# TECHNICAL BULLETIN OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

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ORGANIZED 1922

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VOLUME 7, 2006

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## ORGANIZED IN 1922

The Florida Mosquito Control Association, Inc. is a non-profit, technical, scientific and educational association of mosquito control, medical, public health and military biologists, entomologists, engineers, and laypersons who are interested in the biology and control of mosquitoes or other arthropods of public health importance.

TECHNICAL BULLETIN OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

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# MOSQUITO CONTROL AND ARBOVIRUS SURVEILLANCE WORKSHOP

*A volume of selected papers*

March 23-25, 2004

*Edited by:*
Rui-De Xue

*Sponsored by:*
Anastasia Mosquito Control District
St. Johns County, St. Augustine, Florida

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# INTRODUCTION

Arbovirus and mosquito population surveillance are major parts of a mosquito control program. The selection of suitable methods to accomplish these tasks becomes an important one for local districts. Due to the recent upsurge in mosquito-borne disease in Florida (i.e., West Nile virus) and recent advances in mosquito research, including rapid development of new techniques for control and disease surveillance, there is always a need for mosquito control field staff to update their knowledge in a timely manner. It is with this in mind that the Anastasia Mosquito Control District (AMCD) of St. Johns County, Florida, organized and held its first Arbovirus Surveillance and Mosquito Control Workshop, March 23-25, 2004.

The first day's session included presentations on mosquito population surveillance techniques, attractants, repellents, and mosquito behavior as it affects operational programs. The second day's session featured presentations on interagency collaboration for mosquito-borne disease, malaria surveillance and biology, as well as recent arbovirus research, testing, and surveillance. The third day's session included papers about mosquito control operational programs, biological control methods, new control techniques for mosquito larvae, water management programs, aerial application of adulticides, non-target impacts of adulticides, as well as nuisance midge control. The selected papers in this volume are designed primarily as reference material from the Workshop for employee training in mosquito control, and medical, or public health Entomology.

We are especially appreciative of the speakers who gave presentations and those who reviewed previous drafts of the manuscripts. We also wish to acknowledge the support and encouragement of the AMCD Board, administrative office, and District staff.

Rui-De Xue, Ph.D.
Workshop Organizer
Mary T. Willis
Commissioner Chair, 2004

# MOSQUITO POPULATION SURVEILLANCE TECHNIQUES

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## I. INTRODUCTION

Surveillance of mosquito populations is an essential component of a comprehensive management program. To perform and evaluate an effective mosquito control program in any area, it is critical to know the local species composition, relative abundance, seasonality and spatial distribution of mosquitoes. Obtaining this information is the major objective of mosquito surveillance. Surveillance includes a good understanding of habitats that support mosquito populations, species distribution, and abundance of potential vector species present in the community in order to develop effective mosquito-borne disease prevention and control programs. The recent introduction of West Nile virus (WNV) encephalitis into the United States emphasizes this need. Service (1993) presents an extensive literature review of most known mosquito surveillance techniques. This paper focuses on some of the common techniques utilized by mosquito control programs within the United States to conduct surveillance on all life stages of a mosquito (egg, larvae, pupae and adults).

## II. EGG SURVEILLANCE

Procedures for sampling mosquito eggs can be divided into two main categories (Service 1993). The first involves the detection and collection of eggs from natural habitats. This method primarily involves removing samples of soil from areas that are subject to inundation from rain or tides and washing them through a series of sieves. Because it is very labor intensive, few mosquito control districts do this, thus this paper will not spend any time on this topic. The second category utilizes oviposition traps (ovitraps) which are artificial containers baited with an infusion that acts as an attractant. This method is widely used to sample populations of aedine mosquitoes such as *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) and culicine mosquitoes such as *Culex quinquefasciatus* Say and *Cx. nigripalpus* Theobald.

Ovitraps were developed based on the premise that egg-laying (gravid) females must look for oviposition sites. Little black jars (LBJs) are an example of an ovitrap that collects eggs of container-inhabiting species, such as *Ae. aegypti* and *Ae. albopictus*. Each trap consists of a glass jar painted glossy black on the outside, 3 in. diameter at the top, about 5 in. high with tapered sides and, a capacity of about 1 pint. Water, to a depth of about 1 in., is added to the jar and a ¾-in. wide, 5-in. long hardboard paddle having a smooth and a rough surface is attached vertically with a paper clip to the inside of the jar (Service 1993). Eggs are usually deposited just above the water line on the rough side of the paddle which faces towards the center of the jar. Paddles were originally made of the hardboard used for interior decorating as this is more absorbent than the exterior-type hardboard, and thus present a more suitable oviposition surface (Thaggard and Eliason 1969). Because of difficulties in obtaining hardboard with the correct absorbent properties, various types of paper paddles have been substituted. Brown or grey velour paper paddles were found to be as efficient as the hardboard paddles (Jakob et al. 1970). Kloter et al. (1983) used red velour paper paddles with some success. Brown seed germination paper is also commonly used today.

Ovitraps have also been developed to attract gravid females that primarily develop in permanent water sites, such as many *Culex* spp. Females lay their eggs on water within artificial containers such as wash tubs, buckets, or clay pots. Often, an infusion mixture is added to the container which is more attractive than plain water to attract the gravid female of various species (Reiter 1983, Reiter et al. 1991, Scott et al. 2001). Egg rafts are then collected from the water's surface.

## III. LARVAL MOSQUITO SURVEILLANCE

Mosquito larvae are entirely aquatic and have adapted to a wide variety of habitats, including permanent ponds, marshes, tree holes, and artificial containers such as tires. Mosquito eggs are deposited either in permanent water sources or in the mud at the edges of temporary water sites to hatch when flooding occurs. Larvae are rarely found in lakes and ponds with deep water and clean shorelines or in flowing water such as streams or rivers. Larvae generally take four to ten days to complete their development and then pupate. The pupal stage lasts from one to ten days after which the adult mosquito emerges.

Larval surveillance involves sampling a wide range of aquatic habitats for the presence of immature mosquitoes. The sampling procedure is relatively simple and involves collection of larvae with a dipper or plastic pipette. Standardizing the procedure is only necessary if the goal is to determine population densities over time. Collected larvae and water are placed in a suitable container for transport such as plastic vials, zip-loc bags or jars.

Collected larvae are maintained in plastic trays of habitat water and covered with cheesecloth. Emerged adults are collected and can be placed in plastic vials to await identification.

## IV. ADULT MOSQUITO SURVEILLANCE

Adult surveys are the most frequently conducted mosquito surveillance activity because adult mosquitoes are easier to locate and identify than larvae. Adult mosquito surveillance is undertaken to determine species composition, seasonal incidence, relative abundance, spatial distribution, and collection of samples for arbovirus analysis. Different types of adult surveillance include complaints (service requests), landing/biting counts, traps and/or resting boxes.

*Complaints (service requests).* Complaints can be an efficient surveillance tool for mosquito control agencies, but the data are frequently overlooked in many management programs (Romanowski and Huggins 1989). Most complaints are received by phone. All complaints should be logged on a preprinted complaint form, with follow up and responses as quick as possible. Complaints are an excellent source for finding previously unknown or new mosquito developmental habitats. Some agencies use the number of complaints to determine when to adulticide established areas. The volume of complaints pre- and post-treatment may give a reliable indicator of the success or failure of an adulticiding operation over the entire treated area. In this case, the public is actually being used as a major surveillance tool. Tracking complaints is often used in tandem with other surveillance methods. Some mosquito control programs use the complaint process as a verification of their other surveillance methods.

*Landing rate/biting counts.* The term "landing rate" should be used when an individual records the number of mosquitoes that land on the observer over a designated period of time. The term "bite count" should be used when an individual captures/records each mosquito that comes to bite over a designated time interval. Specimens captured by aspirator (battery or lung powered) can be identified to species. There are many variations on how different mosquito control programs conduct their landing rates and bite count studies. Schmidt (1989) provides very useful guidelines for conducting bite counts and landing rates in terms of clothing to wear, duration of count, how many people to use and how much of the body to collect from. Lately, this surveillance technique has fallen into disfavor among some people due to the risk of exposing individuals to disease agents.

*Traps.* Adult surveillance takes advantage of mosquito attraction to light, carbon dioxide, other olfactory, physical, and visual attractants or the need to find "resting" and oviposition locations. There are many types of traps that have been developed based on these behavioral activities. Service (1993) is an excellent source of information on the diversity of traps available. Basic types include light traps, gravid traps, and resting boxes. Traps should be located in areas where adult mosquitoes are suspected to occur and where the effects of wind and excessive temperature will not hamper trapping success. Different types of traps can be somewhat selective for certain species of mosquitoes, while others may select for females rather than males. Carbon dioxide baited traps, for instance, attract a disproportionate number of females, which is the segment of the population of most interest in arbovirus studies.

*Light traps.* Historically, two basic types of light traps have been used in mosquito surveillance programs, the New Jersey Light trap and the CDC miniature light trap. Many variations of these basic trap designs have been developed by mosquito abatement programs and commercial businesses. Proper location of traps is particularly important. In general, the best catches are made where cover is good and the humidity is relatively high. Locations a short distance into the margins of wooded areas and swamps are very desirable; traps over open water or in open pasture are typically less productive. Traps should be suspended 5-6 feet above the ground, preferably 30 feet or more from buildings. To be avoided are areas near other sources of artificial light, sites exposed to strong winds and places near buildings housing animals. For mosquito control operations, one or more traps should be located between known larval developmental sources and inhabited areas; others are best located in critical spots such as near residential and recreational areas. A single trap usually reflects mosquito activity only within a few yards of its location. A may represent an area as large as a but this information is not always reli and a sufficient number of traps must be lized to assure a representative sample. actual number required will depend up number of factors including the degre accuracy required, the manpower avail size of area involved, etc. Light traps sh be operated on a regularly scheduled This may vary from 1 to 7 nights per depending on a mosquito control gram's resources.

*New Jersey Light Trap.* This trap was nally described by Mulhern (1942). De ment began in 1927 and went thr several modifications before the ver used today was adopted in 1933 as the dard trap for adult mosquito surveillance New Jersey. Several companies produce ations of the original design. One of major points in favor of the New Jersey Li Trap is its simplicity and ease of opera (Reinert 1989). The trap consists of a diameter vertical metal cylinder with a diameter conical roof fitted above the the cylinder. At the apex of the underside the roof, a socket is provided for a 25 light bulb which attracts the mosquitoes the trap. Although many different type light bulbs have been tried in the light (e.g., many people have used a 40 bulb), the clear 25 watt bulb is most used. For consistency and historic acc this remains the bulb of choice in New sey. The entrance to the cylinder is co with 1/4 or 5/16 in. mesh screen, to excl larger insects such as moths and bee Within the cylinder, an 8 in. diameter tric fan sucks in mosquitoes that fly to light. Below the fan is a fine mesh fu which leads to a collection jar. A venti paper or plastic cup (Rupp 1984) is pla within the collection jar to separate insects from the killing agent. The traps normally run on household curr although they can be modified to run 12v battery. In many cases, the trap is tur on by a photoelectric switch, or timer, before dusk and turned off shortly dawn. New Jersey light trap collections contain large numbers of flying insects other than mosquitoes and are difficult to sort.

*Centers for Disease Control (CDC) Light Trap.* The New Jersey light trap was designed primarily for maximizing adult surveillance results while minimizing human labor and bias (McNelly 1989). At present, this trap remains a useful tool in mosquito surveillance but its design places certain restrictions on its use. As stated above, conventional usage requires electric current to power a trap that is expected to remain at a location for long periods of time. As a result, the trap has proven to be inefficient as a short-term monitor of mosquito populations, particularly in areas where electric current is inaccessible. The CDC miniature light trap was developed by Sudia and Chamberlain (1962) for arbovirus surveillance and other short-term mosquito investigations. This trap mimics the New Jersey light trap in the principle of attracting mosquitoes with white light and capturing them with the down draft produced by a motor and fan. The CDC trap utilizes lightweight components, a 6-volt battery and a live capture net.

The overall design of the CDC trap has remained intact since 1962, with only minor differences in its construction (McNelly 1989). The trap has been modified to accommodate the use of different types of batteries. Sealed gel cell batteries are commonly used. Other modifications include the substitution of a kill jar for the live net, a photoswitch option that automatically turns the trap on and off, and an air-actuated gate system.

*Attractants.* Equally important to a discussion on traps is the development of attractants. Rudolfs (1922) first suggested the possibility of carbon dioxide being a mosquito attractant and Headlee (1934) was the first to explore its potential in conjunction with a mechanical trap. Newhouse et al. (1966) used carbon dioxide (as dry ice) to augment the CDC trap. The addition of the dry ice greatly enhances the CDC trap's capabilities; Newhouse et al. (1966) reported an increase of 400-500% in overall catch. If the CDC trap is used with dry ice, removal of the light bulb will actually improve the collection by eliminating nontarget insects, such as beetles and moths that fly readily to light (Carestia and Savage 1967). This eliminates the tedious sorting process that is a prerequisite for identification of most light trap collections. Without the light, the trap is also less noticeable, a consideration in areas where traps may be subject to theft or vandalism.

The amount of dry ice, as well as the type of container used to hold it, will affect the carbon dioxide release rate. In most instances, a five lb. block of dry ice is sufficient to cover the normal dusk to dawn trapping period (McNelly 1989). This delivers between 400-500 ml of carbon dioxide per min., a rate that is comparable to the amount released by a large mammal (Morris and DeFoliart 1969). Insulated containers are available from the trap manufacturers, but they are easily constructed. In regions where dry ice is difficult to obtain, there are other options. One would be the purchase of a dry ice maker; another requires construction of a cylinder delivery system similar to that described by Parker et al. (1986). An advantage of using cylinders for releasing carbon dioxide into traps is that its discharge can be regulated. Uniform release of gas, however, necessitates a sensitive regulatory valve system and meters to control and measure flow rates (Service 1993). This system and the cost of buying or renting compressed gas cylinders can be more expensive and bulky than dry ice. Recently, ICA Tri Nova (Forest Park, GA) developed granules that release carbon dioxide and can be packaged in sachets for use with mosquito traps. The sachets were developed for mosquito surveillance purposes and are designed to release over 70% of their carbon dioxide output within 24 hours, with an average sustained release rate of about 1-3 ml/min over 24 hrs (personal communication, Joel Tenney, ICA). These Granular Media $CO_2$ Sachets are commercially available from John W. Hock (Gainesville, FL).

Other attractants are becoming available to use with traps. Takken and Kline (1989) were the first to demonstrate the usefulness of 1-octen-3-ol (octenol) as a mosquito attractant. Basically very few species are attracted in any numbers to octenol alone, but when octenol and carbon dioxide are used together there appears to be a synergistic effect with most species of *Aedes*, *Ochlerotatus*, *Anopheles*, *Psorophora*, *Coquilletidia*, and *Mansonia*. With *Culex* species, however, there is little attraction to octenol alone or in combination with carbon dioxide; an exception is *Cx. salinarius* Coquillett. Other potential attractants under investigation are acetone, ammonia, lactic acid and phenols.

*Novel traps.* In the past few years various models of traps have been developed for use as backyard mosquito control devices. Two models (Mosquito Magnet-X [aka counterflow geometry or CFG] and Mosquito Magnet PRO produced by American Biophysics Corporation (ABC) (North Kingston, RI) have been incorporated into the surveillance programs of some mosquito control districts. Both traps utilize patented counterflow technology. Counterflow technology utilizes two fans to create two air currents that move in opposite directions. Mosquito attractants are dispensed into a downdraft current, which creates an attractant plume of decreasing concentration as it moves away from the trap. Mosquitoes follow the attractant plume to the trap where they are sucked into the trap by an updraft current. The Mosquito Magnet-X trap (Kline 1999) utilizes $CO_2$ supplied from 9-kg (20-lb) compressed gas cylinders. Control of $CO_2$ flow rate is achieved with a pressure regulator with an output at 15 psig, a 10-μm line filter, a 500 ml/min flow control orifice, and quick connect luer fittings. The Mosquito Magnet PRO utilizes catalytic combustion of propane to produce its own attractants ($CO_2$, heat, and water vapor) (Kline 2002). Octenol is usually used with both trap types.

*Gravid traps.* Gravid female mosquito traps are designed to collect mosquitoes, particularly *Culex* spp., that are attracted to a potential oviposition site containing water high in organic matter (Reiter 1983, 1987). The trap attracts females by means of the oviposition medium (infusion) contained in a pan below the trap. A small suction fan creates an upward current of air from within the confines of the pan, so that the mosquitoes are blown into a collection bag during their preoviposition examination of the oviposition medium. If the trap is not present, females will lay their eggs on the water surface. These traps have been reported to collect from 57% (Ritchie 1984) to 95% of gravid females (Reiter et al. 1986) and are often used in conjunction with light traps for surveillance (Tsai et al. 1989, Savage et al. 1993, Nayar et al. 2001, Nasci et al. 2002). Commercial gravid traps differ in basic design, including size and color of pan for oviposition media provided with the traps (Allan and Kline, in press). Trap efficiency is increased with large pan size and darker color. Various infusions can be used. Reiter (1983) suggested a hay infusion made by adding 1 lb (0.5 kg) of hay and 1 oz (5 gm) each of dried brewer's yeast and lactoalbumin powder to 30 gal (114 liters) of tap water and allowing the infusion to incubate for 5 days. The trap is placed at the desired collection site at least 1 hr before sunset and the pan filled with 0.5 gal (2 liters) of infusion solution. Captured mosquitoes are removed early the following morning to ensure maximum survival of the insects. New medium is used each night.

*Resting boxes.* Various types of artificial shelters have been used to sample the resting population of mosquitoes. These have been reviewed by Crans (1989) and Service (1993). Many of these artificial shelters are species specific for mosquitoes or even a specific physiological stage. Edman et al. (1968) developed a resting box for collecting blood-fed adults of *Culiseta melanura* (Coquillett) in Florida. The trap consists of a plywood box 30 in. long, 18 in. wide and 12 in. high, painted matte grey on the outside and matte black inside. A plywood concave frame, painted matte grey outside and matte black inside, with a 28 × 52-in. opening tapering to a 10 × 16 in. base is placed just inside the opening of the box. These boxes have been operated with and without a red cotton collecting bag on the inside. Boxes without a cloth bag caught similar numbers of mosquitoes as those with the bag, but it [illegible] to remove them with an aspirator than by removing the entire catch within the bag. In addition to *Cs. melanura*, at least 14 other mosquito species have been collected from the boxes. *Cx. nigripalpus* and *Anopheles quadrimaculatus* Say have been caught in large numbers. Resting boxes are not routinely used by mosquito control programs because they are not a reliable indicator for most nuisance species.

## V. SUMMARY

A good surveillance program will use a combination of techniques to monitor all life stages of mosquitoes. The actual scope of any surveillance program, however, will be determined by the fiscal, physical and human resources available to each mosquito management program. Whatever techniques are utilized they need to be done in a consistent manner (preferably in the same location) on a routine basis to be effective.

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# LABORATORY RESEARCH AND DEVELOPMENT OF ATTRACTANTS, INHIBITORS AND REPELLENTS

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## I. INTRODUCTION

This paper describes some of the past and current research findings, and speculates upon future developments conducted primarily by the USDA-ARS-Center for Medical, Agricultural, and Veterinary Entomology, Mosquito and Fly Research Unit in Gainesville, FL, in the area of biting fly attractants and repellents. Topics addressed are the fundamentals and methodology of this research covering the chemical and biological methods used to discover and verify the efficacy of attractants, inhibitors, and repellents. Current and future applications of newly discovered attractants and inhibitors are described.

Haematophagous Diptera use various cues to locate hosts for bloodmeals. Some of these host-seeking cues are carbon dioxide, heat, moisture, vision, and semiochemicals. Possibly, semiochemicals in the host odor plumes are the most significant mediator of attraction of biting flies at short and medium distances. The discovery of the attractants eliciting this behavior can be used to improve lures in insect trapping devices for more accurate surveillance. An understanding of the attractants and sensory receptors employed by the mosquitoes may also hasten the discovery of new repellents and other personal protectants for use against these insects of medical and veterinary importance.

## II. FUNDAMENTALS AND METHODOLOGY

Figure 1 illustrates ideal cases of behavioral responses of female mosquitoes to attractants, repellents and attractant-inhibitors. Assume that the mosquitoes are at rest upon the far screen prior to their exposure to any odors. In Fig. 1(a), when a human subject introduces the arm and hand into the test cage containing female mosquitoes, the mosquitoes take to flight, orient, fly toward the odor source (taxis), and will presumably land on the odor source. Those landing on the exposed skin are likely to take a bloodmeal.

Application of a suitable repellent to the exposed skin surface should produce the response in Fig. 1(b). In this case, a repellent such as deet (N,N-diethyl-3-methylbenzamide) does little to mask the attractive odors. The mosquitoes, upon initial exposure to the attractant-repellent combination, will become excited to flight and orient toward the source of the odors. In a test cage, such as that depicted in Fig. 1, mosquitoes will often brush against the surface bearing the repellent or land briefly and retake to flight. The repellent affects the ability of the mosquito to remain on the treated exposed skin surface and deters feeding. Although this implies that a repellent functions by contact only, it is noticeable that the same mosquitoes will become less excited to flight upon repeated exposures to the repellent. This occurs until the residual repellent dissipates or is removed or absorbed into the skin to a point where the remaining repellent falls below the threshold for activation of mosquitoes to flight, which precedes the eventual failure point for landing and biting.

Attraction-inhibitors have been considered for many years, but only recently have effective inhibitors been reported (Kline et al. 2003). Inhibitors can be classified as "masking agents" and "spatial repellents." The United States Department of Agriculture (USDA) initiated a search for spatial repellents in 1948 and did not identify any promising compounds from the many submitted as (contact) repellents. An inhibitor may not cause mosquitoes to move away, but it inhibits the ability to detect kairomones by a mechanism not yet understood. When present as shown in Fig. 1(c), a perfect inhibitor will mask all attractive odors such that the source is essentially "invisible" to the mosquitoes. As illustrated in Fig. 1(c), the mosquitoes continue their normal (resting) behavior in the absence of any attraction stimuli. In practice, however, some will still be able to detect attractive odors even in the presence of an inhibitor. Of those mosquitoes that detect the attractive odors, a low percentage of those can locate the attractive odor source.

*Attractants and Attractant-Inhibitor Research*

The discovery of these compounds are dependent not only on biological assays, but in the chemical techniques used to discover them. It seems that there are many more attractive (host) odor sources than there are easily identifiable repellents. To best assay repellents, and particularly inhibitors, there is a need for attractive odors to test the efficacy. Whereas repellents have routinely been discovered throughout the years from synthetic sources, with some reliance upon structure-activity relationships with preceding repellent structure data, the search for inhibitors is tied heavily to the analysis of either animal hosts or plants.

*Chemical Analysis of Host Odors*

The analysis of host odors for the purposes of attractants is straight forward in reasoning, but not as simple when it comes to the methodology. Female mosquitoes require blood meals for oogenesis to complete the reproductive cycle. There are a number of attractive stimuli that these mosquitoes can use, e.g. light, heat, moisture, carbon dioxide, and perhaps most importantly, host odors (Takken 1991). If mosquito visual acuity is not highly adept, then a strong argument can be made for their ability to locate and discern their (desired) hosts mainly by the emanated odor profile.

Some of our experiments lead us to believe that when mosquitoes are locating hosts, they are very discriminatory of the compounds present, and do choose primarily on the basis of the compound abundances that are important to the particular mosquito species. However, their discernment is less discrete when it comes to detection of a particular compound, for example acetone compared to butanone or dimethyl disulfide compared to diethyl disulfide, because we suspect that they detect and use a broad range of the "bouquet" of host emanations. Even if some compounds are not optimal structurally, or if exogenous compounds are present in the bouquet plume, mosquitoes will still orient towards the host. Therefore, the approach to the chemical analysis of these kairomones is different than the typical chemical separations approach to the identification of pheromones.

When isolating a sex pheromone, chemical separation by fractionation is usually the best approach because it is often a single compound that is eliciting the mating behavior. This is not the case for the host-seeking process. Not only are there multiple compounds that attract, and do so via synergism, but there are some compounds that inhibit host-seeking (Bosch et al. 2000, Bernier et al. 2003). This greatly complicates any means of chemical separation in an attempt to isolate the kairomones. When there is synergism, there is a risk that testing of single compounds, or even fractions will isolate, for example, two vital compounds in different fractions. Granted that this problem could be overcome by bioassaying combined fractions, but what if there are 3 or 4 compounds? This can become exponentially more complex as the number of attractant compounds in host emanations increases. In addition to the difficulty that can arise from multiple synergistic compounds, unlike pheromones there is less structure specificity for attraction to

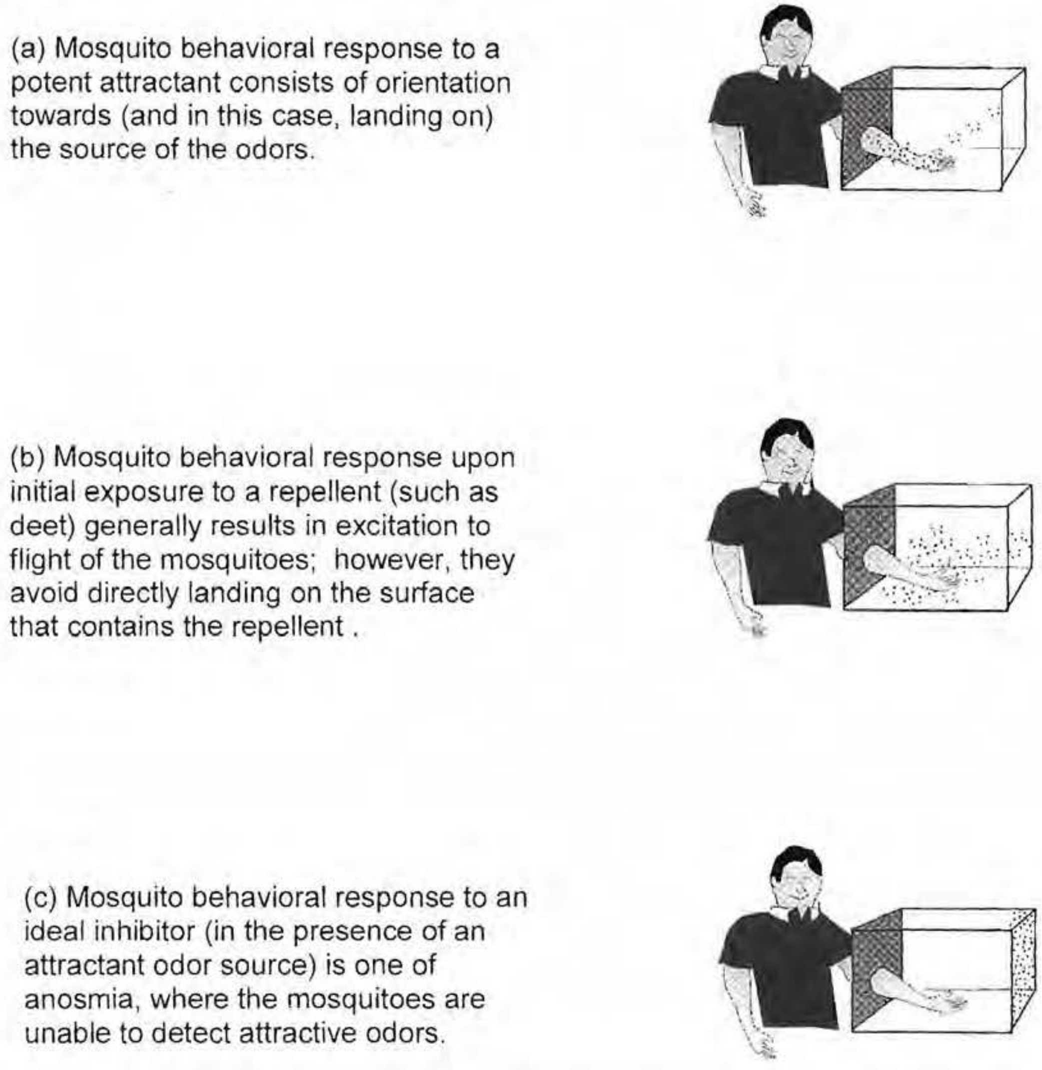


Figure 1. Depiction of mosquito behavioral responses upon exposure to (a) an attractant, or attractive odor source, (b) a classic (contact) repellent, such as deet, and (c) an attractant-inhibitor.

kairomones. For example, if acetone is the best kairmone to add to L-lactic acid (synergistically) to attract *Aedes aegypti* (L.) (Bernier et al. 2003), then L-lactic acid and butanone will be almost as effective a blend.

Finally, the "bouquet" of host odors may contain compounds that inhibit mosquito host-seeking. This is certainly true of human emanations. Unfortunately for humans, the relative amounts of these substances are minute enough (trace level in GC/MS analysis) to where they will be overcome by the synergistic attractant odors. An approach involving fractionation chemically or physically may isolate an inhibitor with an attractant in a particular fraction. If we were to suppose that this attractant alone would produce a noticeable but weak response in a bioassay, then there is a chance that coupled with an inhibitor, this fraction would demonstrate no response. If that is true, then the fraction will be deemed to contain no attractants, and in this approach, we may have just lost the key synergist vital to an attractant blend for mosquitoes.

Therefore, fractionation is not the best approach. There are other approaches that can be used to reduce the number of chemicals that need to be identified (and later bioassayed). One approach is to handle glass beads as a medium to collect samples from human skin (Bernier et al. 1999). However, the drawback is that even though some of the attractive odors are collected, some may still be lost. Every chemical sampling method has advantages and disadvantages and a single sampling method will likely not be sufficient to identify all of the chemicals emanated by a host.

We first realized the possibility of inhibitors originating from humans after two sets of different experiments that took place in the mid 1990s. In the early part of that decade, we were analyzing emanations from two humans that differed markedly in their attraction of *Ae. aegypti* (Bernier et al. 2000, 2002). Instead of focusing only on the chemicals found in greater abundance on the host that was more attractive to *Ae. aegypti*, we noted the compounds that were more abundant on the host that was less attractive to them. The existence of inhibitors, and subsequently, their powerful effect on the overall attraction was realized in 1997, after we assembled a concoction of approximately 30 compounds. This mixture of 30 compounds elicited no attraction, nor any flight activity in *Ae. aegypti* when bioassayed. Yet, as blends of some of these same compounds were tested, we observed activation to flight, orientation and attraction to the port containing the blend. This procedure resulted in identification of L-lactic acid, acetone, and dimethyl disulfide as a highly attractive blend for these mosquitoes (Bernier et al. 2001).

The reason we chose glass beads as a carrier to transfer human emanations, after cryofocusing in the gas chromatograph (GC) injection port, into a GC/mass spectrometer (GC/MS) was because this method minimized the collection of water. The disadvantage was that we were capturing only some of the compounds involved in attraction, and certainly not collecting the most volatile components. We later identified the more volatile compounds using headspace "grab-sampling" by allowing emanations from human skin to equilibrate in an enclosed Tedlar bag. The emanations were then transferred onto a microscale purge-and-trap GC/MS system which removed the high level of moisture in the samples. The disadvantage was a bias imparted against the most polar compounds, as part of the water removal process (water is a polar molecule). However, the complementary data acquired from these two techniques identified the attractants used in our blend for *Ae. aegypti* (Bernier et al. 2001).

### *Chemical Analysis of Plants (Oviposition Attractants)*

Analysis of plant matter, particularly hay infusions are conducted to identify chemicals that are involved in mosquito oviposition (Millar et al. 1992, Du and Millar 1999). The concern here, as with host odor sample collection, is whether or not all chemicals of interest are collected (and desorbed of a sorbent, if applicable) such that they can be identified by chemical methods, such as GC/MS. In our work thus far, we analyzed Bermuda grass hay infusions as a function of fermentation time, using both direct headspace sampling techniques and extractions by impinging the volatiles into a solvent. Because traps baited with hay infusion are effective for collecting gravid *Cx. quinquefasciatus* (Say), (Reiter 1983, 1986) an important mosquito species in the Southeastern U.S., we are focusing our efforts in that direction.

### *Chemical Analysis of Other Attractants (Blood)*

Chemistry efforts to analyze volatiles in blood is not novel by any means. However, we are attempting to determine, and identify, chemicals that may play a role in the ability of laboratory mosquitoes to locate blood contained in a feeding membrane. We are not seeking to identify the feeding stimulant, but rather the attractant(s) that function at short range that the mosquitoes cue upon to locate the bloodmeal. We attempted to collect volatiles on solid phase microextraction (SPME) fibers, using a range of coated sorbent phases. Unfortunately, the ability to produce enough volaeach port. As discussed prior with repellent tests in the first paragraph of the "Attractants and Attractant-Inhibitor Research" section, without an attractant in each port, one cannot truly compare the inhibitors. What would result is a comparison of whether each compound is an attractant in itself (and most likely a very weak one at that).

*Repellents and Toxicant Research*

Repellents and toxicants will be mentioned briefly in this report because there is a research effort in our laboratories at this time to synthesize and test new repellents. There is a collaborative effort with the University of Florida to use quantitative structure-activity relationships (QSAR), with a training set of known repellent structures and protection times, to identify promising structures of candidate repellents for synthesis. Also, ARS, Beltsville is developing 1-[3-cyclohexen-1-ylcarbonyl]-2-methylpiperidine (also known as AI-37220, specifically the active S,S-isomer, SS-220) as a repellent to replace deet (Klun et al. 2004). Currently, the development of new repellents is cost prohibitive because of the toxicological and registration requirements.

The toxicant used most commonly on clothing is permethrin. Permethrin treated uniforms are used extensively by deployed military troops (Young and Evans 1998). This compound is used as a non-topically applied repellent and its most beneficial quality is its excitorepellency, rather than toxicity to biting flies. Mosquitoes and other bloodsucking insects need to come in contact with the compound to have it affect the organism. Quite often, this results in an inability to take a bloodmeal and prolonged exposure can result in death. In this way, protection is afforded to the person wearing the permethrin-treated garment. Military combat utility uniforms use a treatment application rate of approximately 0.125 $mg/cm^2$ of fabric material.

*Assays for Repellents*

It should be noted that there are other protocols to assay repellents in the laboratory; some of which rely on $ED_{95}$ or $ED_{100}$ protection; for more information on this topic, see the WHO position paper (Barnard, 2000). Our assay is conducted until biting occurs, as described below. Direct topical application is an option if the candidate is toxicologically safe; otherwise, it is applied to a patch and that is affixed on the arm so that there is no direct contact between candidate and skin. Candidate repellents are compared against the standard repellent deet. For the compounds that are applied topically, the percentage of control applied is matched closely to the application dosage of the active ingredient in the candidate repellent. Treatments are applied on the forearms and gloves are placed on the hands during tests. Tests are made at 30 and 60 min, and then hourly up to 8-hr after treatment, or until 2 bites are received during a 3-min test period. Field assays follow a treatment similar to that for the laboratory assays. However, human volunteers do not apply any additional repellent on other exposed body surfaces except for untreated protective attire to prevent additional bites outside of the test areas on the arms. A complete description of this protocol for mean complete protection time can be found in USDA (1977).

*Assays for Insecticides (Toxicants)*

Laboratory assay for insecticides is performed by forcing mosquitoes into contact with swatches of treated fabric material. The petri dish method used in our laboratory uses an inverted dish with the center drilled out and a screen flap glued onto the dish to cover the hole. This allows for a small number of mosquitoes, typically 5, to be held under the dish as test specimens. A card is placed on top of the fabric and below the dish holding the mosquitoes to prevent premature exposure of the treated fabric. The card is then removed and the mosquitoes are exposed to the fabric for 2-min. During this time, the petri dish is agitated carefully to force mosquitoes to fly against or land on the fabric. The percentage of mosquitoes "knocked down" (KD%) is recorded at 15 and 60 min post-exposure. Any mosquito incapable of feeding is considered "knocked down" and it is assumed that it will die (the 24 h mortality can be measured according to standard WHO protocols). Essentially, this procedure determines if the amount of treatment on the fabric is present at a sufficient or threshold level to result in effective knockdown of the test specimens.

## III. APPLICATIONS AND FUTURE USES

### *Uses of Attractants*

Biting fly attractants will most likely be used as lures employed in insect traps for surveillance and in conjunction with inhibitors, possibly for control as described below. Carbon dioxide is the best universal attractant to use as a bait in traps because living organisms that supply bloodmeals exhale a higher level of this gas than background, and increased abundance of carbon dioxide in plumes is detectable by biting flies. The drawback to the use of carbon dioxide is that it requires deployment in either a compressed gas cylinder, dry ice, or a means of generation such as combustion of propane. If a highly potent chemical blend released at a low rate such that the use of the blend is not cost-prohibitive could be used in these systems in place of $CO_2$, it would be beneficial to our surveillance capabilities.

The choice of blends may also determine the species of mosquito or biting fly collected in the trap. For example, a blend based on human odors could selectively capture anthropophilic mosquitoes over zoophilic species. In contrast, the success of developing a zoophilic species attractant blend may be easier than the development of an anthropophilic species blend, with respect to capturing mosquitoes in the presence of humans. Even though mosquitoes are capable of efficient discrimination between host (type) odors, it is believed that a zoophilic-based odor blend, even if does not contain all the compound necessary to make it competitive against the preferred host of the mosquito species, may still be able to compete well in proximity of human odors.

It is unknown and perhaps not fruitful to attempt to develop a blend that is more attractive than any human. Such a "superblend" may never be achieved, and even then the use of it in a surveillance trap may not be enough to provide local control of mosquitoes in a small area. However, in the absence of humans, a series of efficient surveillance traps has produced encouraging field reduction of mosquito populations in a small area.

### *Uses of Inhibitors*

As a means of personal protection, inhibitors provide a way to "cloak" humans and animals from mosquitoes. Linalool was identified recently as an inhibitor (Kline et al. 2003) and there are several other inhibitors (both human and animal based) that are being investigated by our laboratory. One possibility is to couple inhibitors with repellents to provide an added dimension of personal protection. Provided that the inhibitor was toxicologically safe, it could be applied topically. The current thought, however, is to disperse these compounds into the environment as an aerosol. Other applications might involve binding these compounds into fabric, similarly to that done currently with toxicants for slow release or contact efficacies. Finally, the release of inhibitors inside a local area surrounded by baited traps might provide a "push-pull" type system where control of a small area can be realized. In our bioassays, there are promising results using the olfactometer that demonstrate how well humans can be "cloaked" by increasing the amount of a natural human-based inhibitor compound in proximity of the human odors, and having this compete against an attractant blend.

### *Uses of Repellents*

The use of topical repellents, such as deet, continues to be the most effective means of personal protection for the general population. Deet was developed in the 1950s for the military and it remains one of the best and safest repellents, despite its

# ADULT MOSQUITO BEHAVIOR IN RELATION TO OPERATIONAL MOSQUITO CONTROL PROGRAMS

RUI-DE XUE

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For survival and reproductive success, adult mosquitoes depend on a series of characteristic behaviors including foraging (sugar feeding and blood feeding), mating, and oviposition, which are governed by internal factors (genetics and physiology) and external cues or stimuli (temperature, light, and relative humidity) (Takken and Knols 1999). Mosquito population surveillance, public education for prevention of disease transmission and personal protection from mosquito bites, larval and adult control are major operational programs in organized mosquito control districts. These programs are based, in part, on mosquito biology and natural history. Therefore, understanding the behavior of mosquitoes, especially adult mosquitoes, will benefit and enhance operational mosquito control programs.

## I. HOST-SEEKING BEHAVIOR IN RELATION TO ADULT MOSQUITO SURVEILLANCE

Adult mosquito surveillance is a common activity in many mosquito control districts. The purpose of an adult mosquito survey is to determine species composition, seasonal activity, abundance, as well as to provide samples for arbovirus testing. There are several different methods used for adult mosquito surveillance, including trapping, landing/biting rate counts, and citizen complaints regarding nuisance mosquitoes. These methods provide information about host-seeking female mosquitoes.

The host-seeking behavior of female adult mosquitoes is regulated by endogenous hormones and mediated by host odors (Bowen 1991). Male mosquitoes respond primarily to plant odors, but several species respond to animal odors (Clements 1999). Female mosquitoes respond to human and animal hosts through olfactory cues, vision, and generalized upwind searching behavior (Clements 1999).

### *1. Landing/Biting Rate Counts*

The human landing rate count is a standard method for collecting host-seeking anthropophilic mosquitoes (Service 1987). Volunteers stand in a designated area for 1 to 3 minutes and collect or count the number of mosquitoes landing on selected areas of the body. Usually, landing rates are conducted 1-2 hours before or after sunset. Counts may vary due to many factors such as species, ambient temperature, time of the day, site vegetation and amount of cover, rain, humidity, and individual odor cues produced by the volunteers. Adult mosquito landing rate counts allow a district to make informed decisions about the application of adulticides or other avenues used for mosquito control. In Florida, a landing rate count of more than 5 mosquitoes per minute warrants control action (Chapter 5E-13: Mosquito Control Program Administration. Florida Mosquito Control Handbook).

### *2. Service Requests*

Female mosquitoes can be an annoying nuisance, when seeking human hosts for a blood meal. During the mosquito season, mosquito control districts receive a number of complaints about biting mosquitoes and a number of requests for control. The District's field inspectors and sprayers are primarily responsible for following up on these requests by inspecting problem areas and assessing them for possible treatment. In addition, inspectors will conduct landing rate counts, and locate larval habitats, such as containers and other sources of standing water. Also, inspectors help residents identify mosquito production areas in their yard and point out the importance of emptying water holding containers to eliminate the development of mosquito larvae. If necessary, inspectors may apply adulticides, such as AquaReslin© and malathion to kill adult mosquitoes. More importantly, field staff will educate residents about basic mosquito biology, disease prevention, and personal protection against biting arthropods.

*3. Traps for Adult Mosquito Surveillance*

Adult mosquito surveillance is one of the major operational programs in mosquito control districts. Several types of traps have been developed based on mosquito host-seeking and oviposition behavior. These traps employ olfactory cues as well as physical and visual attractants. Basic trap types include light traps, gravid traps, resting boxes, and ovitraps. In most mosquito control district surveillance programs, light traps (usually CDC) are often combined with attractants (either with carbon dioxide and/or octenol). Surveillance programs with baited light traps have been used throughout North American for many years.

The development of efficacious attractants is as important as the development of traps. Host-seeking mosquitoes usually are attracted by certain substances, such as carbon dioxide (delivered as either dry ice, pressurized gas from cylinders, or granular media in a sachet) and 1-octen-3-ol (octenol) (Kline et al. 1991). Traps, when used in combination with attractants, usually capture more mosquitoes than traps run without attractants. In Florida, a count of more than 25 mosquitoes per trap per night is considered the threshold for a treatment decision (Florida Administrative Code, 5E-13.036).

Attractant-baited traps are being evaluated as an alternative to the application of chemical insecticides for mosquito management in selected habitats (Kline 2002). This has stimulated private industry to develop new traps for arthropod surveillance and control (Kline 2002). Currently, there are several types of traps on the market, including the Mosquito Magnet©, Coleman mosquito Deleto©, Champion©, Lentek©, and Skeeter Vac©. Some of these traps use propane which when run through a catalytic converter produces heat, moisture, and $CO_2$ as byproducts, all of which serve as mosquito attractants.

Host-oriented behavior of mosquitoes is mainly limited to dawn, dusk, and sometimes nighttime, when their vertebrate hosts tend to be more quiescent, wind speeds are calmer and humidity higher. Most diurnal species remain under cover of vegetation or in shady areas, presumably for protection from predators and desiccation (Gibson and Torr 1999). As a result, traps baited with attractants collect mainly host-seeking mosquitoes.

## II. VISION IN RELATION TO ADULT MOSQUITO SURVEILLANCE

Vision plays a complex role in mosquito host location and mating. Visual components are often integrated along with other cues, especially olfaction for complex mosquito behaviors (Allan et al. 1987). Vision, including simple responses to illumination levels as well as the perception of objects, is an important component of host-seeking, dispersal, mating, appetitive flight, location of sugar sources, resting, oviposition, and location of over-wintering habitats. Numerous reports document that flying mosquitoes depend on visual input for orientation. Visual factors are also involved in the daily selection of suitable resting sites. Light traps, (e.g. New Jersey and CDC) and resting boxes, (e.g. trash cans and paper boxes) are some of the types of equipment that have been developed for adult mosquito surveillance.

## III. DAILY BEHAVIORAL PATTERNS IN RELATION TO ADULT MOSQUITO CONTROL

Daily activity patterns in adult mosquitoes are mediated and regulated by an logenous circadian clock (Klowden )0) and exogenous environmental cues. e patterns of host-seeking, blood-feeding, ar-feeding, mating, and oviposition in nale mosquitoes vary with species and graphic area. In general, most mosquito cies show greater activity at dusk and vn, however, some species (*St. albopictus*) active during daytime hours.

*Prevention and Personal Protection*

Understanding the daily patterns of bit- activity can greatly help individuals in ir approach to vector-borne disease pre- tion and personal protection against squitoes. Avoiding or reducing outdoor vity, especially at dawn and dusk, wearing g pants and long-sleeve shirts, and apply- mosquito repellents containing DEET is e way of reducing contact with host seek- mosquitoes.

Biting activity of mosquitoes is signifi- tly affected by light intensity (Chadee, )4). For example, the activity of several cies of *Anopheles* mosquitoes peaks just ore midnight. Circadian activity will vary 1 species and local weather conditions, h as relative humidity, rainfall, and wind. e most active period for host-seeking navior in *Culex* mosquitoes is during the ning and morning crepuscular periods hmidt 2003).

*Adult Mosquito Activity Patterns and ulticide Application*

Understanding the daily activity patterns idult mosquitoes will help to identify the t times to attempt adulticiding applica- 1. Activity patterns may be influenced by rition, digestion, gonotrophic condition, , body size, and mating status. The nutri- nal state of a mosquito determines its for- ng response to host stimuli. External s, such as temperature, light, moisture, d, and host availability activate mosqui- s to engage in particular behaviors. Also, owledge of how these external cues influ- e behavior can be exploited by applying lticides when a target species is most ve. Most Florida mosquitoes control districts apply insecticides for adult mosquito control during the evening and early morning hours when targeted mosquito species are most active and people are at home or sleeping. Also, knowledge of the daily behavior of adult mosquitoes can help a mosquito control district better respond to service requests.

## IV. SUGAR FEEDING BEHAVIOR IN RELATION TO ADULT MOSQUITO CONTROL

Adult female mosquitoes do not live by blood alone. Female, as well as male, mosquitoes require carbohydrates for flight energy and daily maintenance. These carbohydrates are obtained from nectar, fruit, and honeydew (Foster 1995). Sugar feeding is an opportunistic, non-random, circadian activity undertaken by adult mosquitoes to fulfill their energy requirements and is a fundamental characteristic of mosquito life (Yuval 1992, Foster 1995). In general, both sexes seek a sugar meal shortly after emergence and continue to consume it throughout their lifetime. The actual time of sugar feeding varies and is species specific (Foster 1995). Some species start to seek a sugar source at dusk and dawn following changes in light intensity. For these species, peak activity occurs during the hour following sunset or before dawn. Many *Aedes* mosquitoes consume sugar during daylight hours.

The sugar feeding habits of adult mosquitoes can be manipulated for ecological studies (i.e., determining flight range through feed mark-release-recapture methods) and control purposes. While sugar alone does not attract mosquitoes, the kinds of chemical odors from plants and flowers that may attract them to these sources remains to be addressed. Such attractants could be incorporated with toxins for control. For example, Xue and Barnard (2003) added $CO_2$ and/or octenol to a sugar source mixed with either boric acid or Fipronil® in order to control adult mosquitoes. Studies on attractants for sugar seeking mosquitoes seem promising and may lead to the design of novel vector control products.

## V. RESTING BEHAVIOR IN RELATION TO ADULT MOSQUITO CONTROL

Pates and Curtis (2005) reviewed mosquito behavior in relation to vector control and emphasized that the resting behavior of *Anopheles* mosquitoes was a key factor when house spraying and insecticide-treated bed nets were used to reduce malaria incidence in Africa. Most adult mosquitoes usually rest or hide in shaded areas and vegetation. Some species require locations with high moisture. However, *Anopheles* and *Ae. aegypti* rest in shaded areas with low humidity, such as homes. Several other mosquito species may rest in outdoor shaded areas. *Culex nigripalpus* and *Ae. albopictus* usually rest in vegetation with high humidity. The latter 2 species generally spend more time feeding on sugar and water. Understanding the resting behavior, and type of vegetation that mosquitoes prefer to inhabit, will benefit a district's control program by reducing area-wide application of pesticides.

## VI. OVIPOSITION BEHAVIOR IN RELATION TO MOSQUITO CONTROL

Location and selection of an oviposition site is an essential part of the life history of all mosquito species and usually involves visual, olfactory, and tactile stimuli (Bentley and Day 1989). While this behavior is linked with endogenous regulation, environmental factors, such as rainfall, relative humidity, ambient temperature, and wind speed also are influences. Oviposition site selection involves behaviors associated with landing, substrate contact, substrate evaluation, egg laying, and departure from the oviposition site. The entire process requires a complex integration of physical and chemical cues that is a critical factor for species survival and have important implications with respect to mosquito control (Bentley and Day 1989). Moreover, behaviors associated with oviposition site selection and oviposition can vary considerably by mosquito species.

Gravid female mosquitoes orient to substances referred as "attractants and stimulants" and "repellents and deterrents". An oviposition attractant is defined as a substance that causes mosquitoes to make oriented movements toward the source, while an oviposition stimulant is a chemical that elicits oviposition. An oviposition repellent is a chemical that causes mosquitoes to make oriented movements away from the source, and a deterrent inhibits oviposition. If we understand the oviposition habit and site selection processes used by gravid mosquitoes, we could then select suitable surveillance/control strategies to known oviposition sites. A number of gravid traps and ovitraps (including lethal ovitraps) have been tested and developed for such use (Perich et al. 2003, Xue et al. 2000).

As stated earlier, attractants and stimulants can influence the oviposition site selection by gravid mosquitoes. Researchers have extracted and analyzed a variety of substances from larval production sites (i.e., water, soil, and plants) containing different stages of mosquitoes (Bentley and Day 1989). Some substances have been evaluated in laboratory and field studies while others previously isolated from egg rafts have been marketed for population surveillance and control in Europe. In additional, a number of topical repellents have been tested as oviposition deterrents and repellents with some demonstrating promising control (Xue et al. 2000).

In summary, the effectiveness of adult mosquito surveillance and control is directly influenced by the behavior of the target species in question. When based on mosquito biology and natural history, host-seeking behavior may be manipulated and exploited to benefit clients of control districts which directly results in increased personal protection from nuisance and disease-bearing mosquitoes.

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# MOSQUITO SURVEILLANCE IN PALM BEACH COUNTY TO DETERMINE THE PRESENCE OF LARVAL *ANOPHELES* DEVELOPMENTAL SITES DURING THE 2003 MALARIA OUTBREAK

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## I. INTRODUCTION

Malaria is one of the world's leading causes of arthropod-borne human morbidity and mortality worldwide, with up to an estimated 500 million clinical cases every year, according to the World Health Organization (2000). The disease is caused by a parasite in the genus *Plasmodium* and is transmitted to humans through the bite of an infected mosquito. Malaria was eradicated from the United States in 1949, but imported cases are still reported annually. Most malarial infections in the United States occur in persons who have traveled to countries where transmission is ongoing (Kachur et al. 1997). Occasionally, these infected persons are exposed to biting mosquitoes before seeking medical treatment. Competent resident mosquito vectors ingest the parasite while taking a blood-meal and may, in turn, transmit the parasite to other people, resulting in locally acquired human malaria cases.

Permanent reintroduction of malaria into the United States is of some concern. Two recent outbreaks of locally acquired, mosquito-transmitted malaria in Virginia in 1998 and 2002 demonstrate the continued risk of endemic mosquito-transmitted malaria in heavily populated areas of the eastern United States (Robert et al. 2005).

In the fall of 2003, there were 8 locally acquired human malaria cases confirmed in Palm Beach County, Florida. The strain was determined to be *Plasmodium vivax* from an unknown immigrant from Central or South America. In response to this localized outbreak, surveillance for larval developmental sites was performed by Florida Department of Agriculture and Consumer Services (FDACS) with the assistance of several other organizations. Malaria is only transmitted through the bite of an infected *Anopheles* sp. mosquito. In Florida, the primary vector is *Anopheles quadrimaculatus* Say, although a few other species in this genus are also capable of transmission. The goal of the surveillance effort was to locate any larval developmental sites producing *Anopheles* mosquitoes, to capture adult female *Anopheles*, and test them for the presence of malaria parasites.

Surveillance efforts focused on pinpointing possible development sites in the neighborhoods where the patients lived. Information available indicated that most of the patients remembered being bitten by mosquitoes in the evening outside their homes. Several people had also traveled to different areas of the county. Local travel history information was very general, and none of the information obtained suggested that there was a common area of the county where all the patients had traveled during their incubation periods.

All case residences were located within an 8.0 square mile area in Palm Beach County, Florida. This area is considered too large for the flight range of *An. quadrimaculatus*, which is generally no greater than 2.0 miles. This suggested that there were multiple pockets of infected *Anopheles* in the county, or not all patients acquired malaria at their residences. The latter was considered the most likely scenario. In addition, surveillance for larval development sites was conducted throughout the 8 square mile area to determine the most likely *Anopheles* production source.

## II. TRAPPING AND SURVEILLANCE METHODS

Larval *Anopheles* mosquitoes develop in fresh water, such as ditches, ponds or other bodies of standing water generally with some vegetation or algae mats. Expansive ditch networks run both east-west and north-south for water drainage throughout Palm Beach County. Most of these ditches are well maintained, contain ample numbers of fish, and upon inspection were usually devoid of any mosquito larvae. Some ditch lines are no longer maintained and have become choked with vegetation, causing them to become semi-permanent or temporary waters which produce mosquitoes. Larval dipper samples were conducted extensively in such habitats favorable for *Anopheles* development.

Two different methods were used to collect adult mosquitoes. The first method used resting boxes. Five red plywood resting boxes were placed on the ground under vegetation in shaded areas. *Anopheles* mosquitoes are night feeders, and as the sun rises they search for dark, humid, and quiet places to rest. The resting box provides an artificial hiding place, and mosquitoes were aspirated out of the boxes in the morning. Another type of makeshift resting boxes were also used and fashioned out of plastic (black and green) trash cans. The openings of all resting boxes faced west, so that they would remain shaded from the morning sun. Some *Anopheles* were captured by this method, but not in great numbers.

The second method for collecting adult mosquitoes used CDC-type miniature light traps baited with dry ice. Each trap was equipped with a photo-sensor so that the trap would only run from dusk until dawn. Each trap was equipped with flaps that would close once the trap shut off to keep mosquitoes from escaping before morning collections. All traps were located in areas that were likely to capture high numbers of mosquitoes.

## III. SURVEILLANCE RESULTS

Surveillance was conducted throughout Palm Beach County from September 3, 2003 to September 14, 2003, focusing on areas near case residences. *Anopheles* adults and active production sites were only found near the residences of cases 1, 2, 4 and 8 (Table 1). These sites were within a 2.0 square mile radius of each other and were the only *Anopheles* larvae encountered after extensive surveillance throughout the 8.0 square mile area. At the three outermost and southerly case locations (Cases 3, 5 and 6), no sources for *Anopheles* production were found after careful inspection. Travel histories for these patients were the most unspecific. It is unlikely that those people were infected at their residences.

Three cases were tightly clustered geographically (Cases 1, 2 and 4). Cases 1 and 2 were located on the same street and were likely infected at the same outdoor function. Cases 4 and 8 were probably infected at a nearby homeless camp. We found some *Anopheles* larvae in 2 different ditches near these locations. Another pond nearby was also a good source of *An. crucians.* While not the primary vector, this species could not be ruled out as a possibility.

Traps and resting boxes were set by production sites where *Anopheles* larvae were found, as well as at other reasonable sites

Table 1. Overview of adult and larval mosquito surveillance results near case residences and onset date of malaria symptoms, Palm Beach County, FL, 2003.

| Case # | 1 & 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| Symptom Onset Date: | 7/18 & 7/20 | 7/12 | 8/18 | 8/12 | 8/17 | 8/19 | 9/14 |
| Homeless camp nearby? | Yes | No | Yes | No | No | No | Yes |
| Ditch networks nearby? | Yes | No | Yes | No | No | Yes | Yes |
| *Anopheles* nearby? | Yes | No | Yes | No | No | No | Yes |
| Known exposure? | Yes, at residence | Unknown | Yes, at camp | Unknown | Unknown | Unknown | Unknown |

within the county, in order to collect adults. A total of 1,103 *An. crucians*, 24 *An. quadrimaculatus*, and 133 *Anopheles* spp. were captured from sites in Palm Beach County during the trapping period. All mosquitoes caught were identified at the Palm Beach County Mosquito Control facility and all *Anopheles* mosquitoes were shipped on dry-ice to the Centers for Disease Control (CDC) for testing for the presence of malaria parasites. All of the specimens collected were found negative for parasites.

## IV. RESULTS AND DISCUSSION

It can be difficult to find an infected mosquito even when infection rate is high. Perhaps if more mosquitoes could have been tested, a positive mosquito would have been found. Unfortunately, other trap types which may have been more successful, such as UV-light traps, were not available for use. Backpack aspirations were unsuccessful in capturing *Anopheles* mosquitoes. Overall trap collections were extremely low. Reasons for this may be due to nightly truck spraying by mosquito control where the cases occurred.

Another major obstacle was the lack of detailed local travel history from patients to determine whether patients had visited similar locales during their incubation periods. Reasons for this may have simply been patient difficulty remembering recent activities and travel; however, this information is crucial to surveillance efforts in locating common exposure sites.

The 2.0 square mile area where *Anopheles* larvae and adults were found was also the area where the cases were most clustered. This area is within the flight range of an *Anopheles* mosquito. Another interesting observation was that *Anopheles* larval production was found only near areas where active homeless camps existed. It is a possibility that a homeless and/or illegal immigrant worker residing in such a camp may have been the introductory, or *index* case. Such a person would have high exposure to biting mosquitoes, and may have been infected with malaria or had a relapse which infected the local mosquito population. Such a person may also not have sought out medical treatment, perhaps due to fear of deportation, lack of medical coverage, or cultural beliefs. Area hospitals were on alert for additional cases through 2004. No more cases were ever confirmed and the outbreak was deemed officially over by November 2003. It is unlikely the index case will ever be discovered.

The findings from the surveillance efforts in Palm Beach County helped mosquito control personnel to focus their control efforts and increase efficiency in reducing mosquito populations and prevent new cases. Surveillance for active larval production sites and testing for the presence of parasites in adult mosquitoes can help pinpoint the source of the outbreak, and is a crucial component of malaria control strategies.

## V. SPECIAL ACKNOWLEDGEMENTS

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# AN OVERVIEW OF MALARIA: A MAJOR THREAT TO GLOBAL HUMAN HEALTH

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## I. INTRODUCTION

Malaria is one of the most debilitating diseases afflicting people, and has played a major role in shaping entire civilizations and the outcomes of wars throughout human history. According to the World Health Organization (2004), the incidence of human malaria is estimated to be 300-500 million clinical cases each year in Africa, south of the Sahara, and is thought to kill more than 2.7 million people worldwide each year. Most fatalities are children under the age of 5 years, and these childhood deaths account for nearly 25% of total child mortality in Africa (World Health Organization 2000).

The disease is caused by an intracellular protozoan in the genus *Plasmodium* and is transmitted to people through the bite of an infected *Anopheles* sp. mosquito. Of the more than 50 species of *Plasmodium* infecting a wide variety of animals, only 4 will cause malaria in humans. These 4 species are *Plasmodium vivax*, *P. falciparum*, *P. malariae*, and *P. ovale*. Of these, *P. falciparum* (also called "falciparum malaria") is the most common, most deadly, and most difficult to treat. According to the World Health Organization (1997), *Plasmodium falciparum* occurs in 92 of 100 countries where malaria is considered endemic. It is the main cause of severe clinical malaria and mortality striking young children, non-immune adults, and women during their first pregnancy. *Plasmodium falciparum* is also the predominant malarial species in tropical Africa, eastern Asia, Oceania, and the Amazon area (World Health Organization 1997).

In most malaria-free countries, the risk of sustained (re)introduction of malaria transmission is minimal: either transmission historically never occurred, or socioeconomic development is so advanced that reintroductions can be identified and eliminated in a timely manner (World Health Organization 1997). However, in developing and third world countries low standard of living and governmental instability are major contributing factors to the persistence of malaria transmission. Even when public sector treatment facilities exist, inadequate use of these facilities for the management of malaria is the rule because of their poor quality (World Health Organization 2000). The challenge to malaria control operations is to overcome these obstacles and reach the populations at risk.

## II. MOSQUITO VECTORS

While only *Anopheles* mosquitoes can transmit human malaria, and only female mosquitoes will blood feed, there are several different species which transmit malaria in different parts of the world. In Florida, the historical vector is *Anopheles quadrimaculatus*. In many areas of South and Central America, the vector of major concern is *An. darlingi*. The geographical range of this mosquito is spreading, and the entry of this species into the ecology of new areas has been associated with increases in malaria transmission (Aramburu et al. 1999).

In Africa, mosquitoes in the species complex *Anopheles gambiae* are the principle vectors for malaria. The complex is comprised of six named and one unnamed morphologically similar species. In sub-Saharan Africa, this complex is responsible for approximately 80% of global malaria morbidity and mortality (Levine et al. 2004). These are among the best malaria vectors in the world, although vector capacities vary between species and populations (Léong Pock et al. 2003).

*Anopheles gambiae*, and many other *Anopheles* vector species, develop in temporary fresh waters exposed to sunlight, such as residual pools in drying river beds, ponds, or drainage ditches. *Anopheles* larvae can also readily be found in irrigation ditches and flooded rice paddies. Workers who spend long hours farming such fields or cutting lumber in the forest near larval production sites are at highest risk for infection, due to their close and extended proximity to vector habitats.

While larval production sites are usually located outside of village perimeters, the vector *An. gambiae* sensu strictu prefers to feed on humans versus animals (Levine et al. 2004), and is exquisitely adapted for entering houses (Lindsay et al. 2002). *Anopheles gambiae* (and other *Anopheles* vectors) are active at night when people are asleep and unable to defend themselves from being bitten. The mosquitoes can detect host odors drifting out of the eaves and other openings of houses (Lindsay et al. 2002). They get indoors because of one simple behavioral trait: when gap at the top of the wall (Lindsay et al. 2002). Once inside the house, the mosquito follows the scent trail of host odors, including carbon dioxide emitted by breathing, to find their sleeping host.

## III. ORIGINS AND EMERGENCE OF MALARIA

Human malaria parasites probably originated in Asia or Africa and spread throughout the Old World along with human migrations. There was no record of malaria in England until the 15th century, and it is possible that malaria was introduced into England from soldiers returning from the Crusades. According to Kuhn et al. (2003), historical records show that a malarious illness caused high levels of mortality in the British marshlands and fens from the 15th to the 19th centuries. Malaria remained endemic throughout many marshland communities in southern England throughout the 18th and 19th centuries.

The manner in which malaria was introduced into the Western Hemisphere is uncertain. Writings from ancient civilizations make no reference to any malaria-like diseases (Bogitsch and Cheng, 1998). It is most likely that Spanish conquistadors and African slaves first brought the parasite into the New World. Malaria was endemic throughout the Western Hemisphere, including the United States, and even areas in Canada up until the 20th century. Once malaria was established in North America, it became a major contributor to annual morbidity and mortality, especially during military conflicts. During the Civil War and the Spanish-American War, U.S. troops were severely incapacitated by the disease. More than 25% of all hospitalizations during those conflicts were malaria patients (Bogitsch and Cheng, 1998).

Perhaps no other disease has played as much a role as malaria has played in causing human casualties during wartime and military conflicts around the world. In World War II malaria epidemics critically affected both the Japanese and Allied forces in the Pacific Islands and in Southeast Asia (Fenner 1998). It is claimed that during the Vietnam conflict, malaria was second only to battle wounds as the most common cause for hospitalization among American forces (Bogitsch and Cheng, 1998). As of 1989, most of Africa, south of the Sahara, continued to face an increasingly serious public health crisis as a result of the disease. Numerous civil wars and social unrest in various African countries, combined with fluctuating meteorological and ecological changes favorable to malaria transmission, contributed to these outbreaks (World Health Organization 2000).

## IV. FACTORS CONTRIBUTING TO ERADICATION OF MALARIA IN THE U.S. AND U.K.

By the 1820s malaria incidence began to decline in Great Britain, as well as in some areas in the United States. The decline is ttributed to improved living conditions, as ouses became less suitable for resting mosquitoes. Dark and damp houses were eplaced by light, white-washed walls, and ater, electricity became commonplace. People started to sleep in different rooms and ipstairs, making it more difficult for mosquitoes to find a sleeping host (Lindsay et al. 2002). Increasing livestock densities may ave diverted biting from humans toward cattle, pigs and horses (Kuhn et al. 2003). Cattle numbers rose and were kept outside he home, providing an alternative blood ource (Lindsay et al. 2002). By the mid 1900s, the advent and widespread installation of air-conditioning and window screens furher helped to reduce malaria transmission. The development of drug treatments and the widespread application of DDT, along with draining of swamps and other mosquito production habitats, helped to eliminate malaria completely from the United States and Europe by the mid-20$^{th}$ century.

## V. FACTORS CONTRIBUTING TO MALARIA IN DEVELOPING COUNTRIES

One of the most common problems in countries where malaria remains endemic is he lack of access to adequate and expedient health care because many people nhabit rural areas far from clinics and hospitals. If adequate facilities do exist, they are often located too far for people to make the ourney on foot.

Another factor is that many rural workers engage in regular forest activity or farming that involves extensive time in forested or irrigated agricultural areas, which ncreases malaria risk (Erhart et al. 2004). n a 2004 community study in Vietnam conducted by Erhart et al. (2004) forest activity was the primary means of exposure to malaria. People who engaged in this activity experienced a 10-fold increase in the probability risk factor of acquiring an infection with a subsequent 4-fold increase in probability of exhibiting clinical signs of disease.

Increased agricultural and forestry production increases the economy and mproves human nutrition, but these activities are linked with increased risk for acquiring diseases such as malaria. The links are complex, and risks depend on the different malaria vectors and transmission potential, different health systems, and different agroecologies (Elsevier 2003). Irrigation systems and submerged rice paddies often contribute to *Anopheles* spp. production. The agricultural and natural resource management practices, as well as vector control strategies, that might be implemented to reduce malaria transmission risks have been described in general terms, but specific interventions have yet to be developed (Elsevier 2003).

In altitudes higher than 1500 m and rainfall below 100 cm per year, malaria endemicity decreases but the potential for epidemic outbreaks increases (World Health Organization 1997). This is because populations experiencing continuous pressure from *Plasmodium* parasites tend to build natural immunities which help to reduce mortality and morbidity in those affected areas. Populations living at elevations where temperatures are too cold to support malaria vectors have no exposure to the parasite, and hence will have no natural immunity to the disease. Consequently, during unseasonably warm periods, or periods of increased precipitation, malaria may move into higher non-endemic elevations causing outbreaks.

Another cause of malaria moving into a previously non-endemic area is due to the mass movement of people such as refugees from war-torn areas. High densities of people may inhabit refugee camps where living conditions can be very poor, with tents open to the elements and lack of adequate healthcare or drug treatments.

## VI. WAYS TO PREVENT MALARIA IN DEVELOPING COUNTRIES

One of the easiest and cost-effective ways to prevent malaria transmission is by screening homes to limit mosquito entry. Studies conducted by Konradson et al (2003) have shown that malaria risk is higher among people who live in poorly constructed houses. One of the first intervention trials against malaria was conducted by Angelo Celli in 1899, which showed an average of 96% protective efficacy against malaria when windows were covered with thin muslin and doors were screened with a metal net (Lindsay et al. 2002). Sir Patrick Manson conducted studies in 1900 in which volunteers lived in a screened hut under bed-nets in a malarious area near Rome and not one of them contracted malaria, unlike most of their neighbors, who fell sick with the disease (Lindsay et al. 2002). By 1910, screens were used to protect Europeans living in the tropics and those building the Panama Canal (Lindsay 2002).

Another practical method to keep mosquitoes from entering homes is to install ceilings to close the gap between the top of the wall and the rooftop eaves, where mosquitoes are most likely to enter. The simplest method to accomplish this is to fill the eaves with mud and build homes off the ground (Lindsay et al. 2003). Mosquitoes fly close to the ground when searching for a blood meal, and homes built up on stilts allow the mosquitoes to by-pass the home completely.

One of the most studied, and widely implemented methods, is the use of insecticide treated bed-nets (ITNs) to reduce malaria mortality and morbidity in a range of environments throughout the African and Western Pacific regions (World Health Organization 2000). Erhart et al. (2004) showed that using ITNs properly can significantly reduce malaria infections, and even untreated bed-nets can have a protective effect ($p<0.001$ in one study). However, there are problems with implementing long-term usage of bed-nets by communities to prevent malaria transmission. One of the most commonly encountered obstacles is the discontinued use of bed-nets once local transmission rates have decreased (Binka et al. 1997). People are less willing to use bed-nets when mosquito nuisance problems are reduced, either due to low temperatures or living further from mosquito production sites. Studies have also shown that people may exhibit greater morbidity when they cease using the bed-nets reliably (World Health Organization 2000). Similar changing patterns of ITNs use by season, and difficulties in encouraging community sustainable approaches to ITN use were also described in Tanzania (Winch et al. 1997). Additional reasons for not using ITNs, among households where malaria transmission is both perennial and intense, include lack of availability of bed-nets and other social or technical reasons, such as the net being too small, no room to hang a net, forgetting to hang the net, or the net causing disruptions of sleeping arrangements (Alaii et al. 2003). In order for bed-nets to be effective in preventing malaria transmission, they must be readily available to all residents and must be used regularly even when mosquito biting nuisance is low.

The use of indoor residual insecticide spraying continues to be the main vector control measure implemented in malarious areas (World Health Organization 2000). Reliance on spraying with conventional residual insecticides, such as DDT, is being decreased while new-generation insecticides such as the pyrethroids are now used as replacements (World Health Organization, 2000). These new, although safer, insecticides are considerably more costly to control programs. Another fear is that continued wide-spread applications of only one class of pesticides, such as pyrethroids, may select for mosquito resistance to the insecticides in that class.

One area of major focus is for improvement of healthcare and healthcare reforms in developing countries. Organizational reforms have included restructuring the ministry of health, decentralization of planning, budgeting authority, control of financial resources and responsibility for implementation of program activities (World Health Organization 2000). Increased efforts are being made to implement community-based, sustained education, and disease prevention programs. Governmental reforms and improvements are long-term efforts and take considerable time. Community-based programs are effective, but there is difficulty in sustaining interest and funding for such long-term programs.

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# THE ARBOVIRUS LIFECYCLE: LINKS IN A CHAIN...

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Arthropod-borne viruses are maintained and transmitted in nature by susceptible hematophagous insect hosts. This biological interaction commonly takes place within female mosquitoes and provides an integral link in the natural transmission cycle (Chamberlin 1980). Arboviruses can and do pose significant threats to humans as well as many bird and horse species. While the CDC reported the reemergence of dengue in Cuba during 1997, eastern equine encephalitis virus has also been more prevalent in recent years. By transposing the virus life cycle, the infection of the host organism, and the environment, a holistic picture of the virus-host interaction can be visualized (Van Regenmortel 1998).

In a simple generic enzymatic reaction: $A + B \rightarrow C$. Both A and B are substrate molecules that react to produce the product, C. For this reaction to go to completion, all components must be present in a particular quantitative amount, interact in the correct linear order, and reside in the proper ambient environment. All links in the chain are essential to the production of product. The transmission of virus in nature is the product of the successful reaction between virus and host organism. This interaction is potentially undesirable for susceptible vertebrate and invertebrate hosts, but represents a highly successful virus strategy. Mosquito-arbovirus transmission cycles exhibit much complexity and continue to challenge biologists as they combat emerging and reemerging mosquito-associated diseases. Links at the molecular/cellular, host/organismal, and surrounding environment, can present opportunities for identifying weak points in the chain of transmission. By understanding these links, scientists hope to learn how to institute breaks in the infection continuum.

Sindbis virus (SIN) is the prototype Alphavirus belonging to the Togaviridae family, first isolated in Sindbis, Egypt (Taylor et al. 1955). This arbovirus presents a cubic morphology; bounded by a biological membrane, studded with glycoprotein spikes that surrounds a nucleocapsid containing a plus sense, single stranded RNA genome. In our lab, we use this virus to infect the cells of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) (both species originated from Lake Charles, LA). Our objectives are to A) investigate virus-associated and host-associated responses to arbovirus infection, B) describe the biology and pathology of persistent arbovirus infections of the mosquito host, and C) determine differential response to virus infection, at the tissue-level, in order to study differences between these two related mosquito species.

## I. VIRUS INFECTION OF A CELL

Adsorption/attachment, entry/penetration, uncoating/disassembly, replication, assembly and egress/exit are linear sequential steps essential for the permissive infection of a cell (Knipe 2001). Each step is a vulnerable link in the chain of infection, which can be highly variable between cell types and specific viruses. It is frequently stated, "specificity is at the level of the receptor". This indicates that adsorption/attachment, many times a receptor dependent step, can be quite specific. During a productive infection, the virus enters and uncoats inside the cell, usurps the replication machinery of the host cell, replicates genome, translates proteins and assembles progeny virus. Self-assembly, a virus-specific highlight of molecular construction and packaging is followed by exit of mature progeny ready to infect neighboring cells.

Variations in the implementation of these steps exist and depend upon virus genome and host cell type. Each of these generic steps represents essential . . . links in a chain of infection.

## II. VIRUS INFECTION OF A HOST ORGANISM

Entry into a host, primary replication, spread within a host, cell and tissue tropism, cellular injury, host immune response, and/or persistent infection represent the pathway of virus infection in a host organism. Most arboviruses are blood-borne and the route of entry is via the bite of the female mosquito resulting in horizontal transmission of virus that alternates between vertebrate and invertebrate hosts. Research in our lab focuses on the infection of the mosquito host by either membrane feeding on a viremic blood meal or intrathoracic inoculation of viable virus. Primary replication requires negotiation of the mosquito gut following a blood meal. Inoculation of virus via the intrathoracic route permits spread of virus in the hemolymph that bathes most host cells, thereby maximizing the exposure and interaction of virus and receptors.

Our model investigates SIN infection in the mosquitoes *Ae. albopictus* and *Ae. aegypti,* two container-inhabiting mosquitoes indigenous to the state of Florida (O'Meara 1995). We found that both mosquito species remain persistently infected with SIN for their lifespan and detected a peak in virus titer at day 2 post infection (p.i.) in the whole insect. Although the virus titer decreased dramatically by day 3-4 p.i. it was still detected at day 18 p.i. by plaque assay on BHK-21 cells (Bowers et al. 1995). Because this titer persisted until the end of our experiments, it was of interest to determine what tissues supported virus growth. Investigations to identify the tropism of SIN in the whole insect, following intrathoracic inoculation, revealed both virus-permissive and virus-refractory tissues\organs as detected by an immunoassay of virus proteins (Bowers 1995). Head ganglion, thoracic muscle, gut-associated muscles (i.e., anterior midgut, posterior midgut and hindgut), salivary glands, tracheoles, and fat body cells all expressed virus antigens. However, during the same 18 days p.i., virus antigens were not detected in malphigian tubules or ovarioles. This suggests the presence of virus receptors on permissive tissues, and the absence of receptors on refractory tissues. Alternately, if receptors are present, a block in replication and/or protein production could explain the absence of virus antigen. Undoubtedly, this infection is relatively pantropic and it is apparent that SIN replicates in a variety of different host cell types (Brown and Condreay 1984).

Detection of virus antigens in fat body cells, hindgut-associated muscle and tracheoles appeared as intense at day 18 p.i. as day 3 p.i., suggesting persistent support of virus replication. We believe that these cells may represent an arbovirus reservoir in the insect. Virus antigens were completely cleared from the head ganglion and partially cleared from thoracic muscle, midgut-associated muscles, and salivary glands by day 18 p.i. Virus clearance is an immune response that is antiviral in nature (Ahmed et al. 1991, Ulug et al. 1987). We suspect that this differential clearance is driven by a host response to rid the virus. Molecular mechanisms associated with this clearance are unknown, but may involve an antiviral factor (Luo and Brown 1993).

Evidence for pathologic response to arbovirus infection in the mosquito host is gaining momentum in the literature (Moncayo et al. 2000). Interestingly, SIN appears to persist and present pathology in the midgut and salivary glands that are considered integral to virus transmission in nature (Bowers et al. 2005). Destruction of lateral lobes of the mosquito salivary glands and misalignment of myofibrils in the midgut-associated muscles were detected in SIN-infected *Ae. albopictus* (Bowers et al. 2003). Destruction of these structures was co-localized with the detection of virus antigen and appeared by day 5 p.i. Salivary gland destruction was gross in nature, while the pathology in the midgut-associated muscles was observed at the ultrastructural level. It is suspected that these virus-associated effects could adversely impact insect behavior and survival in the wild.

In response to the ultrastructural evidence supporting virus-associated pathology in mosquito gut-associated muscles, we are investigating actin distribution in these cells. Actin is a major muscle cell molecule and we hypothesize that virus infection of this tissue may alter its highly precise spatial distribution. Comparison between actin patterns in mock-infected (uninfected) mosquitoes and SIN-infected insects will eventually be assessed. Figure 1 demonstrates the distribution of the actin protein in uninfected insects, and this distribution was equivalent in *Ae. albopictus* and *Ae. aegypti*. Longitudinal and circular muscle bundles were quite apparent and this documentation of normal histology is prerequisite to our understanding of virus-associated alterations in the insect. The steps from virus entry into a host organism until clearance, persistence or pathology represent a series of obstacles to virus success. The insect is an influential microenvironment and may modulate virus replication, enhance tissue-specific clearance thus permitting survival of both host and infectious entity.

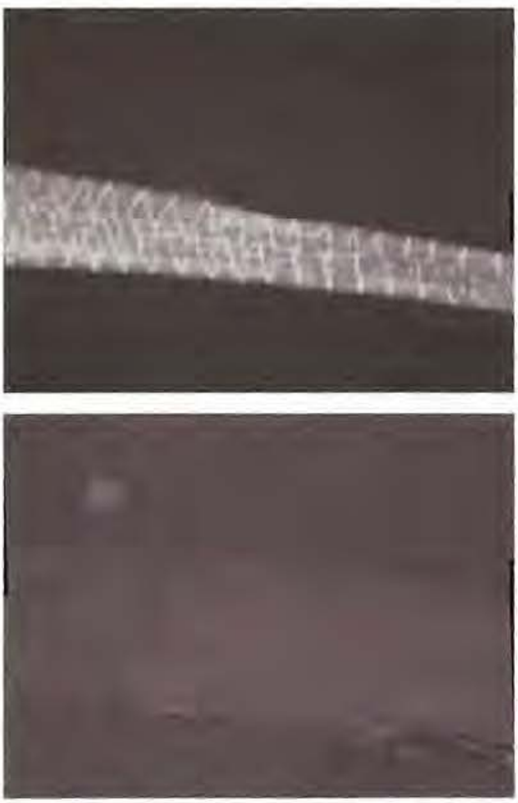

Figure 1. Epifluorescent micrographs of whole mount preparations of anterior midguts of uninfected adult female *Aedes albopictus*. Top photo: Represents the distribution of actin as in circular and longitudinal muscle bundles that were specifically labeled with phallodin. Bottom photo: Displays the negative control in which phallodin was not applied to the tissue. Micrograph image 100× magnification. (Photomicrograph by Mai Vo, undergraduate research student in the Department of Biology, University of North Florida.)

## III. SUMMARY & CONCLUSIONS

The natural cycling of an arbovirus in its arthropod host is a complex process. The interaction of two lifecycles, i.e., virus and mosquito, must be transposed upon a permissive environmental background. In nature, each step in the chain of events from cell infection, host infection, and subsequent host transmission is essential and linked. Just as an enzymatic pathway, these steps are generally linear, proceed as influenced by vector quality, do not skip steps, and continues to completion, i.e., successful transmission. In this regard, it is our responsibility to understand, investigate, and break these links, wherever possible, in order to interrupt cycling of viable virus in the environment.

## IV. ACKNOWLEDGMENTS

The authors thank the Florida Mosquito Control Association Foundation for providing summer funding to Mai Vo, awardee of the 2003 T. Wainwright Miller, Jr. Scholarship. Dr. Paul Linser, University of Florida, Whitney Laboratory, is appreciated for his advice on the phallodin study. Membrane feeding studies were conducted by Kristina Lambert and Andrea Moses, undergraduate research students, Department of Biology, University of North Florida.

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urban, suburban, and rural components, and socio-economic status. With so many different entities involved with WNV surveillance, it was important to provide information prior to the isolation of WNV in Georgia, and to create lines of communication to facilitate continued sharing of information. This included not only providing information to the public, but also sharing data with all of the partners involved in WNV surveillance, education, and control.

## IV. EARLY SURVEILLANCE

In general, surveillance is important for early detection of problems, but without baseline data, it can be difficult to determine when a problem arises. Because it was unknown when WNV would reach Georgia, early surveillance not only provided some baseline data, it also provided a way to work out any difficulties with newly established protocols. In 2000, reports were taken on 2,344 dead birds, with 195 being sent to SCWDS for testing. There were no positives.

## V. SURVEILLANCE 2001-2003

### *1. Human Disease Surveillance*

As previously stated, WNV was first recognized in Georgia in July 2001. That year there were 6 human cases of WNV encephalitis in Georgia, including one death. Initially, human disease surveillance concentrated entirely on neuroinvasive disease. In 2002, WNV was again detected in Georgia and the disease definition was expanded to include milder forms of the disease. Forty-four human cases of illness with 7 deaths were reported. In 2003, the disease definition was changed again to include all acute WNV infections, and Georgia reported 55 verified cases of West Nile virus infection, including 4 deaths. Not surprisingly, human cases tended to be clustered in urban centers throughout Georgia, although cases did occur throughout the state.

Analysis conducted on human cases in 2003 showed the following:

- WNV neurologic illness (flaccid paralysis, encephalitis, meningitis, or meningoencephalitis)—29 (53%)
- WNV fever—21 (38%)
- Asymptomatic WNV infections—5 (9%)
- The average age of cases was 51 years (range 5-83 years).
- The average age of people who experienced neurologic illness was 54 years (range 5-83 years).
- The average age of fatal cases was 65 years (range 51-83 years).
- Thirty-seven (67%) of the 55 cases were male.

### *2. Dead Bird Surveillance*

In 2001, 126 counties submitted birds for testing (Table 1). Fifty-eight counties had birds test positive for WNV, Eastern Equine Encephalitis (EEE), or both. Of the 1,611 birds submitted for testing, 322 tested positive for WNV and 7 tested positive for EEE. In 2002, 113 counties submitted birds for testing. Ninety-three counties had birds test positive for WNV, while 2 counties had birds also test positive for EEE. Of the 2,424 birds submitted for testing, 934 tested positive for WNV while 2 tested positive for EEE. In 2003, 114 counties submitted birds for testing. Sixty-five counties had birds test pos-

Table 1. Dead bird surveillance, Georgia 2001-2003.

| Year | Count | | | Date | | |
|---|---|---|---|---|---|---|
| | # submitted | # counties | # WNV positive | # positive counties | 1st WNV positive | Last WNV positive |
| 2001 | 1611 | 126 | 322 | 58 | 7/9/01 | 11/1/01 |
| 2002 | 2424 | 113 | 934 | 9 | 5/15/02 | 12/12/02 |
| 2003 | 2131 | 114 | 479 | 65 | 1/8/03 | 11/28/03 |

*melanura* potentially increase the risk of EEE transmission in birds, increasing the likelihood that bridge vectors, like *Ae. vexans*, will come into contact with and spread EEE to horses and humans. Increases in *Psorophora* spp. multiples the nuisance impact of mosquitoes and may lead to a need for increased mosquito control activities.

Minimum infection rates (MIR) were calculated for adult mosquito surveillance sites where positive mosquito pools were detected. MIRs were calculated at a specified site, during a specific time period, for *Cx. quinquefasciatus*, using the formula:

$$MIR = \# \text{ positive pools} \div \text{total number of that species of mosquito} \times 1000$$

While an MIR of 6-8 positive per thousand mosquitoes sustained over 3-4 weeks had been set as a level that indicates high risk of human disease (CDC, personal communication), it was found that human cases occurred prior to reaching this level. Moreover, WNV transmission appeared to be more closely associated with increases in *Cx. quinquefasciatus* populations.

In 2003, one county in Georgia submitted 2157 mosquito pools and 366 dead birds to SCWDS for testing. That year in that county, there were 67 WNV-positive mosquito pools, 1 EEE-positive mosquito pool, 27 WNV-positive birds, 1 WNV-positive horse, and 9 human WNV cases. Because of the EEE outbreak, mosquito control efforts were concentrated in areas where this virus was being isolated, while surveillance continued throughout the rest of the county. Although WNV-positive birds had already been found in the county, the first human case occurred in this county prior to the site having isolated virus in mosquitoes. In analyzing the data, it was found that although high sustained MIRs were spatially associated with human cases, there was no temporal correlation. Instead, *Cx. quinquefasciatus* populations were observed to increase prior to the isolation of virus in mosquito pools and prior to detection of human cases. Sites where high MIRs were detected without human cases were found to have *Cx. quinquefasciatus* populations that did not increase until later in the season or populations that were early and short-term in duration.

## VII. CONCLUSION

After 3 years of surveillance, several conclusions could be drawn from the available data. First, Georgia is a big state. Second, our surveillance data are very inconsistent. And finally, three years of data are not enough. However, the GDPH did use the available data to revise surveillance guidelines, these include:

- Monitoring and mapping calls about dead birds (and educating people who call in).
- Shipping a limited number of dead birds throughout the year, especially early in the season, late in the season, and where there are clusters of calls.
- Setting gravid traps where there have been positive birds and/or a number of calls about mosquitoes or dead birds; using map data to avoid clustering trap sites.
- Where possible, setting light traps at the same sites to monitor overall mosquito populations.
- Checking to see if the number of *Culex* spp. are rising starting in April or May. Shipping mosquitoes for testing starting in July.
- Mapping positive horse sites and providing education for horse owners.

Available data were also used to make recommendations about risk reduction. These are:

- Larviciding should start as early in the year as is possible.
- There is a NEED TO PERSONALIZE RISK—personal contact has been found to be more effective than media reports for getting the personal protection risk reduction messages to the public.

# THE MOSQUITO CONTROL PROGRAM IN HERNANDO COUNTY, FLORIDA

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## I. HERNANDO COUNTY

Hernando County is located on the western side of Florida's peninsula and is bordered to the north by Citrus County, to the east by Sumter County, to the South by Pasco County, and to the west by the Gulf of Mexico. It covers approximately 477 sq. miles including the cities of Brooksville (county seat) and Weeki Wachee. Unincorporated areas include Spring Hill, Ridge Manor, Ridge Manor West, Bayport, Aripeka, Lake Lindsey and Hernando Beach. The county stretches 37 miles from East to West and 18 miles from North to South. Within Hernando County's jurisdiction there are 120 sq. miles of environmentally sensitive lands including Chassahowitzka National Wildlife Refuge (owned by Florida Department of Environmental Protection), Weeki Wachee Swamp (owned by Southwest Florida Water Management District), and Withlacoochee State Forest (owned by Florida Department of Forestry). The population in Hernando County was 17,004 in 1970, and has risen to nearly 150,000 in 2005 with most of the current residents coming from out of state.

## II. HERNANDO COUNTY MOSQUITO CONTROL

The mosquito control program of Hernando County consists of the Mosquito/Aquatic Weed Control Division within the Department of Public Works. It is located at the Department of Public Works/Fleet Compound, Brooksville, approximately 10 miles west of I-75. The current year's (2005) operational budget for mosquito control is approximately \$640,000 (including state funding). The Division administers an aquatic weed control program with an annual budget between \$110,000 and \$500,000 (depending upon the level of aquatic weed infestation in local waterways).

Mosquito control staff consists of a director, a coordinator/surveillance technician, four full-time field technicians, and 5-10 part-time seasonal spray truck operators. Equipment utilized for mosquito control includes five spray trucks, an ATV (All Terrain Vehicle), an airboat, a Jon boat, and a Polaris 6-wheel Ranger. The cost of mosquito control, per capita, is \$4.27 per person. This is considerably less than many other Florida mosquito control programs in counties with populations greater than 100,000. The mosquito control program in Hernando County consists of surveillance, source (habitat) reduction, larviciding, biological control, and adulticiding.

## III. SURVEILLANCE

**Service Requests:** Mosquito surveillance is carried out by service requests, light traps, landing rate counts, and larval sampling. Service requests received via phone calls (handled by the receptionists at the Department of Public Works), e-mails, and website posts from the public are logged into a computer database. They are then distributed to field technicians for inspection and treatment. Service requests are the best indicator of increasing mosquito populations, particularly when it comes to daytime biters such as *Aedes* and *Psorophora* species. The annual number of service requests in Hernando County varies from 500 to 2,000. Requests have increased with the introduction and awareness of West Nile Virus in Florida (public panic?). Service requests are received year round, but peak during July-October.

**Larval Populations:** Larval and pupal sampling is conducted to determine the need for treatment. Permanent sites are monitored for mosquito larvae year-round by the staff. Transient sites, due to flooding, are inspected immediately following the start of the rainy season. Samples of larvae are brought back to the laboratory and placed into a "mosquito breeder" (BioQuip Products, Gardena, CA) where they are allowed to emerge into adult mosquitoes for identification.

**Landing Counts:** A total of 100 sites across the county are chosen for landing rate counts. Landing rate counts are important for making decisions on the need for spraying when and if trapping data is not available. In Florida, a landing count of more than 5 mosquitoes per minute warrants control action (Florida Administrative Code, 5E-13.036).

**Trapping:** Hernando Co. Mosquito Control Division operates 16 unbaited CDC light traps 24 hours a day, seven days a week. Specimens are collected, from these traps, three times a week for identification. In 2003, the most abundant species collected was *Anopheles crucians*, which comprised 34% of the collections from a total of 25,000 specimens. Other common species collected, in descending order, were: *Culex nigripalpus* (16%), *Cx. erraticus* (14%), *Uranotaenia sapphirina* (12%), and *Ur. lowii* (11%). *Anopheles crucians* occurs year round. *Culex nigripalpus* peaks in late August through October. *Uranotaenia lowii* and *Ur. sapphirina* are abundant from March to November. The five species, above, comprised the majority (87%) of the specimens collected during 2003. Also, the floodwater species *Psorophora ferox* sometimes occurs in collections and peaks during July through September.

## IV. CONTROL OF LARVAL MOSQUITOES

Hernando County Mosquito Control Division is using Integrated Pest Management measures for controlling pest and disease-carrying mosquitoes in an environmentally safe manner by using a combination of source reduction, larviciding, biological control, and adulticiding.

**Source Reduction:** While servicing clientele requests, mosquito control field technicians often educate property owners about source reduction. Source reduction methods can include turning over water holding containers and cutting down or trimming high vegetation to eliminate mosquito habitat and harborage. Field staff also recommends that clogged ditches be cleaned or modified to drain better. Additional avenues of source reduction include collecting and removing waste tires an important step in eliminate eliminating mosquito larval production sites. Hernando County Mosquito Control Division also conducts aquatic weed control on waterways. Mosquito control benefits from the aquatic weed program through the treatment of cattails, water lettuce, water hyacinth, and other aquatic vegetation that offers habitat and harborage for mosquito larvae. The Mosquito Control Division has invested in a slope mower to cut high vegetation along banks of lakes, rivers, canals, drainage areas, and ditches. In this manner, vegetation management not only reduces mosquito habitat, and harborage sites, but also field inspectors can gain access, if need be, to treat the water for larval control.

**Larviciding:** Chemicals used to kill mosquito larvae are in four categories 1) organophosphates (Abate©), 2) biorational products (*Bacillus thuringiensis* var. *israelensis* [*Bti*] and *B. sphaericus*), 3) insect growth regulators (Altosid©), and 4) monomolecular films (Agnique© MMF). These substances are applied strictly according to label rates and requirements, and usage is based on each type of larval developmental site. Abate is used to kill mosquitoes in temporary waters that have no likelihood of sustaining fish, wildlife, cattle or pets, and which have no direct access to canals, lakes, or rivers. Altosid products are primarily used to target early instars or pre-treat known developmental sites. In this latter case, Altosid XR© briquets are used in an attempt to prevent larval production at the source. Agnique

MMF is used to treat late instars and pupae, especially in defined areas (small bodies of stagnant water). *Bti* products are applied extensively against mosquito larvae in most water bodies while *B. sphaericus* is used in water with high organic content, such as sewage, to control *Cx. nigripalpus* and *Cx. quinquefasciatus* larvae.

**Biological Control:** We obtain mosquito fish, *Gambusia*, from the Florida Fish Hatchery in Sumter County and release them in permanent bodies of water for larval control. We also have these minnows stocked in two ponds and a tank at our facility, and in a condemned swimming pool on a church property. Over the years, our Division has been very successful in placing *Gambusia* throughout the county in drainage retention areas, ponds, and any bodies of water that stand for fairly long periods of time capable of producing mosquito larvae. In addition to considering mosquito larvae a delicacy, *Gambusia* reproduce very rapidly once placed into a new environment, so they provide continuous larval control. Using mosquito fish reduces the need to use chemicals, which is a big plus for both the environment and our budget. Each year, more than 300 bodies of water receive mosquito fish.

In addition, *Gambusia* have drawn tremendous public interest after they were advertised by the local media as a biological control agent available free of charge to the public. Many residents have called our office requesting the "mosquito-eating fish" be released into their ponds and animal water troughs.

## V. ADULTICIDING

When adult mosquitoes are abundant and mosquito-borne disease threats are high, adulticiding is the only control approach available. Hernando County's mosquito control program uses only ground ultra-low-volume (ULV) machines for adulticiding. The ULV machines are mounted on trucks, a Polaris 6-wheeler, and a Polaris ATV (all terrain vehicle). Truck-mounted machines (London Fog@) are connected to an Adapco Monitor III (coordinated with GPS systems) and operated on a 4-hour shift, ½ hour before sunset, five days a week. Due to a limited number of ULV equipped vehicles, the adulticiding crew will sometimes run a second shift between 2:00 a.m. and 6:00 a.m., daily. The incorporation of double-shifts gives us twice the ground coverage without increasing the number of vehicles and ULV machines. Reducing cost and increasing effectiveness is very important to mosquito control operations, especially for programs with very limited budgets. Our program also conducts sporadic spraying in parks and sites where large outdoor events sometimes occur during the day and/or night.

In addition, floodwater sites produce numerous nuisance mosquitoes, such as *Psorophora* and *Aedes* species, which are active during the day. To battle these pests, the crew may adulticide where these species occur in large numbers. Daytime spraying has given a quick knockout of nuisance mosquito populations, enabling residents to continue outdoor activities.

Malathion has been used previously for ULV applications, but due to mosquito resistance, corrosive damage to vehicles and equipment, and the chemicals' strong irritating odor, we have replaced it with the pyrethroid products, Biomist® and AquaReslin®.

## VI. PUBLIC EDUCATION

Reaching out to the public and media about mosquitoes and their control are two very important tasks for mosquito control professionals. The educational materials developed by the Hernando County Mosquito Control Division staff on personal protection and transmission of West Nile virus include a comprehensive website (www.hernandocounty.us/mosquito), PowerPoint presentations, posters, flyers, door hangers, booklets, fact sheets, display bulletins, news articles, and news releases with regular updates. Educational materials are disseminated by email, website posting, door-to-door visits, distribution to community centers, schools, special interest groups, as well as water bill insertions.

Also a public education network in Hernando County has been formed consisting of county commissioners, the administrator, attorneys, department heads, and local media representatives. The mosquito control director regularly updates this group with information on West Nile virus and interacts with them on urgent mosquito issues. Moreover, through a coordinated effort by the county's communication coordinator, director of mosquito control, personnel from environmental services and county extension office, and the county administrator, several video shows have been produced and regularly broadcasted throughout the mosquito season on the county's government television channel. By educating our public and county officials about the impact of mosquitoes on public health, mosquito control personnel feel they have increased the cooperation from residents and gained more support from the county government. With a greater understanding of mosquito control, county officials are more likely to approve future financial requests.

We also invited a local artist to create artwork representing mosquito control and West Nile virus. Over the past year, he has published several cartoons in the local newspaper. These cartoons, which depict a variety of mosquito topics, were received favorably by the general public.

Electronic and print media have also helped us spread mosquito-related information to the general public, these included four TV channels in the Tampa Bay area (Bay News 9, CBS News, Fox News, ABC News), two radio stations (WWJB in Brooksville and PBS in Inverness and Gainesville), two local newspapers (Hernando Today and Hernando Times), several community publications, and county departmental newsletters.

In 2004, the public education effort of the Hernando County Mosquito Control Division staff gained recognition by several federal and state officials. In that year, Hernando County received an Achievement Award from the National Association of Counties (NACo) for *Multi-Media Effort on Public Education to Battle West Nile Virus*, a Superior Award and a Meritorious Award from the National Association of County Information Officers (NACIO) for a mosquito control booklet and a PowerPoint mosquito control presentation, respectively.

## VII. ACKNOWLEDGMENTS

I thank Drs. J. Howard Frank and George F. O'Meara for their critical review of the manuscript, and Citrus County Mosquito Control for providing our Division *Gambusia* when the State Fish Hatchery in Sumter County underwent facility reconstruction.

# SALT MARSH MANAGEMENT IN FLORIDA: BALANCING MOSQUITO CONTROL AND NATURAL RESOURCE INTERESTS

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## I. INTRODUCTION

Salt-marsh mosquito control programs in Florida typically employ a combination of techniques which target the salt-marsh mosquitoes *Ochlerotatus taeniorhynchus* (Weidemann) and *Oc. sollicitans* (Walker). This Integrated Pest Management (IPM) approach usually includes source reduction (the elimination of larval mosquito production sites), larviciding (chemical applications to kill mosquito larvae) and adulticiding (chemical applications to kill adult mosquitoes). It is widely agreed that when it can be used, source reduction is the most effective of these three techniques (Florida Coordinating Council on Mosquito Control 1998).

The distinction between low marsh and high marsh habitat is important in understanding salt marsh ecology. Low marshes are flooded by twice daily high tides. Along the Indian River Lagoon on Florida's east-central coast, these marshes can be vegetated with *Spartina alterniflora* (smooth cordgrass) and *Rhizophora mangle* (red mangrove). Low marsh habitat does not produce salt-marsh mosquitoes.

High marsh habitat is flooded by rainfall and exceptionally high tides. It is frequently vegetated by *Avicennia germinans* (black mangrove), *Batis maritima* (saltwort), *Salicornia virginica* (glasswort) and *Distichlis spicata* (salt grass). High marshes in Florida are capable of producing salt-marsh mosquitoes in large numbers thus requires the focused energies of mosquito control agencies, especially from spring through fall.

## II. SALT MARSH SOURCE REDUCTION TECHNIQUES

Mosquito control agencies were first created in the 1920s in east-central Florida to control salt-marsh mosquitoes. The first source reduction technique used was ditching. Ditches were usually evenly-spaced regardless of where mosquito production sites occurred. Over time without proper maintenance, many of the ditches filled with debris and vegetation. Also, silting frequently closed the ditches along the estuary edge thus hindering their effectiveness.

Dredging of estuary sediments and using this material to fill marshes eliminated the mosquito problem by transforming salt marshes to upland habitat. However, this method proved to be too slow and costly. When this work was undertaken, the ecological value of salt marshes was not appreciated and resulted in the loss of wetland habitat.

Impoundment construction began in the mid-1950s by building earthen dikes around known mosquito-producing high marshes. Flooding impoundments during the summer eliminates oviposition sites for salt-marsh mosquitoes that lay their eggs on the moist mud because they will not oviposit upon standing water. Over a 15 year period, approx. 40,000 acres of salt marshes and mangrove swamps were impounded along east-central Florida making this the most widely used source reduction technique in the state. 192 impoundments were created over 5 counties, much of it carried out on privately owned land (Rey and Kain 1991).

Impounding is highly effective in controlling salt-marsh mosquitoes and can virtually eliminate the need for chemical control in those areas. It has been shown that resident fish can be very abundant in impoundments. These fish can can provide an abundant food source for wading birds and other predators including threatened and endangered species. Also, impoundments can be managed for waterfowl and wading bird enhancement which frequently occurs on national wildlife refuges such as Merritt Island National Wildlife Refuge in Titusville (Carlson et al. 1991).

On the negative side environmentally, research and management experience has shown that impoundment dikes prohibit marsh access to aquatic organisms which must use the high marsh during a portion of their life cycle such as snook, ladyfish and tarpon. Excessive or prolonged flooding of impoundments can also stress or kill native high marsh vegetation.

## III. INTERAGENCY COOPERATION

By the early 1980s, aerial adulticiding and impoundment management were causing tremendous tensions between mosquito control and resource agencies in Florida. To try and resolve these contentious issues, Governor (now Senator) Bob Graham appointed two committees to promote cooperation. They were the Florida Coordinating Council on Mosquito Control (FCCMC) and it's Subcommittee on Managed Marshes (SOMM). In 1986, these two committees were formally created in the Florida Statutes' mosquito control legislation (Chapter 388.46) (Carlson et al. 1991).

SOMM's role is to provide guidance and review for salt marsh management plans taking into account both the mosquito control and natural resource issues involved. It consists of 13 salt marsh management experts from: 1) agencies responsible for wetlands resources, 2) organizations involved in salt marsh research and permitting, and 3) agencies involved in mosquito control. SOMM meets quarterly at different locations around Florida, with meetings typically including a field trip to relevant sites, followed by a business meeting. SOMM also serves as an information source for salt marsh management. In this regard, at four year intervals SOMM sponsors a "Workshop on Salt Marsh Management and Research" (Carlson et al. 1999). The fifth meeting in this series was held the week of February 14, 2005 in Cocoa Beach, Florida. It was held in conjunction with the "4th Biennial Mosquito Lagoon Conference".

Research since the early 1980s has led to Rotational Impoundment Management (RIM) and Ditching/Open Marsh Water Management (OMWM) as being environmentally acceptable salt marsh management techniques.

## IV. ROTATIONAL IMPOUNDMENT MANAGEMENT

RIM involves installing culverts with water control structures through impoundment dikes to seasonally reconnect the impounded marsh and estuary. Culverts are closed in late spring and the marsh is flooded by pumping estuarine water into the impoundment during the mosquito producing summer months. Risers are set at the minimum height necessary for mosquito control. This allows excess water from rain or upland runoff to spill over the risers into the estuary, thereby protecting marsh vegetation from possible overflooding damage. In the early fall, culverts are opened enabling the annual fall high tides and associated organisms to enter the marsh. Culverts remain open through the spring allowing tidal flow between the marsh and estuary. Thus, RIM allows source reduction mosquito control with a minimum of pesticide use. It also allows for the exchange of nutrients and organisms between the marsh and lagoon during the fall, winter and early spring. During the approximately 7 month open period, soil oxidation/consolidation and vegetative regrowth can occur (Brockmeyer et al. 1997).

## V. DITCHING/OMWM

In some marsh situations, shallow ditches constructed with rotary equipment can be used to provide source reduction benefits. Rotary ditches function in one of two different ways. 1) Ditches can be connected to permanent water bodies (either isolated ponds or estuarine waters), thus providing access for larvivorous fish to known mosquito producing locations. In some situations maintaining a ditch connection to permanent water prevents mosquito oviposition by keeping the site too wet.

2) Alternately, a properly designed ditching system can provide a drainage function allowing insufficient time for immature mosquitoes to complete their development. OMWM originated in the northeastern United States and has had its greatest applicability in north Indian River Lagoon counties (northern Brevard & Volusia). Like RIM, OMWM can result in a greatly reduced need for pesticide use while maintaining most natural marsh functions.

## VI. SURFACE WATER IMPROVEMENT AND MANAGEMENT ACT (SWIM)

In 1987, the Florida legislature established the Surface Water Improvement and Management (SWIM) program. SWIM acknowledged that the water quality of many of Florida's water bodies have degraded, and instructed water management districts to design and implement plans and programs for the improvement and management of surface waters. Concerning salt marsh habitats, SWIM is attempting to reverse the loss of emergent wetlands and reintegrate isolated marshes to the lagoon, thus restoring many of their natural functions. Providing improved habitat for native plants, fish and wildlife has high SWIM priority and implementing RIM in these marshes is being aggressively pursued. Currently, two water management districts (St. Johns River Water Management District and the South Florida Water Management District), with cooperation from local mosquito control agencies, are providing funds for the improved management of salt-marsh impoundments. To date, approximately 27,000 acres of impoundments have been reconnected to the Indian River Lagoon making this program an excellent example of mosquito control-natural resource agency cooperation at the State level (R. Brockmeyer, pers. comm.).

## VII. PESTICIDE ENVIRONMENTAL STEWARDSHIP PROGRAM (PESP)

In December 1994, the EPA, in association with the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA), initiated the PESP program. This was created because of the nationwide need for an approach to pesticide application that also considers environmental stewardship. PESP's primary goal is to reduce pesticide risk. Source reduction mosquito control is a good means to accomplish this goal. A key component of the program is the development of a public/private partnership. The American Mosquito Control Association (AMCA) became a PESP partner in 1997 (Carlson 1997). The Florida Mosquito Control Association also participates in this program. Partnership requires the development of a Strategy Document and yearly reports to the EPA demonstrating how goals are being quantitatively met. In 1999, the AMCA received EPA's "PESP Excellence Award for Pesticide Risk Reduction" and in 2003 AMCA was chosen as a "PESP Champion" for diligence in meeting the goals of this program (DeChant et al. 2004).

## VIII. SUMMARY

Tremendous progress has been made over the past several decades in the wise management of Florida's salt marshes. The public purchase of salt marshes has been an important initiative. We have learned that through research, cooperation and compromise at the Federal, State and local levels, both mosquito control and natural resource goals can be met when managing these environmentally important habitats.

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# BIOLOGICAL CONTROL OF MOSQUITOES

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Biological control is generally defined as the manipulated reduction of an insect population by natural enemies (predators, parasites and pathogens). This is in contrast to natural control where the reduction of an insect pest population is by naturally occurring organisms and environmental factors without human input. Diverse complexes of natural enemies including predators, parasites and pathogens have been reported for mosquitoes and many have been evaluated as biological control agents (Chapman 1985, Lacey and Undeen 1986, Lacey and Orr 1994). All mosquito control methods have advantages and limitations with best use depending on an in-depth understanding of the mosquito species to be controlled and the control agents.

Biological control usually involves augmentative release of predators, parasites or pathogens. Augmentative biological control is the release of new or additional numbers of a natural enemy when too few are present to control a pest effectively. This usually relies on the ability to produce sufficient numbers of the natural enemy in the laboratory for a particular strategy. Inoculative augmentative releases are those in which small numbers of natural enemies are introduced that increase over time to an effective level for control and hopefully become established to exert long term pressure. Inundative augmentative releases are those that involve the mass release of high populations of a natural enemy that provides immediate and effective control of the target pest but may not persist. Inundative augmentative releases of biological control agents have proven to be the most effective in reducing mosquito populations to desired levels.

## I. CHARACTERISTICS AND SELECTION OF BIOLOGICAL CONTROL AGENTS

While there are natural enemies of adult mosquitoes, the majority of biological control agents are directed at the immature stages. Desirable attributes of a biological control agents for mosquitoes include, 1) the ability to regulate the host at low densities; 2) possess a high reproductive rate; 3) the life cycle of the natural enemy should be well synchronized and adapted to that of the host; 4) persistence in the environment with the ability to disperse; 5) host specific; 6) easily cultured or colonized to produce sufficient numbers; 7) have a good shelf life. Not all candidates will possess all of these attributes and selection of a candidate agent for a particular application will depend on a variety of factors. These include the efficacy of the agent, possible environmental impacts, compatibility with other control strategies, and costs.

There are three main groups of organisms investigated for mosquito control. Generalist predators are those that feed on a broad range of mosquitoes and include numerous invertebrates and fish that feed on larvae and/or pupae. Parasites of mosquito larvae are essentially restricted to several species of mermithid nematodes. A large number of mosquito pathogens have been isolated from natural populations of mosquitoes including bacteria, fungi, microsporidia and viruses.

## II. PREDATORS FOR BIOLOGICAL CONTROL

Where large numbers of larval mosquitoes are found, there is usually a complex of natural predators that exert considerable pressure on the population. Recognition of this natural control is an important consideration in selecting tactics that would supplement, conserve, or enhance this natural mortality (Lacey and Orr 1994). Numerous species of fish are the most common vertebrate predators and there are a large number of invertebrate predators that feed to some extent on mosquito larvae including species of Odonata, Hemiptera, Coleoptera, Diptera and Copepoda. The most widely utilized agents from this group are predacious (larvivorous) fish, mosquitoes, and copepods.

*Predacious Fish*

The most widely introduced larvivorous fish to control mosquito larvae is *Gambusia affinis* commonly called the mosquitofish (Meisch 1985). This fish is native to the southern and eastern US, has demonstrated broad tolerances for salinity and organic pollution and is generally more effective against permanent water mosquitoes than against floodwater species. Mosquitofish are opportunistic feeders and are generally more effective in controlling culicine mosquitoes and less effective against anopheline species. Effectiveness of mosquitofish generally decreases as the density of vegetation increases. *Gambusia* can be mass reared in the laboratory or collected in nature to augment problem mosquito developmental sites. The ability of *Gambusia* and other predacious fish to persist after introduction, even when mosquito populations are low or absent, is a key factor for successful control. There are numerous other larvivorous fish that have been utilized for mosquito control such as the common guppy (*Poecilia reticulate*) that are effective in highly polluted water and the least kilifish *Heternadria formosa* that are effective in very shallow water habitats. Concerns do exist for unintended adverse effects on native fish species with the introduction of predacious fish such as *Gambusia*. For example, Courtenay & Meffe (1989) listed reports that implicated *Gambusia* in the decline of various native fishes.

*2. Predacious mosquitoes*

Species within the genus *Toxorhynchites* have successfully been utilized for control of container-inhabiting mosquito larvae (Fox 1985). Larvae of *Toxorhynchites* are large, ravenous predators of mosquito larvae in natural and artificial containers. Dispersal to new, often cryptic, containers is effectively accomplished by gravid adult females which is an important attribute when the target mosquitoes are domestic species such as *Aedes aegypti* (L.) or more rural species like *Ae. albopictus* (Skuse). In addition, these adults do not require a blood meal to produce eggs but utilize natural sugar sources such as plant nectar. One problem with introductions has been that *Toxorhynchites* prefer natural larval habitats (such as tree holes) over artificial containers where many of the target species develop. Inoculative release strategies where *Toxorhynchites* spp. would establish, spread, and persist was an early goal but studies have shown that the most effective strategy has been through regular inundative releases. This requires mass production of *Toxorhynchites* in the laboratory which is labor and time intensive requiring living prey mosquitoes but procedures have been developed for several species (Focks et al. 1985). One of the more thorough investigations with *Tx. amboinensis* (Doleschall) was conducted by Focks et al. (1985) in New Orleans, LA where weekly releases of about 100 females/residential block reduced *Ae. aegypti* densities by 46%. In a subsequent study, ULV ground applied malathion provided about 29% reduction of *Ae. aegypti* and when combined with releases of *Tx. amboinensis*, a 96% reduction was reported (Focks et al. 1986).

*3. Predacious copepods*

Copepods are small aquatic microcrustaceans commonly found in a wide variety of aquatic habitats. Freshwater cyclopoid copepods consume a wide variety of prey types including young instar mosquito larvae. Copepods, mostly *Mesocyclops* and *Macrocyclops* species have been introduced to control larvae of container-inhabiting

*1. Bacteria*

The most successful biopesticide yet developed for applied mosquito control is *Bacillus thuringiensis* var. *israelensis* (BTI) registered by EPA in 1983. This bacterium (that acts as a stomach poison) produces parasporal inclusions that are digested in the larval gut to release a number of toxic proteins. These fast acting toxins cause rupturing of the cells of the midgut epithelium and results in larval death. BTI has a broad mosquito host range and has been used to control many species of medically important and pestiferous mosquitoes around the world. BTI is also very effective against black fly larvae. BTI has been formulated for a variety of habitats to be applied with conventional equipment including pellets, granules, slow release briquettes and flowable concentrates. BTI has minimal impact on non-target organisms and safety for vertebrates is well established (Lacey and Siegel 2000). Because BTI does not have any negative effects on predators, rapid control of mosquito larvae by BTI with prolonged control provided by natural enemies can be a highly effective strategy. The major drawback to BTI is the relatively short duration of larvicidal activity especially in organically enriched habitats necessitating fairly frequent application when mosquito development is continuous. This will increase cost required for effective control because more product and applications will be required. Slow release formulations have improved residual activity, particularly in containers and small larval development sites, but additional improvements would greatly increase the effectiveness of this important microbial insecticide. There are currently about 26 BTI products registered for use in the United States. Aquabac, Teknar, Vectobac, and LarvX are examples of common trade names for BTI mosquito control products. The active ingredient in these products varies from 0.2% -10% with more than 1 million pounds of product used in Florida 2002-2003 (http://www.flaes.org/aes-ent/mosquito/reports.html)

*Bacillus sphaericus* (BS), registered for mosquito control by EPA in 1991, is similar to BTI but has a narrower host range within mosquitoes; it is also not active against black fly larvae. *Psorophora* and *Culex* species are highly susceptible to BS toxins while species of *Anopheles* and *Mansonia* larvae are less susceptible. BS is not effective against *Aedes aegypti* and some other *Aedes* spp. A major advantage of BS over BTI is prolonged larvicidal activity in highly organically environments, a prime developmental site for many *Culex* spp.

Unfortunately, there have been reports of resistance by mosquitoes both in the laboratory and in the field to BS. Extensive use in India, Brazil, and other locations have resulted in extremely high levels of resistance to BS in larvae of *Culex quinquefasciatus* Say, an important vector of diseases such as West Nile Virus (Rao et al. 1995). Both BTI and BS can be mass produced by fermentation and are stable with prolonged shelf lives. VectoLex CG and WDG are registered BS products and can be effective one to four weeks after application. The active ingredient in these products varies from 7.5-51.2% with more than 120,000 pounds of product applied in Florida 2002-2003 (http://www.flaes.org/aes-ent/mosquito/reports.html).

*2. Fungi*

*Lagenidium giganteum* is a facultative parasite of mosquito larvae (McCray 1985). It infects a broad range of mosquito larvae via motile zoospores which can form either sexually or asexually. The zoospores attach to the larval cuticle, penetrate and enter the hemocoel, where mycelial growth occurs, eventually developing into septate hyphae. With larval death, asexually produced zoospores are formed and released through vesicles formed on the larval cadaver surface to complete the cycle.

Alternatively, a sexual phase can occur producing oospores that can resist desication and may remain viable for up to seven years. Upon activation (usually flooding of the habitat), oospores produce and release infective zoospores. Artificial culture methods have been developed for the sexual oospore stage (the dormant phase) which makes *L. giganteum* amenable to commercial production (Lacey and Orr 1994). *Lagenidium giganteum* was registered as a mosquito control agent in 1991. Positive attributes include a broad mosquito host range, its facultative parasitic nature, the ability to recycle and mass production and storage capabilities. Several studies have demonstrated that *L. giganteum* is more effective in clear freshwater habitats between 15-35°C. It is also limited by organically polluted water and saline environments. A commercial product was developed (Laginex®) and is not currently available.

*3. Microsporidia*

Microsporidia are some of the most common pathogens found in mosquitoes worldwide (Becnel and Andreadis 1999). While microsporidia play a role as natural regulators of many aquatic Diptera, the only attempts to utilize microsporidia as control agents in aquatic systems have been against mosquitoes. The attraction of microsporidia for management of mosquitoes lies with their ability to cause larval epizootics that continuously cycle within a host population and spread to new habitats.

Infections in mosquito larvae can be recognized by the porcelain white appearance of the fat body or sometimes in regions of the midgut and Malpighian tubules. This represents large masses of spores that are the infectious stage of the pathogen. The spore is a complex structure that contains a coiled polar filament. Spores are ingested by the host and germinate within the midgut. When spores germinate, the polar filament is expelled and inverted to become a tube for transport of the sporoplasm into the host cell. Some microsporidia, such as members of the genus *Nosema, Vavraia,* and *Edhazardia,* are directly infectious to the mosquito host while members of *Amblyospora* require an intermediate copepod host. Two species evaluated in the field are *Vavraia culicis* against *Cx. pipiens fatigans* Say on the South Pacific island of Nauru (Reynolds 1972) and *Nosema algerae* against *Anopheles albimanus* Wiedemann in Panama (Anthony et al. 1978). The approach taken in these releases was to inundate the habitat with spores for short-term control. While these pathogens could be transmitted to larvae in the field, infection levels were generally low and did not persist. Because it does not require an intermediate host *Edhazardia aedis* has been proposed as a classical control agent for *Ae. aegypti. Edhazardia aedis* affects two successive generations of the mosquito host. This microsporidian parasite is well adapted to *Ae. aegypti* having evolved a number of strategies to ensure long term survival. Two of these strategies involve the highly efficient mechanisms of vertical and horizontal transmission. Vertical infection spreads the pathogen to new, often cryptic habitats and horizontal transmission amplifies the infection by producing additional infected adults and larvae. A field study that involved the inundative release of *E. aedis* produced high larval and adult infections and successfully eliminated the population of *Ae. aegypti* within 11 weeks of the introduction (Becnel and Johnson 2000). Limitations to incorporating *E. aedis* into an integrated control program are high costs involved in production in host mosquitoes and methods to store the fragile spore stage. The only possible field application of this microsporidian parasite would be as part of a classical biological control program to establish *E. aedis* in populations where it does not exist for long term control.

*4. Viruses*

Mosquito pathogenic viruses such as baculoviruses and iridescent viruses have been known since 1963 but none have shown any potential due to the inability to transmit these pathogens to the host. Those with the best potential for mosquito control are the nucleopolyhedroviruses (NPVs) within the family Baculoviridae (Federici 1985). NPVs have been isolated from ten mosquito species within the genera *Aedes, Anopheles, Culex, Ochlerotatus, Psorophora, Uranotaenia* and *Wyeomia.* Historically, mosquito baculoviruses have been extremely uncommon, difficult to transmit and epizootics in field populations were rarely observed. Recently, a newly discovered NPV, designated CuniNPV, has been discovered that was responsible for repeated and extended epizootics in field populations of *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* in Florida, USA (Becnel et al. 2001). CuniNPV infects and destroys the larval midgut of *Culex* mosquitoes causing patent infections within 48 hr post-inoculation (p.i.) and death 72-96 hr p.i. Infections produce small globular occlusion bodies (OB's) that measure approximately 400 nm in diameter and contain four, sometimes up to eight, singly enveloped virions per occlusion body. These particles are ingested by mosquito larvae where the OB's dissolve in the alkaline conditions of the larval midgut. This releases the infectious virions that cross the mosquito peritrophic membrane, attach and enter midgut cells and initiate replication in the nuclei. While initial attempts to transmit this baculovirus to mosquitoes in the laboratory were unsuccessful, further investigations revealed conclusively that transmission is mediated by divalent cations: magnesium is essential for transmission whereas calcium inhibits virus transmission. This has been an important finding in understanding transmission of baculoviruses in mosquitoes and can explain, in great part, conditions that support epizootics in natural populations of mosquitoes and allow evaluation of CuniNPV as a new microbial control agent.

## V. CONCLUSIONS

While there are few alternatives to chemical pesticides for adult mosquito control, there are options for control of mosquito larvae including biological and natural control. Currently, most biological control agents play a secondary or supplemental role to other interventions but could have a greater impact by combining them with each other or more conventional control methods for use in well defined habitats. For example, BTI could be used in conjunction with *Gambusia* for control of mosquitoes in permanent water habitats or with copepods in artificial containers. This strategy would exploit the quick action of BTI to knock down the mosquito population with extended control as a result of predation. Identification of biological agents that are compatible with other components of an integrated program would serve to compliment these control strategies by maintaining effectiveness but in a more environmentally responsible manner.

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# LARVAL CONTROL USING ANTI-DIGESTIVE ENZYME AND TRANSPORTER COMPOUNDS

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The stomach of mosquito larvae is simi-r to the stomach of higher organisms in any respects. It is a long straight tube that ıns from the mouth to the anus and in osquito larvae it is called the midgut. The idgut is divided into three main regions: astric caeca, anterior, and posterior regions ılso called anterior and posterior midgut). Iosquito larvae use mechanical movements transport food from the mouthparts to ıe midgut. These movements are con-olled by specific sets of muscles that form a ircular basket" that run along the length f the midgut.

Larvae digest their food (i.e., plant detri-s, microorganisms, and small particles) sing chemical processes similar to those sed by higher organisms. However, instead f using acid for digestion, larvae take advan-ge of an alkaline environment (high pH) side the anterior midgut. pH varies depend-g on the species but it is maintained within range of 10.5-11.0 (Clements 1992). It has een demonstrated in *Aedes aegypti* (L) larvae at maintenance of pH depends on a func-onal plasma membrane $H^+$ V-ATPase and a gh concentration of bicarbonate/carbon-e ions (Zhuang et al. 1999, Boudko et al. )01). In contrast, bicarbonate/carbonate vels are much lower in the posterior mid-ıt, lumen, and hemolymph (Boudko et al. )01). We have identified carbonic anhydrase CA) as a possible candidate responsible for aintaining the high pH in the anterior mid-ıt. In addition to CA, chloride/bicarbonate changers and chloride channels may also involved. Evidence of CA involvement in e alkalization of the midgut has been pro-ded by time-lapse video assays of pH profiles *vivo*. These assays revealed that ingestion of etazolamide (a CA specific inhibitor) at $10^{-4}$ M eliminated lumen alkalization (Boudko et al. 2001). It has also been previously shown, using Hansson's histochemical staining and *in situ* hybridization, that CA activity and mRNA are localized preferentially in the posterior midgut and gastric caeca of *Ae. aegypti* larvae. Inhibition of the pH gradient within the midgut of this species can be accomplished with acetazolamide and another CA inhibitor, methazolamide (Corena et al. 2002). Furthermore, CA has been cloned from the midgut of *Ae. aegypti* (Corena et al. 2002). We have also measured CA activity in *Aedes albopictus* (Skuse), *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Anopheles quadrimaculatus* Say, *Oc. taeniorhynchus* (Wiedemann) and *An. albimanus* Wiedemann larvae and found that these species followed the same distribution pattern and alkalization of the anterior midgut as *Ae. aegypti*. Methazolamide and acetazolamide also affected midgut alkalization and caused larval mortality in all six test species (Corena et al. 2004).

These combined results suggested that the presence of CA is crucial for the maintenance of alkalization in the midgut and survival of larvae. However, different species were affected to different degrees by the inhibitors. Hansson's histochemical stain, a technique specific for CA activity detection, showed that CA is present in the midgut of other species of mosquito larvae besides *Ae. aegypti*. But this stain inhibited methazolamide in *Ae. albopictus*, *Cx. nigripalpus*, *Cx. quinquefasciatus*, *An. quadrimaculatus*, *Oc. taeniorhynchus* and *An. albimanus*.

We have also measured CA activity in the gastric caeca, anterior, and posterior midgut of larvae of these six species using $^{18}O$ isotope-exchange coupled to mass spectrometry. This method is based on the original method developed by Mills and Urey (1940) that was adapted to measure intracellular CA activity in cell suspension (Itada and Forster 1977). The result was a theoretical expression that allowed calculation of CA activity in intact red blood cells. We have adapted this method to measure CA activity in mosquito larvae tissue homogenate (Corena et al. 2002). We found that the histochemistry results were positively correlated with those from mass spectrometry and that localization of CA was, in fact, species dependent. In terms of CA localization in the different regions of the mosquito larval midgut, it is now apparent that all of our test species exhibited CA activity in the midgut although to various degrees. CA activity was consistently detected in the gastric caeca of all species (Fig. 1) while localization in the posterior and anterior midgut appears to be species dependent. We detected CA activity in the anterior midgut for most species with the exception of *Ae. aegypti* and *Cx. nigripalpus*. In the posterior midgut, we were unable to detect CA activity in An. albimanus and An. quadrimaculatus (Fig. 1). The greatest enzymatic activity appeared to be associated with the gastric caeca in *Cx. nigripalpus*, *An. quadrimaculatus*, and *Ae. albopictus*. Moreover, *Ae. albopictus*, *Cx. quinquefasciatus*, and *Cx. nigripalpus* exhibited significantly greater activity in the posterior midgut.

It has been postulated that the activity of gut cells in the first three abdominal segments is responsible for the high alkalinity of the lumen and that this region extends to the posterior midgut by the backward movement of the gut contents as well as the anterior flow of the fluid produced by the Malpighian tubules. Evidence of this anterior flow has been linked to the observation that gastric caeca contents become acidic when larvae are ligated just behind the thorax (Zhuzhikov and Dubrovin 1969). In these studies, larvae treated with methazolamide decreased the pH of the midgut. Although

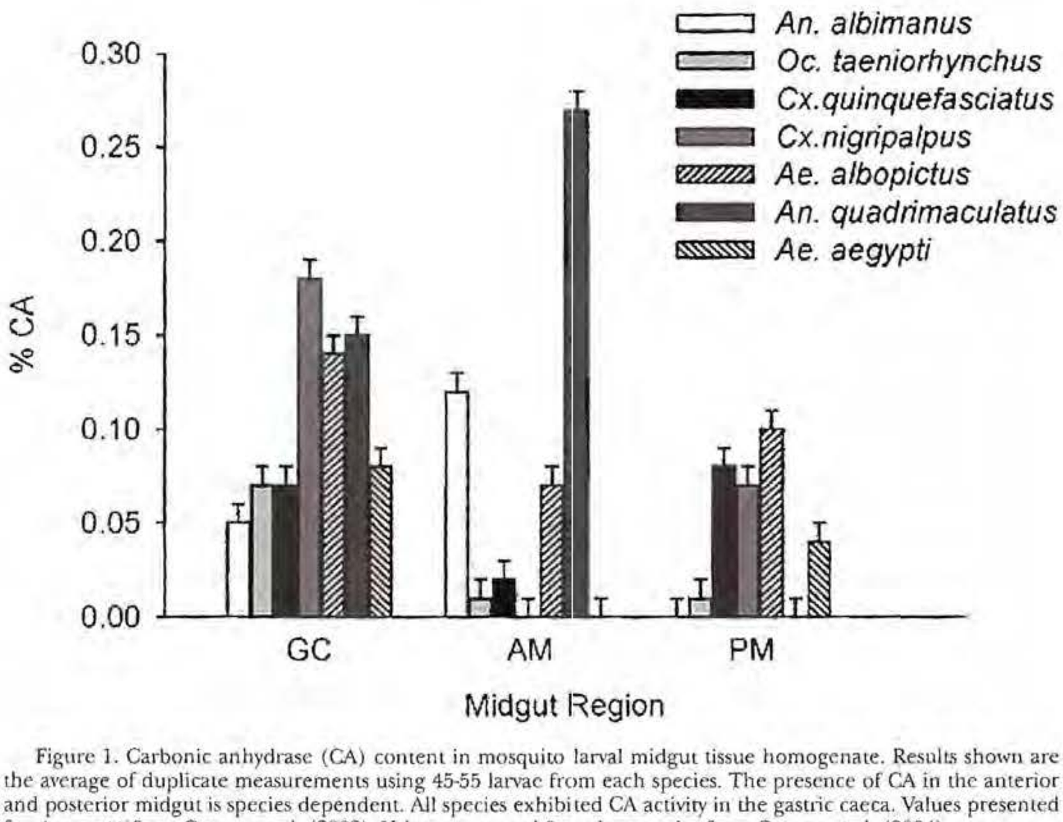


Figure 1. Carbonic anhydrase (CA) content in mosquito larval midgut tissue homogenate. Results shown are the average of duplicate measurements using 45-55 larvae from each species. The presence of CA in the anterior and posterior midgut is species dependent. All species exhibited CA activity in the gastric caeca. Values presented for *Ae. aegypti* from Corena et al. (2002). Values presented for other species from Corena et al. (2004).

the majority of the species were affected by the presence of inhibitor, the extent of the decrease in pH in the anterior midgut was different among species. These observations suggest that CA is crucial in the maintenance of pH within the midgut. Carbonic anhydrase inhibitors interrupt the production of bicarbonate and interfere with ion transport processes that occur in the midgut epithelium ultimately altering the alkalization mechanism. The extent of decrease in pH in the anterior midgut was species dependent, suggesting that perhaps the mechanism of ion transport in this region is more efficient in some species than in others. $LC_{50}$ and $LC_{90}$ values for each species using methazolamide or acetazolamide are shown in Tables 1 and 2. It appears that some species are more susceptible to methazolamide and acetazolamide than others. Remarkably, *Ae. albopictus* larvae were not susceptible to these compounds. Furthermore, there is no apparent relationship between the localization of CA and mortality when larvae were treated with inhibitors. For instance, *Oc. taeniorhynchus* and *An. quadrimaculatus* larvae exhibited different patterns of distribution but were affected in similar ways by the inhibitors. *Culex nigripalpus* larvae appear more sensitive to the effects of CA inhibitors than *Ae. aegypti* even though CA localization in the midgut of both species is similar. Interestingly, both species show a similar pattern of change in alkalization when treated with methazolamide. In *Ae. albopictus*, the pattern of alkalization was similar in methazolamide-treated and untreated larvae with only minor differences. These results indicated that the inhibitors do not have a substantial effect in the alkalization mechanism of the anterior midgut in this species. Because this species showed no mortality when treated with the inhibitors, it is possible to hypothesize that *Ae. albopictus* larvae survived because the alkalization mechanism in the midgut was not affected. Consequently, larvae of other species susceptible to CA inhibitors exhibited signs of pH imbalance in the midgut. Perhaps, inhibition of CA in the midgut resulted in this imbalance along with the inhibition of ion transport processes that led to larval mortality.

Methazolamide (N-(4-methyl-2-sulfamoyl-$^{2}$-1,3,4-thiadiazolin-5-ylidene) acetamide) and acetazolamide (N-(5-[aminosulfonyl]-1,3,4-thiadiazol-2-yl) are sulfonamides primarily used to control intraocular pressure in the treatment of glaucoma in humans. These drugs reduce the transport of ions and decrease aqueous secretion through a local osmotic effect most likely due to their interaction with CA (Parasrampuria and Gupta 1989). Although there are multiple reports on the clinical use of methazolamide and acetazolamide in vertebrates and invertebrates, including their use to study ion transport and acid-base regulation, there were no comparative reports on the use of CA inhibitors to study their effect on the physiology of the midgut in different mosquito larvae species until now (Corena et al. 2004).

Other sulfonamides, namely benzenesulfonamide and *p*-amino benzenesulfonamide (known as sulfanilamides), have been used in the past to determine larvicidal activity (Beesley and Peters 1971, Beesley 1973). Studies on the effect of methazolamide and acetazolamide on mosquito larval physiology may contribute to the development of novel safe larvicides that can target specific mosquito larvae with minimum impact on non-target species. These substituted sul-

Table 1. $LC_{50}$ values of laboratory-reared third and fourth instar larvae to methazolamide and acetazolamide in the laboratory 48 h post treatment. Values from Corena et al., 2004.

| Species | Methazolamide ($LC_{50}$) | Acetazolamide ($LC_{50}$) |
|---|---|---|
| *Anopheles quadrimaculatus* | $10^{-4.1}$ | $10^{-4.6}$ |
| *Culex quinquefasciatus* | $10^{-2.9}$ | $10^{-3.1}$ |
| *Culex nigripalpus* | Could not calculate | $10^{-4.2}$ |
| *Ochlerotatus taeniorhynchus* | $10^{-4.1}$ | $10^{-4.5}$ |
| *Aedes albopictus* | No mortality | |
| *Aedes aegypti* | $10^{-3.5}$ | $10^{-3.3}$ |

Table 2. $LC_{90}$ values of laboratory-reared third and fourth instar larvae to methazolamide and acetazolamide in the laboratory 48 h post treatment. Values from Corena et al., 2004.

| Species | Methazolamide ($LC_{90}$) | Acetazolamide ($LC_{90}$) |
|---|---|---|
| *Anopheles quadrimaculatus* | $10^{-2.7}$ | $10^{-3.6}$ |
| *Culex quinquefasciatus* | $10^{-2.5}$ | $10^{-2.2}$ |
| *Culex nigripalpus* | *$10^{-3.0}$ | Could not calculate |
| *Ochlerotatus taeniorhynchus* | $10^{-2.8}$ | $10^{-3.2}$ |
| *Aedes albopictus* | No mortality | |
| *Aedes aegypti* | $10^{-1.7}$ | $10^{-1.2}$ |

*This value was calculated 24 h post treatment. After 24 h all larvae were dead.

fonamides are very different from the ones mentioned earlier in the studies by Beesly and Peters (1971). In terms of the potential use of CA inhibitors as larvicides, acetazolamide and methazolamide have been shown more effective than benzenesulfonamide and *p*-aminobenzenesulfonamide in our study and that reported previously by Beesley (1973). There are several differences between methazolamide and acetazolamide and the antibacterials benzenesulfonamide and *p*-aminobenzenesulfonamide. The primary difference is chemical structure and lack of antibacterial activity of methazolamide and acetazolamide. In addition, $LD_{50}$ values obtained by Beesley (1973) were reported as percentages (g/ml) in the range of 0.01-0.05% for *An. stephensi* Liston and *Ae. aegypti*. The values determined in our study were 0.002% for *Ae. aegypti* and 0.00002% for *An. quadrimaculatus*. $LD_{50}$ values obtained for the other species in our study were similar. Since the development of methazolamide and acetazolamide, novel and more potent CA inhibitors have been developed (Scozzafava et al. 2000). Perhaps by taking advantage of the highly alkaline environment (pH >9.0) inside the midgut, and these novel inhibitors, it might be possible to develop new larvicides containing CA inhibitors that become active only at very high pH. Since different CA isozymes are present in living organisms, an effective mosquito larvicide based solely on CA inhibition would have to selectively target the mosquito CA without affecting other species. In order to develop such larvicides, further studies are necessary to have a better understanding of the structure of the mosquito CA and the environment that surrounds it as well as the impact of CA inhibitors on non-target species (Corena et al. 2005). The effect of methazolamide and acetazolamide on sheepshead minnows has been studied in order to determine their environmental impact (Corena et al. 2005). The degradation of these two compounds in estuarine water also has been investigated (Corena et al. 2005).

The effect of chloride and bicarbonate transport inhibitors on the alkalization mechanism and survival of different species of mosquito larvae is currently being explored. It appears that a protein responsible for transport of bicarbonate in exchange of chloride is present in the midgut according to our *in situ* hybridization experiments. A similar chloride/bicarbonate exchanger has been identified in *Drosophila*, as well as in four different mosquito species using a polymerase chain reaction and these genes are being cloned at the Whitney Laboratory from each one of six mosquito species mentioned in the previous CA studies. In addition, the mRNA that codes for this protein in the midgut is being localized using *in situ* hybridization experiments in *Ae. aegypti* and *An. gambiae* larvae.

The effect of chloride channel inhibitors, such as fipronil, in the alkalization mechanism has also been investigated in our laboratory. Fipronil has a profound effect not only on larval movement but also on pH inside the midgut. Treatment with this insecticide decreases the pH inside the anterior midgut in *Ae. aegypti* larvae to 7.4 or lower. Chloride channels sensitive to fipronil are modulated by gamma aminobutyric acid (GABA). This compound plays a fundamental role as an inhibitor of neurotransmission in the invertebrate nervous system. Little is known about the properties of invertebrate GABA receptors and the channels modulated by this chemical. Binding of GABA to specific receptors triggers the opening of GABA-gated chloride channels. These channels have important functions in ionic homeostasis, cell volume regulation, transepithelial transport, and regulation of membrane excitability. Through phylogenetic analysis four GABA-gated chloride channels in the *An. gambiae* genome have been identified and a beta-subunit from a GABA-gated chloride channel from the gastric caeca and posterior midgut of *An. gambiae* larvae has been cloned (unpublished results). This beta-subunit in *Ae. aegypti* fourth instar larvae and the signal for this subunit has been localized in the gastric caeca, posterior midgut, longitudinal thoraxic muscles and trachea in both species. Additionally, the mRNA coding for this channel has been detected in the central nervous system (CNS) of *Ae. aegypti* but not in the CNS of *An. gambiae.* Expression and characterization of this channel is underway in our laboratory and results indicate that this channel could also be used as a target for the design of mosquito specific larvicides. Inhibition of this channel might have an impact not only on larval physiology, and cell function particularly in the midgut, but also in survival. Future work will include an investigation to understand what processes will be affected if function of this GABA-chloride channel is blocked in mosquito larvae.

In summary, we will continue our efforts to compare the differences in physiology and protein expression among mosquito species in order to discover innovative ways to control pestiferous and disease-bearing populations. Our experience in unearthing key proteins involved in the mosquito midgut alkalization process appears promising. The isolation and quantification of novel proteins from the larval digestive system and the profound effectiveness of enzyme inhibitors to interfere with that system illustrates potentially novel targets for the design of specific larvicides.

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# REDUCING NON-TARGET IMPACTS FOLLOWING AERIAL ULV INSECTICIDE APPLICATION

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Adult mosquito control efficacy can be increased, and non-target organism impact minimized, if aerial ultra-low-volume (ULV) application of insecticides is conducted at the **right place** (by increasing retention time of mosquitocide droplets in the air in order to enhance their contact with flying mosquitoes), at the **right time** (dusk, dawn or night when adult mosquitoes are actively flying), and at the **right dose** (proper application rate to kill mosquitoes but not non-target organisms).

## I. THE RIGHT PLACE

Following ULV application, insecticide droplets need to remain airborne for effective control of adult mosquitoes. The probability of droplet impingement onto flying adult mosquitoes is increased if droplet retention time in the air is increased. Smaller droplets (<30 µm volume median diameter [VMD]) are retained in the air column longer. Larger insecticide droplets (>100 µm VMD) will deposit on the ground more quickly after application, thereby reducing the likelihood of contact with mosquitoes. Insecticide that is deposited on the ground is not only wasted, but may adversely affect non-target organisms. Environmental contamination can be reduced by adopting application techniques that maintain droplets in the air, promote controlled downwind movement of the insecticide cloud, while minimizing ground deposition (particularly in environmentally-sensitive areas). This concept is different from agricultural applications, which need to coat the surface of crops with insecticides. Moreover, agricultural applications try to reduce insecticide drift off the target zone (crop field) rather than maximize droplet suspension in the air column.

## II. THE RIGHT TIME

Applying an adulticide at the right time is fundamental to reducing non-target organism impact while maintaining effective control. The best time of the day for such applications (i.e., spray window) is at dusk, dawn, or nighttime. These are the time periods when most non-targets are resting (and therefore, protected from insecticide exposure) while most mosquitoes are actively flying. Currently, many mosquito control programs have adopted such spray windows. This practice is a significant achievement that protects many non-targets, such as butterflies and honeybees, from being exposed to the insecticide spray cloud.

Spraying at the right time also means spraying under optimal meteorological conditions. Weather conditions are dynamic and complex. Understanding as well as achieving 'optimal' meteorological conditions for mosquito spraying is often difficult. Current mosquito spray technology can accurately calibrate the amount of insecticide output from nozzle systems. But after the insecticide is released from the spray system, the aerosol is in the hands of Mother Nature. The spray cloud, as it is carried by wind and influenced by gravity, starts its journey from an altitude of 100-300 feet to ground level. Wind speed and direction greatly affects the distribution of the spray cloud. Downwind movement and deposition of insecticide residue can vary greatly from one spray mission to another. This situation creates significant variation in control efficacy and can often influence whether the effects on non-target organisms are minimal or substantial.

## III. THE RIGHT DOSE

Increasing the application rate may increase the risk of non-target mortality due to escalating exposure levels. In reality, it is sometimes very difficult to apply the proper dosage to achieve adequate mosquito control without causing non-target mortality. Tolerance differences in non-targets may be the result of physiologic as well as geographic differences within and among those organisms. Also natural topographic barriers such as trees, bushes, and grasses can provide refugia for non-targets to escape exposure from the insecticide aerosol. However, the level of mosquito control in vegetated areas may be less than open areas and if the application rate is increased to compensate for this, adverse impacts on non-targets may occur.

To determine the proper application rate, the efficacy of that insecticide against the target mosquito species has to be determined. Then, non-target impacts at that application rate should be evaluated. If the application rate is adequate to kill the majority of adult mosquitoes and low enough to spare the non-targets of concern, the rate will be appropriate. This process will ensure the delicate balance between effective mosquito control and minimal non-target organism impact.

## IV. NEW SPRAY TECHNOLOGY

Mosquito control programs, worldwide, are continuing to develop new spray technology to promote better mosquito control efficacy and lessen damage to non-targets. In the late 1990s, James Robinson's group at Florida's Pasco County Mosquito Control District, led an effort to develop a high-pressure nozzle system to deliver small insecticide droplets (<30 µ VMD). Zhong et al. (2003a) compared the insecticide residue deposition from a high-pressure system with that of the conventional flat-fan nozzle system. Using fenthion as the test material, heavy ground deposits were found within 1 mile downwind of the application with the flat-fan nozzle system and resulted in 80% mortality of caged fiddler crabs. On the other hand, minimal fenthion ground deposits were detected during the high-pressure nozzle trials. No fiddler crab mortality was observed within the 5-mile downwind test area following three single swath applications repeated during three consecutive nights.

In other experiments, the impact of naled on honeybees, *Apis mellifera* L., was investigated by exposing beehives to night-time aerial ULV applications using the flat-fan nozzle system (Zhong et al. 2003b), and later with the high-pressure nozzle system (Zhong et al. 2004). The tests were conducted during routine mosquito control missions in Manatee County, Florida. Honeybees, which clustered outside of the hive entrances, were subjected to naled exposure during these spray missions. The high-pressure nozzle system substantially reduced bee mortality. Also the average honey yield at the end of the season was not significantly reduced for those hives in the naled application area using this nozzle system.

## V. INSECTICIDE RESIDUE MONITORING

At present, bioassay techniques are widely used to measure both mosquito control efficacy and adverse non-target organism impact. Bioassays will only answer yes or no (i.e., dead or alive); it does not answer where or how much insecticide is present following the ULV application. But when insecticide residue monitoring is used in conjunction with bioassays, mosquito control programs will have a powerful tool to combat mosquitoes while protecting non-targets.

Insecticide residues cannot be seen by the human eye. However, they can be detected and quantified by modern analytical techniques such as gas chromatography or high performance liquid chromatography. Through monitoring of insecticide residues we can: 1) determine the actual concentration of insecticide in the air; 2) determine the actual concentration of insecticide deposited on the ground; 3) determine the distance of aerosol movement downwind; 4) establish appropriate thresholds for determining non-target impacts and mosquito control efficacy; and 5) compare different application equipment or operational scenarios. This will facilitate the proper use of insecticides for mosquito control.

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# POPULATION MONITORING, ECOLOGY AND CONTROL POSSIBILITIES FOR NUISANCE MIDGES (DIPTERA: CHIRONOMIDAE)

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## I. IMPORTANCE

Large numbers of adult midges that emerge from suburban and urban aquatic habitats in some situations result in severe nuisance, economic, and in some cases, medical problems to the nearby residents and businesses. It has been estimated in Florida (Anonymous 1977) that the recurring costs associated with adulticiding and removal of unsightly and odorous dead midges, cleaning, washing and maintenance of equipment, stained buildings, including business losses to motel, recreational, and tourism industries may amount to several million dollars annually. Dense mating swarms can restrict or preclude outdoor activities. For some situations in the world, midge swarms can cause direct traffic hazards; accumulations of dead midges have the potential to make roads and airport runways slippery and dangerous to be operational (Ali 1995). Resting midges attract large numbers of spiders, resulting in unsightly webs in and around affected structures. In Florida, at least eight species of Chironomidae have been identified to cause a variety of nuisance and economic problems (Ali 1996).

Medical problems associated with midges are primarily allergy-related and are associated with larval hemoglobins, which persist on the epidermis of metamorphosed adults (Gad El Rab et al. 1980); symptoms include dermal irritation, allergic rhinitis, and in some cases asthma. While currently not confirmed to vector any disease organisms (Ali 1996), midge egg masses have been reported to support non-pathogenic strains of the cholera pathogen, *Vibrio cholerae*, which utilize the gelatinous sheath of the egg biomass as a carbon source (Broza and Halpern 2001, Halpern et al. 2004). Moreover, emerging adults have been implicated in the incidental transport of bacteria or residual organic insecticides from polluted habitats (Ali 1995).

## II. MIDGE BIOLOGY

Chironomid midges are holometabolous insects in the order, Diptera. Their life stages include egg, four larval instars, pupa, and adult. Eggs are typically laid on the water surface and sink to the bottom of the habitat. Larvae of most species are bottom dwelling, often building tubes and the larval stage constitutes the majority of the midge lifespan. Adults tend to be short lived and females are capable of producing one or two clutches of eggs. Laboratory studies have indicated that *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards, the two predominant pest species in Florida, have a 4-5 week immature lifespan during summer (Frouz et al. 2002, Lobinske et al. 2002a).

Immature chironomids are often an important component of food-chains in natural aquatic ecosystems. Historically, large swarms of midges in Florida have occurred. Indeed, John James Audubon reported such swarms from the St. Johns River as early as 1831. In recent times, the construction of many man-made water bodies has provided suitable habitats for nuisance midges. In natural and artificial systems, eutrophication from anthropogenic sources can result in reduced invertebrate diversity and increased abundance of pollution-tolerant organisms, such as many species of chironomids, which often predominate. An important aspect of eutrophication is appearance of phytoplankton blooms that may serve as the primary food source of larval nuisance midges (Ali 1996). This increased food supply results in greater survival and enhanced growth. As a result, those chironomid species that have large-sized larvae produce larger adults (females), which are more fecund than smaller females (Xue et al. 1994).

## III. PROBLEM HABITATS

Chironomid emergence at nuisance or pest levels has been reported from a wide variety of aquatic habitats, natural or man-made, as well as fresh or saltwater (Ali 1996). These midge sources can range in surface area from <1 ha to several thousand hectares. Freshwater lentic sources include natural and man-made lakes, ponds, and other habitats, such as reservoirs, golf course ponds, aquaculture ponds, phosphate mining pits, and water-spreading basins or flood-control channels used for aquifer recharge. At wastewater processing facilities, sewage filter beds, sewage oxidation ponds, sludge lagoons, and other wastewater holding and processing enclosures at treatment plants can produce very large numbers of midges. Similarly, water control structures, such as irrigated agriculture canals, effluent discharge channels, flood control conduits, and storm drains, may produce midges in phenomenal numbers. Lotic habitats with midge nuisance potential include rivers, streams, and ditches of various kinds. Flooded wetlands can also support dense larval populations of midges. Saltwater habitats include marine fish ponds, tidal coves, small and large coastal lagoons, and brackish water impoundments.

## IV. SAMPLING

Larval populations can be monitored in small, shallow (<1 m deep) lentic habitats with a simple scoop sampler (Ali 1996). Standard Surber or kick samplers can be used in shallow, lotic habitats. For deeper water habitats, benthic dredge samplers, such as the Ekman, are often used. Benthic samples should be washed through appropriate pore-sized sieves to retain midge larvae and pupae. Taxonomic identifications are normally made in the laboratory using available keys to determine population estimates of nuisance species. Emergence of adults from habitats can be measured with submerged cone traps or floating traps (Ali 1996). New Jersey light traps are effective in monitoring prevalence and dispersal of adult populations in areas with midge nuisance.

Simple random sampling normally provides reliable estimates of larval populations in relatively small habitats with homogenous substrates, but can become tedious, labor-intensive and time-consuming in large lakes with heterogeneous substrates. For these larger systems, stratified sampling methods (Cochran 1963) are required to reduce and economize the sampling effort and yet obtain reliable population estimates of statistical integrity. Information concerning larval ecology of the target species is required, as is a detailed knowledge of the ecological geography of the habitat. Stratified sampling allows the investigator to concentrate the survey efforts on areas of the problem habitat with the greatest statistical variance, while allocating less effort to regions of low variance; the latter regions commonly are those that support very low or no larval populations. To facilitate this process, selected environmental parameters influencing larval distributions (Ali et al. 2002, 2003, Lobinske et al. 2002b), were used by Lobinske et al. (2004), to assemble simulation models for estimating likely population distributions of larval *G. paripes* in a large problem lake in central Florida. When tested against an independently-collected data set, 65 to 73% of the samples with nuisance densities (>1,000/m$^2$) of larval

*G. pemphe* were within the modeled estimation of nuisance stratum (Lobinske and Ali, unpublished data).

## V. CONTROL POSSIBILITIES

After obtaining reliable population estimates, as well as identifying the sections of a habitat that support high or nuisance levels of a midge species, control measures can be applied. This strategy allows one to restrict control measures to locations supporting nuisance levels, thus reducing overall cost of application, as well as minimizing adverse impact on non-target aquatic organisms and the environment at large. Current avenues of chironomid control practice and research depend upon chemical, biological, and physical/cultural means.

### 1. *Chemical*

Chemical larvicides for chironomid control registered within the United states include the organophosphate, temephos (Abate®), insect growth regulators (IGRs): s-methoprene, a juvenile hormone analog (Strike®), and diflubenzuron a chitin synthesis inhibitor (Dimilin®, 24-C, California State Registration). Temephos (Abate 5CG at 0.28 kg AI/ha) reduced total chironomid larvae in a residential recreational lake by 87-97% for 37 days posttreatment (Xue et al. 1993). Pellets of s-methoprene at 0.22 kg AI/ha gave 64-98% control of total midges for 7 weeks posttreatment in experimental ponds (Ali 1991b). For prolonged midge control (up to 49 days posttreatment), Ali et al. (1994) recommended diflubenzuron at 0.05 kg AI/ha (25 WP), followed in 2-3 weeks by a tablet formulation (Diflox Compresse), based on results from experimental ponds. For a review of chironomid chemical control see Ali (1991a).

### 2. *Biological*

The only biological control agent currently registered for midge control is *Bacillus thuringiensis* serovar. *israelensis* (*Bti*). Recent field trials with *Bti* in a sand mix (applied at 1,000,000 ITU/m$^2$) resulted in good control of *G. pemphe* and *Goeldichironomus* spp. for up to 3 weeks in a complex of man-made lakes in Ponte Vedra Beach, FL (Ali and Lobinske, unpublished data). Proposed uses of predatory fish and birds have been examined, but generally these have been partially effective (Ali 1995). The comparatively slow reproductive response of these predators, in relation to overwhelming reproduction rate of most chironomids, renders them incapable in terms of controlling midge outbreaks. Other biological control methods investigated involve several parasites and pathogens, as well as the use of predatory flatworms. Although some are potentially useful, little additional research has been conducted on these midge control possibilities (Ali 1995).

### 3. *Physical and Cultural*

Manipulation of physical or cultural conditions can be a valuable adjunct to other control methods, as well as potentially useful as a sole control technique in some situations. Surface films can provide an effective emergence barrier in relatively small and protected habitats. The monomolecular surface film, Agnique® MMF at 0.5 gal/acre provided 79-97% emergence suppression of chironomids for 1 week posttreatment in experimental ponds (Ali 2000). Many midge species are strongly phototactic, a behavior that can be manipulated to divert midge swarms away from areas of human activity, and aggregate them for adulticiding purposes (Ali and Lobinske 2000). Associated with this control strategy, knowledge of their seasonal abundance, diel periodicity of adult eclosion, and information on adult dispersal, can be useful in planning the timing and location of implementing adult control measures (Ali 1996).

## VI. SUMMARY

Effective and efficient control of midges in a nuisance situation can be enhanced by integrating the different control options mentioned earlier. Efficient long-term control also includes a systematic distributional survey of the larval habitat. After the initial investment to properly map and scout larval populations, a regular scouting program will alert managers to the time and location of nuisance populations. As a result, only those locations supporting nuisance populations should be treated in a timely and economical manner in order to suppress mass emergences of adult midges.

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## The First Mosquito Control and Arbovirus Surveillance Workshop, Anastasia Mosquito Control District (AMCD) of St. Johns County, St. Augustine, Florida, March 23-25, 2004

### PROGRAM

Tuesday, March 23, 2004

| | |
|---|---|
| 8:00-10:00 am | Registration |

**Moderator: Dr. Rui-De Xue**

| | |
|---|---|
| 10:00-10:10 am | **Introduction**<br>**Mr. Steve McEvoy**, Acting Director of AMCD<br>Welcome<br>**Mrs. Mary T. Willis**, Commissioner Chair of AMCD |
| 10:10-11:00 am | **Mosquito population surveillance techniques**<br>**Dr. Dan Kline**, Research Entomologist, USDA/CMAVE/Mosquito & Fly Unit, Gainesville, FL |
| 11:00-11:40 am | **Mosquito attractants and repellents**<br>**Dr. Ulrich Bernier**, Research Chemist, USDA/CMAVE/Mosquito & Fly Unit, Gainesville, FL |
| 11:40-12:00 | **Discussion** about traps with attractants for population surveillance with **Dr. Kline and Dr. Bernier** |
| 12:00-12:40 pm | Lunch break |
| 12:40-1:10 pm | **Mosquito control in Hernando County**<br>**Dr. Guangye Hu**, Director, Hernando Mosquito Control, Brooksville, FL |
| 1:10-2:00 pm | **Field testing of traps, attractants, and sentinel chickens**<br>**Ms. Melissa Doyle**, Biological Technician, AMCD, St. Augustine, FL |
| 2:00-2:50 pm | **Adult mosquito behavior related to operational programs**<br>**Dr. Rui-De Xue**, Entomologist, AMCD, St. Augustine, FL |
| 2:50-3:00 pm | **Discussion** |

Wednesday, March 24, 2004

**Moderator: Ms. Melissa Doyle**

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| 10:00-10:30 am | **Interagency collaboration for mosquito-borne disease surveillance**<br>**Dr. Terry O'Reilly**, Department of Health, Duval County, Jacksonville, FL<br>**Ms. Marah Clark**, City of Jacksonville, Mosquito Control Division, Jacksonville, FL |
| 10:30-11:15 am | **Malaria outbreak in West Palm Beach**<br>**Ms. Jennifer Simpson**, Environmental Specialist II, Department of Agricultural & Consumer Service, Tallahassee, FL |

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| 11:15-12:00 pm | **Arbovirus life cycle: links in a chain...** **Dr. Doria Bowers**, Assistant Professor, Department of Biology, Univer of North Florida, Jacksonville, FL |
| 12:00-12:30 pm | Lunch break |
| 12:40-1:30 pm | **Arbovirus surveillance and sentinel chickens** (Slide show by M. Doyle **Mr. James Burgess**, Lee County Mosquito Control District, Ft. Myers, |
| 1:30-2:15 pm | **Arbovirus test techniques** **Mr. Gerardo Medrano**, Entomologist, East Flagler Mosquito Con District, Palm Coast, FL |
| 2:15-3:00 pm | **Georgia mosquito and arbovirus surveillance programs** **Dr. Rosmarie Kelly**, Medical Entomologist, State Department of Hea Atlanta, Georgia |
| 7:00-9:00 pm | Dinner at Holiday Inn (Beach Blvd.) |

Thursday, March 25, 2004

**Moderator: Dr. Rui-De Xue**

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| 9:30-10:00 am | **Overview of mosquito control program in the City of Gainesville** **Ms. Kellie Etherson**, The president of the FMCA, Director, Gainesv Mosquito Control, Gainesville, FL |
| 10:00-10:30 am | **Adulticides and adult mosquito control** **Mr. John Boone**, Supervisor, AMCD, St. Augustine, FL |
| 10:30-11:00 am | **Optimized aerial ULV application** **Dr. James Brown**, Director, Jacksonville Mosquito Control Division, Ja sonville, FL |
| 11:00-11:50 am | **Biocontrol of mosquitoes** **Dr. Jimmy Becnel**, Research Entomologist, USDA/CMAVE/Mosquit Fly Unit, Gainesville, FL |
| 11:50-12:00 pm | **Altosid product introduction** **Mr. Mel Whitson**, Sales Representative, Zoecon Company, Florida |
| 12:00-12:30 pm | Lunch break |
| 12:30-1:00 pm | **Ecology and control of nuisance midges** **Dr. Richard Lobinske**, Senior Biological Scientist, Mid-Florida Resea and Education Center, University of Florida/IFAS, Apopka, FL |
| 1:00-1:30 pm | **Larval control using anti-digestive enzyme compounds** **Dr. Maria Corena**, University of Florida, Whitney Laboratory, St. Aug ine, FL |
| 1:30-2:00 pm | **Impacts of insecticides on non-targets** **Dr. Harry Zhong**, Assistant Professor, PHEREC/FAMU, Panama City, |
| 2:00-2:30 pm | **Marsh management and mosquito control** **Mr. Douglas Carlson**, Director, Indian River Mosquito Control Distr Vero Beach, FL |
| 2:30-2:40 pm | **Concluding remarks** **Mrs. Emily Hummel**, Commissioner Vice Chair of AMCD, St. Augustine, |
| 2:40 pm | END |