

SURVEY IN THE SOUTHEASTERN USA REVEALS THE NEED FOR IMPROVED INVASIVE MOSQUITO SURVEILLANCE

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ABSTRACT. Invasive mosquito species play an important role in transmitting pathogens that cause diseases in humans and animals around the world. In the last decade, arboviral pathogens transmitted by invasive mosquito species have increased substantially in the southeastern region of the USA (“the Southeast”). Early detection of invasive mosquitoes is an important component of an integrated mosquito management (IMM) plan. To determine the capacity of the southern region of the USA to conduct invasive mosquito surveillance, the Mosquito Biodiversity Enhancement and Control of Non-native Species (BEACONS) working group conducted a survey in 2021 in seven US southern states: Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina. A total of 348 mosquito control agencies were contacted, and of those, 90 agencies (26%) responded. Here we report the results about the status of an IMM program and the techniques used for mosquito and pathogen surveillance in the Southeast. Results reveal several gaps in surveillance for invasive mosquito species, compromising the ability for early detection and rapid response. Further, we identified a lack of arbovirus testing, which could result in inadequate arboviral risk assessment and may increase the risk of human and livestock to acquire arboviral infections. This survey data can assist decision makers at the county, regional, and state levels to ameliorate gaps in surveillance capacity in the Southeast.

KEY WORDS Invasive mosquito species, mosquito and arbovirus surveillance, mosquito BEACONS working group, southeastern USA, survey

INTRODUCTION

In recent years, the number of invasive mosquito species that vector human and animal pathogens in the southeastern region of the USA (hereafter the Southeast) has increased (Zohdy et al. 2018, Wilke et al. 2020). The presence and range expansion of

these invasive species increases the risk of arbovirus transmission to humans and livestock (Kendrick et al. 2014, Hinojosa et al. 2020) and places an additional burden on the response capacity of state and local mosquito and arbovirus programs. For example, the presence of 2 invasive species, *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), in the US southern region (hereafter the South) has resulted in autochthonous transmission of chikungunya (Kendrick et al. 2014), dengue (Jones et al. 2024), and Zika viruses (Likos et al. 2016). Eight southern states and 1 territory, Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Puerto Rico, have been burdened by an average of 47.4% of the total dengue cases (travel related and local transmission) in the USA between 2014 and 2024 (CDC 2024). Since 2022, the situation has worsened, with these regions bearing over 60% of the nation’s dengue cases (CDC 2024). *Aedes japonicus* (Theobald) and *Culex coronator* (Dyar and Knab) are competent vectors of West Nile virus (Sardelis et al. 2001, Alto et al. 2014) and have extended their range into the southern states (Sames et al. 2019, Kelly et al. 2023). Range expansions of *Mansonia titillans* (Walker) and *Ae. scapularis* (Rondani), which are medically important to human and veterinary health, have also been reported in the South (Moulis et al. 2015, Cartner et al. 2018, Reeves et al. 2021). In some foci within the South, *Ae. albopictus* and *Ae. japonicus* serve as secondary vectors of the La Crosse encephalitis virus, the most prevalent cause of pediatric neuroinvasive arboviral disease in the USA (Westby

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et al. 2015, Tamini et al. 2021, Vahey et al. 2021). With ongoing global environmental change and greater connectivity through travel and commerce, a thorough and multifaceted approach is needed to effectively monitor and detect new species in an area.

Some mosquito species have expanded their range and invaded new locations due to an increase in the transportation of goods and international travel, as well as climate change and urbanization (Kolimenakis et al. 2021, Lahondère and Bonizzoni 2022, Semenza et al. 2022, Salkeld et al. 2023).

Adaptation of invasive mosquitoes to urban environments may be a selective advantage due to limited natural predators, greater availability of artificial containers, and a higher density of humans for blood feeding (Wilke et al. 2020). Consequently, the arboviral pathogens they transmit are also more abundant in urban areas than in rural habitats (Smith et al. 2009, Rose et al. 2020, Wilke et al. 2020). Arbovirus surveillance can provide early detection of invasive mosquito species and the pathogens they carry. These findings can lead to the suppression of vector populations through control interventions, thus reducing biting pressures and consequently the transmission of arboviruses (Roiz et al. 2018). For example, the use of an integrated mosquito management (IMM) approach including the treatment and removal of larval habitats, adulticide applications, routine property inspections, and public education, allowed Delta Vector Control District in Exeter, CA, to detect an early invasion of *Ae. aegypti* in 2014 and to eliminate the newly found population (Kelly et al. 2021). Similarly, the South Walton County Mosquito Control District in Florida detected and eliminated a newly established population of invasive *Ae. aegypti* by responding to citizens' service requests (T. Ratliff, personal communication). They deployed surveillance traps more densely to identify the extent of introduction and conducted property inspections to dump any containers holding water and wash containers with soap to reduce any eggs that could be hatched later in combination with spraying insecticides for adult control.

However, there are limitations and challenges in monitoring for and controlling invasive and non-native mosquito species. Collections can become laborious due to the need of specialized surveillance tools for the different stages and behaviors of mosquitoes (Reiter and Gubler 1997). Implementing the use of multiple surveillance tools can be costly, and it requires specialized staff training. In addition, increased effort is required to coordinate deployment, identify mosquito collection specimens, tabulate the results, and evaluate decision making. Depending on budget and staff allocations, a multitrap surveillance approach may not be feasible for many control programs. In addition, without the appropriate surveillance tools for the invasive species known, monitoring the impact of control efforts can be a challenge. Resource limitations often result in gaps in the ability to detect and identify

the presence of invasive species when they first arrive in a new geographic area.

To determine the capacity for non-native and invasive mosquito surveillance and control in the Southeast, the Mosquito Biodiversity Enhancement and Control of Non-native Species (BEACONS) working group conducted a survey in Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina in 2021. The BEACONS working group is a multistate committee group created in 2020 to increase the capacity for non-native and invasive mosquitoes in the South with members in these 7 states (Giordano 2021). In 2023, portions of this survey were published and included results revealing training and resource needs (Nguyen et al. 2023). This study presents data focused on the technical aspects of the survey related to non-native and invasive mosquito surveillance capacity and provides insights into future work needed to improve surveillance and control capacity in the Southeast.

MATERIALS AND METHODS

Analysis of survey results was conducted as previously reported (Nguyen et al. 2023). Briefly, an anonymous survey created using Qualtrics software version 05.2021 (Provo, UT) was distributed from August to December 2021 to 348 mosquito surveillance and control programs across 7 states: Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina. The survey was determined to have minimal risk and was exempt from review by the University of Florida Institutional Review Board on June 22, 2021 (IRB202101286). Survey responses were compiled using Microsoft Excel (Microsoft Corp., Redmond, WA) and organized using Excel pivot table functions. The 95% confidence intervals (CI₉₅) were determined using the Wald formula (LaPlace 1812). Qualitative comments from open-ended questions were grouped according to conceptual similarities.

The mean monthly low and high temperature in degrees Celsius was obtained from timeanddate.com (1998). The mean monthly low and high temperature was aggregated from weather reports collected during 1992–2021 around the county where each mosquito control program is located. The weather reports used for the mean monthly temperature calculations are from CustomWeather, which uses the airport weather stations in addition to the World Meteorological Association and Meteorological Assimilation Data Ingest System weather stations. Figures were generated in R version 4.1.2 (R Core Team 2021) and Python version 3.10.2 using Matplotlib version 3.5.1 (Hunter 2007), numpy version 1.22.3 (Harris et al. 2020), and Pandas version 1.4.1 libraries (Pandas Development Team 2020).

RESULTS

This survey was provided to 348 mosquito control agencies in 7 southeastern states (Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina). Of these, 90 agencies (26%) responded.

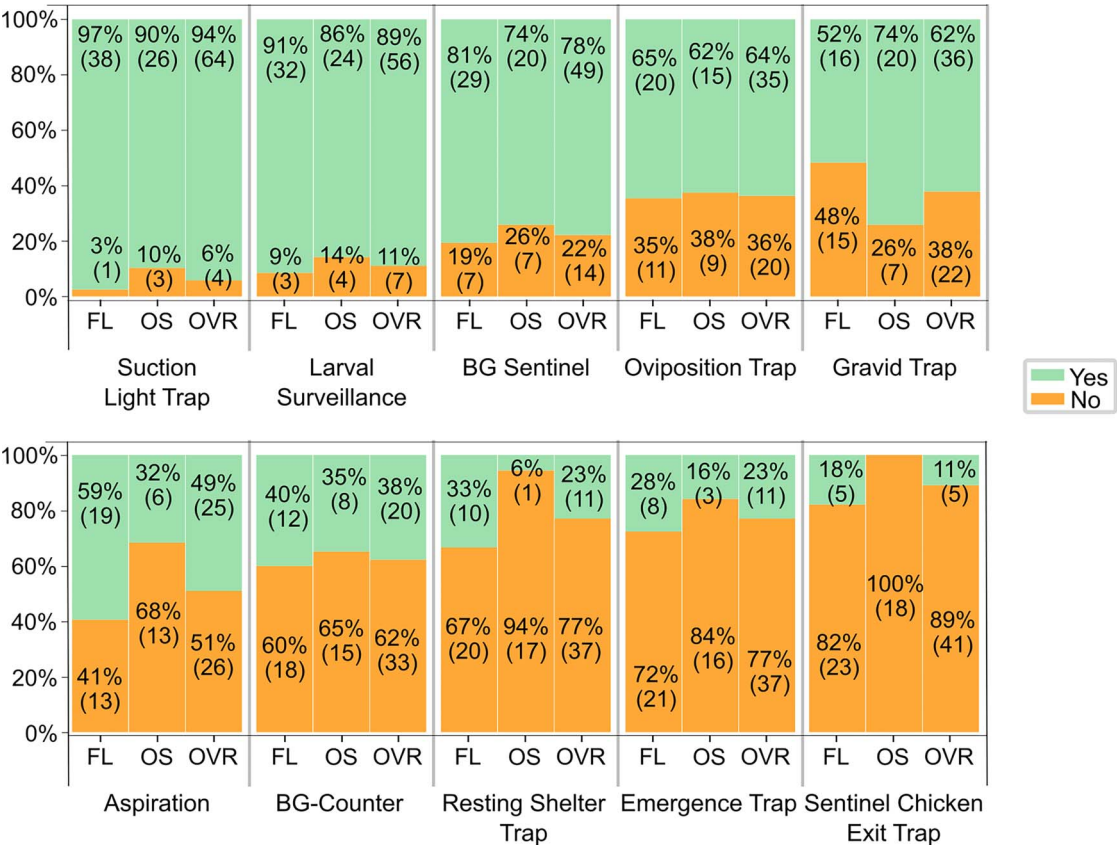


Fig. 1. The utilization proportion of various surveillance tools in the Southeast according to Florida (FL), other states (OS), and overall states (OVR) in 2021. Other states include Alabama, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina. The numbers in parentheses indicate the number of agencies.

Most of the survey participants were from Florida (42/90). Since other states had low numbers of participants per state (2–14), for analysis purposes, we aggregated those to the “Other States (OS)” group (see Fig. 1 in Nguyen 2023).

Traps and lures commonly used for invasive mosquito surveillance: Mosquito collection traps are essential surveillance tools. Among 90 agencies, 78% (CI₉₅ = 69.4–86.6%) reported conducting mosquito surveillance and/or mosquito control. Most agencies (77%, CI₉₅ = 68.3–85.7%) agreed that using specific trapping methods is important for invasive mosquito surveillance. However, only 59% (CI₉₅ = 48.8–69.2%) indicated having access to mosquito traps required to monitor invasive mosquito species. Among the agencies that have access to mosquito traps, suction light traps (typically called “Centers for Disease Control and Prevention [CDC] light traps”) were the most commonly used surveillance tool (94%, CI₉₅ = 88.4–99.6%) in the South (Fig. 1). The second most commonly used surveillance tool was larval surveillance (89%, CI₉₅ = 81.5–96.5%), followed by the BioGents® (BG)-Sentinel trap (78%, CI₉₅ = 67.8–88.2%), oviposition traps (64%, 51.3–76.7%), gravid traps (62%, 49.5–

74.5%), and BG-Counter traps (38%, 13.1–24.9%). Only 23% (CI₉₅ = 11.1–34.9%) of the agencies used resting shelter traps and aquatic emergence traps. The sentinel chicken exit trap, a specialized trap attached to the top of sentinel chicken coops (Kobilinsky 2006), was the least utilized tool (11%, CI₉₅ = 2.0–20.0%), used only by Florida agencies that also had the highest count of tools used in every type of trap except for gravid traps (Fig. 1). Most of the agencies (86%) that have access to traps use 3 or more types of traps, 9% used 2 types of traps, and 5% used only 1 type of trap.

Lures can be used in combination with traps to increase the efficacy and diversity of mosquito collection, and Table 1 shows the lures utilized with the most common traps reported by the participants. Since CO₂ was the most utilized lure, agencies were asked if their facilities have access to pressurized gas cylinders or dry ice. Forty-six percent (CI₉₅ = 35.7–56.3%) responded that they had access to dry ice, 28% (CI₉₅ = 18.7–37.3%) had access to a CO₂ tank, 16% (CI₉₅ = 8.4–23.6%) had access to both, and 11% (CI₉₅ = 4.5–17.5%) had no access to either one (Fig. 2). Respondents were not asked to report CO₂ flow rate from compressed gas tanks.

Table 1. Percent lure usage with the most common traps reported in this survey in the Southeast in 2021. The top section shows lures utilized with suction light traps and BG-Counter/BG-Sentinel traps. The lower section shows lures utilized with gravid traps and oviposition traps. The numbers in parentheses indicate the number of agencies.

Lure type	Suction light traps	BG-Counter/BG-Sentinel
CO ₂	71% (41)	52% (38)
Light	19% (11)	
BG-Lure	2% (1)	30% (22)
Octenol	8% (5)	7% (5)

Lure type	Gravid traps	Oviposition traps
Infusion	78 (22)	56% (13)
Water	7% (2)	35% (8)
Fish base lure	11% (3)	9% (2)
Other traps	4% (1)	

A critical part of invasive taxa surveillance is the identification of mosquitoes, which can be completed utilizing specific morphological features and/or molecular tools (Yssouf et al. 2016). Sixty-one percent (CI₉₅ = 50.9–71.1%) of the agencies identified their collected mosquitoes internally. Of these, 18% (CI₉₅ = 7.8–28.2%) of the agencies only identified their mosquitoes to genus (11% from Florida agencies and 7% from OS agencies), 73% (CI₉₅ = 61.3–84.7%) identified their collected mosquitoes to species using only morphology (44% from Florida and 29% from OS), 2% (CI₉₅ = 1.7–5.7%) identify their mosquitoes to species using only molecular tools (2% from Florida and 0% from OS), and 7% (CI₉₅ = 0.3–13.7%) identified their mosquitoes to species using both morphology and molecular tools (0% from Florida and 7% from OS; Fig. 3).

Viral testing of the collected invasive mosquitoes is an important tool to determine if mosquitoes carry arboviruses that threaten human and/or veterinary health. Participants were asked whether they perform viral testing on their collected mosquitoes, and if they do, if the test was done on site or if they submit samples to a state or federal laboratory for testing. They were not asked to specify if they tested for invasive pathogens and whether they tested invasive mosquito species. Only 29% (CI₉₅ = 19.6–38.4%) of the responding agencies conducted viral testing, including 1% of agencies conducting mosquito viral testing on site, 20% sending mosquitoes to a state or a federal laboratory for mosquito viral testing, 2% conducting both mosquito viral testing on site and sending mosquito samples to either a state or a federal laboratory, and 6% determining seroconversion in sentinel chicken flocks. Table 2 shows the number of agencies that conduct mosquito viral testing.

Mosquito surveillance frequency in the South: Sixty-nine percent (CI₉₅ = 59.4–78.6%) of the agencies reported the frequency of their surveillance program. The typical mosquito surveillance occurred between March and November (21%, CI₉₅ = 12.6–29.4%).

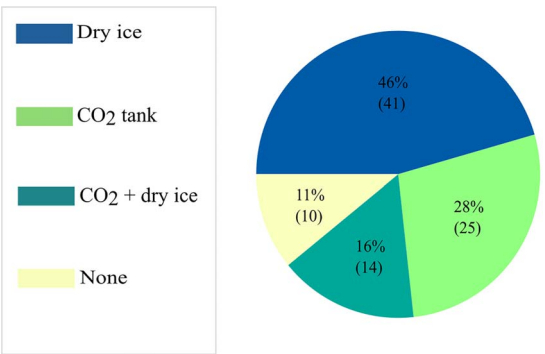


Fig. 2. The percentage of CO₂ availability in their facility in the Southeast from either dry ice, a CO₂ tank or both in 2021. The numbers in parentheses indicate the number of agencies.

Twenty percent (CI₉₅ = 11.7–28.3%) of the agencies had year-round surveillance (Fig. 4A). Of these, 79% were in Florida. Consistent with this practice, 82.4% of Florida agencies expressed that year-round surveillance is either important or very important, while 63.3% of OS value year-round surveillance. Agencies that considered year-round surveillance very important or important have a higher rate of year-round surveillance, whereas those that answered surveillance is not important or had no opinion did not conduct year-round surveillance, regardless of the mean of monthly low temperature (Fig. 4B).

DISCUSSION

Invasive mosquito species vectors of arboviral diseases have increased substantially in the last decade in the South. The presence and range of expansion of these invasive and non-native species raises the risk of arbovirus diseases impacting public and veterinary health (Kendrick et al. 2014, Hinojosa et al. 2020). Early detection of invasive mosquitoes using specific surveillance methods and providing comprehensive training is essential for prompt and effective responses. The mosquito BEACONS working group conducted the first survey of mosquito control agencies in the Southeast to determine the capacity of seven states in this region to perform invasive mosquito surveillance. Our results have the limitation of low participation in this survey. Nevertheless, they revealed important gaps in the monitoring of invasive mosquitoes that are worth considering.

Mosquito collection traps provide valuable information about mosquito abundance, diversity, distribution, and infection rates, and more importantly, they can detect the presence of new introductions and range limits of invasive species at the early stages of encroachment. Our results show that most of the South utilizes CO₂ as the most commonly used lure and suction light traps for detecting important invasive *Aedes* species. However, this type of trap is less effective for

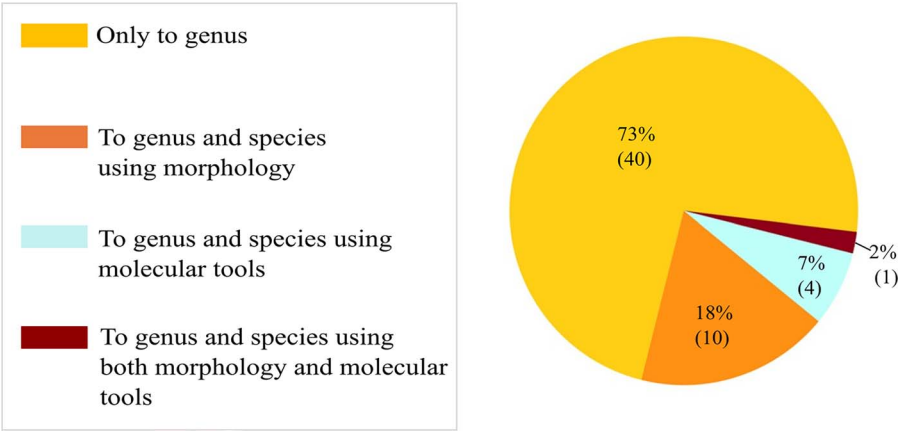


Fig. 3. Use of morphology and/or molecular tools to identify mosquitoes to genus or to genus and species used by all state respondent agencies in 2021. The numbers in parentheses indicate the number of agencies.

the collection of important invasive *Aedes* species such as *Ae. aegypti* and *Ae. albopictus* than the BG-Traps in combination with BG-Lure or CO₂, which are considered the most effective traps for container inhabiting *Aedes* species surveillance to date (Hardwood et al. 2015, Ngape et al. 2021).

The use of less effective surveillance methods may hinder the detection of rare or new invasive species, further contributing to increased transmission risks (Cornel et al. 2016). Since all traps create a bias for mosquito species collection, increasing expenditures for multiple trap types may not be perceived as cost-effective. In addition, the implementation of multiple traps also requires increased labor, trained staff to identify mosquito collections, and other daily expenditures that add to the total cost of an effective surveillance program. Therefore, a multi-trap approach may not be feasible for low-budget agencies. It should also be considered that some agencies do not have any mosquito surveillance or control programs, most likely due to economic restraints. As reported previously in Nguyen et al. (2023), agencies with more resources to conduct surveillance had more diverse trapping methodologies and usage compared to agencies with fewer resources.

Table 2. Number of agencies that conduct mosquito viral and chicken sentinel seroconversion testing in the Southeast in 2021. FL = Florida; OS = other states participating in this survey, which included Alabama, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina.

State group	Mosquito viral testing			No viral testing
	On site	At state or federal laboratories	Chicken sentinel seroconversion	
FL	1	8	5	29
OS	2	12	0	35

In accordance, our results showed that Florida agencies reported the greatest diversity of surveillance tools compared to agencies in OS (Fig. 1) while also reporting the highest budget for mosquito surveillance (Nguyen et al. 2023). Developing alternative, more affordable, and accessible surveillance tools may facilitate broader adaptation of these tools among mosquito control programs in the Southeast. Some studies have been conducted to test different traps and lures. For example, Li et al. (2016) reported that the use of a black light trap with either octenol or BG-Lure collected similar numbers of mosquitoes compared to the CDC light trap but with fewer non-target insects. Eastwood et al. (2020) reported that the use of 2 lures made of different concentrations of octenol and ammonium bicarbonate with BG-Sentinel traps was more efficient, collecting higher numbers and higher diversity of key *Aedes* arbovirus vectors than the BG-Sentinel trap with the BG-Lure. Another alternative is the modification of the already widely used suction light traps with different lures that could attract more diverse species of invasive mosquitoes, which may facilitate a change in surveillance methods without substantial changes in operational infrastructure. Moreover, academic programs or citizen programs could also be leveraged to enhance the detection of invasive mosquito species (Uelmen et al. 2023).

Most agencies rely only on morphological identification rather than on molecular techniques. However, mosquito samples that become weathered or damaged during collection or storage and species that are morphologically similar are difficult to identify correctly based on morphological characteristics alone. For these collections, molecular tools may be required for the correct taxonomic determination of unknown mosquitoes. However, we found that only 9% of agencies use molecular tools for species identification, demonstrating a need for access to these approaches when species taxonomic identification is difficult to determine and may represent a species new to the region.

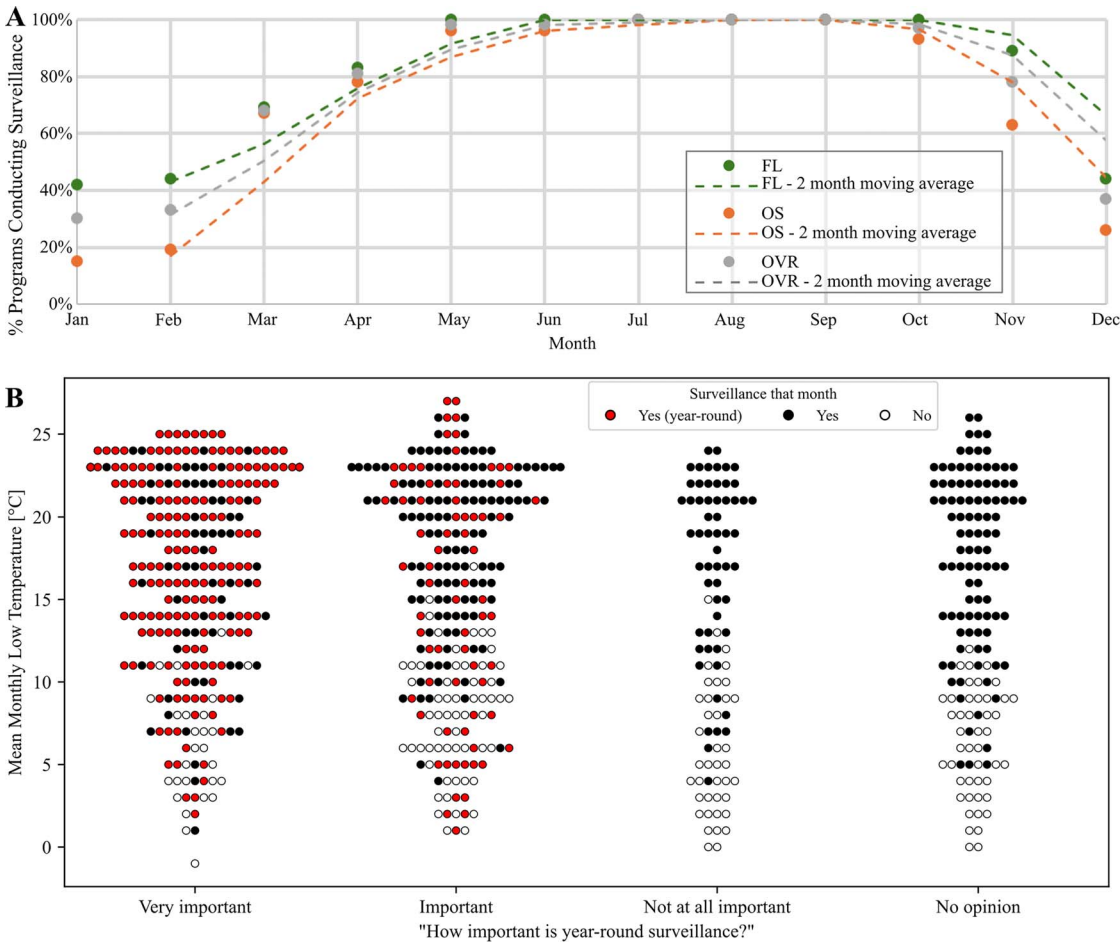


Fig. 4. (A) Months of mosquito surveillance reported by participating agencies in Florida (FL), other states (OS), and overall states (OVR). The lines indicate the moving average of the percent of programs conducting surveillance over a 2-mo period. (B) The relationship between mean low temperature each month in a county where a mosquito control program is located and the decision to conduct surveillance that month categorized by the expressed importance of conducting year-round collections by the program.

Testing for pathogenic viruses in mosquito collections is an important step to determine if mosquitoes are infected with arboviruses that threaten human and/or veterinary health. If a virus is detected, operators can initiate prompt and proactive IMM responses to control mosquito populations, as well as inform public health programs to help reduce risk. Viral testing determines the presence of viral antigens and/or viral nucleic acids (Lequime and Lambrechts 2014). Viral antigens can be detected using immunological assays testing mosquitoes directly (Tsai et al. 1987) and/or in blood samples from sentinel chicken flocks (Riles et al. 2022). In our survey, only 29% of the agencies conduct viral testing for mosquito collections, which may result in an incomplete risk assessment for pathogen transmission. The lack of molecular identification to species and viral testing may be due to economic reasons because training and methods involved for processing identifications

using molecular tools as well as viral testing are expensive (Ajamma et al. 2016, Ramirez et al. 2018). Lack of personnel trained in taxonomic identification and/or molecular approaches may be an additional barrier for agencies to conduct molecular identification and virus testing. Understaffing and budget constraints have been a persistent issue for mosquito control organizations and may lead to suboptimal performance (Moise et al. 2020, Nguyen et al. 2023). Greater cooperation across mosquito control programs promoting arbovirus surveillance can help to improve timely and effective arbovirus surveillance.

Climate is an important driver of the spatial and temporal distribution of mosquito species in different locations. A seasonal distribution study conducted in Florida reported that some vector species showed peak abundance even during the dry winter season, suggesting the need for year-round surveillance in southern areas of our study region (Giordano et al.

2020). Invasive species introductions can be missed if mosquito surveillance is not conducted at the appropriate frequency for a given site. However, we found only 20% of the agencies participating in our survey reported conducting mosquito surveillance year-round. Mosquito control programs appear to begin and end collections on specific calendar dates, even if conditions may be suitable for mosquito activity. This is evident by the lack of strong correlation between mean monthly low temperature of any particular month and whether a mosquito control program conducts surveillance or not (Fig. 4B). This suggests that there appears to have other factors in play when a program decides when to conduct mosquito surveillance. The survey results show that perception of the importance of year-round surveillance is one of the factors. Their experience and results of year-round surveillance may have led to the perception that year-round surveillance is needed.

In summary, our survey results show that some southeastern states require increased resources, training, and education for an effective early detection surveillance program to reduce the public health threat that invasive mosquito species pose. This analysis should be used to appropriately identify deficiencies and create a dialogue to address them at the regional and state levels. Our results highlight resource gaps between current methods used for mosquito surveillance and what is required to adequately detect invasive mosquito species. Low-cost traps that can collect a greater diversity of mosquito species as well as greater access to molecular identification and virus testing is needed to improve early detection of invasive species and potential pathogen threats. Further research and development will be needed to reduce the time and cost of surveillance to improve detection and control. Overall, reducing the barriers for invasive mosquito surveillance will improve IMM approaches and have a positive impact on public health and well-being.

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