Bromeliads, *Aedes* and Zika

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With the return of summer to the southern regions of the United States, we can expect mosquito populations to reach high levels and the incidence of mosquito-borne diseases to follow in step. A new level of anxiety accompanies this yearly event due to the recent emergence and rapid spread of a new disease caused by the **Zika** virus in Latin America and the Caribbean Islands. Zika has torn through Latin America with especially great ferocity because the many humans living in the region have had no recent exposure to the virus, and no resistance to the virus.

Outbreaks of other mosquito-borne virus diseases, such as **Dengue Fever** and **chikungunya** during recent mosquito seasons, prove that we can expect **Zika** to spread in areas of the United States where the main carrier, *Aedes aegypti*, and a secondary carrier, *A. albopicta*, have established populations. Dozens of travelers infected by Zika in tropical America have already been reported from Florida and other Gulf Coast states. Eventually, one or more of these infected travelers will bring the virus into contact with mosquito colonies capable of serving as vectors for the pathogen and local outbreaks will occur. Fortunately, recent history also suggests that current mosquito control regimes, in combination with the active health care system, are sufficient to keep any outbreaks short and confined to a small area. Dengue Fever and chikungunya are transmitted by the same two *Aedes* species, and have been present in the southern US for some years now, but outbreaks have been limited in geographical extent and numbers of victims.

Current mosquito control efforts in Florida, unfortunately, are poorly designed to control the known Zika vectors. Spraying pesticides that kill on contact during the night hours is not likely to have any effect on the populations of *Aedes aegypti* and *A. albopictus* – both species fly during daylight hours and spend nights resting in areas that will generally protect them from contact with the spray.

Despite - or perhaps because of - this, officials charged with mosquito control have been quick to suggest bromeliads are a major risk factor in the spread of Zika, some even going so far as to suggest removing all bromeliads from yards. Of course, should residents of southern Florida try to follow those suggestions, they would quickly be confronted with the fact that many houses are surrounded by large numbers of native bromeliads that are protected by state law. In any case, there has been quite a lot of research on the relationship between mosquitoes and bromeliads that suggests removing bromeliads from the landscape will not affect the population of Zika carrying mosquitoes in any noticeable degree

A short summary of the threat posed by mosquito borne diseases was prepared by Dr. J.H. Frank a few years ago for the web site of the Gainesville Bromeliad Society. Text of this overview is reproduced in its entirety as Figure 1. If you would like to see a photograph of an *Aedes* mosquito greedily feeding on a human arm, a visit to the website (www.gainesvillebromeliadsociety.org) would be in order. A much larger, more

***Aedes albopictus* (common name: Asian tiger mosquito)** is an invasive species of mosquito from Asia that arrived in Texas in the 1980s and spread widely in the southeastern USA. It now occupies virtually all of Florida and has largely displaced *Aedes aeqypti* (common name: yellow fever mosquito). Adult mosquitoes of both these species are black with white markings. Behavior of these two species is similar.

Adult females of these mosquitoes lay their eggs glued at the waterline of small water containers (such as saucers under plant pots, scrap tires, empty cans, jars and bottles, water barrels, water dishes for pets, birdbaths, boats, and bromeliad leaf axils). The eggs hatch when they are inundated by water (such as during rain or when the container is topped up). The larvae hatching from the eggs feed in the water and, when they are large enough, become pupae. Pupae are comma-shaped and do not feed – after a couple of days, adult mosquitoes emerge from them. The females fly off to find a blood meal, from you or your family or friends or neighbors. The male mosquitoes feed on plant nectar.

The real problem is that both species are capable of transmitting dengue fever, chikungunya and Zika . Please search Google for information on chikungunya. Your best source is CDC (Centers for Disease Control). It is a disease that you, your family and neighbors do not want. It can be painful and debilitating and there is no vaccine. The disease was first detected in the Americas on St. Martin (a small Caribbean island) in December 2013. By late June 2014 it had expanded its range to 11 Caribbean Islands and parts of Central America and South America. Tourists returning from the Caribbean to the USA have been found infected. All it would take is for someone from your neighborhood to visit the Caribbean, return with an infection, be bitten by an *Aedes albopictus* mosquito from your yard that then bite someone else, and bingo, your neighborhood will be a focus of infection. Your local Mosquito Control District will likely send inspectors to your yard, and if *Aedes albopictus* adults or larvae or pupae are found, you will be in violation of Florida Statutes (1987. 386.041. Nuisances injurious to health). Your violation by producing these mosquitoes in your yard gives the Mosquito Control District various powers.

Until now, bites produced by *Aedes albopictus* mosquitoes in Florida were mainly just a nuisance. Now it is time to get serious by controlling these mosquitoes in your yard. Be prepared to show Mosquito Control District employees that your bromeliads are not producing mosquitoes.

Suggested control methods: (see facing page)

Figure 1 (continued on facing page). Brief review by Dr. J. H. Frank of the threat posed by *Aedes*-borne diseases in Florida, and suggestions on controlling unwanted mosquitoes in your own yard.

comprehensive article on the relationship between mosquitoes and bromeliads, also by Dr. Frank, can be found on the University of Florida web site (http://entnemdept.ufl.edu/frank/bromeliadbiota/mosbrom4.htm). Much of the detail that follows is taken from Dr. Frank's article.

Over 80 mosquito species have been recorded in Florida, but the number commonly encountered is about 25. Of these, only a handful are responsible for the great majority of diseases spread to and between people. *Aedes aegypti* and *A. albopictus* are of particular interest because they are the only mosquitoes known to be capable of transmitting Zika between humans at this time. In Brazil, *A. aegypti* is believed to be responsible for almost all of the transmission to humans, and it is not known how ef-

1) Methoprene. This is an insect juvenile hormone analog. It interferes with development of insect larvae so they die before they reach the adult stage. It does the same to immature crustaceans (shrimp, crab, lobster) but has no effect on vertebrate animals including people and pets. Buy Altosid (a trade name) methoprene mosquito granules and sprinkle them by hand into the water in your bromeliad axils. Their effect is supposed to last up to 30 days. You can order them online under the name Altosid methoprene mosquito granules. Read the directions.

2) *Bacillus thuringiensis* serovar *israelensis*. This is a bacterium that kills larvae of aquatic flies (mosquitoes, black flies, and chironomids). This strain (israelensis) is specialized to aquatic fly larvae. Other strains are specialized to caterpillars of butterflies and moths and beetles. It is harmless to vertebrate animals including people and pets). You can buy this product as Bayer Advanced Garden Mosquito Preventer as granules in 1lb plastic containers at garden stores. Directions for larger containers: “sprinkle one teaspoon per 25 square feet” – so obviously a tiny amount per bromeliad leaf axil if you intend to apply by hand.

3) Sweeper nozzle on a garden hose. Blasts from a hose thus fitted should wash out debris (including the food of mosquito larvae) from bromeliad axils and perhaps some mosquito larvae and pupae, too. Sweeper nozzles can be bought at garden stores.

4) Hydrogen peroxide. Dave Johnston, our speaker on 22 June, recounted how he buys small (8 oz) bottles of hydrogen peroxide at CVS, dilutes with water in a 4-gal spray tank, and sprays the tank content on his bromeliads. The concentration should not be above 3% because higher concentrations (e.g., 10%) have been used to kill weeds. This is perhaps the easiest method if you have thousands of bromeliads.

Please try these methods and learn which one works best for you. The test is: How many black and white mosquitoes (*Aedes albopictus* or *Aedes aegypti*) bite you at (say) 6 pm a biting hour in the shade near your plants in the summertime. Could you demonstrate to a Mosquito Control District Inspector the absence of these mosquitoes after you begin routinely using one of these methods?

Note that the situation differs in southern Florida where little mosquitoes (*Wyeomyia mitchelli* and *Wyeomyia vanduzeei)* which are not disease vectors are dominant in bromeliad axils (their larvae tend to outcompete the *Aedes* larvae for food). Adult females of *Wyeomyia* do bite people, but they do not transmit diseases to people.

Copied from the Affiliates page of the Bromeliad Society of Gainesville web site (accessed Feb 2016). Printed with permission of the author and the Bromeliad Society of Gainesville, Florida.

fective *A. albopictus* will be as a vector. This is important in Florida because the more recently introduced *A. albopictus* has largely driven *A. aegypti* out of the state, and taken over the range formerly inhabited by the latter. To the extent that *A. albopictus* is a less effective vector, we will see less opportunity for an outbreak of Zika

Many of the cultivated bromeliads that adorn our lawns and gardens (as well as native bromeliads growing in wild populations) hold water for extended periods of time in tanks, or cups, formed by the overlapping leaf sheaths at the base of the rosette. These pools of water can be used by mosquitoes to lay eggs for the next generation. Many different mosquito species can lay eggs in this water and expect successful larval and pupal development. *Aedes aegypti* and *A. albopictus* have been found breeding in bromeliads, but these two *Aedes* species prefer to lay eggs in more-enclosed water-holding containers with darker walls. You are most likely to find the larvae and pupae of these species in jars and jar lids hidden beneath shrubs or partially buried in soil, in clay pots that hold water for a week or two following rain, in water that collects in discarded tires or unattended bird baths. In habitats lacking the human-made containers, they are typically found in small, open chambers formed in tree stems by fungi. In favorable conditions, *A. aegypti* can develop from egg to adult in about a week, so it is not necessary to have a constant source of water.

In the southern half of the Florida peninsula, there are two native mosquito species that are bromeliad specialists – they only lay eggs in bromeliad tanks. *Wyeomyia vanduzeei* and *W. mitchellii* are the predominate species found in bromeliad tanks for areas south of Lake Okeechobee – they are able to effectively exclude most competing mosquito larva. Neither of these *Wyeomyia* species has ever been implicated in the transmission of any human disease. This is not to say that *Aedes aegypti* and *A. albopictus* are not present in southern Florida, but the populations of those mosquitoes are not coming out of bromeliad tanks.

In addition to the two links mentioned above, further information can be found at various places on the internet. For example, a booklet prepared for homeowners battling mosquitoes is available from the University of Florida Institute of Food and Agriculture (IFAS) website (http://edis.ifas.ufl.edu/pdffiles/in/in104500.pdf)

The Florida Medical Entomology Lab (part of the IFAS system) maintains a Mosquito Information Website that covers the mosquito fauna of Florida in detail (http://mosquito.ifas.ufl.edu/Index.htm). This website also provides current information on mosquito-borne diseases, such as Zika.

Further detail on the larval preferences of *Aedes* can be found in Donald A. Yee. 'What Can Larval Ecology Tell Us About the Success of *Aedes albopictus* (Diptera: Culicidae) Within the United States?', Journal of Medical Entomology Advance Access published June 28, 2016 found in the Asian Tiger Mosquito Collection of Oxford Journals. (http://jme.oxfordjournals.org/page/Asian_Tiger_Mosquito_Collection)

The above review has dealt exclusively with Florida because information for other states likely to have problems with Zika in the coming years is not readily available online. At least, I did not find much more than lists of mosquitoes recorded from each state. Texas has a publication on diseases transmitted by mosquitoes, originally published as an AgLife Bulletin, that should be of interest, although it is pre-Zika. It can be downloaded at http://www.austintexas.gov/sites/default/files/files/Health/Environmental/mosquitoes_diseases.pdf

Aside from the current mosquito control regimes, three long-term approaches to controlling the spread of Zika by suppressing populations of *Aedes aegypti* have been reported in the national press during the past 6 months. A fourth approach aims to control disease transmission by interfering with the ability of *A. aegypti* to carry and transmit the viruses responsible for Dengue and Zika. All four approaches specifically target *Aedes aegypti* populations, but, in theory could also be directed against populations of *A. albopictus* with only minor modifications.

There are actually two approaches utilizing different aspects of special strains of bacteria in the genus *Wolbachia* being pursued. The first approach, carried out by researchers at Monash University in Australia, is to raise large numbers of male and female *Aedes aegypti*, infect them with a mosquito-specific strain of *Wolbachia* that has been found to decrease the ability of *Aedes aegypti* to transmit the virus responsible for Dengue Fever to humans. The Australian group that developed this approach has more recently turned its attention to the closely related Zika virus. If the strain of *Wolbachia* responsible for the decrease in Dengue transmission has a similar effect on Zika, the idea is to release mosquitoes carrying the proper *Wolbachia* strain into local mosquito populations harboring Zika.

The selected *Wolbachia* strain will spread to other members of the population through natural sexual behavior, leading to a decline in the effectiveness of the mosquitoes as Zika vectors. One great advantage of this approach over current chemical (pesticide)-based control methods is that the *Wolbachia* is carried directly to the uninfected females of *Aedes aegypti* by male mosquitoes who are exquisitely equipped to locate females of the same species, no matter where they may be hidden. This particular approach does not aim to decrease the mosquito population. This approach has shown promising results in field trials in Cairns, and is currently in large-scale field trials in Brazil.

Another approach using *Wolbachia* exploits the observation that two mosquitoes can successfully mate only if they both carry the same strain of *Wolbachia*. In this case, a sample of mosquitoes from a population that is targeted for control is used to raise a large number of male *Aedes aegypti* in the lab. The *Wolbachia* strain carried by female mosquitoes is also raised in the lab and subjected to treatments, such as exposure to different antibiotics, that effectively change it into a different strain. The lab-raised *Aedes* larvae are injected with the modified *Wolbachia* strain. They carry the modified strain in their sperm when they mature. When these males are released back into their populations, mating between a female mosquito carrying the common local strain of *Wolbachia* bacteria and one of the males with altered *Wolbachia* will result in nothing more than dead embryos. If sufficient numbers of altered males are released, this can lead to a drastic population crash in the targeted population. Field tests of this approach are underway at present.

A popular review article on both of these approaches by Erin Weeks was published last year in Entomology Today, and is available at https://entomologytoday.org/2015/03/23/wolbachia-bacteria-can-control-mosquitoes-with-fewer-chemicals/

Another approach involves the release of large numbers of genetically-altered, lab raised male mosquitoes whose offspring with wild-type females do not survive to reproduce. Altered mosquitoes, including the offspring between altered mosquitoes and wild-type mosquitoes require regular doses of tetracycline in their diet for survival. Without the supplemental tetracycline, the offspring die before reaching adulthood. If sufficient numbers of modified males are released, this approach could lead to dramatic reductions in the sizes of targeted mosquito populations over a short time period. The group that developed this approach, Oxitech, has conducted field trials in Brazil and has proposed field trials in the Florida Keys later this year. This overall approach has run into vocal opposition and has been the subject of conspiracy theories due to the genetic modification of the mosquitoes. In February this year, Chelsea Harvey produced a summary entitled 'What it would really take for GMO mosquitoes to stop Zika' that is available at: http://www.ndtv.com/world-news/what-it-would-really-take-for-gmo-mosquitoes-to-solve-the-zika-crisis-1273851. The Oxitech website (www.oxitec.com) contains more details on the genetic modification used in their program.

The final approach to controlling *Aedes aegypti* populations is modeled on the successful campaign to eradicate the screwworm fly, *Cochliomyia hominivorax* (Coquerel), as a serious threat to cattle-ranching in the southern United States and Mexico. This program starts with raising large numbers of the male screwworm flies in the lab. These males are sterilized through a treatment such as exposure to ionizing radiation. Sterile males are then released in large numbers at the edges of the population. If the number of sterile males in an area is much larger than the number of females, the sterile males will effectively exclude any fertilization of the females by fertile males. Success of this approach depends on some details in the life cycle of the insect: a female screwworm fly mates only once during its life, and, if mated to a sterile male, lays non-viable eggs. Over time, the screwworm population borders shrink as populations on the margins are eliminated. Extirpation of entrenched populations was amazingly fast using this technique. After some preliminary tests carried out during 1956-1957, a large-scale effort was made to eliminate the screwworm fly from Florida in the following years. The last verified record of screwworm fly infestation in Florida was in 1959. Similarly rapid progress was seen in the push to eliminate the fly from the Southwestern United States. However, the continued presence of screwworm flies in areas of Mexico adjacent to Texas, Arizona and New Mexico led to continuing reinfestation of the previously cleared areas. To combat this, a program to eliminate the pest in Mexico was begun as a joint project between USDA and the Mexican government. Over the years, this program has been extended progressively further south. The current southern border of the pest-free zone is in Panama. Outbreaks north of the border are prevented by continuing releases of sterile males within a buffer zone

If you are interested in more details, a history of the screwworm fly eradication program through 2000 by John H. Wyss can be found at www.oie.int/doc/ged/D5672.PDF and another history that brings the story to the current date by James E. Novy is available from http://www.fao.org/docrep/U4220T/u4220T0a.htm. If you are interested in the history of techniques for using ionizing radiation to render male insects sterile, look to the following link: http://www.intechopen.com/books/evolution-of-ionizing-radiation-research/ionizing-radiations-in-entomology.

With this record of success, it is clear that a program based on using male mosquitoes to interrupt normal mating behavior could be highly effective unless the life history of the target mosquito includes traits that allow fertile males to successfully mate with females already mated to the sterile males. One of the potential barriers to this approach, or any other approach relying upon modified males, is the need to produce vast quantities of these males on a continuous basis. Much is already known about raising industrial quantities of mosquitoes, but you really need to flood target populations with a great excess of altered males to be effective. Given the potential numbers of mosquitoes in a single population, a massive rearing program would be needed over an extended period. An extended treatment period is especially important in the case of *Aedes egypti* because these mosquitoes can survive for several months in the egg stage. If you stop treatment before the last of these eggs hatch, the population could quickly rebound.

You might have noticed that only three of the research programs discussed above have the suppression of *Aedes* populations as the final goal. The program to reduce the effectiveness of Zika (or Dengue) transmission by infecting *Aedes* populations with specific strains of *Wolbachia*, would presumably not have any effect on population densities. There is some controversy about suppressing, or even extirpating, populations using the other three methods. However, neither *A. aegypti* nor *A. albopictus* is native to the Americas. The only concerns about eliminating them are possible unforeseen consequences. For instance, an even more troublesome mosquito species might take over the habitat niches opened up if these two species were locally eliminated. However, the number of mosquito species that efficiently spread dangerous human diseases is apparently quite small. It seems likely that any more troublesome mosquito species that takes over could itself be controlled by one of the proposed methods.

One final note, the approach to control that depended upon releasing lab-raised males with an altered *Wolbachia* strain, has apparently shifted in recent months. As noted on the Mosquito Mate website (mosquitomate.com), the focus is now on controlling *Aedes albopictus* populations present in the United States. Also, a single *Wolbachia* strain that is incompatible with all known *Wolbachia* strains found in female *Aedes albopictus* in the United States is now incorporated into the male mosquitoes. This eliminates the step of producing a unique modified strain of *Wolbachia* for each population treated. A new approach is also under development by the same company. Again, the starting point is using male mosquitoes of the target species to find the females in the target population, but here the male mosquitoes deliver a chemical larvicide to the small pools of water favored by the female *Aedes* for egg-laying. In other words, this approach is more akin to the sprays typically used in mosquito control programs, but the 'spray' in this case is directed much more effectively to the target species and much lower volumes are needed for effective control.