques instead of pesticides to manage mosquito production from coastal salt marshes and mangrove forests. The FMEL staff also have been active in developing, and implementing wetlands policy, as well as addressing coastal management issues of widespread importance.

More recently, work at FMEL has resulted in improved understanding of the mechanisms responsible for distribution patterns of *Ae. albopictus* and *Ae. aegypti* attributable to satyrization and evolution of satyrization-resistance in mosquito populations in Florida. Collaborations with industry are providing improved detection methods for the surveillance of emergent arboviruses (Zika, chikungunya, and dengue viruses) in mosquito and clinical samples.

Other contributions include improved understanding of lethal and nonlethal effects of insecticides on life history and population growth of vector mosquitoes, enhanced understanding of susceptibility to infection and transmission of Florida mosquito vectors for emergent arboviruses including Zika, chikungunya, and dengue viruses, discovery of new mosquitoes such as *Ae. pertinax, Culex interrogator, Cx. panocossa, and Aedeomyia squamipennis* in Florida and elsewhere by FMEL faculty, development of honey-card methods for arbovirus surveillance in Florida, determination of insecticide resistance status of Florida mosquito populations.

Current research programs at the FMEL span disciplines from molecular biology, biochemistry, physiology, virology, and genetics to population level disciplines including population biology, ecology, and epidemiology. The FMEL is well known for its strong field components and the integration of its research with mosquito control and public health programs. The fact that FMEL scientists work on both the vectors and the pathogens allows staff to ask unique questions about the interactions between hosts, pathogens, vectors, and the environment. Research collaborations include partnerships with leading researchers and institutions throughout the world and have included collaborative projects in Africa, Central and South America, Europe, Asia, and Oceania.

The FMEL Applied Mosquito Research Program conducts research related to pesticides and mosquito control. The FMEL extension program emphasizes gathering information, distributing important research findings, providing training to clientele in mosquito control, public health, and the general public, and conducting biological studies at the mosquito control agencies.

Examples of current FMEL areas of research include:

- Susceptibility of Florida mosquitoes to arboviruses
- How infection alters mosquito biology
- Role of larval ecology in determining adult traits (longevity, vector competence, fecundity)
- Biology and ecology of container mosquitoes
- Use of novel autodissemination techniques to control *Ae. aegypti* and Ae. albopictus.
- Arboviral surveillance and risk assessment
- Mathematical modeling of mosquitoes and sand flies – and the diseases they carry.
- Development of diagnostic techniques for disease agents. Accurate and rapid identification of disease agents is a critical component of surveillance and control of mosquito-borne arboviruses. We developed a single-tube PCR amplification assays that can identify 22 mosquito-borne RNA viruses. Additionally, this assay system has the ability to distinguish between closely related viruses within the Flavivirus genus as well as the California serological group. This assay was highly sensitive as demonstrated by the ability to detect as few as 6-10 dengue virions in a single mosquito. FMEL scientists also are pioneering the use of FTA (Finders Technology Associates) cards, which preserve viruses on honey-coated surfaces from which female mosquitoes sugar-feed, both for detecting arboviruses circulating in nature and for measuring the capacity of laboratory-infected vectors to transmit.
- Assessment of how different triggers for antiviral responses affect transmission of arboviruses from a single mosquito. Virus movement through the mosquito, invading and replicating in different tissues and interacting with immune pathways, likely varies between virus-mosquito species combinations, affecting the dynamics of an individual's ability to transmit virus in saliva. Understanding these processes will improve our understanding of virus epidemiology.
- Explore how ecological and environmental interactions affect mosquito population dynamics, movement, and arbovirus transmission. Arbovirus

transmission cycles are complex and often involve many species interacting with each other and the environment. This research investigates which factors are most important in invasion, transmission, and intervention strategies to better understand these cycles and highlight high priority areas for further research.

- Investigate how contact rates between vertebrates affect the transmission of directly transmitted viruses. Individual level interactions are important in transmission but are not well understood. We are exploring individual contact rates under different interaction rules to understand the effect on transmission. This research area has been expanded to also consider the effect of individual level behavioral decisions on mosquito movement and control.

- Ecology of Mosquito Vectors of Arboviruses: Objectives are to explain patterns of distribution and abundance of vector species, especially invasives, in relation to their potential for transmission of pathogens. *Aedes aegypti* and *Ae. albopictus*, two important vectors of the dengue and chikungunya viruses to humans, often come in contact in their invasive ranges. In these circumstances, a number of factors influence their population dynamics, including resource competition among the larval stages, prevailing environmental conditions, and reproductive interference in the form of satyrization. As the distribution and abundance of *Ae. aegypt*i and *Ae. albopictus* have profound epidemiological implications, knowledge of competitive interactions must be coupled with spatial, seasonal, and environmental preferences of vectors to assess disease risks.

- Mathematical modeling of mosquitoes and sand flies – and the diseases they carry.

- Wetlands ecology.

- Biology and ecology of estuaries and tidal wetlands.

- Field ecology of container mosquitoes with emphasis on factors affecting spatial distribution and habitat and oviposition site selection. For example, the geographic distributions of *Ae. aegypt*i and *Ae. albopictus* overlap in tropical Asia, North and South America, and a few African nations. Spatial segregation because of differences in habitat preferences by the two species has been proposed as one mechanism promoting geographical coexistence Typically, *Ae. aegypti* predominates in urban areas, and *Ae. albopictus* predominates in rural areas, however, our knowledge of habitat preferences of these species remains qualitative and subjective because the environmental determinants have not been identified or quantified. The latter information is essential for predicting *Ae. aegypt*i and *Ae. albopictus* incidence and abundance, and for risk assessment of disease transmission.

- Characterization at the gene level of mosquito pesticide resistance mechanisms to reduce the development of insecticide resistance in mosquito populations. Breakdown in mosquito control strategies due to insecticide resistance would severely impact our ability to manage arbovirus outbreaks. We are studying the mechanisms used by mosquitoes to circumvent mosquito control efforts to devise strategies to prevent or minimize development of insecticide resistance.

- Characterization of gene expression variations in mosquitoes due to blood feeding and infection with human disease-causing pathogens. It is well known that a mosquito infected with a pathogen experiences changes that impact its life history traits including changes in gene expression and cellular function, and some of these costs have been studied. What is not well understood is the molecular mechanism that contributes to a successful transmission ability of these arboviruses by mosquitoes. Our research focuses on the expression changes in the midgut of the mosquito as this is the first site of entry of all pathogens that enter via a blood meal. Because the midgut tends to be an active environment, the presence of a parasite in the blood source could be predicted to alter mosquito gene expression. It is likely there are detectable molecular changes resulting from the interaction of midgut tissues with an invading pathogen like a virus. Investigating the interactions may allow the isolation of factors that aid infection and may also provide novel disease transmission control measures.

- Ecology of Everglades virus (an endemic relative of Venezuelan equine encephalitis) in Florida.

- Genetics of vector competence for arboviruses.. Characterize the population genetics and evolution of *Ae. aegypti*. Transcriptome analysis of *Ae. aegypti* to identify controlling genes for vector competence.

- Strategies for containment of infected arthropods and/or arthropods containing novel transgenes and/or driving transgenes.

- Providing handbooks, videos, fact sheets, technical bulletins, brochures, computer tutorials, traveling displays, exhibits, and newsletters concerning mosquitoes, mosquito control, and public health.

- Measurement of Vectorial Capacity in cooperation with Florida Mosquito Control agencies. The vectorial capacity equation allows local assessments of disease risk attributable to the abundances and vector competence of *Ae. aegypti* and *Ae. albopictus*. A new project by multiple FMEL investigators, developed in cooperation with Monroe, Miami-Dade, Martin, South Walton, Manatee, and Orange counties, couples vector competence measures in FMEL's BioSafety Level 3 (BSL-3) for ZIKV of the potential vectors in these counties with field measures of blood-feeding, abundance, and adult female survivorship. These results will be incorporated into epidemiological risk models for Florida.

Examples of research being performed by Applied Mosquito Research Program faculty include:

- Working with RNA interference-mediated knockdown expression of genes related to vector competence or antiviral defense mechanism. This RNAi technology with the targeted genes will be applied in the field as a non-pesticide control method using chitosan nano-particles.

- Using RNA sequencing (RNAseq) to detect genes that interact with chikungunya virus (CHIKV) and potentially interfere with infection. The results of this and future studies will be used to attempt to suppress CHIKV vector populations in Florida by the manipulation of these functions in mosquito populations with molecular biological techniques.

- Identifying particular genes or pathways that play a role in the mosquito's defense against dengue virus (DENV). This knowledge will be used to interfere with development of DENV and other *Flaviviruses* such as Zika virus that has recently spread throughout the Americas.

- Studying permethrin resistance and its associations with vectorial capacity along with a genomic approach to investigate differentially expressed genes in permethrin-resistant populations will inform risk assessment for arbovirus transmission in Florida and management of insecticide resistance.

- Obtained the full-length sequence of the "*Temsha esterase*" gene, which is responsible for organophosphate insecticide resistance in *Cx. nigripalpus* populations, and are studying *Temsha* in other mosquito species to determine its role in their resistance and to develop appropriate strategies that might mitigate insecticide resistance in natural mosquito populations.

- Evaluating the efficacy of new and existing larval control products on mosquitoes and nontarget organisms.

- Investigating microbiota (bacteria and archaea) associated with the St. Louis encephalitis vectors to identify potential symbionts/pathogens that may be used in the development of new control strategies and help us understand variability in susceptibility to pathogens or pesticides between different mosquito populations.

- Understanding the underlying mechanisms of unintended impacts of larval control on chief aquatic food web components such as on primary production and microbiota.

- Initiated investigations on the role of microeukaryotes (ciliate protists and rotifers) on mosquito production and their potential use for delivery mechanisms of effective control strategies.

- Performing molecular analysis of pesticide resistant mutant mosquito strains to provide information for understanding the response of mosquitoes to pesticides in the field.

- Using RNAi methodology to develop new products and safer pesticides for mosquito control.

- Developing a new diagnostic method for detecting dengue virus serotypes, chikungunya virus and West Nile virus, as well as *Plasmodium falciparum*, employing biotinylated antibodies and the streptavidin (SA) sensors using Bio-Layer Interferometry (BLITz) technology and a portable BLI-equipped BLItz device.

- Developing ultra low volume (ULV) tests for potential natural products as new pesticides.

11.2.2.4 University of Florida, Whitney Laboratory for Marine Bioscience

The Whitney Laboratory for Marine Science (Whitney Lab) is located in St. Augustine and has several faculty investigating the physiology of mosquito disease vectors. The research is primarily on the malaria vector *Anopheles gambiae* and the yellow fever mosquito, *Ae. aegypti*. Projects involve studies on transport physiology and ion exchange in mosquito larvae, the physiology of amino acid transport in the mosquito midgut, and anion regulation in the mosquito midgut. The research at the Whitney Lab utilizes functional genomic approaches to characterize physiological mechanisms in mosquitoes particularly those dealing with digestion. Projects employ molecular biology, biochemistry, electrophysiology, and laser scanning confocal microscopy. The goal of the work is to ultimately provide new targets for larvicides.

11.2.2.5 University of Miami

Several faculty at the University of Miami (UM) in Coral Gables are investigating the epidemiology, ecology, and control of vector-borne infectious diseases with emphasis on international and overseas projects. Current research projects are conducted in Kenya and other countries in Africa and the Latin America Caribbean Region. Projects include studies on African malarial vectors, their larval ecology, behavioral and chemical ecology, and their vector competence for malaria. The mosquito research program is part of the UM Global Public Health Program. This interdisciplinary program involves faculty throughout the UM system and emphasizes the development of international disease control programs.

11.2.2.6 University of North Florida

Research at the University of North Florida (UNF) in Jacksonville is focused on studies of mosquito-arbovirus interactions. The program is using a well-studied virus, Sindbis virus (SINV), to study its interactions in various tissues of *Ae. aegypti* and *Ae. albopictus*. The infection process of an arbovirus is being evaluated using SINV to characterize SINV-associated pathology, persistence, and tissue specific clearance of the virus in the mosquito.

11.2.2.7 University of South Florida

Vector borne pathogen research at the University of South Florida (USF)is primarily located within the Department of Global Health in the College of Public Health at the University of South Florida. The Department of Global Health, founded in 2005, began its concentration on vector borne diseases with the hiring of four senior investigators in 2006-2008 including two investigators named State of Florida World Class Scholars. The research arm of the Department of Global Health is located in the Interdisciplinary Research Building (IDRB) on the USF campus, a state of the art research building completed in 2007. Facilities in the Global Health Research laboratories include a BSL2 certified insectary, an ABSL2 animal facility, select agent certified ABSL3 and BSL3 facilities for arbovirus research, a high content imaging facility, a next generation sequencing facility, a live animal imaging facility, and high throughput screening equipment for drug discovery.

Since its beginning in 2007, the vector borne research effort in Global Health has attracted over $51 million in external research funding from NIAID (National Institute of Allergy and Infectious Diseases), National Science Foundation (NSF), the Department of Defense (DoD), Center for Disease Control (CDC), the Bill and Melinda Gates Foundation, The Carter Center, World Health Organization (WHO), and FDACS. Recently, USF's department of Global Health became one of the four Florida institutions to be named as part of only four CDC Regional Centers of Excellence for Vector Borne Diseases in the U.S. The faculty in the Global Health vector borne disease have published 238 papers in peer reviewed journals since 2007.

Currently, the vector borne research program consists of 9 faculty (5 tenured) and 22 support staff. The program collaborates with a number of other institutions in the state, including Anastasia, Polk, Citrus, Volusia, North Walton, and South Walton mosquito control programs, the UF, the Florida Department of Health (FDOH), and the FMEL. Current research projects include:

- Developing a saturation map of the malaria genome for identification of essential genes and new drug targets

- Drug discovery for malaria

- Vaccine development against vivax malaria

- Development of an *in vitro* liver stage model for malaria

- Investigation of the potential of the ecdysone receptor as a novel drug target in filarial parasites

- Development of reverse genetic methods for the study of filarial parasites

- Use of remote sensing techniques to identify breeding sites for the vectors of vector borne diseases, including onchocerciasis, malaria and Zika virus

- Development of novel traps and community directed vector control methods to accelerate elimination of onchocerciasis throughout Africa

- Studies of the ecology of wintertime transmission of eastern equine encephalitis virus (EEEV) in Florida

- Development of risk maps for transmission of EEEV in Florida in both wintertime and peak season transmission times

11.2.3 Local

Many Florida mosquito control programs conduct research on issues that address local needs.

Anastasia Mosquito Control District (AMCD) conducts applied research. Research ideas/projects usually are generated from residents and employees during operation of surveillance and control. Applied research emphasizes screening/selection of new formulations of insecticides and new equipment for surveillance and control of mosquitoes and mosquito-borne diseases. AMCD is participating in and collaborating with federal and state agencies, universities/institutes, and private companies to conduct applied research about surveillance and control of mosquitoes. Many applied research projects have been done by graduate students, interns, and visiting scientists under AMCD staff supervision and through collaborations with multiple agencies at state, national, and even international levels.

Beach MCD evaluated aerial permethrin applications and barrier treatments in cooperation with PHEREC.

Collier MCD has been involved in ongoing research to improve ULV aerial application equipment and techniques, testing of new mosquitocides, resistance testing of adult mosquitoes, and the integration of GIS/GPS into surveillance and aerial operations.

East Flagler MCD has been evaluating PCR technology for EEE virus detection.

Florida Keys MCD has conducted research on dispersal of salt marsh mosquitoes, resistance to pesticides, new product trials including products utilizing sterile insect techniques, and habitat use by mosquito larvae.

Indian River MCD has been developing a GIS using ArcView for its saltmarsh management, inspection, and larviciding program. Field inspectors will use hand held GPS data loggers. Collected information will be joined with the IRMCD historic larviciding data base. Studies continue to assess the age and blood feeding status of *Cx. nigripalpus* populations in assessing risk from mosquito-borne pathogens, particularly SLEV and WNV.

Jefferson County provides basic research with trapping and counts for vector control if needed during extraordinary events. The staff is small and dedicated to public service at this time.

Lake County Mosquito Management collaborated with CMAVE on the spatial distribution of mosquitoes utilizing the program's mosquito surveillance database.

Lee County MCD has actively participated for over 450 years in the improvement of larval and adult mosquito control through field and laboratory research programs. The research programs have produced publications, patents, and products related to larval parasites and pathogens, chemical larvicides and pupacides, granular and liquid active-ingredient delivery systems, invasion of exotic species, disease epidemiology, adulticide efficacy, and aerial adulticide spray nozzle characterization and efficiencies. Other research efforts have involved new surface films for control of mosquito larvae and pupae, adjuvants to facilitate the efficacy and spreading of larvicides injected into the water stream during ditch truck applications, development of control-delivery granular formulations of larvicides, laser-based wind tunnel characterizations of nozzles for aerial adulticide nozzles, and validation of drift models for aerial application of adulticides. Spin-off research also has produced new technologies in the areas of bioremediation and lubricants.

Manatee County MCD has worked on the development of GIS/GPS for mosquito control applications and optimum droplet sizes for aerial mosquito control.

Okaloosa County Mosquito Control (MC) has participated in a cooperative project with PHEREC to study the surveillance and ecology of WNV and EEEV in wild birds and mosquitoes.

Orange County MC has supported a FMEL M.S. graduate student to evaluate WNV surveillance strategies in the County. The program evaluated factors that effect the movement of adult *Cx. nigripalpus* in Orange County to determine a window to spray for the adults to achieve maximum kill.

Pasco County MCD has developed a computer-based adult mosquito surveillance program and innovative spray equipment.

Pinellas County MC has worked with FMEL in evaluating groundwater levels using hydrologic modeling to predict freshwater and floodwater mosquito hatchings. The program used rotator traps to evaluate peak activity periods for target mosquito species to tailor their adulticiding missions to coincide with these times. Pinellas County MC also has worked with PHEREC on a spectral imaging prediction evaluation project. They have analyzed trap data for a five year period to determine seasonality of mosquito species, comparability of similar habitat trap results, and evaluation of trap placement.

Sarasota Mosquito Management has conducted research on the behavior, ecology, and control methodologies of container-inhabiting mosquito species, on assessing arbovirus detection techniques for operational use, and on the ovipositional preferences of *Psorophora columbiae*.

St. Lucie County MCD studies have included Penaeid shrimp life history, benthic ecology of tidal creek restoration areas, wetland post-hurricane restoration evaluations, ecological impacts of RIM practices on mangroves, mangrove life history in post-wetland restoration areas, impacts of wetland restoration on spotted seatrout spawning, and the use of impoundments for marine fish and clam stocking in the Indian River Lagoon (IRL). The program also has worked on impoundment management using enhanced tidal circulation and evaluating mosquito magnets and the attractants used with such traps. Future work will study tidal dynamics in the estuary as it relates to inter-inlet dynamics and their role in water quality. The program plans to provide tidal data collected using telemetry to the South Florida Water Management District as part of a cooperative hydrodynamic monitoring program.

Santa Rosa MC has participated in a cooperative project with PHEREC to study the surveillance and ecology of WNV and EEEV in wild birds and mosquitoes.

South Walton MCD has participated in a cooperative project with PHEREC to 1) identify vector species responsible for transmission of WNV and EEEV and 2) evaluate the residual effectiveness of insecticide-treated vegetation as a barrier against mosquitoes.

Volusia County MC has conducted research on novel control techniques for non-biting midges, *i.e.*, outdoor lighting alteration and larval habitat water chemistry manipulation. Other studies have been conducted on the impact of salt marsh spoil restoration excavation on vegetation and fiddler crab utilization of altered sites.

11.2.4 Private

11.2.4.1 Harbor Branch Oceanographic Institution, Inc.

Harbor Branch Oceanographic Institution, Inc., a not-for-profit organization located on approximately 500 acres along the IRL in Ft. Pierce, has a commitment to understand and protect the oceans, estuaries, and adjacent coastal regions. In past years, several scientists were involved in the following activities related to mosquito control:

- Water control systems and their hydrological and biological impact on impounded marshes and fish communities

- Determination of the effectiveness of various artificial means of marsh management, such as culverts and weirs, and the compatibility of water management schedules with habitat requirements for different life history stages of important fish species

- Effects of organophosphorus mosquito insecticides on hatching fish larvae and other estuarine zooplankton

11.2.4.2 Mote Marine Laboratory

Mote Marine Laboratory, a non-profit private institution in Sarasota, with funding support from Florida Department of Health and Rehabilitative Services (FDHRS) and Lee County Mosquito Control District, has studied the effects of mosquito larvicides on non-target invertebrates and vertebrate larvae. They conducted studies to determine if aerial application of temephos (Abate®) is detrimental to non-target organisms in a mangrove-fringed salt marsh in southern Florida. The test organisms used in the studies were the marsh fiddler crab, *Uca rapax,* and the mangrove tree crab, *Aratus pisonii*. Their conclusions are reported in their Final Report for 1993, Mote Marine Laboratory Technical Report No. 333.

11.3 THE NEED FOR COMPETITIVE EXTRAMURAL FUNDING FOR FLORIDA'S RESEARCH LABORATORIES TO SUPPORT MOSQUITO CONTROL

In 1984, Florida mosquito control directors realized that additional funding for research on mosquito control was needed to supplement shrinking budgets for mosquito control research. Following a recommendation of the Research Advisory Committee of the Florida Mosquito Control Association (FMCA), Florida's mosquito control programs decided to assign a portion of their annual state-appropriated operational funds to research. Through a competitive grant program, up to $500,000 annually was contracted to various institutions to support needed mosquito control research. This program was eliminated in 1991 due to a statewide budget shortfall but was re-instated in 1996 at $250,000. At that time, it was determined that only the two state laboratories, FMEL and John A. Mulrennan, Sr. Public Health Entomology Research and Education Center (PHEREC), would be eligible to submit proposals for funding. Currently, all State Universities in Florida are allowed to apply for funding under this program.

A review of the Research Chapter in the first and second editions of the *Florida Mosquito Control White Paper* will show that there have been great strides in the past twenty years on many of the issues listed in that chapter as research needs. Unfortunately, many important needs have yet to be addressed and are again listed in this chapter. The inability to address many of the listed needs is largely due to the lack of adequate monetary support for research.

FMEL and CMAVE are mandated to perform research on mosquito control to reduce mosquito pests and mosquito-borne disease. All have faculty, staff, and facilities to investigate mosquito biology and provide research for new effective, economical, and environmentally sound control methods. Since its establishment, FMEL has secured federal, state, and county grants and contacts for its support. FMEL ranks among the leading UF/IFAS units in productivity and extramural support but has experienced reductions in its state general revenue for technical and operational support. Both laboratories need additional revenues to accomplish their respective mandates and provide Florida mosquito control and public health professionals essential new information to improve Florida's capabilities to control mosquitoes and mosquito-borne pathogens. It is imperative that the state mosquito research program administered by FDACS, Bureau of Scientific Evaluation and Technical Assistance and the Division's administrative staff, maintain the current funding levels of \$500,000 or more in order to meet the growing demands for more research to improve Florida's capabilities for mosquito control. The current spending level of \$500,000 can support approximately five to ten projects out of fifteen to thirty projects submitted annually.

FAMU announced in 2010 that the PHEREC in Panama City, Florida, would close. After PHEREC was closed in 2011, some of the pesticide research mission of that Center was transferred to the FMEL with an accompanying line item allocation from the State of \$500,000. Those funds must be reauthorized each year and are used to pay for the salaries of three faculty, technical assistance, equipment, and supplies for pesticide related research. See the "Applied Mosquito Research Program" under FMEL for more details.

Florida experienced a Zika virus outbreak in 2016 with 1,122 travel-associated cases and more than 285 known cases of local transmission by mosquitoes in the Greater Miami area alone. In response, Governor Scott released \$25 million to spur research efforts to combat the disease, but these funds mostly were directed at clinical concerns such as the development of vaccines and diagnostics.

Without strong funding support for research, the scientists at the university laboratories will be forced to seek research support in other more readily funded areas that may be removed from the immediate needs of Florida mosquito control and public health. Already, mosquito control is losing some of its effective insecticidal weapons.

Mosquito control does not have a promising future for protecting Florida's public without researchers searching for innovative technology and verifying their safe use. The threat of more mosquitoes and the pathogens they carry will affect the well-being of Florida residents and tourists. It is certain that Florida is at great risk from emerging pathogens, like WNV, Zika, dengue, chikungunya, and other arboviruses, which demands Florida be better prepared to mitigate effectively an impending, potentially catastrophic epidemic.

Without a strong mosquito control effort backed by a superior dynamic research effort, Florida will become an increasingly uncomfortable and dangerous place to live and visit.

Chapter 12

EDUCATION, EXTENSION, AND OUTREACH

Chapter Coordinators: *Neil Wilkinson* and *Dr. Roxanne Connelly*

2009 Coordinator: *Dr. Roxanne Connelly*

1998 Coordinator: *Dr. Charlie Morris*

Summary

This chapter describes the needs for and the implementation methods used to educate and inform mosquito control workers, related professionals, and the public about matters related to mosquito control in Florida. We describe the organizations involved and the techniques used for extending knowledge appropriate to select audiences.

12.1 INTRODUCTION

An important component of Florida mosquito control is to increase the understanding of mosquito control workers, other professionals, and the general public on matters related to mosquito biology, ecology, disease transmission, and control. This effort is accomplished through education, extension, and outreach.

12.2 EDUCATION

Education focuses on increasing the professionalism of all mosquito control workers. Three agencies dedicate significant time to this effort:

- Florida Department of Agriculture and Consumer Services,
  Division of Agricultural Environmental Services,
  Bureau of Licensing and Enforcement

- Florida Mosquito Control Association

- University of Florida, Institute of Food and Agricultural Sciences,
  Florida Medical Entomology Laboratory

12.2.1 Florida Department of Agriculture and Consumer Services, Division of Agricultural Environmental Services, Bureau of Licensing and Enforcement

The Florida Department of Agriculture and Consumer Services (FDACS), Bureau of Licensing and Enforcement is the principle certifier of mosquito control personnel in the public health category of certified pesticide applicators. The Bureau offers three to five one- or two-day workshops throughout the state each year. The purpose of these workshops is to prepare people to take and pass the Public Health Certification exam.

The Bureau offers a basic exam which is needed to become certified. The purpose of offering/requiring certification is to insure mosquito control professionals in the state of Florida have at least a minimum level of knowledge and understanding of mosquito biology, ecology, disease transmission, and mosquito control.

FDACS, through the Bureau of Scientific Evaluation and Technical Assistance administers a proficiency exam for directors of mosquito control programs. Passing the exam is a prerequisite for all mosquito control directors in the state of Florida.

12.2.2 Florida Mosquito Control Association

The Florida Mosquito Control Association (FMCA), through its Dodd Short Course Committee, provides training in mosquito biology and control beyond the basic level.

The Dodd Short Course Committee, organizes and presents three types of courses: Annual, Regional, and Specialty. The Annual Dodd Short Courses consist of 15 to 30, ½- to 4-day courses, all of which are held during one week in January or February. The courses are designed for specific groups such as new employees, clerical staff, biologists and entomologists, inspector-sprayers, administrators, technology specialists, mechanics and equipment operators, elected commissioners, and directors of mosquito control programs. The courses cover a wide range of topics related to mosquito control, and each year a few non-mosquito related courses are offered to increase the general abilities of mosquito control staff. Examples of such courses include Public Speaking and Critical Thinking. Most courses have maximum enrollments and emphasize student participation. Fieldwork is included in many of the biology courses.

Employees of mosquito control programs, university faculty and staff, state agency staff, manufacturer and distributor representatives, and other individuals volunteer to teach at the annual Dodd Short Courses. The non-mosquito related courses are often contracted with a private consultant for a fee.

Each course carries one to sixteen continuing education units (CEUs) for recertification in either the public health or the aquatics categories of the Florida Pesticide Applicator Certification program that is managed by FDACS, Bureau of Licensing and Enforcement. Annual attendance averages over 285 people. The course fees are used to finance the annual Dodd Short Courses.

The Dodd Short Course Committee also organizes Regional Short Courses and Specialty Short Courses. The Regional Short Courses supplement the annual courses and are intended for mosquito control programs with limited travel budgets that prevent them from taking advantage of the annual courses. Regional courses are designed for a specific group of mosquito control personnel, particularly veteran inspector-sprayers, who work in a region of the state such as the southwest or Panhandle. Courses can be arranged for any group needing training. While attendance is not limited to employees in the region, most attendees work within a one-hour commute to the course site. Regional courses are organized and presented when requested by mosquito control program directors, have tuition, and are designed to provide CEUs for certified applicators.

Specialty Dodd Short Courses deal with specific topics such as “Finding Zika in all the Right Places” and “Conducting CDC Insecticide Resistance Bottle Bioassays”. The instructors are typically out-of-state experts who are not available during the annual short course week or whose subject is of interest to many people who teach at the annual courses. Attendance is open, but most specialty course attendees are directors, supervisory personnel, and specialists. Specialty courses have a tuition, usually carry CEUs for certified pesticide applicators, and are organized as opportunities arise or by request to the Dodd Short Course Committee.

The Aerial Training Committee is responsible for developing training courses and manuals, fly-ins, and specialty short courses that meet the mosquito control related needs of pilots and other aerial application related personnel. The FMCA Aerial Short Course is a three-day short course held every January that provides information on basic aerial application techniques, new technologies, current research, changes in regulations/label language, and unique perspectives from a wide range of mosquito control programs. The course attracts attendees not just from Florida but from all parts of the United States, as well as Canada, Australia, and Europe. A number of pilots bring their aircraft, many with unique spray equipment setups. These courses offer CEUs in the public health and/or aerial categories.

12.2.3 University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory

The University of Florida (UF), Institute of Food and Agricultural Sciences (IFAS), Florida Medical Entomology Laboratory (FMEL) celebrated sixty years of dedicated research on mosquitoes and biting flies in 2016. The FMEL is committed to training the next generation of scientists that will advance research and training in medical entomology in the future and to continue training of mosquito control personnel in Florida to help maintain efficient and professional mosquito control operations in the State.

Graduate students at the FMEL earn M.S. and/or Ph.D. degrees in the University of Florida's Department of Entomology and Nematology. Students take advantage of the unique laboratory and field opportunities offered by the FMEL and obtain state of the art training through contact and collaborations with the faculty of the FMEL, all of whom are leading authorities in medical entomology.

Graduate work at the FMEL is planned in close collaboration with a FMEL faculty member who serves as the Chair of the student's graduate committee. A graduate committee will be include other FMEL faculty, other faculty in the Department of Entomology and Nematology, and/or faculty in other relevant departments.

The FMEL offers the Advanced Mosquito Identification and Certification Course each spring. This two-week course provides intense training in the identification of adult and larval mosquitoes of North America. Students who pass the exams receive a certification from the UF. FMEL also provides many opportunities to earn CEUs in public health through their Lecture Series which includes seminars from national and international guest speakers on topics of importance to medical entomology and mosquito control. Other specialty courses and training events are regularly offered by FMEL faculty and staff based upon demand and opportunities. The FMEL maintains a website of interest to mosquito control professionals. Http://fmel.ifas.ufl.edu has content related to the FMEL research programs, faculty and staff, and FMEL publications.

The IFAS Electronic Data Information Source, EDIS, is a collection of fact sheets written for the general public on a wide variety of topics (http://edis.ifas.ufl.edu). FMEL faculty contribute extensively to this collection on subjects including *St. Louis Encephalitis in Florida, West Nile Virus in Florida, Lyme Disease in Florida, Saltmarsh Mosquito Management,* and *Mosquito Repellents.* Extension Specialists continually add new topics that are important for Florida residents such as dengue, chikungunya, Zika, container mosquitoes, and bromeliads. FMEL fact sheets are available in English and Spanish.

A basic mosquito identification course for the staff of mosquito control operations became so popular that it outgrew the space available at FMEL. This and other courses are now offered yearly at the FMCA's annual Dodd Short Courses where FMEL faculty and staff frequently serve as course organizers and lecturers.

12.2.4 Industry Short Courses

Some mosquito control product distributors offer free *ad hoc* short courses open to their customers and to other mosquito control personnel. These courses often also carry CEUs for the Public Health Certification.

12.3 EXTENSION

Beyond courses, the FMCA and FMEL offer other extension services to assist Florida mosquito control programs. These services vary from producing a bi-monthly newsletter to conducting multi-year research on specific problems.

The *Wing Beats* Magazine Committee of the FMCA publishes a quarterly trade magazine for mosquito control professionals in conjunction with the American Mosquito Control Association (AMCA). It is currently distributed to over 3,900 people in the United States, Canada, Latin America, and overseas. This 40-page color magazine contains articles of interest to operational mosquito control personnel. Advertising has fully supported the production and distribution of the magazine since its inception in 1990, yet advertisements take up no more than half the pages. *Wing Beats* has been well managed over the years and typically breaks even in terms of income and revenue.

Since 1990, the FMEL, in cooperation with the FMCA, has produced and distributed a bi-monthly newsletter, *BuzzWords*. It is currently distributed to over 1,000 mosquito control professionals in Florida and throughout the United States. The newsletter contains short communications on all aspects of mosquito biology and control, including announcements of meetings, significant changes in personnel, employment opportunities, news items, obituaries, and official mailings from the FMCA to its members. It is automatically sent to FMCA members and is available by request to anyone in the United States free of charge.

12.4 OUTREACH

FMEL faculty, scientists at the USDA Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, and mosquito control personnel from across the state work with or assist many other mosquito control programs on research and demonstration projects of mutual interest. Projects are numerous and varied, ranging from computer system setup, equipment repair and calibration, characterizing mosquito problems, evaluating control methods to conducting arbovirus surveillance and program operations and administration.

Florida mosquito control programs across the state utilize a variety of approaches depending on the size of the program, resources available, and specific community needs to inform the public about the nature of their programs, the needs for and benefits of mosquito control, and the relationship between mosquito control and environmental and health agencies. These efforts include school programs, presentations to civic groups, exhibits at local events, literature, house calls, and public service announcements.

School programs in Florida range from support for three full-time teachers who are in schools each week of the school year to providing staff to offer *ad hoc* presentations to classes upon request. Educational programs for each mosquito control district will vary with the individual teaching, the mosquito control district, and also with each school district being served. Manatee County Mosquito Control District (MCMCD) pays the salary of one full time employee who serves the District as an Educator/Biology Lab Tech. The educator at MCMCD teaches Kindergarten through fifth grade using mosquitoes to support the Sunshine State Education Standards. The program not only aims to re-enforce what the students are learning in the classroom but also to educate the community about the role mosquitoes play in overall public health. The program at

MCMCD is an hour-long program for first through fifth grades; The Kindergarten program is 30 minutes. The lessons for each grade starts with a traditional teaching portion using power point slides or videos, relating mosquitoes to lessons such as the water cycle and how that affects the mosquitoes. A portion of this program is also hands-on, utilizing various activities with open discussions between students and the educator. The program is offered to elementary schools by the MCMCD Educator via direct contact with teachers throughout the school year.

The Lee County Mosquito Control District (LCMCD) pays the salary of two full-time teachers employed by the Lee County School District and a third full-time instructor employed by Florida Gulf Coast University. These educators teach week-long environmental science/health units in a case study approach using mosquitoes and mosquito control as the focus. Programs are presented to fifth and seventh grade science classes and high school biology and chemistry classes. They also offer a one day lesson to kindergarten classes using trained university students to provide the instructional activities. More typical school programs are one- to two-hour presentations by one or more mosquito control employees who have other duties. These lessons often have a field component and typically focus on third-, fourth-, and fifth-grade classes. The lessons are as varied as the people who teach them.

The Pasco County Mosquito Control District (PCMCD) pays the salary of one full-time teacher who aids the District as the Public Education Specialist. The teacher uses a two-day program, with ties to the Next Generation Sunshine State Standards, to educate fifth grade students about mosquitoes. The program covers mosquito anatomy, life cycle, disease transmission, and even PCMCD source reduction efforts. The program is 45 minutes with each fifth grade class and includes a presentation with an interactive lecture and ends with a hands-on lesson for the students. Students are encouraged and challenged to share what they learn with their families and friends to help spread information. The Public Education Specialist also stays involved around the community by attending events to spread awareness, as well as making presentations as requested by special interest groups.

Most mosquito control programs have staff members who make presentations to civic groups and special interest groups upon request.

All programs have a telephone number that citizens may call to request spraying or find out more about the mosquito control program. Several programs advertise this number and even notify the media of when and where spraying will be conducted for the next day or week.

Many programs develop their own literature or use literature developed by the FDACS, FMEL, and other organizations to inform citizens how they can assist in controlling mosquitoes, what services they offer, and how citizens can take advantage of their services. Literature may be distributed as door knob hangers, bookmarks, or fact sheets and is made available in a variety of places including bookstores, libraries, schools, banks, and other locations where the public may encounter the literature.

The thrust of all these public education and outreach programs is to let the public know what mosquito control is all about, why it is so important and what they can do to reduce mosquitoes in their own yards. When the public is adequately informed about mosquitoes they are in a better position to support mosquito control efforts. It is essential that outreach and educational efforts are created with a clear message. For instance, each locale has many species of mosquitoes and each species has their own requirements for survival and not all species pose a problem to humans, pets, and wildlife. The species that do pose a threat need to be understood as controlling them depends on their specific needs and habits. Up to date information is required to know when conditions are optimal for control. Collecting this information to drive control efforts is a science-based approach. Mosquito control professionals are well trained and are constantly updating their understanding of mosquitoes and control methods.

Chapter 13

HOW FLORIDA MOSQUITO CONTROL IS REGULATED

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Summary

The regulation of mosquito control in Florida poses a unique set of circumstances. In Florida, mosquito control frequently requires ground and/or aerial applications of pesticides in highly populated areas. Thus, the potential for human exposure to the pesticides exists. Also, mosquito control is frequently carried out near water bodies or wetlands, where mosquitoes breed and rest which may be areas considered environmentally sensitive. Since the success of an adulticide application relies heavily on drift to reach the target, label violation concerns about off-target movement and environmental impacts are common. Enforcement of the label is necessary to ensure that no unreasonable adverse effects occur. However, the same enforcement and registration requirements designed to protect human health and the environment can limit mosquito control's effectiveness. This chapter will explore the various agencies and laws which regulate mosquito control in Florida.

13. 1 AGENCY INVOLVEMENT AND ENFORCEMENT

13.1.1 Florida Department of Agriculture and Consumer Services

Chapter 388 Florida Statutes (F.S.) addresses mosquito control by stating that Florida's policy is to achieve and maintain adequate arthropod control to protect human health and safety, foster the quality of life, promote economic development, and allow for the enjoyment of its natural attractions. This policy is to be achieved by the creation of mosquito control programs, at the local level, to reduce populations of pestiferous and disease-vectoring arthropods and is to be carried out in a manner consistent with protection of the environment. Thus, Chapter 388 F.S. first establishes the necessity of mosquito control and goes further in requiring that control measures be consistent with environmental laws. Enforcement of mosquito control activities is necessary to ensure that all regulatory requirements are met.

Chapter 388 F.S. authorizes mosquito control offices to do whatever is necessary to control all species of mosquitoes and other arthropods of public health importance as long as that work is not inconsistent with Chapter 388 or other pertinent legislation. Control can be achieved through reducing locations where mosquitoes are produced or by the application of chemicals which are approved by the Florida Department of Agriculture and Consumer Services (FDACS). The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as well as Chapters 388 and 487 F.S., establish the primary requirements which FDACS must enforce.

13.1.2 United States Environmental Protection Agency

FIFRA governs the distribution, sale, and use of pesticides in the United States with the U.S. Environmental Protection Agency (EPA) administering these regulations. FIFRA grants significant regulatory latitude to the states, and many states have laws which mirror the Federal regulations. Through a cooperative effort, the EPA and the states work together to ensure that regulatory compliance is achieved.

13.2 REGISTRATION

Under FIFRA, the EPA is responsible for registering new pesticides and ensuring that, when used according to label directions, they will not cause unreasonable adverse effects to human health or the environment. Only pesticides registered under FIFRA or those exempted under FIFRA Section 25(b) by the EPA can be distributed or sold. Further, all pesticide products, even those exempted by EPA from federal registration, must be registered by FDACS in the Bureau of Scientific Evaluation and Technical Assistance.

Pesticide registration decisions are based primarily on EPA's evaluation of the test data provided by the applicants. This test data, which is based on EPA's standards for reporting, allows for a determination of whether a pesticide has the potential to cause adverse effects to humans, wildlife, fish, and plants, including endangered species. A registrant is required to submit to the EPA all factual information regarding unreasonable adverse environmental effects. FIFRA further grants EPA the authority to cancel the registration of a pesticide if it causes unreasonable risks to human health and the environment. However, the EPA is required to weigh the risks and benefits of each product before acceptance for registration.

FIFRA's Section 24(a) allows each state to regulate the sale or use of any federally registered pesticide or device. In Florida, this regulation is enabled through Chapter 487 F.S. and requires biennial renewal. Regulations pertaining to pesticide registration in Florida appear in Chapter 5E-2 of the Florida Administrative Code (F.A.C.).

13.3 AUTHORITY

Section 388.361 establishes FDACS as the lead agency to administer and enforce all rules under the Mosquito Control Law. Rules pertaining to mosquito control appear in Chapter 5E-13 F.A.C. FDACS also is charged with adopting rules providing for the following:

1. Criteria to demonstrate that arthropod population levels constitute a public health or nuisance problem.

2. Criteria regarding aerial spraying of pesticides on private lands which minimize deposition and the potential for substantial adverse effects.

3. Requirements that all arthropod control pesticides, including adulticides and larvicides, be used only in accordance with the registered labeling or be otherwise accepted by the EPA or FDACS.

4. Protection of the health, safety, and welfare of arthropod control employees, the general public, and Florida's natural resources.

FDACS can adopt rules which are more stringent than the EPA's label requirements. FDACS establishes criteria for licensing of all private and public arthropod control applicators and program directors and requires that applicators report their activities, as a condition of being a Chapter 388-approved program. Licensing or certification is not required for private individuals controlling arthropods on their own residential or agricultural property. FDACS-authorized inspectors can enter upon any property to inspect records or lands in order to investigate complaints, and FDACS has the authority to cooperate with federal and other state agencies as appropriate.

Florida's legislatively-established Coordinating Council on Mosquito Control (FCCMC) has the responsibility to develop and implement guidelines to assist FDACS in resolving disputes arising over the control of arthropods on publicly owned lands. Another key issue on which the FCCMC provides recommendations is aerial spraying in which a goal is to minimize environmental harm.

FCCMC has the authority to designate subcommittees to assist in carrying out their responsibilities. The Subcommittee on Managed Marshes (SOMM) is such a committee charged with providing technical assistance and guidance on salt marsh management plans and developing and reviewing research proposals for mosquito source reduction techniques.

13.4 ENFORCEMENT ACTIONS AND VIOLATIONS

Section 388.3711 outlines enforcement actions that FDACS may take:

1. FDACS is authorized to enforce Chapter 388 including requesting that a circuit court grant an injunction.

2. It can deny, suspend, or revoke any license or certification, or the disbursal of state aid, in accordance with the provisions of Chapter 120.

3. If FDACS finds a violation to be severe, it can deny, revoke, or suspend a certification or license or the disbursal of state aid. It also can place the offending party on probation for up to 2 years.

4. It may impose an administrative fine not exceeding $500 for each violation of any of this Chapter’s provisions.

When determining a penalty, factors such as the severity of the violation or the probability that death or serious harm may occur are considered. An arthropod control program may cooperate with another county, district, or municipality, but it must first be approved by FDACS.

13.5 STORAGE AND HANDLING REQUIREMENTS

Part 19 of FIFRA addresses storage, disposal, transportation, and recall of pesticides. This chapter allows the EPA to require registration information regarding safe storage and disposal. It also allows the EPA to establish requirements for the transportation, storage, and disposal of the pesticide, any container of the pesticide, any rinsate containing the pesticide, or any other material used to contain or collect excess or spilled quantities of the pesticide. In addition, the registrant may be required to show evidence of sufficient finances and resources to carry out a recall and subsequent disposition if necessary.

Pesticide Management and Disposal Regulations were addressed initially in the 1988 amendments to Section 19 of FIFRA. These amendments expanded the authority of the EPA to regulate the storage, transportation, and disposal of pesticides, containers, rinsates, and contaminated materials. This amendment also ended EPA's requirement to accept canceled and suspended pesticides for disposal and directed the Agency to develop new regulations governing the recall of pesticides. New container design and residue removal regulations also were implemented.

Three phases were established to handle the task of writing pesticide management and disposal regulations:

- Phase I established recall plans, requirements for storage of recalled pesticide, storage and disposal plans, indemnification procedures, and transportation requirements for suspended and canceled products.

- Phase II focused on pesticide container and containment requirements and established residue removal and container refilling procedures, design standards to promote closed systems, construction standards that will encourage recycling, and containment standards for bulk storage and product transfer.

- Phase III regulations covered pesticide package storage, management of excess pesticides and rinsate, mixing/loading spill control procedures, and additional transportation requirements.

Pesticide mixing/loading facilities are built to reduce the potential for soil, groundwater, and surface water contamination. In general, facilities that are designed and operated with environmental protection in mind must comply with the four basic laws pertinent to pesticide mixing/loading operations. The four fundamental laws are:

- FIFRA
- Resource Conservation and Recovery Act (RCRA)
- Comprehensive Environmental Response, Compensation and Liability Act (CERCLA)
- Clean Water Act (CWA)

While there are state regulations that specify how a pesticide mixing and loading facility is to be built and operated, there are no federal regulations.

Chapter 487 F.S. prohibits the handling, transportation, storage, display, distribution, or disposal of pesticides in such a manner which will endanger human beings, the environment, food, feed, or any other products.

Concerning the application of pesticides, FIFRA and Chapters 388 and 487 F.S. prohibit the use of any registered pesticide in a manner inconsistent with its labeling. The label includes enforceable language concerning label rates, target sites, and disposal requirements.

FDACS requires storage of pesticides used by mosquito control operations to meet certain requirements established in rule 5E-13.0331(4) F.A.C. These pesticides must be stored and maintained so they are not accessible to unauthorized persons. Secured storage can be: Fences with a minimum six feet of height, door locks, valve locks, electronic security systems, or any other reasonable method to prevent or deter theft or unauthorized access or use. Buildings used to store pesticides should be of rigid construction so unauthorized entry can not be achieved without the use of heavy machinery or equipment. If a portable building is used for storage of pesticides, the building must be secured in place so it can not be towed or otherwise removed by unauthorized persons.

13.6 OTHER REGULATIONS AND INITIATIVES

13.6.1 Clean Air Act

Although the Federal Clean Air Act (CAA) has little effect on the mosquito control industry, the potential for regulating application drift does exist. Each individual state, based upon its State Implementation Plan, could potentially regulate methods which release pesticides, either as particulates or as organic emissions, into the air.

13.6.2 Comprehensive Environmental Response Compensation and Liability Act

Numerous pesticide ingredients and formulations are regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), commonly known as Superfund. However, pesticides applied as per label instructions are exempt.

13.6.3 Department of Transportation

Before a material is shipped domestically, it must be determined if it meets one or more of the Department of Transportation (DOT) hazard class definitions. Pesticides are frequently subject to DOT regulations. If a pesticide is classified as hazardous, it must be properly packaged, described, and certified on shipping papers. DOT also requires training to be provided to all HAZMAT employees regarding the safe transportation of hazardous materials including emergency response.

13.6.4 Resource Conservation and Recovery Act

Mosquito control pesticide wastes can be regulated under the Federal Resource Conservation and Recovery Act (RCRA). Examples include: Unused pesticides that are listed or are considered hazardous waste, certain discarded residue or rinsate from containers, nonempty pesticide containers or pesticide residue consisting of contaminated soil, water, or other debris resulting from the cleanup of a spilled pesticide.

13.6.5 Reduced Risk Pesticides Initiative

To reduce human health and environmental risks, the EPA developed the Voluntary Reduced Risk Pesticides Initiative to encourage the registration of lower risk pesticide products containing new active ingredients. The long-term strategy will develop criteria for identification of such pesticides, streamline the registration process, improve the availability of information to users, and reward those who develop such reduced risk pesticides.

13.6.6 Public Lands

Chapter 388.4111 F.S. addresses arthropod control on Florida's publicly-owned lands by recognizing that some environmentally sensitive and biologically productive public lands may be subject to arthropod control measures. Such activities must be approved by the appropriate land managing agency and performed by the local arthropod control agency using methods and materials which are minimally necessary yet economically feasible to abate a public health or nuisance problem, while causing the least hazard to natural resources. If the local arthropod control agency proposes an arthropod control plan to the agency and if it is not acceptable and they cannot agree on the elements of the plan, then the FCCMC can recommend a control plan. Chapter 388.4111© F.S. outlines the steps that can be taken if an agreement cannot be reached.

13.7 RECOMMENDATIONS FOR STORAGE AND HANDLING OF PESTICIDES

At times it is necessary to store or transport pesticides in a different container than that in which it was shipped. This different container is frequently referred to as a "service container." When using such a service container, as pursuant to chapter 5E-13.0331(3), F.A.C., the following information shall be securely attached to it:

Pesticide Concentrate

1. The name, address, and telephone number of the county or district program
2. Product name
3. EPA Registration Number from product label
4. Name and percentage of active ingredient(s)
5. Signal word from the registered label

Use-Dilution Preparation

1. The name, address, and telephone number of the county or district program
2. Product name preceded by the word "diluted"
3. EPA Registration Number preceded by the words "derived from"
4. Name and percentage of active ingredient(s) as diluted
5. Signal word from the registered label

A reference copy of the EPA-approved labeling for the product must be kept at the county or district's office. Also, the pesticide in the service container cannot be sold or distributed for use by any other person.

13.8 CERTIFICATION AND TRAINING

In 1992, the Florida Department of Health and Rehabilitative Services, Pest, Mosquito and Dog Fly Control program was transferred to the FDACS. This program falls within the authority of the Division of Agricultural Environmental Services (AES).

Certification of pesticide applicators in Florida is conducted by the Bureau of Licensing and Enforcement (BLE) within FDACS. Through Chapter 388 F.S., the BLE is responsible for the regulation of mosquito control workers through Public Health Pest Control licensure and certification. They also enforce applicable laws and rules.

Certification exams are administered with the help of the University of Florida, Institute of Food and Agricultural Sciences. FDACS has established criteria for the certification of all private and public arthropod control applicators and program directors and requires appropriate record keeping and reporting. No certification is required of private applicators controlling arthropods on their own individual residential or agricultural property. Chapter 5E-13.040 F.A.C. establishes the criteria for certification of applicators.

To comply with FIFRA requirements, two categories are included within the Public Health Pest Control License.

1. **Public Health**: The Public Health category includes public applicators using or supervising the use of pesticides in public health programs for the management and control of pests having medical, public health, or nuisance importance. To obtain this license, practical knowledge of vector disease transmission as it relates to application programs is required. In addition, the recognition, life cycles, and habitats of relevant pests and knowledge of environmental conditions that may affect public health arthropod control, and knowledge of the importance and employment of non-chemical control methods, such as sanitation, waste disposal, and drainage, are required.

2. **Aerial**: The Aerial category applies to public applicators who apply any pesticide used for public health arthropod control from an aircraft. Licensure in this category is issued only in conjunction with licensure in the Public Health Category. To obtain the aerial license, practical knowledge must be demonstrated of the principles and practices of aerial pest control and the safe application of pesticides by aerial methodologies.

To obtain a license, a passing grade of 70% is required. Individuals who score below 70% may retake the test as often as once a day, in accordance with testing schedules.

The Division of AES sponsors training programs for preparation to take the exams. This includes training in the core area (*i.e.*, pesticide handling and safety) from the University of Florida County Cooperative Extension Service offices. Training in public health pest control is offered by FDACS and through the FMCA Dodd Short Courses.

Licenses must be renewed every four years with sixteen continuing education units (CEUs) required for renewal of the licenses. Re-examination is required if the license is not renewed within 90 days of the expiration date. Chapter 5E-13 F.A.C. requires Public Health Pest Control applicators to keep accurate pesticide use records. These records must be retained for a period of three years and made available to FDACS upon request.

Chapter 5E-13 F.A.C. further requires that all new mosquito control directors must already have or obtain Public Health Pest Control licensure and director certification. Within six months of being hired, all new mosquito control directors must take and pass a directors' exam, which is a comprehensive evaluation of the knowledge required to administer a mosquito control program in Florida including budget planning and pesticide calibration.

Funds are appropriated annually by the Florida Legislature to support the Public Health Pest Control Program, which is administered by the FDACS Division of AES. There are no fees collected in administration of this program.

13.9 AERIAL REGULATIONS

Aerial mosquito control operations are regulated under the Code of Federal Regulations (CFR) Title 14, Part 137, which governs agricultural aircraft operations within the United States. Aircraft owned and operated by government organizations, such as mosquito control districts, are entitled, but not required, to be operated as “public aircraft.” “Public aircraft” are exempt from many, but not all, of the requirements of Part 137.

Mosquito control organizations operating “civil” (*i.e.,* not “public”) aircraft are required to obtain an Agricultural Aircraft Operator Certificate under Part 137, which entails presenting for inspection at least one “certificated and airworthy aircraft” and appropriately rated aircraft pilot who must pass a knowledge and skill test to demonstrate competency in aerial agricultural operations. Additionally, the pilot is required to meet certain prior experience requirements before conducting operations over a congested area.

Part 137 Subpart C outlines general operating rules for agricultural aircraft, many of which are not applicable to “public” aircraft. Of note, however, is that this is the *only* regulation which allows agricultural aircraft “during the actual dispensing operation” to operate at altitudes “required for the proper accomplishment of the agricultural aircraft operation.” All aircraft, *civil* and *public*, are otherwise required to maintain the *minimum* safe altitudes outlined in Part 91 (“General Operating and Flight Rules”) of the regulation, which are *significantly* higher than would be practical for any sort of mosquito control aerial operations.

In sections 137.43 through 137.47 allowances are made for agricultural aircraft which are not equipped with radios, transponders, position lights, or basic instruments to permit them to operate in some areas of controlled airspace after prior coordination with the appropriate authorities.

Arguably the most noteworthy part of this regulation is Section 137.51, which delineates requirements for operation over “congested areas” and does *not* exempt “public” aircraft from compliance. In addition to obtaining “prior written approval” from an appropriate government official having jurisdiction over each municipality involved (usually accomplished by way of an open-ended letter of approval), the organization must provide prior notice of each operation to the public through newspapers, radios, etc., and have each operation approved by the local Federal Aviation Administration (FAA) Flight Standards District Office (FSDO).

To circumvent the redundancy of submitting a complete plan with all of the required information for each and every operation, most mosquito control organizations opt to publish a yearly "congested area plan" which meets all of the requirements outlined in the section and submit it to the local FSDO for approval. Once approved, this plan serves as a kind of "contract" between the mosquito control organization and the FAA, defining exactly how the organization will conduct its business and usually then only requires that a facsimile notification be sent to the FSDO advising them of the intended treatment area, times, pilot(s), and aircraft before each night's flight activities.

It should be noted that each FSDO's designated representative has complete discretion in approving a specific aerial operation over his or her jurisdiction's congested areas. Accordingly, although some sections of Part 137 exempt "public" aircraft from being certified as airworthy, or pilots from being appropriately licensed, or the organization from being inspected, such organizations may be prohibited from operating over congested areas if the FAA official with jurisdiction feels the operation cannot be conducted with an appropriate degree of safety. Additionally, the FAA gives no precise definition of a "congested area." Operators who are unsure of whether or not they are operating over a congested area should consult their local FSDO to make this determination.

There is often some confusion as to what information is required to be included in a congested area plan. Apart from the features briefly outlined in Part 137.51, *i.e.* "consideration of obstructions to flight; the emergency landing capabilities of the aircraft to be used; and any necessary coordination with air traffic control," the regulation does not give specific details as to the required content. A more complete description can be found in the FAA Inspector's Handbook 8700.1 Chapter 120, Evaluate a Part 137 Congested Area Operations Plan, Section 2, Paragraph 3) D. Operators may request a copy of this paragraph from their FSDO for clarification. The same chapter of the Handbook requires the FAA designated representative, or "Principal Operations Inspector," in accepting the plan, to provide a written approval and to stamp, date, and sign each page of the plan.

Of further note, some mosquito control organizations, although operating regularly as "public" aircraft, opt to additionally obtain a Part 137 Agricultural Aircraft Operator Certificate. This certificate allows the operator, should the need arise and with the approval of FDACS and in compliance with Florida Statute, to accept reimbursement for assisting other organizations with aerial mosquito control applications. Acceptance of any kind of reimbursement for these services, to include fuel and pesticide, negates the "public" status of government-operated aircraft and requires the operator to hold a Part 137 certificate, except in circumstances where "the government on whose behalf the operation is conducted certifies to the Administrator of the FAA that the operation is necessary to respond to a significant and imminent threat to life or property (including natural resources) and that no service by a private operator is reasonably available to meet the threat." This arrangement is a significant "gray area" and should be navigated carefully, with prior consultation and approval of appropriate FAA representatives having jurisdiction over both organizations' areas, to avoid incurring considerable fines and disciplinary action.

13.9.1 Aircraft Registration, Security, and Storage

As pursuant to Chapter 5E-13.0371, F.A.C., FDACS requires that aircraft used for mosquito control in Florida be registered annually with the Bureau of Licensing and Enforcement on or before June 30 of each year. In addition, these aircraft shall be secured when not in use, either by storage in a locked building, locked in place, mechanically disabled from flying, or any other reasonable method that would prevent or deter theft or unauthorized use. Any purchase, sale, rental, leasing, or transfer of ownership of a mosquito control aircraft required to be registered with the Department must be reported to the Department within 24 hours of the transaction.

Appendix I

ACKNOWLEDGMENTS AND AWARDS

Over the past several decades, Florida mosquito control programs and professionals have been honored for their commitment to saltmarsh source reduction programs. As a result of these efforts to benefit wetland resources, several individuals and programs have received environmental awards of high distinction.

U.S. Fish and Wildlife Service Conservation Service Award to Jack Salmela
Under the direction of Leon Jack Salmela, Brevard County undertook the state's largest source reduction program. Jack's care, perseverance, and success in maintaining these marshes for both mosquito control and wildlife resources was highlighted in 1986, when he received the U.S. Department of Interior, Fish and Wildlife Service's Conservation Service Award.

As described by the U.S. Fish and Wildlife Service:

> *This award is the highest honor bestowed by the Secretary to private citizens and groups for direct contributions to the mission and goals of the Department. It was presented to Mr. Salmela for his endless contributions to wildlife conservation through effective mosquito control techniques and his personal dedication to effective management of wildlife resources.*

Florida Department of Environmental Regulation Secretary's Environmental Award to the Saint Lucie County Mosquito Control District
In January 1990 for their innovative impoundment management program, the St. Lucie County Mosquito Control District (Frank Evans, Director, and James David, Assistant Director) received the Florida Department of Environmental Regulation Secretary's Environmental Award. This award was for Wetland enhancement and management, which has significantly contributed to protection, conservation, or restoration of the air, water, or natural resources of the State.

Florida Mosquito Control Association's Provost Award
The Florida Mosquito Control Association's (FMCA) Provost award is given to FMCA members who have devoted their careers to mosquito control, while at the same time showing profound concern for the environment. For four recipients of this award, their individual commitment to environmentally sound saltmarsh management was a large factor in their receiving this prestigious honor. In 1987, Jack Salmela (Director of

Brevard Mosquito Control District) was awarded this honor largely for his work in the wetlands management and conservation efforts in association with the U.S. Fish and Wildlife Service on the Merritt Island National Wildlife Refuge.

In 1994, the Provost award was presented to E. John Beidler, Director of the Indian River Mosquito Control District, who was instrumental in initiating and overseeing impoundment research undertaken in the 1980s to identify the impoundment management techniques which are most environmentally compatible. This research led to the general acceptance and implementation of Rotational Impoundment Management (RIM), which is the impoundment management technique most commonly used along the Indian River Lagoon. John also was a long-time member of the Florida Coordinating Council on Mosquito Control since 1986.

Dr. Jorge Rey, a wetlands scientist at the University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory (FMEL), received the Provost Award in 2001. Dr. Rey has worked on saltmarsh management issues since he arrived at FMEL in 1978. In the early 1980s, he was one of the scientists who approached the Indian River Mosquito Control District (IRMCD) suggesting a cooperative project with the Harbor Branch Oceanographic Institution and IRMCD to investigate the ecological effects of impoundment management. This federally funded project lasted for eight years and was the foundation on which RIM was based. Jorge has been a member of the Subcommittee on Managed Marshes since it was created in 1986.

In 2007, Dr. George O'Meara received the Provost Award. A Full Professor at the FMEL, Dr. O'Meara is a mosquito biology expert and a tireless visionary whose goal was to provide mosquito control research-based information to allow for more efficient, effective, and environmentally proper management. His work has contributed to reduced pesticide usage, minimizing habitat alterations while reducing mosquito populations, evaluating wastewater and stormwater treatment impacts on mosquito populations, and ensuring that mosquito control impacts are taken into consideration as Florida continues to provide strategies to manage water resources.

National Association of Counties Achievement Award

St. Lucie County Mosquito Control District received the 1991 National Association of Counties Achievement Award for the restoration of tidal flushing to their coastal impoundments.

Appendix II

BEST MANAGEMENT PRACTICES FOR MOSQUITO CONTROL IN STORMWATER MANAGEMENT FACILITIES

VOLUSIA COUNTY MOSQUITO CONTROL

The surface storage of stormwater required by state and local regulations has created mosquito larval habitats. Our goal is to eliminate larval mosquito production from stormwater management facilities. This effort requires a basic understanding of mosquito life cycles and habitats. The immature stages of mosquitoes (eggs, four instars of larvae, and pupae) use permanently or intermittently wet habitats. The most pestiferous mosquitoes – *Aedes* and *Psorophora* species - lay eggs on damp ground that periodically floods. These eggs may survive for years between floodings. Primary disease vectoring mosquitoes – *Culex* species - lay eggs on the water's surface. Some mosquitoes complete development to adults in as few as six days. Minnows, such as *Gambusia* species, are the most effective predator of immature mosquitoes in permanent or semi-permanent water bodies that are free of vegetation. The use of this information can result in proper design, construction, and maintenance of stormwater management facilities to prevent mosquito production.

Three elements are important to assure that a stormwater management facility does not produce mosquitoes:

1) **Design** of the proper facility for the site – based on soils and other site constraints
2) Proper **construction** and certification by the designer
3) Guaranteed **maintenance** of the system

In combination, these three elements can achieve the goal of <u>no</u> mosquito production from stormwater facilities.

Design

The three basic types of designs are wet, dry, and intermittent systems. Designs should be based on site characteristics and sound engineering principles and include consideration of soils, seasonal high water table, and pre-development drainage characteristics. The <u>Soil Survey of Volusia County</u> provides good, general information that must be field validated. Slopes should support ease of maintenance and enable children and other citizens to extricate themselves. Maintenance plans should be carefully reviewed to prevent additional problems. The soil technician at the Volusia County Agriculture Center is available for free consultation on soil and water tables.

Wet system retention or detention facilities typically are used in high water table environments. The permanent water table will help to maintain populations of top feeding minnows, provided there is approximately eighteen inches of water during the driest periods. The bottom should be graded to avoid isolated pockets of standing water. A maintenance easement of at least fifteen feet should surround the facility above the high water line. Wetland plantings are discouraged as they can cause mosquito or maintenance problems.

Dry systems (retention areas or swales) are best used in low water table environments with permeable soils. These areas should be designed to be dry within three days of a rainfall event based on a twenty-five year frequency storm of twenty-four hour duration. A good rule of thumb is to place the bottom one foot above the seasonal high water table. Where this minimum freeboard cannot be achieved by raising the retention area, a wet system should be used. We do **not** recommend the use of underdrains to control water elevations because of their expense, susceptibility to failure, and frequency of maintenance. As an alternative to surface storage, we have regularly approved underground exfiltration systems in low water table soils. The pipe should be placed at least one foot above the seasonal high water table. The inlet sumps may produce mosquitoes; However, the maintenance value outweighs an easily treated mosquito production site. These systems are readily maintained on a consistent basis by jetting the pipe with a Vac-type truck unit.

The least desirable system is an intermittently wet/dry system. This system is used at a site where overriding design criteria exist - such as tree preservation - in a high water table soil environment. These undesirable systems can be significantly improved by utilizing minnow reservoirs, constructed of Florida Department of Transportation type "C" catch basins. The tops of the catch basins are installed at ground level with a minimum depth of two feet with a solid bottom and grated top. The catch basin becomes a protected refuge for minnows and tadpoles when the retention facility is dry. Each individual depression within the retention area will require one reservoir. Maintenance of these reservoirs can be performed by hand or with a jet/pump system. A small, permanent pond in one part of the facility could perform a similar function. Retrofitting existing facilities that function as intermittent wet/dry systems, contrary to the original design, would benefit from this revised design.

Construction

Inlets and outlets should be constructed with erosion protection devices. Construction should be done with hydraulic excavators or similar equipment to avoid depressions. A professional engineer should certify that the facilities have been constructed according to the proposed plans.

Maintenance

An agreement in the stormwater management permit should specifically identify the party responsible for maintenance. A maintenance schedule – and a procedure to ensure that maintenance is carried out – are vitally important. Mandated maintenance is an important element in local stormwater regulations. Side slopes should be kept free of weeds; Grass should be properly managed to prevent erosion. Weed management (chemical and/or physical removal) should be used in permanent water facilities. Requests to stock minnows in wet facilities can be made to Volusia County Mosquito Control. Tire tracks in roadside swales and other activities that cause ruts and depressions in dry facilities should be avoided.

This guidance allows professionals involved in stormwater management to proactively prevent issues with some relatively simple solutions. It is possible to solve existing problems using the same information. It is our hope that this document will convince people to become a part of the solution – not part of the problem. Proper surface storage of stormwater to eliminate mosquito production is one of the most inexpensive and environmentally sensitive approaches to mosquito control available.

APPENDIX III

HISTORY OF RESISTANCE

Chronology of Insecticide Resistance in Florida Mosquitoes[1]	
1943	• Dade County uses DDT to control mosquitoes.
1945	• Pinellas County uses DDT in thermal aerosols to control *Aedes taeniorhynchus.*
1949	• Florida mosquito control depends almost exclusively on DDT. • Brevard County reports DDT no longer provides adequate control of *Ae. taeniorhynchus*.
1950	• Laboratory tests confirm DDT resistance in *Ae. taeniorhynchus.*
1955	• DDT, BHC, and dieldrin no longer control saltmarsh *Aedes.* • Malathion is shown effective for controlling resistant *Aedes.*
1957	• Malathion use is widespread in the state. • Baseline susceptibility field data with malathion are established in Indian River County. • Florida State Board of Health issues policy that OPs be used only as adulticides.
1958	• No areas of state can be termed non-resistant to DDT. • Shiloh strain of *Ae. taeniorhynchus* is established.
1960	• Baseline susceptibility data for malathion and *Ae. aegypti* are obtained.
1961	• *Ae. aegypti* is shown resistant to DDT in Key West.
1963	• Program is established to monitor insecticide resistance in mosquitoes. • There is no confirmed resistance to any OP in the state. • Fenthion and naled are effective against *Ae. taeniorhynchus.*
1965	• Lee County reports poor results with aerially applied malathion for *Ae. taeniorhynchus* control. • Advanced degree of resistance to malathion (RR 6-20 and 12-74 for LC_{50} and LC_{90} respectively) is detected in offshore islands populations of *Ae. taeniorhynchus* in Sarasota and Lee Counties [RR=resistance ratio (RR)]. • *Culex nigripalpus* and *Cx. salinarius* are susceptible to malathion.

1966	• Malathion resistance is reconfirmed in laboratory tests. • First confirmed report of OP resistance in the state is published. • U.S. Department of Agriculture (USDA) attempts to document the extent of malathion resistance. All field strains tested are resistant to malathion (RR 3-30 and 5-170 for LC_{50} and LC_{90} respectively). • IONA strain of *Ae. taeniorhynchus* is established and shows resistance to malathion.
1967	• Malathion resistance in *Ae. taeniorhynchus* occurs throughout the state. • There are no other confirmed reports of OP resistance. • Field data shows it takes more malathion to control *Ae. taeniorhynchus* than it did in 1959. • Malathion resistance is developing.
1968	• A reversion in malathion resistance (9 to 3 and 52 to 6 for LC_{50} and LC_{90} respectively) occurs in the Sanibel Island population of *Ae. taeniorhynchus.* It is connected to a decrease in malathion usage in Lee County.
1969	• Researchers report a significant difference between glass and polypropylene beakers when testing Abate® and Dursban®. They use only glass beakers. • Baselines are established for Abate®, fenthion, and Dursban®.
1971	• USDA workers confirm that *Ae. taeniorhynchus* from Allenhurst remain highly resistant to malathion (RR 28 and 46 for LC_{50} and LC_{90} respectively).
1972	• Monitoring continues and shows little variation in resistance levels.
1974	• Zoecon Corporation scientists coined the term “biorational insecticide” to describe the approach of developing environmentally safe insecticides based on understanding insect physiology (Djerassi *et al*. 1974).
1975	• First commercial use of methoprene (Altosid IGR).
1976	• *Culex nigripalpus* shows signs in the laboratory of becoming resistant to malathion.
1979	• Cottondale strain of *Cx. quinquefasciatus* is established. • Tampa strain of *Cx. quinquefasciatus* is shown to be resistant to chlorpyrifos (RR3 and 6), naled (RR 3 and 8), fenthion (RR 4 and 1), malathion (RR8 and 17), and temephos (RR 6 and 39) all for LC_{50} and LC_{90} respectively. • The first published report of resistance in this species in the state appears.
1982	• Temephos is used in larval control programs, a deviation from state policy. As a result a program is established to monitor resistance to this insecticide.
1989	• *Culex nigripalpus* continues to be monitored for resistance. • Malathion is recommended as the treatment of choice.

1998	• Report of methoprene resistance in *Aedes taeniorhynchus* on 2 barrier islands, Lee County, FL (Dame *et al.* 1998).
2000	• Report of restoration of susceptibility to methoprene after a 4-year cessation of the use of methoprene (Hornby 2000).
2002	• No indication of methoprene resistance in populations of *Aedes taeniorhynchus* from the Florida Keys in an area where methoprene formulations had been in use continuously for 20 years (Floore *et al.* 2002).
2004	• Liu *et al.* (2004a) reported permethrin resistance in larvae of *Culex quinquefasciatus* from Vero Beach, FL, as well as the ability for this strain to develop cross-resistance to deltamethrin, chlorpyrifos, fipronil, and imidacloprid. This strain demonstrated susceptibility to *Bti* and spinosad. • Liu *et al.* (2004b) reported on south Florida populations of *Aedes albopictus* that exhibited low level resistance to chlorpyrifos and deltamethrin, and low tolerance to permethrin, malathion, resmethrin, spinosad and *Bti.*
2014	• Marcombe *et al*. (2014), working on *Aedes albopictus* from two sites in St. Johns County, found the two populations to be susceptible to the larvicides *Bti,* temephos, and spinosad. One of the strains exhibited resistance to methoprene and pyriproxyfen, and significant resistance to carbamates was seen in both Florida populations. Assays on the adult stages of both strains indicated resistance to DDT and malathion, and susceptibility to deltamethrin.
2016	• The University of Florida, Institute of Food and Agricultural Sseinces, Florida Medical Entomology Laboratory receives funding from the Florida Department of Health to conduct a state-wide analysis of insecticide resistance in *Ae. aegypti* and *Ae. albopictus.* Wide-spread resistance to pyrethroids was reported for *Ae. aegypti.* Testing results posted quarterly: http://www.floridamosquito.info/insecticide-susceptibility-testing-results/
2017	• Richards *et al* (2017) reported populations of *Aedes albopictus* from Bay, Indian River, and Manatee counties to be resistant to malathion and etofenprox, and a Bay County population with resistance to deltamethrin. A population of *Culex quinquefasciatus* from Indian River County exhibited resistance to bifenthrin, deltamethrin, etofenprox, malathion, permethrin, and phenothrin.

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Appendix IV

Acronym List

ACOE	Army Corps of Engineers
AES	Agricultural Environmental Services
AFPMB	Armed Forces Pest Management Board
AGDisp	Agricultural Dispersal
AI	Active ingredient
ARS	Agriculture Research Service
ATV	All terrain vehicle
BG	BG Sentinel trap®
BMP	Best management practice
BSETA	Bureau of Scientific Review and Technical Assistance
BSL	BioSafety Level
Bti	*Bacillus thuringiensis israelensis*
Bs	*Bacillus sphaericus*
CAA	Clean Air Act
CDC	Centers for Disease Control
CDC-AGO	CDC-autocidal gravid ovitrap
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CEU	Continuing education unit
CHD	County Health Department
CHIKV	Chikungunya virus
CFR	Code of Federal Regulations
CMAVE	Center for Medical, Agricultural, and Veterinary Entomology
CWA	Clean Water Act
DENV	Dengue virus
DEF	S, S, S, - tributyl phosphorotrithioate
DoD	Department of Defense
DOT	Department of Transportation
DDT	dichloro-diphenyl-trichloroethane
DEET	N,N-diethyl-meta-toluamide
ECC	Education Coordination Committee
EEE	Eastern equine encephalitis
EDIS	Electronic Data Information System
EEEV	Eastern equine encephalitis virus
EPA	United States Environmental Protection Agency

EPCS . . . . Entomology and Pest Control Section
EPI . . . . University of Florida's Emerging Pathogens Institute
ERC . . . . Entomological Research Center
ERP . . . . Environmental Resource Permit
ESA . . . . Endangered Species Act

FAA . . . . Federal Aviation Administration
FAC . . . . Florida Administrative Code
FAMA . . . . Florida Anti-Mosquito Association
FAMU . . . . Florida Agricultural and Mechanical University
FCCMC . . . . Florida Coordinating Council on Mosquito Control
FDACS . . . . Florida Department of Agriculture and Consumer Services
FDEP . . . . Florida Department of Environmental Protection
FDER. . . . . Florida Department of Environmental Regulation
FDHRS . . . . Florida Department of Health and Rehabilitative Services
FDNR . . . . Florida Department of Natural Resources
FDOH . . . . Florida Department of Health
FGFWFC . . . . Florida Game and Fresh Water Fish Commission
FIFRA . . . . Federal Insecticide, Fungicide and Rodenticide Act
FIT . . . . Florida Institute of Technology
FMCA . . . . Florida Mosquito Control Association
FMEL. . . . . Florida Medical Entomology Laboratory
F.S. . . . . Florida Statutes
FSBH . . . . Florida State Board of Health
FSCBG . . . . Forest Service, Cramer, Barry, Grim
FSDO . . . . Flight Standards District Office

GAT . . . . Gravid *Aedes* trap
GIS . . . . Geographic information system
GMO . . . . Genetically modified organisms
GPS . . . . Global Positioning System
GUP . . . . General use pesticides

HJV . . . . Highlands J virus
HBOI . . . . Harbor Branch Oceanographic Institution

IEI . . . . Idiopathic environmental intolerance
IFAS . . . . Institute of Food and Agricultural Sciences
IGR . . . . Insect Growth Regulator
IPM . . . . Integrated Pest Management
IRL . . . . Indian River Lagoon
ITU . . . . International toxic unit

JAMSARL . . . . John A. Mulrennan, Sr. Arthropod Research Laboratory
JH . . . . Juvenile hormone

kdr . . . . Knockdown resistance

LCMCD. . . . . Lee County Mosquito Control District
LORAN . . . . Long Range Navigation
LSM. . . . . Larval source management

MCD. . . . . Mosquito Control District
MCWA.. . . . . Mosquito Control in War Areas
MFIR . . . . Minimal Field Infection Rate
MFO.. . . . . Mixed Function Oxidases
MMF . . . . Monomolecular films

NASA . . . . National Aeronautics and Space Administration
NECE . . . . Navy Entomology Center for Excellence
NGVD. . . . . National Geodetic Vertical Datum
NIAID .. . . National Institute of Allergy and Infectious Diseases
NJLT . . . . New Jersey light trap
NPDES.. . . . . National Pollutant Discharge Elimination Section
NSF . . . . National Science Foundation

OMRI . . . . Organic Materials Review Institute
OMWM . . . . Open Marsh Water Management
OP . . . . Organophosphate

PESP . . . . Pesticide Environmental Stewardship Program
PHEREC. . . . . John A. Mulrennan, Sr. Public Health Research and Education Center
PRC . . . . Pesticide Review Council
PRRS .. . . Pesticide registration Review Section

RCRA . . . . Resource Conservation and Recovery Act
RED. . . . . Re-registration Eligibility Decisions
RIM. . . . . Rotational Impoundment Management

SES . . . . Scientific Evaluation Section
SDS . . . . Safety Data Sheet
SINV. . . . . Sindbis virus
SLE. . . . . St. Louis encephalitis
SOMM . . . . Subcommittee on Managed Marshes

TMOF. . . . . trypsin modulating oostatic hormone
TPP.. . . . . triphenyl phosphate

UF . . . . University of Florida
ULV . . . . Ultra Low Volume

UM . . . . University of Miami
USDA . . . . United States Department of Agriculture
USF . . . . University of South Florida
USFWS . . . . United States Fish and Wildlife Service

VEE .. . . Venezuelan equine encephalitis
VEEV . . . . Venezuelan equine encephalitis virus complex

WNE . . . . West Nile encephalitis
WNV. . . . . West Nile virus
WPA . . . . Work Projects Administration

YF . . . . Yellow fever
YFV .. . . Yellow fever virus

ZIKV . . . . Zika virus

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