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A Review of the Control of *Aedes aegypti* (Diptera: Culicidae) in the Continental United States

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Abstract

Aedes aegypti (L) is an anthropophilic mosquito involved in the transmission of a variety of viral pathogens worldwide including dengue, chikungunya, yellow fever, and Zika viruses. This species, native to Africa, is well established in the continental U.S. (CONUS) and occasionally contributes to localized outbreaks of viral diseases. In the last seven decades, mosquito control programs in the CONUS have been focused on vectors of eastern equine encephalitis, St. Louis encephalitis, and West Nile viruses, as well as nuisance species. *Aedes aegypti* receives little control focus except during outbreak periods, which has led to a lack of information on appropriate and effective control options targeting *Ae. aegypti* in the CONUS. As such, in the event of an *Ae. aegypti*-borne arboviral outbreak in the CONUS, there are limited evidence-based control recommendations or protocols in place. Autochthonous outbreaks of *Ae. aegypti*-borne pathogens have occurred recently in the CONUS, including dengue outbreaks in 2010 and 2013, a chikungunya outbreak in 2014, and the 2016 outbreak of Zika virus. The increasing frequency of *Ae. aegypti*-borne outbreaks necessitates increased attention and research on control of this species to prevent and mitigate future outbreaks. This review consolidates and synthesizes the available literature on control of *Ae. aegypti*, specifically within the CONUS, focusing on data generated through operational applications as well as field and semifield experiments. The purpose of this review is to identify and highlight areas where additional research is needed. The review covers chemical control and insecticide resistance, biological control, source reduction, trapping, and alternative techniques.

Keywords

Aedes aegypti; mosquito control; continental U.S.

The yellow fever mosquito, *Aedes aegypti* (L) (Diptera: Culicidae), is perhaps one of the most thoroughly studied vector species worldwide. Implicated in the transmission of yellow fever, dengue, chikungunya, and Zika viruses, the control of this species and the pathogens it transmits is of key interest to many nations, including the United States. Most field research

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on the control of this species has been conducted outside of the continental U.S. (CONUS), with few studies that are truly representative of and applicable to the CONUS *Aedes aegypti* populations, environmental conditions, vector control infrastructure, and human lifestyles. The CONUS is composed of a variety of unique geographical features, habitats, housing, and niches and, as such, the assumption cannot be made that control options tested outside of this region will have comparable efficacy.

Aedes aegypti possess a suite of characteristics that allow this species to be an extremely efficient arbovirus vector. The *Aedes aegypti aegypti* (L) subspecies (the invasive subspecies that has spread around the world) is extremely anthropophilic in nature, often living around or inside human dwellings (Scott et al. 2000) and preferring to feed on humans (Scott et al. 1993a). This species has been shown to perform multiple feedings within a single gonotrophic cycle, increasing the opportunity to both acquire and transmit pathogens (Scott et al. 1993b). Although blood is typically imbibed to supplement egg development, *Ae. aegypti* has been shown to use blood as an energy source, preferentially seeking out bloodmeals instead of sugar meals (Edman et al. 1992) or increasingly seeking out bloodmeals in the absence of adequate sugar sources (Foster and Eischen 1987). These factors all contribute to an increased opportunity for *Ae. aegypti* to attain and transmit pathogens to humans (Scott and Takken 2012).

Aedes aegypti also possess a unique set of characteristics that allow this species to effectively infiltrate and establish in new areas. The skip oviposition habit of this species allows one female to deposit eggs in multiple containers per gonotrophic cycle (Corbet and Chadee 1993, Colton et al. 2003), optimizing the chances of successful offspring. The containers used are often small, cryptic, and variable in presentation (Cheong 1967), making identification and removal of containers challenging even for trained personnel. In addition to small, cryptic sites, this species has also been documented using stormwater drains, sewage treatment plants and cesspits, and septic tanks as oviposition sites, which are areas that can be challenging to monitor and treat (Hribar et al. 2006, Barrera et al. 2008, Arana-Guardia et al. 2014). The eggs, once laid, are desiccation resistant in the environment, although exact length of viability is dependent on relative humidity (Kliewer 1961, Sota and Mogi 1992). Viability of *Ae. aegypti* eggs in laboratory studies has, under certain conditions, surpassed 1 yr (Faull and Williams 2015). The ability of the eggs of this species to survive long periods of desiccation and their propensity to develop in small, water-filled containers allowed this species to spread around the world aboard ships (Lounibos 2002). Without this dispersal assistance, this species usually has a relatively short range of dispersion, typically less than 100 m although they have been documented moving up to 800 m (Muir and Kay 1998, Honório et al. 2003, Harrington et al. 2005).

In addition to their short flight range, climatic factors such as precipitation, temperature, and humidity are also thought to limit the range of *Ae. aegypti* (Hopp and Foley 2001). Climate change is anticipated to affect the range of this species, potentially increasing the suitable area for *Ae. aegypti* within the CONUS, although our understanding of this range expansion is limited due to confounding factors associated with the close association *Ae. aegypti* has with humans (Eisen and Moore 2013). For example, in hot, arid environments, people are known to keep water in containers, have yards full of irrigated vegetation providing refugia, and often use evaporative coolers, which may provide crucial humidity for *Ae. aegypti* (Fink et al. 1998), allowing this species to expand its range into previously uninhabitable areas. This trend is projected to continue in the coming decades, with models anticipating *Ae. aegypti* expanding its range into temperate urban areas as far north as Chicago, IL by 2050 (Kraemer et al. 2019). This trend is already being seen in some regions of the CONUS, with unanticipated populations spreading into California (Gloria-Soria et al. 2014), Washington DC (Lima et al. 2016), Nevada (Pless and Raman 2018), and Nebraska (Bucco-White 2019). Small populations of *Ae. aegypti* were found in both 2016 and 2017 in the far southern Canadian city of Windsor (Giordano et al. 2020), indicating that this species has the potential for a much broader spread throughout North America than previously anticipated. However, while climatic factors and human manipulation of habitats may allow the establishment of local *Ae. aegypti* populations, the ubiquitous use of air conditioning and window screens and much lower overall contact with *Ae. aegypti* has prevented large-scale outbreaks of *Ae. aegypti*-borne viruses in the CONUS thus far (Reiter et al. 2003).

This recent range expansion is concerning considering increasing *Ae. aegypti*-borne pathogen activity in the CONUS within the past decade. Local outbreaks of a variety of pathogens have been detected in the CONUS, including dengue virus in Florida in 2010 and 2013 (Rey 2014) and Texas in 2013 (Thomas et al. 2016), chikungunya virus in Florida in 2014 (Kendrick 2014), and Zika virus in Florida in 2016 (Likos et al. 2016). As *Ae. aegypti* continues to spread into new and unanticipated parts of the CONUS, so increases the possibility of additional local outbreaks. As such, a renewed focus should be placed on better understanding the efficacy of control options targeting *Ae. aegypti* in the CONUS.

During World War II, programs to control *Ae. aegypti* were used at major ports of entry to prevent dengue or yellow fever incursion (Bradley and Atchley 1955). From 1965 to 1969, due in part to the success of nations in Central and South America, the United States pursued efforts to eradicate the invasive populations of *Ae. aegypti* in North America (Schliessmann 1964, 1967). The Communicable Disease Center (CDC; name since changed to the Centers for Disease Control and Prevention) was tasked with developing and implementing effective control programs to curb *Ae. aegypti*, resulting in a surge of research into *Ae. aegypti* control during that era. The *Aedes aegypti* Eradication Branch of the Communicable Disease Center developed a program focused on enacting control at the state and local level through collaboration with state public health agencies. These agencies would then coordinate statewide training and surveillance as well as control measures including source reduction, insecticidal applications, community clean-up, and sanitation measures (Schliessmann 1964).

Unfortunately, the program to eradicate *Ae. aegypti* from North America did not receive adequate funding early on (Schliessmann 1964) and ultimately the program was discontinued with little acknowledgement due to a lack of funding (Slosek 1986). The widespread development of insecticide resistance (Busvine and Coker 1958, Camargo 1967, Inwang et al. 1967), negative impact on avian reproduction (Porter and Wiemeyer 1969), and environmental persistence (Risebrough et al. 1967, Beyer 1980) of dichlorodiphenyltrichloroethane (DDT) were also discovered, leading to the removal of one of the most effective control means available at the time. Since then, there has been a relative paucity of research and development focused on control of *Ae. aegypti*, specifically in the CONUS. It is worth mentioning that an increase in surveillance by the CDC and state health departments in response to the introduction of the Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), in the 1980s led to additional data on *Ae. aegypti*, as both species can be monitored using similar traps and occupy similar larval niches (Moore et al. 1986, 1988). Unfortunately, mosquito control programs are increasingly facing budget pressure and lack of funding, resulting in a lack of expertise, surveillance data, and preparation for outbreak events (Herring 2010, Moise et al. 2018). Autochthonous outbreaks are occurring with increasing frequency in the CONUS, a fact which should encourage increased funding of vector control programs to enact preventive programs.

Currently, most mosquito control districts in the CONUS do not focus operational treatments on *Ae. aegypti* specifically, instead focusing on control of nuisance species and vectors of more prevalent arboviruses such as West Nile virus (STPMAD 2019a). While most districts do not focus on *Ae. aegypti* control, some have contingency plans for the local control of populations in response to *Ae. aegypti*-borne pathogen outbreaks and since the introduction of Zika virus, many agencies have updated their response plans. The St. Tammany Parish Mosquito Abatement District has contingency plans in place in the event of travel-associated or autochthonous cases of dengue virus, chikungunya virus, or Zika virus, including increased surveillance, control by aerial and truck adulticiding, and source reduction (STPMAD 2019). Operational mosquito control programs that include *Ae. aegypti* as a target species often rely on source reduction through community outreach, educational programs, and routine agency evaluation and mitigation. The Florida Keys Mosquito Control District includes *Ae. aegypti* as a target species and conducts regular source reduction campaigns, larviciding with *Bacillus thuringiensis israelensis* (*Bti*) and spinosad (biorational bacterially derived toxins), and adulticiding through truck mounted ultra-low volume (ULV) spraying of permethrin (a pyrethroid pesticide) and malathion (an organophosphate pesticide) or aerial applications of naled (an organophosphate pesticide) (FKMCD 2019). Other programs, such as the Consolidated Mosquito Abatement District in Central California are taking aggressive steps towards control of *Ae. aegypti* including operational applications of the incompatible insect technique in collaboration with commercial partners Verily and MosquitoMate (CMAD 2019b).

This review aims to condense and synthesize the current literature on *Ae. aegypti* control in the CONUS (Table 1). We identify knowledge gaps for future investigations and determine the current readiness of the United States to respond to an *Ae. aegypti*-borne pathogen outbreak. The CONUS represents a unique environment for control: the country includes multiple climatic and ecological zones (Daubenmire 1938, Fovell and Fovell 1993, Lugo et al. 1999) as well as variability in housing conditions and local cultures (Reiter et al. 2003, Hayden et al. 2010, Hotez 2011). For this reason, experimental results from other countries may not sufficiently model similar experimental applications within the CONUS. This review will focus on operational (evaluations conducted as part of mosquito control operations), field (evaluations conducted in open air, unconfined conditions), and semifield (evaluations conducted outdoors, but typically in a caged environment) evaluations, as various factors in the field (temperature, precipitation, canopy cover, native bacteria, etc.)

can affect the application and success of control methods that are not reflected in laboratory based studies.

Chemical Control

Chemical control of larval and adult mosquitoes in the CONUS is limited by the number of chemical classes that can be employed due to strict testing and registration guidelines prior to broad use (USEPA 2019a). These testing guidelines often involve extensive and costly investigations into the impact the chemical agent may have on nontarget organisms, vertebrate wildlife, and humans that can take years or even decades. For these reasons, mosquito control agencies have a limited choice of active ingredients at their disposal, and new pesticides with different modes of action are rarely identified and put into production for public health use. Additionally, while many mosquito control programs rely heavily on chemical control, these compounds often have not been evaluated against *Ae. aegypti* in the CONUS and those that have are often only evaluated in limited environments and conditions. In many parts of the CONUS, *Ae. aegypti* is uncommon and control of this species is not being prioritized. As such, many of these pesticides have been tested against vectors of endemic diseases such as *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Culex tarsalis* Coquillett (Diptera: Culicidae) (vectors of St. Louis encephalitis and West Nile viruses) and nuisance species such as the salt marsh mosquitoes *Aedes taeniorhynchus* (Wiedemann) (Diptera: Culicidae) and *Aedes sollicitans* (Walker) (Diptera: Culicidae) and the floodwater mosquito *Aedes vexans* (Meigen) (Diptera: Culicidae).

Larvicides

Larviciding, or the use of pesticides specifically targeting the larval stage, has evolved immensely within the CONUS in the past century. Historically, larviciding against *Ae. aegypti* in the CONUS made use of materials such as kerosene (LeVan 1941) or engine oil (LeVan 1940), which were used to prevent larvae from respiring at the water–air interface. Pellets composed of Paris green (an arsenite) and plaster of Paris were also used historically to control larval *Ae. aegypti* in containers at a Key West cemetery (LeVan 1940). DDT was also important as a larvicidal agent; however, *Ae. aegypti* quickly developed resistance to this pesticide throughout the Americas where eradication programs were underway (Pal and Gratz 1968).

Modern larvicidal agents used in the CONUS include biorational bacterially derived toxins like *Bti*, *Lysinibacillus sphaericus*, and spinosad, insect growth regulators (IGRs) such as methoprene and pyriproxyfen, and surface agents such as monomolecular films and mineral oils. These pesticides are applied to the aquatic larval sites of mosquitoes and are typically either applied as a dunk, briquette, or tablet placed directly into water or as a granular, wettable powder, or liquid formulation applied by backpack sprayer, ULV sprayer, or aerial application. Some larvicidal agents, such as pyriproxyfen, have further been dispersed by mosquitoes themselves during skip oviposition from treated traps to natural oviposition habitats. The historical efficacy of the organophosphate chemical agents temephos and chlorpyrifos will also be discussed, although there are no registrations currently being supported for these products as larvicides in the United States.

Bacillus thuringiensis israelensis is a commonly used bacteria that, when ingested by mosquito larvae, releases toxins that destroy the stomach lining of the mosquito, thereby killing the mosquito through starvation or through septicemia (Gill et al. 1992). This bacterially derived toxin is the active ingredient in many commonly used formulated larvicides. These larvicides have been shown to be efficacious against container-inhabiting *Ae. aegypti* in the laboratory (Mulla et al. 1982) and with a variety of different application methods in the field. Multiple studies conducted in Florida have found *Bti* to be effective against local populations of *Ae. aegypti*. In one study at a site in Key West, Florida, that typically experiences great population growth of *Ae. aegypti* during and after summer rain events, weekly aerial applications of *Bti* (VectoBac WG, Valent BioSciences, Libertyville, IL) resulted in significant decreases of *Ae. aegypti* compared with the control site (Pruszynski et al. 2017). Similarly, in another study conducted in an urban area of the Florida Keys, *Ae. aegypti* larvae placed in cups of water treated at max application rate (1 kg/ha) with VectoBac WDG (Valent BioSciences, Libertyville, IL) experienced 100% mortality at 48 h post inoculation (Knapp et al. 2018). In northern Florida, *Bti* applications at maximum label rates also resulted in 100% mortality 48 h post exposure in open environments (Knapp et al. 2018). Field trials conducted at Camp Blanding Joint Training Center in Starke, Florida, investigated four *Bti* products (two granular: VectoBac GR [Valent Biosciences, Libertyville, IL] and Sustain MGB [AllPro Vector Group, Northville, MI], two liquid: VectoBac WDG [Valent Biosciences, Libertyville, IL] and Aquabac XT [Becker Microbial Products, Parkland, FL]) and observed that each resulted in close to 100% mortality of *Ae. aegypti* within 6 m of the application line with a backpack sprayer (Harwood et al. 2015). Ultimately, the best performing formulation assessed was VectoBac GR, with >80% mortality on average and the highest reduction at elevated oviposition sites (those at 1.5–3 m in height). Finally, *Bti* (Vectobac WGD) applied by buffalo turbine was used during the 2016 Zika outbreak response in Miami-Dade, Florida, in combination with adulticidal agents (naled and deltamethrin, discussed in the adulticide section), which resulted in decreased mosquito counts and led to sustained suppression (McAllister et al. 2020).

Spinosad (Natular, Clarke, St. Charles, IL) is an insecticide derived from the actinomycete soil bacterium, *Saccharopolyspora spi-nose* (Hertlein et al. 2010). The efficacy of spinosad has been tested against *Ae. aegypti* in the CONUS. In one study, application was performed using a ULV backpack sprayer applying a liquid formulation of spinosad (Natular 2EC, Clarke, St. Charles, IL) to cups placed at 1.8–9.1 m from the spray path in an arid region of the Coachella Valley of California at varying vegetation densities (Golden et al. 2018). After treatment, second and third instar *Ae. aegypti* were introduced to the treatment cups. This study found that at mid and maximum label rate (91.3 and 182.6 ml/ha, respectively), 33.8 and 51.6% average mortality, respectively, was achieved, with an average mortality of 79.1% at 1.8 m and decreasing mortality further from the spray path. Further, there was no significant difference in mortality achieved between sparsely and densely vegetated areas (Golden et al. 2018). In North Florida, trials were conducted to investigate the residual use of spinosad (Natular 2EC) at a military training facility employing structures to replicate protected environments (Aldridge et al. 2018). Spinosad was applied at the maximum rate (79.5 ml/acre) using a truck mounted ULV sprayer to empty cups located in front of, within, and behind a building to replicate dry environments prior to a rain event. Upon introducing *Ae. aegypti* larvae within 1 wk of treatment, cups that were placed in front of the structure received the highest mortality (48.7%), those inside the open building had 32.3% mortality, and the cups behind the building had the lowest mortality at just 13.3%. This indicates a residual effect of spinosad when used as a preemptive control option (Aldridge et al. 2018). However, cups were collected, covered, and transported in ideal conditions between treatment and introduction of larvae. Additional studies should be conducted to investigate the residual efficacy of treated cups left in field conditions.

Organophosphate pesticides work by inhibiting the action of cholinesterase, causing overstimulation of nerve endings due to accumulation of excess acetylcholine (Roberts and Reigart 2013). Organophosphate pesticides such as temephos and chlorpyrifos have historically been used for mosquito larval control, although many applicators are phasing out these chemicals due to their broad-spectrum efficacy against nonmosquito organisms (Hughes et al. 1980, Milam et al. 2000). Clarke, the company holding the remaining few registrations in the United States for temephos (product name Abate), filed a voluntary registration cancelation request for all temephos products in 2011. Subsequently, the U.S. Environmental Protection Agency canceled the registration for this product with no new product to be manufactured for U.S. distribution and no sales of existing stocks after 31 December 2016 (US EPA 2011). Although temephos was found to be effective against *Ae. aegypti*, resistance was developing to this pesticide around the world (Bisset 2011, Grisales et al. 2013, Singh et al. 2014), despite a lack of published resistance data for *Ae. aegypti* to temephos in the CONUS. Chlorpyrifos has also been shown efficacious against *Ae. aegypti* larvae. One study conducted in Galveston County, Texas, placed a chlorpyrifos (Dursban 10CR, Dow Chemical Company, Midland, MI) treated ovitrap at every other residence along a two-block area. Researchers found that the number of *Ae. aegypti* positive ovitraps was typically lower than in control plots and that the average number of eggs per treated ovitrap was lower than control plots, reaching 50% lower in September of 1979 (Micks and Moon 1980). The majority of chlorpyrifos usage in the United States is in agricultural production, although nonagricultural uses are also common and include golf courses, turf, green houses, nonstructural wood treatments, roach and ant bait stations, and mosquito adulticides. Presently there are eight federally registered chlorpyrifos mosquito adulticide products, but there are no registered chlorpyrifos larvicide uses. In 2017 and again in 2019, the US EPA denied a petition to revoke agricultural (food) uses of chlorpyrifos (USEPA 2019b). While chlorpyrifos had not been registered for mosquito control in California, the state has initiated cancelation proceedings for chlorpyrifos agricultural and turf products (CalEPA 2019).

IGRs are pesticides that mimic the hormones expressed by larvae and pupae, thereby interrupting the natural metamorphosis process and either preventing emergence of adults or resulting in deformed adults. Two primary IGRs are currently being used for mosquito control in the CONUS, methoprene and pyriproxyfen. Interestingly, the presence of methoprene (Altosid, Central Life Sciences, Schaumburg, IL) was found to be attractive to ovipositing *Ae. aegypti* adults in Louisiana, resulting in greater egg collections in treatment cups than in control cups lacking this IGR (Carroll 1979). In male mosquitoes that do emerge after treatment with methoprene in the fourth instar or pupal stage, laboratory evidence indicates an inability of these males to achieve rotation of the terminalia for successful mating (O'Donnell and Klowden 1997). Unfortunately, field evidence for the efficacy of this IGR against *Ae. aegypti* in the CONUS has not been published.

Pyriproxyfen is another IGR (trade name Nyguard IGR, MGK, Minneapolis, MN) that has been tested for efficacy against *Ae. aegypti* larvae in field trials. This IGR was found to be effective, causing 82 and 87% emergence inhibition of *Ae. aegypti* larvae at 164 and 329 ml/ha, respectively, when applied by ULV truck-mounted sprayer. Furthermore, emergence inhibition of up to 92% was observed in cups at 8 m from the spray path (Doud et al. 2014).

Monomolecular films and mineral oils are different from the other chemical larvicides discussed in that rather than targeting the insect itself, they target the water's surface, reducing surface tension and making respiration of larvae and pupae more difficult. In laboratory studies, monomolecular films (Aquatain Mosquito Formula, Aquatain Products Pty Ltd, Kyneton, Victoria, Australia; applied at 1.0 ml/m^2) have been shown to produce relatively low larval *Ae. aegypti* mortality after 48 h (34% mortality) (Webb and Russell 2009). However, in treated containers, no new fourth instars pupated during this 48-h period and the monomolecular film produced 100% mortality of pupae within 180 min. Field-based evaluations on the effectiveness of monomolecular films for CONUS *Ae. aegypti* populations have not been conducted; however, mosquito control districts in the United States are currently using monomolecular films and mineral oils (CCMC 2019, STPMAD 2019b).

Adulticides

Early adult *Ae. aegypti* control was achieved using DDT, which was a very effective organochlorine pesticide that was responsible for much of the eradication seen throughout North, Central, and South America between the 1940s and 1960s (Schliessmann 1964, Camargo 1967). However, while DDT was effective at controlling *Aedes aegypti*, 70–80% of its use was for agricultural and forest pests, for which it was used ubiquitously (Turusov et al. 2002). Due to the widespread use of DDT, insecticide resistance to this pesticide was found to develop quickly (Abedi and Brown 1961). Furthermore, the environmental persistence and negative effects of organochlorine pesticides on wildlife and humans (Risebrough et al. 1967, Porter and Wiemeyer 1969, Beyer 1980, Roberts and Reigart 2013) led to the suspensions of registrations for this pesticide. There are no organochlorine pesticides being used for mosquito control in the CONUS at this time (Roberts and Reigart 2013).

Currently, adult mosquito control in the CONUS is limited to the use of organophosphate and pyrethrin/synthetic pyrethroid pesticides (USEPA 2019a). Typically, application of adulticides is performed using ULV cold fogging, thermal fogging, or aerial applications. For application over large areas of open land, aerial applications may be necessary (Latham and Barber 2007). Aerial applications have been found to be effective at controlling exposed outdoor *Ae. aegypti*; however, *Ae. aegypti*'s close ties to human habitation means that this species typically does not fly very far to find hosts and may not be as likely to encounter aerial sprays when resting in sheltered sites awaiting a human host (Britch et al. 2018). Although ULV is generally considered the preferred application route for ground application by mosquito control districts due to the smaller application volume required and a less visible plume and quieter application leading to lower perceived impact on the public, there is some evidence that thermal fogging may be more effective in hot-humid and hot-arid environments (Britch et al. 2010). The anthropophilic nature of *Ae. aegypti* adds further complexity to the most effective route of adulticide application.

Common OP adulticides in use by mosquito control districts in the CONUS include malathion, chlorpyrifos, and naled. While there are data on the use of chlorpyrifos against *Ae. aegypti* larvae, the evidence of its efficacy against adults is currently lacking; however, the efficacy of malathion has been assessed to some degree. In one 1987 study conducted in New Orleans, Louisiana, applicators used ground ULV applications of malathion (formulated as 91% malathion with heavy aromatic naphtha at a 2:1 ratio, applied at a rate of 48 ml malathion active ingredient per hectare) for 11 consecutive treatments over 6 d. In the short term, these applications were effective, resulting in significantly lowered egg counts in ovitraps and adult captures. However, these results were short-lived, with populations rebounding within 2 wk of the final treatment to higher levels than in control areas (Focks et al. 1987). Ultra-low volume applications of malathion (formulation and application rate undisclosed) were carried out using a truck mounted ULV fogger to caged *Ae. aegypti* in coastal Texas. These treatments were effective, achieving 90–100% mortality within a half-block area of the spray path; however, beyond the half block, poor control was achieved likely due to the presence of vegetation and other physical barriers to pesticide dispersal (Micks and Moon 1980).

Few studies have evaluated the efficacy of naled against *Ae. aegypti*. This OP pesticide is typically applied aerially, although aerial applications for *Ae. aegypti* are relatively uncommon except during outbreak events. During the 2016 Zika outbreak in Miami-Dade, Florida, aerial applications of Naled (Dibrom Concentrate, AMVAC Chemical Corporation, Newport Beach, CA) were utilized on the southern Miami Beach and Wynwood zones (McAllister et al. 2020). Although mosquito surveillance was not ongoing in these neighborhoods prior to the outbreak, surveillance conducted during the intervention indicated reductions in adult abundance after these adulticide applications. In areas where larviciding with *Bti* (Vectobac WGD) applied by buffalo turbine were also conducted, this suppression effect was maintained.

Pyrethrum is a natural insecticidal agent produced from the extraction of oleoresin of chrysanthemum flowers that causes nervous system paralysis in insects (Roberts and Reigart 2013). Pyrethrum is often used in formulations with the synergist piperonyl butoxide (PBO), which prevents enzymatic detoxification of pesticides by insects. This combination has been tested against *Ae. aegypti* in California, where a pyrethrum + PBO formulation (Evergreen EC 60–6, MGK, Minneapolis, MN) was applied by truck mounted ULV fogger at a rate of 60 g/ha. The greatest mortality was seen at 15.2 m from the spray path (100 and 93.3% mortality in two trials), with decreasing mortality with increased distance from the spray path. The overall average mortality seen across all distances and trials was 58.4% (Cornel et al. 2016).

Pyrethroid pesticides are the synthetic alternative to pyrethrums, which have the same mode of action causing nervous system paralysis in insects (Roberts and Reigart 2013). Pyrethroid pesticides in use for mosquito control within the CONUS include the active ingredients deltamethrin, etofenprox, permethrin, sumithrin, lambda-cyhalothrin, bifenthrin, cyfluthrin, and resmethrin. While there is some data on the efficacy of a few of these pesticides, no CONUS *Ae. aegypti* field studies are published for lambda-cyhalothrin, bifenthrin, cyfluthrin, or resmethrin at this time. In general, there is a lack of field evidence for the use of most pyrethroid pesticides against *Ae. aegypti* in the CONUS.

Field trials using deltamethrin (formulated as DeltaGard, Bayer Inc., Cary, NC) have produced mixed results against *Ae. aegypti*. Trials conducted in California showed high efficacy of deltamethrin (applied at 1.5 g/ha by a truck mounted ULV sprayer) against *Ae. aegypti* in open fields (100% mortality). However, when applied in a residential area containing houses, only 55.6% mortality was observed (Cornel et al. 2016). At max label rate (0.00134 lb/acre), DeltaGard produced 93% mortality in field cage assays using locally collected *Ae. aegypti* during the 2016 Zika virus outbreak in Miami-Dade County, Florida. Following these trials and bottle bioassays, DeltaGard was used in two zones of local Zika transmission in Miami-Dade County (northern Miami Beach and Little River) that did not receive aerial applications of Naled (McAllister et al. 2020). After application, and in conjunction with buffalo turbine applications of Vectobac WDG, decreased mean mosquito counts and continued suppression were observed. It is worth noting that it is unclear whether natural seasonality changes may have impacted these results for the Little River zone, however.

Etofenprox (formulated as Zenivex, Central Life Sciences, Schaumburg, IL) applied at maximum application rates by ULV truck mounted fogger during field assays conducted in response to the 2016 Zika outbreak in Miami, Florida, produced relatively low mortality (57%) (McAllister et al. 2020). Similarly, in trials conducted in California, Zenivex (applied at 4 g/ha by truck mounted cold aerosol ULV fogger) only provided 74.6% control compared with 100% with DeltaGard in open fields (Cornel et al. 2016). The mode of action for etofenprox is very similar to other pyrethroids through its action on the neuronal axon; however, it differs in some of its base chemical composition, which may lead to the overall lower efficacy against *Ae. aegypti*.

There is a lack of field evidence for efficacy of permethrin and sumithrin against *Ae. aegypti*, except from field assays conducted during the 2016 Zika outbreak in Miami, Florida. These assays indicated that permethrin (formulated as Biomist, Clarke, Rosella, IL) and sumithrin (formulated as Duet, Clarke, Roselle, IL) at mid-rate applications were not found to produce significant control overall at 33% mortality and 44% mortality, respectively (McAllister et al. 2020). Further research on the efficacy of these pyrethroid pesticides in various CONUS habitat types is necessary.

Insecticide Resistance

Although insecticide resistance is not a new concept, it is a growing problem worldwide. Insecticide resistance can occur through several mechanisms which can be characterized into two categories: metabolic resistance mechanisms and target site mutations. Metabolic resistance mechanisms are seen with changes in the activity of carboxylesterases, P450-dependent monooxygenases, and glutathione *S*-transferases. Common target sites for resistance development include mutations in acetylcholinesterase receptors, GABA receptors, and voltage-gated sodium channels that ultimately lead to the *kdr*, or knockdown resistance, phenotype. A more in-depth discussion of these mechanisms is available in multiple review articles (Brogdon and McAllister 1998, Hemingway et al. 2004, Liu 2015).

Resistance in *Ae. aegypti* has been very loosely monitored in the CONUS since early acknowledgement of DDT resistance (Camargo 1967); however, recent efforts are improving the collection of insecticide resistance evidence for this species within the CONUS. The CDC instituted a program to provide free materials to perform CDC insecticide resistance bottle bioassays to mosquito control districts in the United States (CDC 2019a). Additionally, the development of the MosquitoNET database to document resistance throughout the United States is allowing closer tracking of the resistance status of CONUS *Ae. aegypti* populations (CDC 2019b). As these programs become more broadly used, the status of insecticide resistance in the CONUS will become clearer.

Currently, there is ongoing resistance testing for CONUS *Ae. aegypti* adult populations. Resistance to a variety of pyrethroid pesticides including permethrin, etofenprox, and bifenthrin was observed in *Ae. aegypti* from Dallas, TX (Richards et al. 2018). This population was also categorized as possibly resistant to deltamethrin (demonstrating between 80 and 97% mortality in bottle bioassays). Newly established California populations were not found to be resistant (>90% knock-down was found in assays); however, the population showed greater time to knock-down for pyrethrum, sumithrin, and permethrin, which was countered by using piperonyl butoxide. The susceptibility of this population to deltamethrin and malathion was comparable to the susceptible population analyzed (Cornel et al. 2016). *Aedes aegypti* populations collected around Miami-Dade County, Florida, showed extremely high resistance to all synthetic pyrethroids tested including sumithrin (<15% mortality), etofenprox (<10% mortality), permethrin (<15% mortality), and deltamethrin (<70% mortality). The same populations were found to be susceptible to the OP pesticides, malathion and naled (McAllister et al. 2020). Populations of *Ae. aegypti* collected from counties around Florida showed moderate to high resistance to permethrin (Estep et al. 2018), and ongoing monitoring of *Ae. aegypti* populations throughout Florida shows increasing resistance to a variety of pyrethroid active ingredients including cypermethrin, deltamethrin, etofenprox, permethrin, and sumithrin (Parker et al. 2020). This variability demonstrates the need for resistance monitoring prior to the application of pesticides to ensure efficacy of the product and to decreased expenditures on ineffective products.

Resistance monitoring methods for larval mosquitoes have been developed by the World Health Organization (World Health Organization 1981) in order to monitor resistance to larvicides; however, there is currently a paucity of available data on larval resistance in CONUS *Ae. aegypti*. Generating these data for *Ae. aegypti* populations throughout the country should be a priority alongside the increased focus on adult insecticide resistance. In the event of an outbreak, it is vital that there are data on all available control options to make sound recommendations on the best strategy.

Managing insecticide resistance in the CONUS *Ae. aegypti* populations should be an important goal for all mosquito control jurisdictions with established *Ae. aegypti* populations. In the event of an *Ae. aegypti*-borne virus outbreak, having knowledge of the most effective control options is critical. Rotating pesticides and pesticide classes is one way to prevent the evolution of resistance. By alternating chemicals with different modes of action for both adult and larval mosquitoes, vector control operations can delay the evolution of insecticide resistance to any one chemical class. Additionally, using an integrated mosquito management (IMM) strategy using a variety of methods in addition to chemical control, many of which are described throughout this work, can remove selection pressure for the evolution of resistance from the population. Finally, increased effort should be placed on the research and development of novel insecticide classes. The limited options of only two classes of adulticides in the CONUS severely restricts our ability to effectively manage resistance.

Biological Control

Biological control (biocontrol) techniques are those that employ natural enemies, predators, parasites, and natural pathogens of an organism in order to control it. A variety of biological control agents have been discovered and employed for mosquitoes worldwide. However, as multiple review articles on the subject reveal, most of the field testing employed for these methods has been conducted outside of the CONUS (Han et al. 2015, Lima et al. 2015). Despite this lack of data, there are benefits of biological control such as a very slow evolution of resistance to these control measures (Rey et al. 2004). Another benefit can be lower costs associated with biological control; however, it is also important to consider the time and infrastructure needs for these types of control options.

Larvivorous Arthropods

One biocontrol technique that has received a lot of attention is the employment of larvivorous arthropods that feed on mosquito larvae. The best studied larvivorous arthropod group for *Ae. aegypti* is the autogenous mosquito genus *Toxorhynchites* (Diptera: Culicidae). *Toxorhynchites* larvae typically prey upon other mosquito larvae to achieve adequate protein provisions for egg production later in life. As such, blood is not required for completion of a gonotrophic cycle. Four *Toxorhynchites* species have been investigated for their efficacy against *Ae. aegypti* within the CONUS including *Toxorhynchites rutilus rutilus* (Coquillett) (Diptera: Culicidae), *Toxorhynchites amboinensis* (Doleschall) (Diptera: Culicidae), *Toxorhynchites moctezuma* (Dyar and Knab) (Diptera: Culicidae), and *Toxorhynchites splendens* (Wiedemann) (Diptera: Culicidae).

Toxorhynchites rutilus rutilus (Diptera: Culicidae) is a native species in the southeastern U.S. that has predominantly been investigated as a control option in field trials in Louisiana. This species was able to provide 74% control of *Ae. aegypti* on average in field trials in New Orleans, Louisiana (Focks et al. 1982). This species was also found to preferentially feed upon fourth instar *Ae. aegypti*, allowing natural density dependent mortality in early instars to occur and then be compounded by the predator effect in late instars (Padgett and Focks 1981). Unfortunately, the sylvan nature of *Tx. rutilus rutilus* may hamper its ability to act as a biological control due to their preference for elevated tree holes as oviposition sites and difficulty in locating oviposition sites at the ground level (Focks et al. 1983a).

Toxorhynchites amboinensis, a *Toxorhynchites* species native to Asia, also shows promise as a biocontrol agent due to their ability to locate and oviposit in a broad range of small containers found in typical urban environments in New Orleans, Louisiana. This species is also effective against anthropophilic species such as *Ae. aegypti* as they have demonstrated a willingness to enter buildings to seek out suitable oviposition sites, preferentially laying eggs in those containers possessing prey 1.6-fold more often than containers lacking prey in field releases in New Orleans (Focks et al. 1983b). When used in an integrated approach with ground-ULV applications of malathion, introductions of *Tx. amboinensis* resulted in 96% suppression of *Ae. aegypti* in field trials conducted in New Orleans, Louisiana, compared with only 29% suppression with malathion alone (Focks et al. 1986). This species seems to be an efficient biocontrol agent, especially when used as part of an IMM strategy. Little data are available on the survival and fecundity of adults of this species specifically in the CONUS, so the frequency and amplitude of releases necessary to enact long-term control is unclear. Additionally, there are issues associated with releasing exotic species into the CONUS without proper evaluation. Any future studies conducted with foreign arthropods must be properly evaluated to prevent unintended ecological consequences.

Other *Toxorhynchites* species that have received less attention include *Toxorhynchites moctezuma* and *Toxorhynchites splendens. Toxorhynchites moctezuma* was tested as a biocontrol option for tire piles in Brownsville, TX, near the Mexico border. This species was found to effectively control mosquito pupae in tire piles, especially when under canopy cover. However, this control waned when prey populations grew beyond the predatory capacity of the *Tx. moctezuma* individuals (Uejio et al. 2014). *Toxorhynchites splendens* was investigated as a biocontrol agent in multiple cities of Florida. Unfortunately, this species was unable to establish local populations and, even after multiple releases of laboratory reared adults, there was no significant reduction in *Aedes* larvae (Schreiber and Jones 1994).

There are some limitations to the use of this larvivorous arthropod group. Rearing *Toxorhynchites* colonies can be labor intensive and requires the rearing of additional mosquitoes to act as larval food sources. Furthermore, distribution of *Toxorhynchites* to treatment areas can be labor intensive, especially if the released individuals are not reproducing naturally to sustain the population.

Larvivorous Fishes

Larvivorous fishes are another biocontrol method used for mosquitoes, employing species such as mosquito fish (*Gambusia* species) and killifish (*Fundulus* species). This method is effective in areas where *Ae. aegypti* are infiltrating larger water bodies, such as cisterns or wells. One investigation of the efficacy of *Gambusia holbrooki* (Girard) in Key West, Florida, found that *Gambusia* were unable to catch up if introduced to a heavily infested water body. However, if an initial control method was used to clear the body of *Ae. aegypti* larvae prior to introducing *Gambusia*, the fish were able to prevent reestablishment of *Ae. aegypti*. Further, in containers with *Gambusia* added, only 0.7% of containers still had *Ae. aegypti* larvae compared with 49.7% of containers without *Gambusia* (LeVan 1941). Although there is no published data on the cost effectiveness or efficacy of mosquitofish for modern *Ae. aegypti* management, many mosquito control agencies have programs to provide mosquitofish to the public for use in private water-holding vessels or water features on their property (e.g., Consolidated Mosquito Abatement District, California; Sacramento-Yolo Mosquito & Vector Control District, California; Salt Lake City Mosquito Abatement District, Utah).

Entomopathogenic Fungi

While studies have been conducted to investigate the effects of various entomopathogenic fungi on *Ae. aegypti* (Scholte et al. 2004), relatively few studies have investigated the field application of this biocontrol option. In the laboratory, *Ae. aegypti* larvae have shown susceptibility to *Lagenidium culicidum* (Umphlett) (McCray et al. 1973), *Crypticola clavulifera* (Humber, Frances, and Sweeney) (Frances et al. 1989), *Culicinomyces clavosporus* (Couch) (Cooper and Sweeney 1982), *Beauveria tenella* (Saccardo) (Pinnock et al. 1973), and *Metarhizium anisopliae* (Metchnikoff) (Daoust et al. 1982). Adults are susceptible to *Entomophthora culicis* (Braun) (Kramer 1982), *Erynia conica* (Nowakowskia) (Cuebas-Incle 1992), and *Beauveria bassiana* (Balsamo)(Clark et al. 1968). *Coelomomyces stegomyiae* (Keilin) has also been shown to interrupt reproduction of *Ae. aegypti* in laboratory studies by preventing egg development and oviposition (Lucarotti 1987).

The major challenge to using entomopathogenic fungi as a biocontrol method is in the delivery of the fungi to the target organism and the longevity of the fungi in nature (Achee et al. 2019). Additionally, many of these fungi have negative effects on nontarget species, including both vertebrates and invertebrates (Scholte et al. 2004). However, as discussed at length later in this paper in the section on Trapping, *Beauveria bassiana* has been successfully tested in the United States as part of the In2Care (In2Care BV, Wageningen, The Netherlands) autodissemination trap to control *Ae. aegypti* (Snetselaar et al. 2014). The successful use of this entomopathogenic fungus provides evidence that further research into the effective deployment and use of other fungi is warranted.

Copepods

Although cyclopoid copepods (Crustacea: Copepoda) have been employed worldwide to control mosquitoes (Lazaro et al. 2015), the available CONUS data for these organisms are sparse. Field and semifield trials have been conducted in Vero Beach, Florida, using the copepod *Macrocyclops albidus* (Jurine) (Rey et al. 2004). This species was found to survive and reproduce effectively in the subtropical climate and resulted in significant control of *Ae. aegypti* in semifield experiments. Results demonstrated a dose-dependent effect with 22% survival of mosquito larvae in containers with 10 copepods versus 1% survival in containers with 100 copepods added. However, one study conducted in Brownsville, Texas, found that when combined with *Tx. moctezuma* in tires, *Mesocyclops longisetus* (Thiébaud) did not result in any additional reduction of *Ae. aegypti* pupae or adults (Uejio et al. 2014).

Mosquito control programs in the United States have employed the use of copepods historically and continue to do so. The New Orleans Mosquito, Termite, and Rodent Control Board produced a manual for the collection, propagation, and distribution of copepods for mosquito control around the city (Marten et al. 1997). Using the colonized copepods, the district found that the presence of *M. longisetus* and *M. albidus* resulted in a 99% reduction of *Aedes* larvae in tires, although a breakdown of which *Aedes* species were found dispatched in each tire was not listed (the paper lists *Ae. albopictus*, *Ae. aegypti*, and *Ae. triseriatus* Say as tire-inhabiting species common in New Orleans) (Marten et al. 1994). Additional research is needed to determine the efficacy of copepods in other habitat types and climatic zones of the CONUS and to identify additional species for testing.

Parasites of *Ae. aegypti*

The microsporidian, *Edhazardia aedis* (Kudo), has shown promise as a biological control for *Ae. aegypti*. Within the laboratory, this parasite has been shown to transmit vertically from mother to offspring and significantly affect the egg batch size and emergence rates of *Ae. aegypti* (Becnel et al. 1995). Furthermore, while this parasite does infect other mosquito species, there is evidence that vertical transmission is restricted to *Ae. aegypti* (Becnel and Johnson 1993). In semifield evaluations of *Edhazardia aedis* conducted in large screened enclosures in Florida, inoculative releases of six infected larvae per container to four out of 26 containers (with four covered, inaccessible containers as controls) resulted in inoculation of all containers by 20 wk post initial inoculation. However, the enclosed *Ae. aegypti* population was not completely removed in this manner and the parasite was unable to successfully overwinter in containers through the following study year. The same study further investigated inundative releases of the parasite, in which 25 infected larvae were placed into each of the study containers, resulted in complete elimination of *Ae. aegypti* by week 11 (Becnel 2000). Andreadis (2007) produced an extensive review of the available literature on microsporidian biology, ecology, and use as biological control agents of mosquitoes.

Gregarine parasites have also shown some promise against *Ae. aegypti*; however, these parasites have only been evaluated in a laboratory at this time. *Ascogregarina culicis*, a Protozoan parasite, resulted in mortality to *Ae. aegypti* larvae and had a sublethal effect of shortening the development time of the mosquito larvae (Sulaiman 1992). This effect was impacted by the geographical location of the parasite strain and mosquito strain, however, indicating that this is a biocontrol that would need extensive field evaluation for CONUS *Ae. aegypti* populations. Additional information on Gregarine parasites and their potential role as mosquito biocontrol can be found in Tseng (2007).

Source Reduction Through Community Engagement

Container Removal

Due to their reliance on small container habitats, there is potential to reduce *Ae. aegypti* through the employment of source reduction through community engagement. The early attempts to eradicate *Ae. aegypti* from the United States identified community source reduction as a major aspect of the eradication efforts, relying on citizens to remove water holding containers from their property (Schliessmann 1967). The U.S. Navy and Marine Corps still consider source reduction as the best method for population reduction of container inhabiting *Ae. aegypti* (Navy and Marine Corps Public Health Center 2016). This 'bottom-up' approach has been identified as an effective means of providing control of *Ae. aegypti* even after governmental resources for 'top-down' approaches have dried up (Gubler and Clark 1996). However, there have been challenges in getting communities worldwide to participate in these programs, often stemming from a disconnect between the message and the perception of the local populace (Service 1993). Further, participation tends to wane during outbreak-free periods, necessitating strong leadership to encourage continued action and diligence (Morrison et al. 2008).

In the United States, community engagement is being used to counter and control *Ae. aegypti* in various regions. The Consolidated Mosquito Abatement District of California has developed outreach brochures and dedicated a section of their website to *Ae. aegypti* surveillance, information, and control for the public (CMAD 2019a). In areas with active *Ae. aegypti* populations, outreach through television and internet campaigns is being used to increase public awareness (Cornel 2016). In California, upon realizing that many new identifications of *Ae. aegypti* were made by the public, greater focus was placed on community outreach through the radio, newspaper, mailers, billboards, and public-school presentations (Metzger 2017). Citizen science is also being used through the Invasive Mosquito Project to provide classroom education on invasive mosquitoes and mosquito-borne diseases and to collect data from around the United States on the presence of invasive *Aedes* and *Culex* mosquitoes (Invasive Mosquito Project 2020).

While source reduction enacted through community engagement is beneficial in reducing the overall burden of *Ae. aegypti* in the landscape, this method will not prevent or remove all larval habitats. Water filled habitats associated with urban areas such as storm drains, septic tanks, and water treatment facilities can still provide harborages for mosquito larvae that cannot be rectified by the public (Hribar et al. 2006, Barrera et al. 2008, Arana-Guardia et al. 2014). This emphasizes the importance of having a cooperative approach to mosquito control with a focus on IMM to control *Ae. aegypti*. Source reduction provides a permanent solution to removing oviposition sites for *Ae. aegypti*. However, a major gap for the CONUS regarding this method is lack of published data on efficacy measures, successful educational campaigns with benchmarks, and other data to explain the effectiveness of this approach.

Trapping

Trapping is sometimes overlooked as a control measure due to the excessive manual labor that can be involved and often low collection rates. Many traps, such as ovitraps, BG Sentinel traps, and CDC miniature light traps are excellent surveillance tools, but have not generally been considered a control option. However, development of new traps and modifications of existing traps are allowing increased collections and mortality of collected mosquitoes, pushing traps from the surveillance only category to surveillance and control (Kline 2006).

In the early 1980s, autocidal ovitraps were designed with removable egg paddles that could be maintained weekly as well as backup mesh that prevented second instar mosquito larvae from accessing the water surface and thereby leading to mortality. These ovitraps were field tested in Houston, Texas, where they resulted in a decline in the Breteau index of 36% compared to an increase of 500% in control areas (Cheng et al. 1982).

Ovitraps were further modified in order to target multiple mosquito life stages. The In2Care trap is an autodissemination ovitrap wherein the mosquito enters the trap to lay eggs and pyriproxyfen adheres to the mosquito body. Due to the skip-oviposition habit of *Ae. aegypti*, the female will carry the pyriproxifen to nearby oviposition sites, often finding cryptic sites that are difficult to find and remove. The female also picks up spores of *Beauveria bassiana* from the trap, which will kill the female in 1–2 wk (Snetselaar et al. 2014). Results of semifield experiments conducted in Palmetto, Florida, using recently colonized *Ae. aegypti* from Manatee County, FL, showed that the In2Care traps were attractive oviposition containers to *Ae. aegypti*, achieved 81% inhibited emergence in pots that received pyriproxifen transferred by females, and significantly decreased the female's survival (Buckner et al. 2017).

The CDC autocidal gravid ovitrap is another modified ovitrap used for the control of container breeding mosquitoes. This bucket style oviposition trap contains infusion water to attract gravid *Ae. aegypti*, an autocidal oviposition substrate to kill offspring, and is coated with an adhesive to catch and kill resting females (Mackay et al. 2013). This trap was employed in field trials conducted in California where one trap was placed at each of 144 households for a total of 12 wk. This effort resulted in a significant reduction of *Ae. aegypti* collections in treatment versus control areas and a significant decline over time within the treatment area (Cornel et al. 2016).

Attractive toxic sugar bait (ATSB) stations are control based units that require little manual labor input. These stations rely on the mosquito's propensity to seek out sugar meals for energy. The mosquito feeds on the sugar and either ingests or comes into contact with pesticide resulting in mortality. The potential for sugar baited insecticide stations was discovered in Florida in the 1960s (Lea 1965), when 0.5 mg/ml malathion mixed with a 20% Karo syrup solution resulted in 100% mortality of caged *Ae. aegypti* compared to 26% in malathion alone treatments. The sugar substrate attracted the mosquitoes and enticed them to remain in contact with the pesticide longer than pesticide alone, resulting in higher mortality. As both male and female mosquitoes regularly seek out sugar meals, this control method targets both sexes. In addition to sugar, host kairomones such as L-lactic acid and 1-octen-3-ol, can be used to increase the mosquitoes approaching the station (Scott-Fiorenzano et al. 2017). This further increases the specificity of the station, reducing the number of nontarget insects attracted. One Florida study involving both semifield and field application of fipronil and boric acid-based bait stations found that while in semifield environments, both compounds resulted in lower landing rates compared with control, similar results were not found in field trials. In the field, there was no significant decrease in either the mosquito populations or in human landing rates after deployment of ATSB stations (Xue et al. 2008).

A series of propane-powered traps have been developed and tested as mosquito management tools. The best known of these is the Mosquito Magnet (Woodstream Corp., Lancaster, PA), which is a commercially available large trap that releases octenol and uses propane to generate a plume of CO_2 that attracts mosquitoes and then uses a strong vacuum to collect them into a reservoir. In early semifield trials conducted in a large outdoor screened enclosure (enclosure dimensions: 9.2 × 18.3 × 4.9 m) in Florida, the Mosquito Magnet was able to collect on average 626.1 of the 1,000 *Ae. aegypti* released into the enclosure, decreasing the landing count to just 2.6 on average within the enclosure (Kline 2002). This study also conducted field trapping; however, no *Ae. aegypti* were collected with the Mosquito Magnet during that part of the experiment. One *Ae. aegypti* individual was collected with another trap tested (counterflow geometry trap) indicating that there were *Ae. aegypti* present in the vicinity and the Mosquito Magnet failed to collect them. It is possible that the competition of alternative hosts in the field study resulted in lower trap captures than in the semifield cage study and additional research is needed to better understand the efficacy of the Mosquito Magnet traps for *Ae. aegypti*.

Sterile Insect Technique

Sterile insect technique (SIT) is a method in which male insects are sterilized and then inundative releases are conducted to increase the chances that the sterile males will outnumber fertile males on the landscape. The sterile males will mate with fertile females thereby making all her offspring nonviable and, in many cases, preventing her from mating again with a fertile male (Dyck et al. 2005). SIT has been used historically with major eradication campaigns for organisms such as the screwworm fly (*Cochliomyia hominivorax* (Coquerel)) (Krafsur et al. 1987, Vargas-Teran et al. 1994) and tsetse fly (*Glossina austeni* (Newstead)) (Vreysen et al. 2000) as well as others. One study found that irradiated sterile *Ae. aegypti* males released in Pensacola, Florida, showed no evidence of reducing the *Ae. aegypti* population and had no effect on the viability of eggs collected in the area (Morlan et al. 1962). This could indicate that SIT is inappropriate for this organism, or this result could have been influenced by other factors. Additional research is warranted based on the success of SIT with other organisms. Currently, mosquito control districts in the CONUS are developing SIT programs (Anastasia MCD 2017, Lee County, Florida MCD 2019).

Incompatible Insect Technique

In addition to traditional SIT, *Wolbachia*-based incompatible insect technique is an alternative method being tested in the CONUS. *Wolbachia* works through cytoplasmic incompatibility; when an infected male mates with an uninfected female, this renders her incapable of producing viable offspring, essentially sterilizing the female. This method has been tested in the field in Florida, with field releases of over 6.8 million *Wolbachia* infected males over the course of 6 mo. This effort resulted in a significant decline in egg hatch of 70% hatch by the end of the releases and a 78% reduction in *Ae. aegypti* females over the course of the study that was maintained through the following month after releases ceased (Mains et al. 2019). This method has also been field tested with *Ae. aegypti* in California through the Consolidated Mosquito Abatement District in collaboration with MosquitoMate and Verily from 2017 to 2019, achieving up to 95% reduction of *Ae. aegypti* females in 2018 and 84% reduction in 2019 in some neighborhoods (CMAD 2019b).

Autodissemination Augmented by Males

A novel approach to control, autodissemination augmented by males (also known as ADAM) relies on males dusted with pyriproxyfen to deliver the larvicide to larval habitat sites and to females through contact during mating. This method has been tested in three different areas of the CONUS including Los Angeles, California, Clovis, California, and Key Largo, Florida. In each of these sites, 10,000 pyriproxyfen dusted male *Ae. aegypti* were released weekly over the course of 5 wk in Clovis, 6 wk in Key Largo, and 8 wk in Los Angeles. While significant larval mortality was observed when larvae were exposed to water collected from ovisites in the three treatment areas, the effects on adult populations varied. ADAM releases did not produce significant reduction of female *Ae. aegypti* in Clovis. In Los Angeles, a 66% reduction in female *Ae. aegypti* was observed, which was maintained for 2 wk after releases ceased. Similarly, in Key West, an 88% reduction was observed, although maintenance of this reduction for 2 wk post treatment was not significant (Brelsfoard et al. 2019). Based on these results, ADAM shows potential as a control method for *Ae. aegypti*. Incomplete control of populations indicate that this method should be supplemented with additional control measures in operational mosquito control applications.

Conclusion

This review provides a synthesized account of the current research available on control of *Ae. aegypti* in the continental U.S.; however, it also highlights many important gaps in our knowledge base on controlling this important vector species. Recent upticks in the frequency of *Ae. aegypti*-borne pathogens in the CONUS (Rosenberg et al. 2018) combined with the expanding range of *Ae. aegypti* necessitates taking preemptive action to identify the most effective control options and have plans in place prior to the next major outbreak. Additionally, increased incidence of insecticide resistance necessitates evaluation and deployment of novel pesticides and integrated pest management strategies. It is unlikely that any single approach will be fully effective against *Ae. aegypti*, and development of IMM strategies will be imperative in effecting control of this species in the CONUS.

Despite the significant role *Ae. aegypti* plays in the transmission of pathogens worldwide, the available literature on control within the CONUS indicates a lag in research between the early eradication efforts and the modern increased incursion of the species. Indeed, early literature on the eradication effort identified that DDT resistance was already being reported in other areas and emphasized the need for, 'continuing studies to provide alternate control methods-for example, by substitution of other insecticides, or by use of chemo- and radiological sterilant or other biological control techniques' (Schliessmann 1964). This mosquito has already pushed the boundaries of its range beyond that which was previously anticipated, forging a path into urban centers such as Washington D.C. (Lima et al. 2016) and Las Vegas, Nevada (Pless and Raman 2018), cities in which both foot traffic and international travel are key elements of the local tourism industries. Our lag in developing and implementing effective control strategies for *Ae. aegypti* within the CONUS has put us at a disadvantage against this species in the face of potential future pathogen threats. As a nation, it is imperative that we establish best practices of *Ae. aegypti* control as well as effective predictive modeling to monitor the spread of this species. This would allow us to not only control this invasive threat where it already occurs but also to prevent further incursions into new regions.

Throughout the United States, mosquito surveillance and control programs face constant threat of losing funding (Kelly 2011, Del Rosario et al. 2014). This is particularly pronounced in poor and underserved communities (Harris et al. 2014). Mosquito control programs not only offer control services but are also tasked with monitoring mosquito populations. One study modeled the cost of responding to a vector-borne disease outbreak using various surveillance scenarios. The study found that without active surveillance at the time of a mosquito-borne disease outbreak, not only is there a lag in the response time to detect the presence of the pathogen, but there was a $380.9 million difference in the cost of the overall response to the outbreak (Vazquez-Prokopec 2010). Public health in the CONUS is at increasing risk for *Ae. aegypti*-borne pathogens despite our readiness for such events remaining stagnant. The evaluation of control strategies should be of paramount importance moving forward to prevent further incursion of vector-borne pathogens and the associated public health and economic consequences of inaction.

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Table 1.

Chemical, biological, trapping, and novel/genetic methods tested against *Aedes aegypti* populations within the United States

Class	Target	Active Ingredient	Product	Application Rate	Application Method	Year	State	Type	Citation
Chemical	Larvae	*Bacillus thuringiensis israelensis*	VectoBacWG	0.56 kg product in 4.7 liter water/ha	Aerial spray	2011	Florida	Field and Operational Experiments	Pruszynski et al. 2017
Chemical	Larvae	*Bacillus thuringiensis israelensis*	VectoBac WDG	1 kg/ha	Handheld thermal fogger	2015	Florida	Semi-field and Field	Knapp et al. 2018
Chemical	Larvae	*Bacillus thuringiensis israelensis*	VectoBac GR	1.1 kg/1,000 m^2	Backpack sprayer	2014	Florida	Semi-field	Harwood et al. 2015
Chemical	Larvae	*Bacillus thuringiensis israelensis*	Sustain MGB	0.9 kg/1,000 m^2	Backpack sprayer	2014	Florida	Semi-field	Harwood et al. 2015
Chemical	Larvae	*Bacillus thuringiensis israelensis*	VectoBac WDG	34 g granules in 7.5 liter watei; applied at 1 1/1,000 m^2	Backpack sprayer	2014	Florida	Semi-field	Harwood et al. 2015
Chemical	Larvae	*Bacillus thuringiensis israelensis*	Aquabac XT	82 ml in 7.5 liter water, applied at 1 liter/1,000 m^2	Backpack sprayer	2014	Florida	Semi-field	Harwood et al. 2015
Chemical	Larvae	*Bacillus thuringiensis israelensis*	Vectobac WGD	0.5 lb/acre	Aerial spray and truck-mounted ULV sprayer	2016	Florida	Operational	McAllister et al. 2020
Chemical	Larvae	Chlorpyrifos	Dursban 10CR	1 pellet/container	Applied by hand	1979	Texas	Operational	Micks and Moon 1980
Chemical	Larvae	Pyriproxyfen	Nyguard IGR	164 ml/ha in spray 1, 329 ml/ha in sprays 2 and 3	Truck-mounted ULV sprayer	2012	Florida	Field-Trial	Doud et al. 2014
Chemical	Larvae	Spinosad	Natular 2EC	182.6 ml/ha	Backpack sprayer	2016	California	Field-Trial	Golden et al. 2018
Chemical	Larvae	Spinosad	Natular 2EC	79.5 ml/acre	Truck-mounted ULV sprayer	2016	Florida	Field-Trial	Aldridge et al. 2018
Chemical	Adults	Deltamethrin	DeltaGard	1.3 liter/km	Truck-mounted ULV sprayer	2013	California	Field-Trial	Cornel et al. 2016
Chemical	Adults	Deltamethrin	DeltaGard	0.0035 lb/acre	Truck-mounted ULV sprayer	2016	Florida	Field-Trial	McAllister et al. 2020
Chemical	Adults	Deltamethrin	DeltaGard	0.007 lb/acre	Truck-mounted ULV sprayer	2016	Florida	Field-Trial	McAllister et al. 2020
Chemical	Adults	Deltamethrin	DeltaGard	0.007 lb/acre	Truck-mounted ULV sprayer	2016	Florida	Operational	McAllister et al. 2020

Chemical	Adults	Etofenprox	Zenivex	0.0035 lb/acre	Truck-mounted ULV sprayer	2016	Florida	Field-Trial	McAllister et al. 2020
Chemical	Adults	Etofenprox	Zenivex	396.9 ml/km	Truck-mounted ULV sprayer	2013	California	Field-Trial	Cornel et al. 2016
Chemical	Adults	Malathion	91% malathion + heavy aromatic naphtha	48 ml/ha	Truck-mounted ULV sprayer	1982	Louisiana	Operational	Focks et al. 1987
Chemical	Adults	Malathion	Undisclosed	Undisclosed	Truck-mounted ULV sprayer	1979	Texas	Field-Trial	Micks and Moon 1980
Chemical	Adults	Naled	Dibrom	0.1 lb/acre	Aerial spray	2016	Florida	Operational	McAllister et al. 2020
Chemical	Adults	Permethrin + PBO	Biomist	0.0035 lb/acre	Backpack sprayer and truck-mounted ULV sprayer	2016	Florida	Field-Trial	McAllister et al. 2020
Chemical	Adults	Pyrethrin + PBO	Evergreen EC 60–6	573.2 ml/km	Truck-mounted ULV sprayer	2013	California	Field-Trial	Cornel et al. 2016
Chemical	Adults	Sumithrin + Prallethrin	Duet	0.0035 lb/acre	Backpack sprayer and truck-mounted ULV sprayer	2016	Florida	Field-Trial	McAllister et al. 2020

Class	**Target**	**Classification**	**Species**	**Additional Agents Used**	**Release Rate/Method**	**Year**	**State**	**Type**	**Citation**
Biological	Larvae	Larvivorous Arthropod	*Toxorhynchites rutilus rutilus*	N/A	1–2 *Tx.* added per container	1980	Louisiana	Field-Trial	Focks et al. 1982
Biological	Larvae	Larvivorous Arthropod	*Toxorhynchites amboinensis*	N/A	10 releases of between 56 and 521 each males and females	1981	Louisiana	Field-Trial	Focks et al. 1983b
Biological	Larvae	Larvivorous Arthropod	*Toxorhynchites amboinensis*	2:1 mixture 91% malathion + heavy aromatic naphtha	1,600 females released/ week for 14 wk	1983	Louisiana	Field-Trial	Focks et al. 1986
Biological	Larvae	Larvivorous Arthropod	*Toxorhynchites moctezuma*	*Mesocyclops longisetus*	Naturally occuring *Tx. moctezuma* populations tested	2007	Texas	Field-Trial	Uejio et al. 2014
Biological	Larvae	Larvivorous Arthropod	*Toxorhynchites splendens*	N/A	13,000 adult *Tx. splendens* released throughout two years of study	1989–1990	Florida	Field-Trial	Schreiber and Jones 1994
Biological	Larvae	Larvivorous Fish	*Gambusia holbrooki*	Kerosene	40–50 *Gambusia*/cistern	1939	Florida	Operational	LeVan 1941
Biological	Larvae	Copepod	*Macrocyclops albidus*	N/A	10 or 100 copepods/ container	1999	Florida	Semi-Field	Rey et al. 2004
Biological	Larvae	Copepod	*Macrocyclops albidus*	N/A	150 copepods added/ container	2000–2002	Florida	Field-Trial	Rey et al. 2004
Biological	Larvae	Copepod	*Macrocyclops albidus*	N/A	Poured/sprayed with backpack sprayers	1994	Louisiana	Operational	Marten et al. 1994

Biological	Larvae	Copepod	*Mesocyclops longisetus*	N/A	Poured/sprayed with backpack sprayers	1994	Louisiana	Operational	Marten et al. 1994
Biological	Larvae	Copepod	*Mesocyclops longisetus*	*Toxorhynchites moctezuma*	~150 *M. longisetus* added	2007	Texas	Field-Trial	Uejio et al. 2014
Biological	Larvae	Microsporidian	*Edhazardia aedis*	N/A	6 infected larvae/ container in inoculative trials; 25 infected larvae/container in inundative release trials	1996	Florida	Semi-Field	Becnel 2000
Class	**Target**		**Trap Type**	**Other Chemical Agents Involved**	**No. Traps Employed**	**Year**	**State**	**Type**	**Citation**
Trapping	Larvae		Autocidal ovitraps	N/A	330 traps, two placed per premisis	1977–1978	Texas	Field-Trial	Cheng et al. 1982
Trapping	Larvae/ Adults		In2Care Trap	Pyriproxifen, *Beauveria bassiana*	Four/enclosure for dissemination trials	2015	Florida	Semi-Field	Buckner et al. 2017
Trapping	Larvae/ Adults		CDC Autocidal Gravid Trap	Autocidal oviposition substrate and adhesive	One trap/household, 144 households	2014	California	Field-Trial	Cornel et al. 2016
Trapping	Adults		Attractive Toxic Sugar Baits	1% boric acid, 0.1% fipronil	Six traps/enclosure	Not Listed	Florida	Semi-Field	Xue et al. 2008
Trapping	Adults		Propane-1 Trap	CO_2	One trap/night for two nights	1996	Florida	Semi-Field	Kline 2002
Trapping	Adults		Portable CO_2 Trap	CO_2	One trap/night for four nights *(Ae. aegypti* only tested one night)	1997	Florida	Semi-Field	Kline 2002
Trapping	Adults		Portable Propane trap	CO_2	One trap/night for 30 nights *(Ae. aegypti* only tested for 19 nights)	1998	Florida	Semi-Field	Kline 2002
Trapping	Adults		Mosquito Magnet Beta-1	CO_2 and l-octen-3-ol	One trap/night for 25 nights *(Ae. aegypti* only tested for six nights)	1999	Florida	Semi-Field	Kline 2002
Trapping	Adults		Mosquito Magnet Beta-2	CO_2 and l-octen-3-ol	One trap/night for 11 nights	1999	Florida	Semi-Field	Kline 2002
Trapping	Adults		Mosquito Magnet	CO_2	One trap/night for 9 nights	1996	Florida	Semi-Field	Kline 2002
Class	**Target**			**Technique**		**Year**	**State**	**Type**	**Citation**
Genetic/ Novel	Adults/ Larvae			Sterile Insect Technique		1960–1961	Florida	Operational	Morlan et al. 1962
Genetic/ Novel	Adults/ Larvae			*Wolbachia-ba.sed* SIT		Ongoing	California	Operational	Consolidated Mosquito Abatement District 2019b
Genetic/ Novel	Adults/ Larvae			*Wolbachia*-based SIT		2018	Florida	Operational	Mains et al. 2019

Genetic/ Novel	Larvae	Autodissemination Augmented by Males (ADAM)	2018	Florida and California	Operational	Brelsfoard et al. 2019

For this paper, semifield experiments are defined as those conducted outdoors in an enclosed or otherwise artificial environment exposed to ambient conditions; field experiments are those that are conducted outdoors using either caged or wild individuals, but strictly for research purposes and not for the control of local wild populations; and operational treatments are those that are employed and evaluated as part of a control procedure with the purpose of controlling wild populations. Only experiments where insects were exposed to ambient conditions in the CONUS are included, as laboratory assays often cannot reproduce these conditions as effectively as field studies.