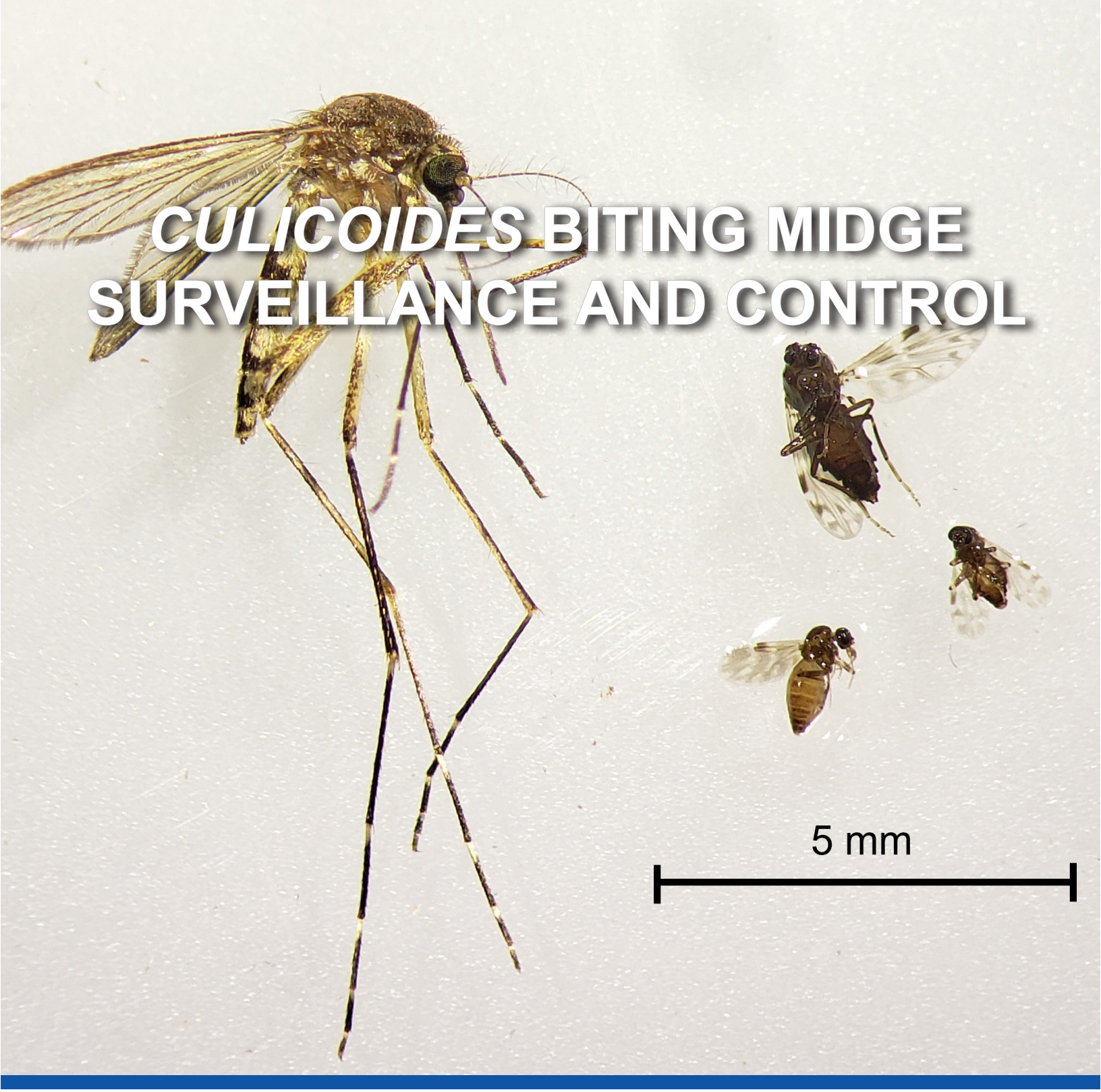


CULICOIDES BITING MIDGE SURVEILLANCE AND CONTROL

THE AMERICAN MOSQUITO CONTROL ASSOCIATION

September 2026

The Centers for Disease Control and Prevention (CDC) funded the American Mosquito Control Association (AMCA) to develop guidance for integrating *Culicoides* biting midge surveillance and control into existing mosquito management programs. To create this manual, the AMCA Board of Directors and Executive Committee engaged a consultant to work with the Technical Advisor and lead the development, writing, and editing of a document containing implementation best practices and an in-depth rationale of the guidance. Additional contributors brought diverse perspectives and practical experience to the creation of this guidance by performing evaluations, sharing protocols, reviewing draft content, and collaborating on the final design and layout.

This document consists of two parts:

1. Implementation guidance for incorporating *Culicoides* biting midge surveillance and management into existing mosquito control programs
2. Scientific background, rationale, and expanded discussion for managers, regulators, and researchers

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AUTHORS

Jennifer R. Gordon, Ph.D.
Author
Principal Entomologist
Bug Lessons Consulting LLC
Wheat Ridge, CO

Daniel Markowski, Ph.D.
Author
Technical Advisor
American Mosquito Control Association
Sacramento, CA

REVIEWERS & CONTRIBUTORS

Corey Brelsfoard, Ph.D.
Reviewer
Professor
Texas Tech University
Lubbock, TX

Vilma Cooper, Ph.D.
Reviewer
Global Staff Operations Manager
Valent BioSciences
Minneapolis, MN

Vincent Corbel, Ph.D.
Reviewer
Research Professor
Institut de Recherche pour le Développement
France

Casey Crocket, Ph.D.
Reviewer
Research Entomologist
Centers for Disease Control and Prevention
Division of Vector-Borne Diseases
Ft. Collins, CO

Aaron Lloyd, Ph.D.
Reviewer
Executive Director
Lee County Mosquito & Hyacinth Control Districts
Lehigh Acres, FL

Peter Obenauer, Ph.D.
Contributor
Technical and Training Manager
Vector Disease Control International
Little Rock, AR

Kevin Pritts
Reviewer
Manager
Fort Bend County Health & Human Services
Environmental Health, Mosquito Control
Needville, TX

Dominic Rose, M.S.
Reviewer
Entomologist
Centers for Disease Control and Prevention
Division of Vector-Borne Diseases
Ft. Collins, CO

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BACKGROUND ON THIS DOCUMENT

From late-2023 to mid-2024, an outbreak of Oropouche virus (OROV) in South America and the Caribbean impacted over 8,000 people.[1] By the end of 2024, the outbreak ultimately resulted in travel-related cases in Canada, Italy, the Cayman Islands, Uruguay[2] and 108 cases in six U.S. states and Caribbean territories.[3] Additionally, local transmission of the virus was recorded for the first time in Cuba,[1] a country located only ~90 miles from Florida. Furthermore, evidence has begun to suggest that pregnant women who become infected with OROV may have negative birth outcomes such as miscarriages or infants born with birth defects.[4] Finally in 2024, public health professionals recorded the first deaths of infected individuals who were otherwise healthy.[5]

Due to the emerging status of OROV and the new risk presented by *Culicoides* biting midges (*Culicoides* spp.), the AMCA obtained funding through the Centers for Disease Control and Prevention (CDC) to execute a series of evaluations between 2025-2026 to help vector control professionals seamlessly incorporate surveillance and control measures into existing integrated mosquito management (IMM) programs to target these vector species. The AMCA's evaluation projects (Figure 1) covered several topics including: spatial repellency, adult and larval control, adult surveillance, age determination, and programmatic evaluation. Ultimately, the evaluation projects focused on improving vector control tools, evaluating operational control methods, and developing new technologies to aid in the collection of surveillance data to better understand *Culicoides* spp. population distributions and their response to control interventions.

To briefly summarize the objectives of the various projects (Table 1), a multi-site, semi-field trial was conducted to evaluate larvicide efficacy against *Culicoides sonorensis* (Brelsfoard's AMCA project). These trials were implemented at study locations in Texas, Louisiana, and Kansas where standardized protocols were used to assess six larvicide formulations. Substrate sampling, larval bioassays, and chemical residue analysis using liquid chromatography–mass spectrometry (LC–MS) were performed to evaluate larval mortality over time and product persistence. Additional field studies evaluated whether standard mosquito ultra low volume (ULV) spraying techniques are effective for controlling *Culicoides* biting midges. Field cage trials (Obenauer's and Pritts' AMCA projects) and operational spray evaluations (Scotts' AMCA Project) were conducted to assess various aspects of field cage trials and efficacy following ULV applications targeting adult *Culicoides* biting midges. Each of these projects also completed complementary work to examine the seasonal abundance and species composition of *Culicoides* spp. populations.

Additionally, laboratory work was conducted to evaluate botanical spatial repellents as potential tools for reducing human exposure to *Culicoides* spp. (Table 1, Olds' AMCA project). Multiple botanical oils and concentrations were tested under controlled conditions to determine relative repellency. Another project evaluated the use of a novel surveillance tool that uses

Figure 1. The AMCA funded projects across the U.S. and South America to generate data driven recommendations to assist with the integration of *Culicoides* biting midge control and surveillance activities into regular mosquito control operations.



Images provided by Christopher Swab, Vincent Corbel, Peter Obenauer, Isik Unlu, and Kevin Pritts.

shortwave infrared spectroscopy to estimate the age structure of field-collected *Culicoides* spp. populations. This work included repeated spectral scanning and biological validation through abdominal pigmentation analysis to determine parity status (Swab's AMCA project).

Furthermore, a multinational collaborative project was initiated to evaluate chemical vector control strategies targeting *Culicoides* spp. in South America (Table 1, Corbel's AMCA project). This project involves coordinated activities among research partners in Brazil, Peru, and Colombia. The results from this work will be added in a future supplement to this document.

The AMCA also partnered with Sac-Yolo Mosquito and Vector Control District to develop a 3D-printed *Culicoides* biting midge trap modification based on a design concept from the Florida Medical Entomology Laboratory (FMEL). Finally, AMCA coordinated the collection of *Culicoides* spp. specimens by mosquito control programs across the country and arranged for those samples to be shipped to CDC for species identification.

This document represents a compilation of the findings from these AMCA funded evaluations, a review of the literature where appropriate, and input from external subject matter experts. As a result, the AMCA recommends incorporating *Culicoides* biting midge-related activities into established IMM programs

Table 1. Studies Funded by AMCA to Develop *Culicoides* Biting Midge Management and Surveillance Guidance

Citation in Document	Project Title	Key Study Personnel and Affiliation
Brelsfoard's AMCA project	The Efficacy of Larvicides on *Culicoides sonorensis* Midges in a Semi-Field Setting	Corey Brelsfoard (Texas Tech University), Lee Cohnstaedt (USDA ARS), & Kevin Caillouet (St. Tammany Parish Mosquito Abatement)
Corbel's AMCA project	Multi-Center Study to Evaluate Chemical-Based Vector Control Products Against *Culicoides* spp. in South America	Vincent Corbel, Jessica Gouvea, Michele Planta, José Bento Lima, Adémir J. Martins, & Maria Luiza Felippe Bauer (FIOCRUZ-RJ, Brasil), Karina Bertazo Martha, Lesliane de Amorim Lacerda (Espiritu Santo Health Centre, Brasil) Quinones, Martha, Martha Liliana Ahumada, & Olga Lucía Cabrer (INS/UNAL, Colombia) & Miriam Palomino Salcedo, Edwin Requena Zuñiga (INS, Peru)
Obenauer's AMCA project	Evaluating If Standard ULV Application Procedures and Droplet Spectrum Targets at Mosquitoes Will Be Effective Against Biting Midges	Peter Obenauer, Broox Boze, & Caroline Card (Vector Disease Control International)
Olds' AMCA project	Evaluating Novel Botanical Repellents For Public Health Protection Against *Culicoides*	Cassandra Olds (Kansas State), Bethany McGregor, Daniel Kline, & Seth Gibson (USDA)
Pritts' AMCA project	Improving Strategies For Controlling *Culicoides* Spp. With Available Ready To Use Adulticides	Kevin Pritts, Jeremy Verde, Bret Nash, & Michael Schaffer (Fort Bend Health & Human Services), & Nina Dacko (Independent entomological consultant)
Scott's AMCA project	Beyond Mosquitoes: Refining *Culicoides* Control in the Field	Jamesina Scott, Chip Markwardt, Jessi Edmiston, Michelle Meighan, Kara Gaylor, Porter Anderson, Julian Chavez, & Brad Hayes (Lake County VCD)
Swab's AMCA project	Assessment of the Age Structure of Field Collected Cohorts of *Culicoides* Using Shortwave Infrared Spectroscopy	Christopher Swab (Cummings School of Veterinary Medicine, Tufts University), Dr. Sam Telford III (Department of Infectious Disease and Global Health, Tufts University), & Daniel Cole (Staff Entomologist, Central Massachusetts Mosquito Control Project)

as opposed to creating new, singly focused control efforts targeting these vector species. The following document provides guidance on how to implement this recommendation and offers a draft disease response plan to help existing mosquito control programs keep their communities safe (Appendix I). To learn more about IMM in general, please see the AMCA's *Best Practices for Integrated Mosquito Management* manual and virtual training program.

PART ONE:
IMPLEMENTATION GUIDANCE TO INCORPORATE *CULICOIDES* BITING MIDGE SURVEILLANCE & MANAGEMENT INTO EXISTING MOSQUITO CONTROL PROGRAMS

QUICK IMPLEMENTATION GUIDANCE

Community Engagement

- If the risk of contracting OROV increases in an area, begin community messaging regarding precautions residents and staff can take to protect themselves and their properties.

Personal Protection

- EPA-registered on skin repellent products contain active ingredients such as (but not limited to) DEET, picaridin, IR3535, or oil of lemon eucalyptus, and many have claims about protection against *Culicoides* biting midges, biting flies, or no-see-ums. However, repellency may vary by product, and users should always consult the individual product label for specific directions and ensure *Culicoides* spp. (often referred to as biting flies or no-see-ums on repellent labels) are listed.
- To help identify tools for reducing human exposure to *Culicoides* biting midges, the AMCA funded work investigating the potential spatial repellent effects of botanical essential oils. Oils tested revealed that repellency varied by concentration and active ingredient. Massoia bark oil, amyris oil, and fir needle oil demonstrated the strongest repellency at several concentrations. Geranium oil and pevilla oil performed well with approximately 70% repellency. Patchouli oil was similarly effective, but at a lower concentration, while cassia oil was among the least effective botanical repellents evaluated (Table 2).

Larval Surveillance

- Larval surveillance, although valuable, may require substantial resources and should be reserved for long-term programs that aim to suppress populations prior to an outbreak.
- During a disease outbreak, conducting surveillance in larval production sites is not recommended due to the time and complexity involved.

Adult Surveillance

- AMCA recommends using modified CDC light traps based on guidance from the Burkett-Cadena laboratory from the University of Florida's Florida Medical Entomology Laboratory (FMEL).
- A 3D printed version of the FMEL trap modification can be used to rapidly separate mosquito and *Culicoides* spp. specimens, allowing for simultaneous surveillance of these vector species.
- The species of *Culicoides* collected by vector control professionals will vary (Table 4) based on multiple factors such as season, trap type, lure, light source, location, and habitat type.

Larval *Culicoides* Biting Midge Control

- Several commercially available larvicides routinely used to control larval mosquitoes demonstrated substantial activity against *Culicoides sonorensis* larvae under semi-field conditions (Table 5). Products containing spinosad, pyriproxyfen, and novaluron generally provided longer-lasting control

than methoprene-based formulations. The evaluation of these products suggests that existing mosquito larvicides may offer practical tools for *Culicoides* spp. management and arbovirus risk reduction.

- Larvicide performance was influenced by environmental conditions. Treatments in the drier Texas location generally maintained efficacy longer than those in Louisiana and Kansas, where higher rainfall may have diluted or displaced active ingredients. Taken as a whole, vector control professionals may need to tailor product selection and retreatment schedules to local habitat conditions and weather patterns.
- Under semi-field conditions, spinosad- and pyriproxyfen-based products showed the greatest persistence, maintaining measurable residues and larval mortality for up to 50 days, whereas methoprene products declined in effectiveness after approximately 20–30 days. These results suggest that longer-residual products may reduce retreatment frequency and improve management of difficult-to-access larval habitats.
- Previous research has shown that *Bacillus thuringiensis israelensis* (*Bti*) is relatively ineffective against *Culicoides* spp. and is not currently recommended for control.

Adult *Culicoides* Biting Midge Control

- Previous research demonstrated that a bifenthrin-based product applied to vegetation around homes effectively reduced *Culicoides* biting midges. When performing barrier treatments, applicators should focus treatments in shaded or protected areas where *Culicoides* spp. will rest and/or most likely encounter people.
- Previous research has shown that aerial and ground-based ULV applications of different active ingredients (i.e., malathion, naled, resmethrin, and permethrin) can reduce *Culicoides* spp. in some areas.
- AMCA funded research suggests that ground-based ULV applications of insecticides may be a useful tool as part of a *Culicoides* biting midge management program. Three pyrethroid products and an organophosphate product were found to be effective against *Culicoides* spp. in Texas and Georgia (Table 6).

Field Cage Trial Procedures

- Standard mosquito ULV applications appear highly effective against adult *Culicoides* spp. during field cage trials, producing rapid mortality at standard operational droplet sizes and distances (up to 300 feet). Droplet spectrum in the 17–36 μm range appears able to penetrate testing cage materials and deliver lethal doses to the smaller vectors.
- However, successful *Culicoides* spp. field evaluations require specialized collection and cage designs. Standard mosquito-testing equipment with larger mesh sizes, collection bags, and standard cage designs may allow escape, alter droplet exposure, or bias efficacy measurements. Verification of droplet deposition at the cage stand with impingers and use of NoSeeUm netting (<0.6 mm or ~0.02 in mesh) in cage design were among the most critical factors for obtaining reliable results.
- Cage shape may significantly influence droplet trajectories and measured mortality. Cylindrical versus "pancake" cage designs may produce different results.
- The modified FMEL CDC trap collection system can improve survival of live specimens needed for bioassays and field

cage tests, if the bottom vial is replaced with fine mesh material rather than alcohol.

- Maintaining live *Culicoides* spp. for extended periods is operationally difficult. Rapid processing after collection, cooler temperatures, and minimizing holding time are critical for successful field bioassays.
- Programs can conduct field cage test trials with *Culicoides* spp. using the protocol found in Appendix II.

Evaluating Program Success

- Vector control professionals may need to reframe success and gather additional data when evaluating control efforts to fully understand the impact of their programs. For example, when using trap data to compare wild population abundance pre- and post-application, a statistically significant reduction in adult *Culicoides* spp. is not always observed. However, alternative evaluation methods (e.g., field cage tests and measuring population age structure pre- and post-treatment) may be useful in determining the application's efficacy.
- Understanding local species composition and seasonal abundance may be just as important as selecting an adulticide product when developing a proper adulticide management strategy.
- *Culicoides* spp. management evaluations should include species-level identifications whenever possible because treatment efficacy may differ among species.
- A promising evaluation tool for the efficacy of ULV applications could be the use of Shortwave Infrared Spectroscopy (SWIRS). In Massachusetts, SWIRS analysis suggested that post-treatment populations contained a greater proportion of younger individuals than pre-treatment populations.
- Control efforts may reduce vectorial capacity by selectively removing older, potentially infectious individuals even if total *Culicoides* spp. abundance quickly rebounds.

PART TWO:
SCIENTIFIC BACKGROUND, RATIONALE, AND EXPANDED DISCUSSION FOR MANAGERS, REGULATORS, AND RESEARCHERS

CULICOIDES BITING MIDGE OVERVIEW

Culicoides biting midges are a group of tiny, blood-sucking, true flies (Diptera) in the family Ceratopogonidae and the genus *Culicoides*.[6] Flies in this group go by many common names such as punkies, biting midges, and no-see-ums. Globally, there are approximately 1,350 species of *Culicoides* biting midges, and more than 150 species in the U.S.[6-9] Published research has documented *Culicoides* spp. in all 50 U.S. states,[2, 9-11] Puerto Rico,[12] and the U.S. Virgin Islands.[13]

As with other dipterans such as mosquitoes, *Culicoides* have four distinct life stages: egg, larva, pupa, and adult.[6] Their eggs are exceptionally small (~0.25 mm), crescent shaped, and turn dark brown as they mature. The resultant larvae are small, thread-like, and have a darker head. Pupae have a cephalothorax, respiratory organs called horns, and abdomens that are flexible and mobile.

Female *Culicoides* spp. generally lay eggs on moist substrates, and the eggs typically hatch within a few days of being laid.[6] While the eggs cannot survive desiccation, they can remain dormant for months until environmental conditions are conducive for successful development. Additionally, some larvae may delay development for up to a year in poor conditions. Under ideal conditions in the laboratory, the time from egg to adult can take less than three weeks.

Culicoides spp. larvae are not fully aquatic like mosquito larvae, but they do require ample moisture.[6] Larvae can utilize many habitats that contain the required conditions of water, air, and food, such as along the edge of a stream, marsh, pond, or puddle or in a tree hole, banana leaf, leaf axils, or other organic containers. However, an individual species will likely only utilize one or two different habitats.

The wings of *Culicoides* spp. have distinct patterns of pigmentation that entomologists often use to identify adults to species (Figure 2).[14, 15] Additionally, adults have mouthparts modified to form a proboscis. Similar to mosquitoes, both males and females consume nectar as a carbohydrate source, but only the females take bloodmeals to obtain nutrients for egg development.[6] Generally, *Culicoides* spp. are active at dusk, evening, or morning; however, depending on species and environmental conditions, bites may occur during the daytime as well.[6]

Figure 2. Compilation of *Culicoides* biting midge wing patterns and their associated species.

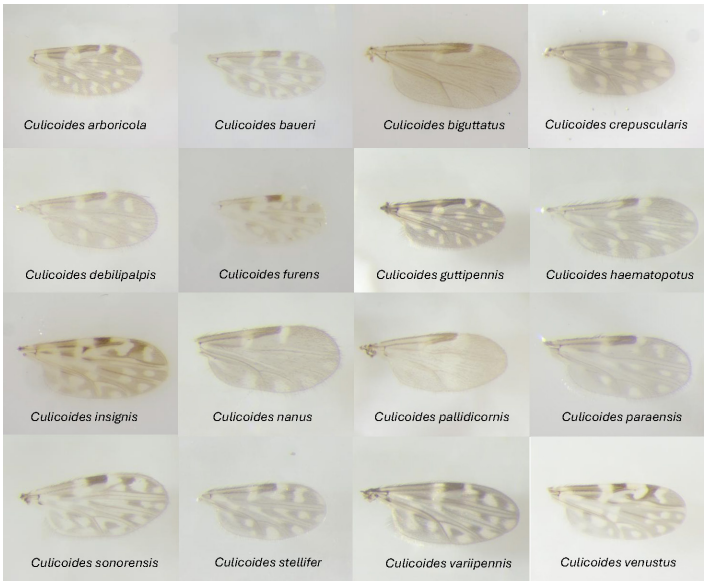


Image provided by Bethany McGregor.

Pests and Vectors

As mentioned above, different species utilize different habitats, but people may encounter large numbers of biting females along coasts and shores, wooded areas, and pastures and prairies.[6] Populations of these potential vectors can grow very large, as exemplified by one group collecting over 35 million individuals over the course of a one year study.[16] As a result of the large numbers and painful bites inflicted by this small fly, the pestiforous impact of these flies can warrant control efforts.

Globally, different *Culicoides* spp. can transmit multiple pathogens to both humans and animals, with the majority identified thus far being viruses.[9, 17, 18] However, isolation of a virus from a field collected sample does not guarantee that the fly is a competent vector of the pathogen.[9,18] In the U.S. and its territories, *Culicoides* spp. are mostly considered vectors of wildlife and livestock disease-causing pathogens.

Oropouche virus (OROV) is a pathogen in the genus *Orthobunyavirus* principally vectored to humans by *Culicoides paraensis*.[9, 19] Symptomatic individuals usually exhibit an acute febrile illness characterized by fever.[20] First identified in 1955 from a patient in Trinidad and Tobago, this virus has likely infected over 500,000 people and caused over 30 outbreaks in Brazil, Peru, Panama, and Trinidad and Tobago between 1960-2009.[20]

In the U.S., *C. paraensis* has been documented in the eastern and central parts of the country, but no collection records currently exist for this vector in Puerto Rico or the U.S. Virgin Islands.[2] When collected in the continental U.S., adult *C. paraensis* occur in areas with deciduous tree holes where the immatures develop.[2] Females of this species bite during the late afternoon or early evening generally in or near deciduous woodlands; however, *C. paraensis* can occur and reproduce in semi-urban environments as long as natural habitats exist that remain wet during dry periods.[2] Though *C. paraensis* is currently the only species confirmed as a vector of OROV,[2] laboratory studies suggest that *Culicoides sonorensis*, a species located throughout North America, could pose a risk as a vector of OROV.[21, 22]

KEEPING COMMUNITIES AND STAFF SAFE

The 2024 outbreak of OROV in South America and the Caribbean resulted in disease cases in areas with no previous history and a trend of recording increasingly worrisome symptoms.[2, 4, 5] As a result, mosquito control programs across the U.S. began preparing to respond should a human case appear or if the virus was documented in the environment (e.g., OROV positive pools of mosquitoes or *Culicoides* spp.). A key first step in any response strategy is to communicate to the public and program staff ways to protect themselves and their loved ones.

Personal Protection Using Repellents

The AMCA recommends people use EPA-registered insect repellents to reduce their risk of exposure to OROV and other arthropod-borne viruses (arboviruses).[23, 24] Products that receive EPA registration have been evaluated and approved for both human safety and efficacy when used according to all label directions.[25] When choosing a repellent, select products that list the specific pest of concern on the label. For example, when choosing a product to protect against *Culicoides* biting midges, make sure the label lists flies, biting midges, biting flies, no-see-ums, *Culicoides*, punkies or one of the other common names for this pest and vector.

There are products on the market that may have repellent claims but are not registered by EPA. These products are either exempt from registration or illegal.[25] Exempt products: 1) contain ingredients that the EPA has previously determined pose little risk to human health at the concentrations found within products on the market and 2) follow all other criteria necessary for minimum risk exemption.[26, 27] However, exempt products have not been evaluated for efficacy. As such, when considering an exempt product, prioritize products from trusted manufacturers with independently generated efficacy data. Illegal products do not meet the requirements for exemption and have not been registered. Illegal products should be avoided as they may pose health risks, not repel biting arthropods as claimed, or both.

Types of Repellents

In the U.S., individuals have multiple repellent options available that roughly fall into three categories: on skin (or topical) repellents, permethrin-treated clothing and gear, and spatial repellents.[28] On skin repellents refer to products applied directly to a person's skin that make the person less attractive to the biting insect or tick.[29] Many different EPA-registered on skin repellents exist that use different active ingredients including (but not limited to): DEET, picaridin, IR3535, and oil of lemon eucalyptus (PMD).[30, 31]

When applying an on skin repellent, make sure to thoroughly apply the product according to all label directions and only apply to exposed skin or clothing (i.e., do not wear repellents under clothes) (Figure 3).[32] Additionally, the percent active ingredient indicates how long a product will provide protection before needing to be reapplied as opposed to how well the product repels.[31] As such, people should reapply the product according to label directions.

Figure 3. A) Always remember to read and follow the label directions when applying on skin repellents. B) Apply repellent to exposed skin.







Images courtesy of the Centers for Disease Control and Prevention Public Health Image Library/ Amy E. Lockwood; Division of Vector-Borne Diseases.

Permethrin-treated clothing and gear are another way to repel *Culicoides* biting midges. Permethrin is a pyrethroid insecticide used for many different purposes, but when applied to clothing following all label directions, it works as an insect repellent that has been used by the military since the 1990s.[33] People can purchase EPA-registered, permethrin-impregnated clothing. Additionally, EPA-registered sprays containing permethrin can be purchased and used to treat clothing, gear, and barriers.[34] As with any pesticide, make sure to read and follow all label directions, and reapply permethrin to items only as described on the label.

Spatial repellents emit chemicals into the air that protect people from biting insects located within the protected area.[35] EPA-registered spatial repellent products use fans and/or heat to disperse the active ingredient.[29] These products may contain different active ingredients, but volatile pyrethroids have been shown effective at preventing mosquito bites.[36, 37] Much of the research on spatial repellency has focused on repelling mosquitoes,[36, 38, 39] although a smaller number of studies have evaluated other biting arthropods.[40] At least one laboratory study found

the pyrethroid transfluthrin to have spatial repellent effects against two different *Culicoides* species.[41]

To help identify more spatial repellent active ingredients efficacious against *Culicoides* spp., Olds' AMCA project investigated seven botanical essential oils against laboratory strains of *C. sonorensis* (Table 2). Oils tested revealed that repellency varied by concentration and active ingredient. Massoia bark oil, amyris oil, and fir needle oil demonstrated the strongest repellency at different concentrations. Geranium oil and pevilla oil performed well with ~70% repellency. Patchouli oil was similarly effective but at a lower concentration. Cassia oil was among the least effective botanical oil evaluated.

Community Engagement

An IMM program will regularly engage their communities as part of routine operations. As such, the *AMCA's Best Practices for Integrated Mosquito Management* manual has a whole chapter on effective community engagement on pages 20-32. Additionally, communication needs arising from increased risk of OROV may constitute a public health emergency. In this case, additional guidance on community engagement as part of an emergency response can be found in the *AMCA's Mosquito Management During a Public Health Emergency* manual on pages 10-13.

There are two main messages to share when risk from *Culicoides* spp. or OROV increases: 1) how to properly protect yourself and loved ones and 2) how to protect your homes and properties. As for information regarding personal protection, this message should be shared directly with staff during all hands meetings and communicated externally to the broader public through appropriate channels. Specific information may include[42]:

- Dawn and dusk are the most active periods for many mosquitoes and *Culicoides* biting midges. Take extra precaution when outdoors during these periods.
- Apply EPA-registered on skin repellents that include both mosquitoes and biting midges on the label. Always read and follow all label directions.
- Wear loose-fitting, long-sleeved pants and shirts.
- Wear clothing and use gear that have been treated with permethrin for additional protection in heavily infested areas.

To protect homes and properties, consider messaging around the following information:

- Control biting insects and mosquitoes in and around your homes by removing standing water and consider applying barrier treatments that target their resting habitat.[43]
- Keep *Culicoides* spp. out of your house by replacing window screens with a mesh size small enough to prevent these very tiny flies from passing through.
- Contact local vector/mosquito control if biting insects become too bothersome.

Table 2. Compounds Investigated as Potential Spatial Repellents against Two Laboratory Colonies of *Culicoides sonorensis*[†]

Active Ingredient	Repellent Type	Permitted in Exempt Product[§]	Application Rate (mcg/cm2)	% Repellency (± S.E.M.) after 60 min[‡]
Massoia Bark Oil	Botanical Essential Oil	No	100	80.6 (± 1.5)
Amyris Oil	Botanical Essential Oil	No	50	78.2 (± 3.7)
Geranium Oil	Botanical Essential Oil	Yes	50	69.6 (± 3.8)
Patchouli Oil	Botanical Essential Oil	No	25	70.2 (± 3.5)
Fir Needle Oil	Botanical Essential Oil	No	50	78.6 (± 3.9)
Cassia Oil	Botanical Essential Oil	No	50	58.3 (± 9.6)
Pevilla Oil	Botanical Essential Oil	No	75	67.0 (± 7.6)
N,N-diethyl-meta-toluamide (DEET)	Synthetic	No	100	78.3 (± 6.0)

[†] Spatial repellent assays used two laboratory-maintained populations of *C. sonorensis*: the Ausman colony (Brighton, CO; established 2001) and the Kansas colony (Manhattan, KS; established 2020). All oils were tested against the Ausman colony, with a subset further tested against the Kansas colony.

[§] Active ingredients listed under 40 CFR 152.25(f)(1) are considered a minimum risk pesticide.[76] The Environmental Protection Agency (EPA) previously determined that these active ingredients pose minimal risk to human health at percentages often found in products on the market.[25]

[‡] Actual repellency measured in laboratory assays using a high-throughput, glass tube method based on Jiang et al. 2019.[77] In short, filter papers were treated with either a potential spatial repellent, acetone as a negative control, or DEET as a positive control. Each end of a glass tube was then fitted with one of the experimental filter papers or the positive and a negative control. A line was drawn in the middle of the tube, and 30 *C. sonorensis* were placed inside. After 60 min, the number of specimens that were on the side of the line corresponding with the treatment were counted.

SURVEILLANCE

Using vector surveillance to make data driven decisions is a fundamental activity of any integrated vector management program. As such, regular surveillance for *Culicoides* spp. must occur for a program to effectively lower the risk from this fly. For an in-depth discussion on best practices for performing mosquito surveillance, please refer to pages 33-43 of the *AMCA's Best Practices for Integrated Mosquito Management*. To learn more about arbovirus surveillance, please refer to pages 44-48 of the same resource.

Immature Surveillance

Larval *Culicoides* spp. surveillance typically focuses on identifying and monitoring semi-aquatic breeding habitats such as mud, manure-enriched soils, pond margins, wastewater areas, salt marshes, and livestock-associated substrates.[44, 45] Because immature *Culicoides* spp. are small and difficult to spot in the field, surveillance programs commonly use soil-core sampling combined with flotation extraction techniques, Berlese/Tullgren funnels, or laboratory emergence methods to recover larvae and pupae from substrate samples.[46] Comparative studies have demonstrated that flotation-based extraction methods using saturated salt or sugar solutions are among the most effective techniques for recovering immature stages from organic soils and marsh substrates, while emergence chambers allow adults to emerge for more reliable species-level identification.[46, 47]

Many surveillance programs also rely on emergence traps placed directly over suspected larval habitats to measure adult production and identify productive breeding sites in agricultural and wetland environments.[44, 45] These approaches are especially important because larval morphology is often difficult and accurate species identification requires skilled entomologists. However, emerged adults are relatively easier to identify through morphological or molecular methods.[46] Studies conducted in livestock systems and wetland habitats have also shown that emergence trapping can help define seasonal abundance patterns, overwintering ecology, and habitat associations relevant to arbovirus surveillance and vector management.[45] Researchers have concluded that combining emergence trapping with direct substrate sampling provides the most accurate assessment of larval habitat productivity and *Culicoides* spp. population dynamics.[44, 46]

Due to the time and expertise required, the AMCA recommends that mosquito programs focus on adult surveillance to assess *Culicoides* spp. population levels in response to a possible public health outbreak such as OROV. Larval surveillance, although valuable, should be reserved for long-term programs that aim to suppress populations prior to an outbreak.

Adult Surveillance

A robust adult surveillance program allows public health professionals to make data driven decisions and is the backbone to any scientific vector management program.[48] Though various traps can collect *Culicoides* spp.,[43, 49] AMCA recommends modifying CDC light traps to

collect *Culicoides* biting midges. Guidance from the Burkett-Cadena laboratory from the University of Florida's FMEL describes how to modify the trap's mesh sleeve and collection chamber to effectively capture these insects.[50] In short, a plastic vial filled with 70% isopropanol is fitted at the bottom of the fine-mesh sleeve and collection chamber to capture the tiny flies as they pass through the upper chamber that contains large insects (i.e., mosquitoes). Materials to complete this modification can be purchased from multiple suppliers. Alternatively, AMCA worked closely with staff at the Sacramento-Yolo Mosquito and Vector Control District to develop a 3D printed version of the FMEL modified light trap chamber for *Culicoides* biting midges. The print files can be downloaded here.

Adult *Culicoides* spp. surveillance should start by establishing fixed trap locations, ideally at sites that overlap with existing mosquito traps and where one would reasonably expect to find both *Culicoides* spp. and mosquitoes (Figure 4). Additionally, traps should be set and collected weekly throughout the entire season. The goal with establishing consistent locations and cadence is to allow personnel to compare data through time and space.[48] Populations may vary throughout the season, and control measures should only occur when data meets or exceeds pre-established action thresholds. Similar to mosquito surveillance, collections should be sorted, speciated (Table 3), and counted.[51]

To collect the primary vector of OROV in the continental U.S., traps should be placed where *C. paraensis* are mostly likely to be found (i.e., tree holes). Typically, adults may be collected in or near deciduous forests that contain mature trees with the desired habitat.[2] This habitat may already be under surveillance for *Aedes triseriatus* or *Ae. sierrensis* mosquitoes. Additionally, collection records for this species show that *C. paraensis* exist generally in the southeastern U.S. but have been documented as far north as Wisconsin and as far west as Colorado.[2]

As with mosquitoes, the actual species collected

Figure 4. A) Location from Obenauer's AMCA project as an example of where to hang a modified CDC Light Trap to perform *Culicoides* spp. surveillance. B) An FMEL-modified CDC Light Trap used to collect and separate *Culicoides* spp. from mosquitoes.





Images provided by Peter Obenauer.

Table 3. *Culicoides* Species Identification Resources

Region of the U.S. and Territories	Year Published	Identification Method	Citation
Continental U.S.	1985	Morphology	Wirth, W.W., Dyce, A.L. and Peterson, B.V., 1985. An atlas of wing photographs, with a summary of the numerical characters of the Nearctic species of *Culicoides* (Diptera: Ceratopogonidae).
Southern U.S., Puerto Rico & U.S. Virgin Islands	1988	Morphology	Wirth, W.W., Dyce, A.L. and Spinelli, G.R., 1988. An atlas of wing photographs, with a summary of the numerical characters of the Neotropical species of *Culicoides* (Diptera: Ceratopogonidae).
North America	2022	Molecular	Shults, P., Moran, M., Blumenfeld, A.J., Vargo, E.L., Cohnstaedt, L.W. and Eyer, P.A., 2022. Development of microsatellite markers for population genetics of biting midges and a potential tool for species identification of *Culicoides sonorensis* Wirth & Jones. Parasites & vectors, 15(1), p.69.
Ontario, CA (possible upper Midwest)	2023	Morphology	Janke, L.A., Vigil, S., Lindsay, K.G., Furukawa-Stoffer, T., Colucci, N., Ambagala, A. and Hanner, R., 2023. *Culicoides* (Diptera: Ceratopogonidae) of Ontario: A Dichotomous Key and Wing Atlas. Canadian Journal of Arthropod Identification, (50), p.1.
Florida	2024	Morphology	Blosser, E.M., McGregor, B.L. and Burkett-Cadena, N.D., 2024. A photographic key to the adult female biting midges (*Diptera: Ceratopogonidae: Culicoides*) of Florida, USA. Zootaxa, 5433(2), pp.151-182.

by vector control professionals will likely vary based on multiple factors[49] such as season, trap type, lure, light source, location, and habitat type. AMCA funded project teams collected 22 species located throughout the U.S. across various habitats (Table 4). Some of these species are known vectors of pathogens to wildlife, but interestingly, no collections contained *C. paraensis*. Regardless, IMM programs that begin *Culicoides* spp. surveillance should expect to collect multiple species. In addition, different trap types will perform better at collecting *Culicoides* spp. than others, as exemplified by rotator traps being relatively ineffective for live specimens (Pritts' AMCA project).

Regarding OROV specifically, the CDC has published a response plan that states surveillance for *C. paraensis* and *Culex quinquefasciatus* should begin if there are probable or confirmed (either single or multiple) locally acquired cases. In these investigations, public health professionals should set traps at locations where the person may have contracted OROV such as around the home, work, or other likely sites of infection (e.g., areas of recreation or where the individual has visited). Additionally, collections may be pooled to perform arbovirus surveillance.

Table 4. *Culicoides* Species Collected by AMCA Funded Evaluation Teams

Species Collected	Research Group	Location	Collection Method	Trapping Habitat
Culicoides avaritia	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides baveri	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides bergi	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake
Culicoides biguttatus	Swab et al.	MA	CDC Light trap with fine-mesh collection cup; CO_2 & UV light: Models used are John W. Hock Co. Stealth Trap Model 2014, and John W. Hock Co. UVA-LED Light Trap Model MN:1612.	Wooded suburban residential area, and a cow pasture (small dairy farm)
Culicoides crepuscularis	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
	Olds et al.	KS	CDC Light trap with modified collection cup; CO_2 & UV light	Cow/calf pastures
	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake
Culicoides freeborni	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake
Culicoides furens	Obenauer et al.	GA	CDC miniature light traps modified with collection cup; CO_2 & incandescent light	Wooded Inland water edge off Atlantic Coast
Culicoides guttipennis	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides haematopotus	Olds et al.	KS	CDC Light trap with modified collection cup; CO_2 & UV light	Cow/calf pastures
	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides haematopotus/ defoliarti	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake

Table 4 (cont.). *Culicoides* Species Collected by AMCA Funded Evaluation Teams

Species Collected	Research Group	Location	Collection Method	Trapping Habitat
Culicoides monoculicoides	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides neopulicaris	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides obsoletus	Swab et al.	MA	CDC Light trap with fine-mesh collection cup; CO_2 & UV light: Models used are John W. Hock Co. Stealth Trap Model 2014, and John W. Hock Co. UVA-LED Light Trap Model MN:1612	Cow pasture (small dairy farm)
Culicoides saundersi	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides (selfia)	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake
Culicoides (silvicola)	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake
Culicoides sonorensis	Olds et al.	KS	CDC Light trap with modified collection cup; CO_2 & UV light	Cow/calf pastures
	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake
Culicoides spinosus	Swab et al.	MA	CDC Light trap with fine-mesh collection cup; CO_2 & UV light: Models used are John W. Hock Co. Stealth Trap Model 2014, and John W. Hock Co. UVA-LED Light Trap Model MN:1612	Cow pasture (small dairy farm)
Culicoides stellifer	Swab et al.	MA	CDC Light trap with fine-mesh collection cup; CO_2 & UV light: Models used are John W. Hock Co. Stealth Trap Model 2014, and John W. Hock Co. UVA-LED Light Trap Model MN:1612	Near livestock corrals, a beef cattle pasture, cow pasture (small dairy farm)
	Olds et al.	KS	CDC Light trap with modified collection cup; CO_2 & UV light	Cow/calf pastures
	Scott et al.	CA	CDC Light trap and resting boxes	Wooded area around lake

Table 4 (cont.). *Culicoides* Species Collected by AMCA Funded Evaluation Teams

Species Collected	Research Group	Location	Collection Method	Trapping Habitat
Culicoides travisi	Pritts et al.	TX	Modified Mini CDC light trap w/ dual chambers collection cup; CO_2 & Visible light	Wooded area next to agricultural land and retention pond
Culicoides variipennis	Swab et al.	MA	CDC Light trap with fine-mesh collection cup; CO_2 & UV light: Models used are John W. Hock Co. Stealth Trap Model 2014, and John W. Hock Co. UVA-LED Light Trap Model MN:1612	Beef cattle pasture, cow pasture (small dairy farm)
Culicoides venustus	Olds et al.	KS	CDC Light trap with modified collection cup; CO_2 & UV light	Cow/calf pastures

CONTROL

Reducing *Culicoides* spp. populations over large areas is notoriously difficult, and the reasons are likely varied. For instance, control professionals may have an incomplete understanding of larval habitats that prevent holistic, targeted control programs.[52, 53] Another possible underlying factor could be that pesticide applications do not make contact with adults. For example, ULV applications may not penetrate as well into vegetation where *Culicoides* spp. may harbor.[54] In the case of ground level applications (e.g., truck-mounted ULV), the product may not reach *Culicoides* spp. that may prefer harboring higher (e.g., in tree canopies).[55, 56] Furthermore, new adults may rapidly repopulate a treated area,[57] giving the impression that control efforts are not killing biting flies. Although generally considered weak fliers (they typically use their own powered flight to move relatively short distances from larval habitats and hosts[18, 58]), *Culicoides* biting midges can disperse surprisingly long distances when assisted by prevailing winds.[18, 59]

Despite these difficulties, vector management professionals do have tools available that can manage different life stages. For an in-depth discussion on managing immature and adult mosquitoes using insecticides, please refer to pages 67-78 of the *AMCA's Best Practices for Integrated Mosquito Management* manual.

Larval Control

To manage larval *Culicoides* spp. populations, two strategies may be used: 1) manipulation or destruction of development sites and 2) application of larvicides. Although both strategies may prove beneficial, there is little published literature supporting the removal or manipulation of larval habitats to manage *Culicoides* spp. populations. Additionally, large-scale habitat modification projects can be difficult for most programs due to multiple factors such as the extreme amount of resources required and the challenge of locating all development sites (e.g., some species may be able to utilize multiple habitats while other species require very specific habitats).[53] Additionally, even when habitats have seemingly been removed, there is no guarantee that the population will be suppressed.[52]

Larvicides, however, may be easier to incorporate into an established IMM program by comparison and have been shown to reduce survival of immature *Culicoides sonorensis* (Brelsfoard's AMCA project). Applicators may be able to simultaneously treat *Culicoides* spp. larval habitats that are adjacent to known mosquito development sites (e.g., along the moist edge of a body of water known to produce mosquitoes) as part of regular mosquito control operations.

To begin using larvicides against *Culicoides* spp., a program should prioritize products that are efficacious against both mosquitoes and *Culicoides* spp., allowing the applicator to use a single product in multiple habitat types. Brelsfoard's AMCA project investigated six different commercial products (Table 5) against immature *C. sonorensis* under semi-field conditions (Figure 5). This team found that all products were lethal to immature *C. sonorensis* up to 20 days post-treatment, and some products may last over 50 days. Products containing

Table 5. Larvicidal Efficacy against Adult Emergence of *Culicoides sonorensis* Applied under Semi-Field Conditions[†]

Larvicide Formulation[§]	Insecticide Class	Active Ingredient (AI)	Application Type	Application Rate (US Standard)	50% Mortality (S.E.M.)	95% Mortality (S.E.M.)
Altosid	IGR	(s) - Methoprene (5%)	liquid	5-20 lb. per acre	38.52 (2.71)	n/a
Sumilarv 0.5G	IGR	Pyriproxyfen (0.5%)	granules	0.35 oz. per 500 gallons	>50 (3.55)	n/a
Natular SC	Spinosyn	Spinosad (22.5%)	liquid	1.2-2.9 oz. per acre	>50 (9.61)	12.91 (5.24)
TEKKO PRO	IGR	Novaluron (1.3%) and pyriproxyfen (1.3%)	liquid	0.04-0.08 lb per 50 gallons	>50 (302)	8.74 (3.64)
Strike Ultra	I/GR	(s)-Methoprene (33.6%)	liquid	3 fl oz/ 1 million gallons	30.22 (3.14)	n/a
Natular G30	Spinosyn	Spinosad (2.5%)	granules	5-12 lbs per acre	27.39 (18.44)	n/a

[†] Plastic tubs that had water located at one end and a manure/soil mixture at the other to resemble a "shoreline" were placed outside in three different locations representing three different ecotypes. Larvicides were applied to these tubs using equipment and methods employed by existing mosquito control programs. Soil samples were taken from the "shorelines" within each of the containers every 10 days. Soil samples were taken back to the laboratory, and researchers placed 30 *C. sonorensis* larvae into the soil samples (bioassays). Adult emergence was then recorded for each bioassay.

[§] Before applying any pesticide, always read and follow all product label directions. Always ensure that the target organism or application site is listed on the label.

[Ÿ] Days post exposure refers to the calculated number of days post treatment that would result in either 50% or 95% mortality of immature *C. sonorensis*.

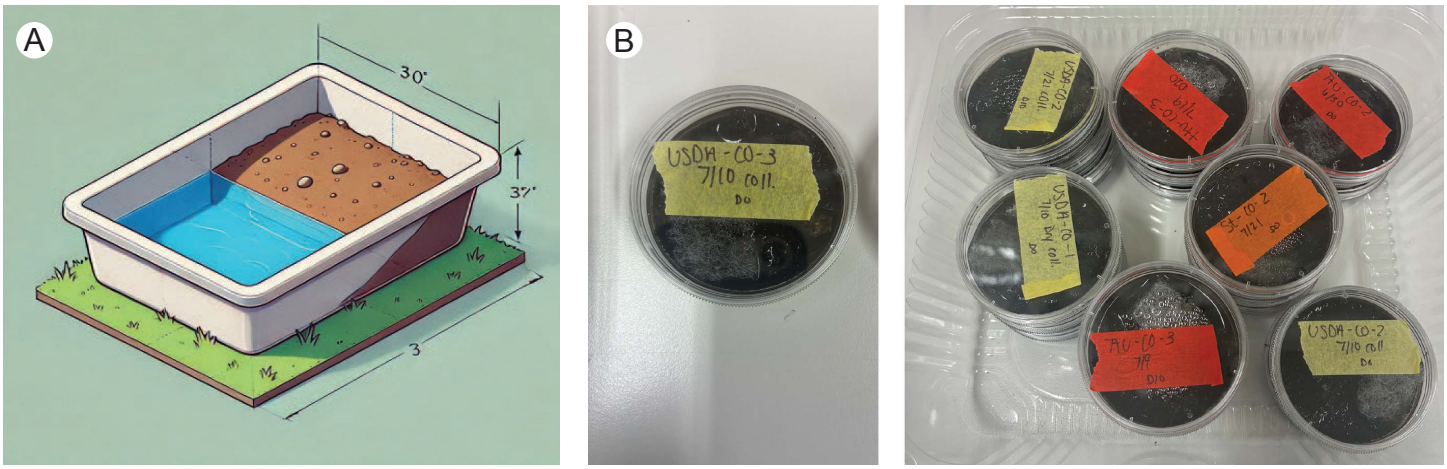
spinosad, pyriproxyfen, and novaluron demonstrated the greatest persistence, while methoprene products declined more rapidly.

Previous research has shown that *Bacillus thuringiensis israelensis* (*Bti*), an active ingredient commonly used in mosquito control, is relatively ineffective against *Culicoides* spp. and, therefore, is not currently recommended for control.[60, 61] Additionally, Brelsfoard's AMCA project showed that environmental conditions such as rainfall likely influence product persistence. As with all pesticide applications, always read and follow product label directions and ensure that the target organism or application site is listed on the label, as per each state's guidelines.

Adult Control

Different types of habitats can produce large populations of *Culicoides* spp. and may require different management strategies tailored to

Figure 5. Experimental setup for Brelsfoard's AMCA project. In short, A) 40 L (10.6 gal) plastic tubs were set up across three ecotypes to mimic semi-aquatic *Culicoides* spp. larval habitats, with water on one end and a soil mixture on the other. Larvicides were applied using standard mosquito-control methods, and substrate samples were collected at the soil water interface every 10 days for laboratory bioassays. B) Substrate samples were collected and placed into 60 mm petri dishes. The substrate samples were used in bioassays to determine lethality of the larvicides tested. Evaluators added 15 *Culicoides sonorensis* larvae and a 2 x 2 cm (0.79 x 0.79 in) piece of dacron to each petri dish and recorded adult emergence to assess larvicide efficacy over time.



Images provided by Corey Brelsfoard.

the environment (e.g., along coasts, in pastures and prairies, or in wooded areas). Regardless, insecticides targeting adult *Culicoides* spp. are another tool to consider as part of an integrated management plan. Just as with a mosquito control program, when populations reach preestablished action thresholds, a program has justification to take adult control measures. In these instances, control professionals should consider two methods of applying adulticides: 1) localized residual, barrier treatments or 2) area-wide ULV applications.

For barrier treatments, professionals apply pesticides to surfaces used by resting adult *Culicoides* spp. with the goal of killing any flies contacting the treated surface. Previous research found that a bifenthrin-based product applied to vegetation around homes effectively reduced *Culicoides* biting midges.[43] When performing barrier treatments, applicators should focus treatments where the pesticide will have the most impact on the target pest such as where they rest or most likely encounter people. Areas to consider treating may include vegetation around the home,[43] window screens, and the backs and undersides of chairs. To protect pollinators and other beneficial insects, avoid applying barrier treatments to blooming plants and vegetation that may attract bees, butterflies, or other pollinating species.

Alternatively, ULV applications of insecticides atomize a small volume of product into the air so that flying *Culicoides* spp. encounter the droplets. Pesticide applications via ULV equipment can be applied aerially or from the ground. Previous research has shown that these types of applications using different active ingredients (i.e., malathion, naled, resmethrin,

and permethrin) can reduce *Culicoides* spp. populations.[54, 62-64] However, results varied based on numerous factors such as the distance from the point of insecticide discharge, level of vegetative growth (e.g., open spaces vs. spaces filled with trees), and active ingredient.

Regarding ground-based ULV (Figure 6), Obenauer's, Pritts', and Swab's AMCA projects generally showed that vehicle-mounted ULV applications may be a useful tool in a *Culicoides* spp. management program. However, this result may be more nuanced based on data from Scott's AMCA project and previously published research.[65] In Texas and Georgia, Obenauer's and Pritts' AMCA projects used field cage tests containing field-collected *Culicoides* spp. to evaluate the efficacy of four different commercially available products that are commonly used in routine mosquito control programs (Table 6). Results showed that all products applied (using standard ULV spray equipment, calibrations, and droplet sizes effective against mosquitoes) were efficacious against *Culicoides* spp. up to 300 ft away from the point of ULV discharge, though Pritts' AMCA project showed declining efficacy as cages moved further away.

Figure 6. Mosquito control truck applying pesticides via ultra low volume (ULV) application equipment.



Image courtesy of the Centers for Disease Control and Prevention Public Health Image Library/ Amy E. Lockwood; Division of Vector-Borne Diseases.

Timing ULV missions is critical to ensure effective control of the target vector while minimally impacting nontarget organisms. Many vector species of mosquitoes are most active at dusk and dawn, which contrasts with peak activity of many beneficial nontarget organisms.[66] As a result, ULV spray missions targeting mosquitoes generally occur during the evenings and nights to reach vectors while simultaneously avoiding beneficial arthropods. Pritts' AMCA project reported that the *Culicoides* spp. collected in his area were crepuscular or nocturnal, which overlaps with the activity periods of many common mosquito species. Additionally, a previous study reported that aerial ULV applications of naled performed at night (and outside of the peak crepuscular activity of the *Culicoides* spp. in the area) successfully suppressed their populations (70-98% reductions).[67] Thus, ULV spray missions performed in the evenings and at night targeting mosquitoes may simultaneously reduce *Culicoides* spp. numbers.

However, Scott's AMCA project evaluated the effectiveness of ground-based ULV using CDC light traps to measure abundance before and after treatment. This team found ground-based ULV to be ineffective at significantly reducing the abundance of collected *Culicoides* biting midges. Taken together with the previous mortality observed in field cage tests, this report would suggest that although *Culicoides* spp. may be susceptible to pesticide applications, measurable reductions in field populations may not be readily observed. This failure to significantly reduce natural populations could be due to factors such as not treating a wide enough area,

Table 6. Ground-Based Ultra Low Volume (ULV) Adulticide† Efficacy against *Culicoides* Biting Midges Using Field Cage Tests§

Study Author	Active Ingredients	Insecticide Class(es)	Trade Name	Location	Application Rate	Droplet Size (µm)	% Mortality (S.E.M.) at 100 ft‡	% Mortality (S.E.M.) at 200 ft	% Mortality (S.E.M.) at 300 ft
Obenauer's AMCA project	Permethrin + PBO	Pyrethroid + Synergist	Permanone 30-30	GA	0.0035 lbs AI/acre	17 - 36	100.0	100.0	100.0
Obenauer's AMCA project	Sumethrin + PBO	Pyrethroid + Synergist	Anvil 10-10 ULV	GA	0.0024 lbs AI/acre	18 - 27	100.0	100.0	97.0 (± 1.2)
Pritts' AMCA project	Malathion	OP	Fyfanon ULV	TX	0.006 lbs AI/acre	4.8 - 30.1	92.3 (± 5.3)	78.2 (± 16.2)	70.0 (± 17.3)
Pritts' AMCA project	Pyrethrins & PBO	Pyrethroid + Synergist	Evergreen 5-25	TX	0.002 lbs AI/acre	4.8 - 30.1	92.2 (± 7.8)	97.7 (± 2.3)	73.1 (± 24.9)

† Before applying any pesticide, always read and follow all product label directions. Always ensure that the target organism or application site is listed on the label.

§ Field cage trials were performed based on a standard design such as that outlined in Stark et al. 2017.78 In short, wild *Culicoides* spp. were collected from the local area and placed inside modified cages. These cages were then placed at 100, 200, and 300 ft away from the application swath.

‡ All percent mortalities expressed were recorded 60 min after ULV application.

continual emergence from untreated larval habitats, immigration from surrounding areas, environmental conditions affecting application efficacy, or limitations in surveillance sensitivity.

Ideally, vector control professionals should only choose products labeled for *Culicoides* biting midges and that have published efficacy data. Obenauer's and Pritts' AMCA projects used field cage trials and found three different pyrethroid products and an organophosphate product effective against *Culicoides* spp. in Georgia and Texas (Table 6). In addition to efficacy data, programs should choose products that include both mosquitoes and *Culicoides* spp. on the label when targeting both vectors simultaneously during spray missions. *Culicoides* spp. may show up on labels under different names such as flies, biting flies, biting midges, no-see-ums, punkies and others, so being aware of the different common names for this fly may help with product selection.

When applying any insecticide, applicators need to take every effort to ensure their treatments do not interfere with nontarget organisms.[48] As stated previously, products should never be applied to blooming flowers used by pollinators and other beneficial insects. Additionally, application timing should target a time when vectors are active but nontarget organisms are resting. Wind speed and direction need to be monitored to avoid applications drifting to unintended locations. Furthermore, program managers should have equipment calibrated every year to ensure the correct droplet size and application rate. As with all pesticides, make sure to read and follow all label directions, and ensure the pest and/or application site is listed on the label.

EVALUATING *CULICOIDES* BITING MIDGE CONTROL PROGRAMS

When incorporating *Culicoides* spp. control metrics into an established IMM program, managers will need to think critically about how to measure success and define metrics before performing any control activities. The *AMCA's Best Practices for Integrated Mosquito Management* manual has several sections to help: Setting Action Thresholds (pp. 54-59), Monitoring Insecticide Resistance (pp. 79-81), and Record Keeping (pp. 82-84).

Evaluation Metrics

Surveillance data is critical to determine if a program is successfully managing *Culicoides* spp. or if operations need to be modified. For mosquito control, program managers may consider the following indicators as metrics of success:

- **Reduced mosquito abundance, customer calls, or positive pools**
- **Changes in species composition**
- **Elimination of known developmental sites compared to historic records**

Whereas all of the above metrics can also be used to evaluate a *Culicoides* spp. control component, results from previous research[65] and Scott's AMCA project suggest that using existing evaluation metrics may prove challenging. As a result, programs may need to reframe their vision of success and consider additional program metrics, such as a change to the population's age structure.

The extrinsic incubation period describes the amount of time required for a vector to consume a pathogen in a bloodmeal and become infectious to a human or animal.[68] Considering this phenomenon, older vectors pose higher risk of arbovirus transmission relative to younger vectors. As such, control efforts that change a population's age structure to have more young individuals may lower the risk of virus transmission even if the overall number of biting individuals is unaffected (i.e., reduce the population's vectorial capacity).[69, 70]

Vector control professionals can estimate the age structure of a *Culicoides* spp. population by assessing parity status (whether a female has laid a clutch of eggs or not[71]). This is done by examining a female's abdomen using a dissecting microscope under 20X magnification and looking for pigment.[72] To better assess parity, Swab's AMCA project developed a new technique using Shortwave Infrared Spectroscopy (SWIRS) (Figure 7). Using this new method, team members assessed parity status of collected *Culicoides* spp. pre- and post-truck-mounted ULV treatment. Preliminary results demonstrated that the population age structure had shifted, suggesting that the ULV applications removed older individuals from the population. In the future, programs may be able to use this method to evaluate programmatic goals more efficiently.

Another important parameter to consider when evaluating adulticides is the timing of insecticide applications. Corbel's AMCA study collected 4.5-times more *Culicoides* biting midges between 4:00 and 5:00 P.M. than between 2:00 and 3:00 P.M. during the same day, indicating that biting activity may vary considerably throughout

Figure 7. Photomicrographs of *Culicoides biguttatus* abdomens taken using Shortwave Infrared Spectroscopy (SWIRS). Abdomens were rehydrated with PBS solution mixed with a small amount of detergent and slide mounted in glycerol. All abdomens are dorsal aspect except for the top right abdomen which is slightly lateral aspect.

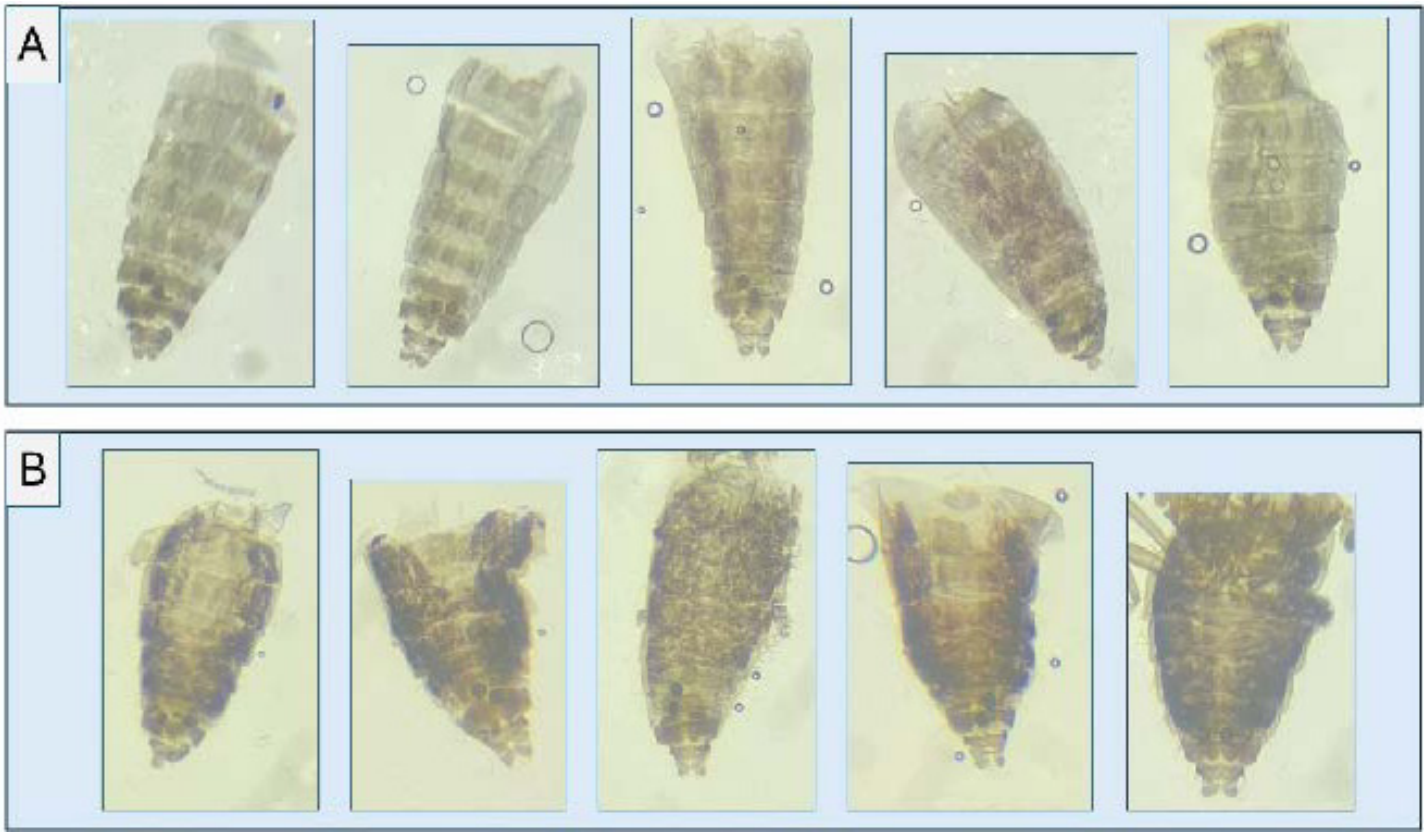


Image provided by Christopher Swab.

the day. Therefore, program managers should identify a baseline and monitor the peak biting period of *Culicoides* spp. in their area to not only optimize the timing of interventions, but also accurately assess impact of control efforts on vector density, activity, and survival.

Field Cage Trials

Whenever possible, programs should add *Culicoides* spp. evaluation metrics to existing IMM program activities as opposed to creating brand new undertakings. For instance, programs should begin collecting field cage test data against *Culicoides* spp. that run concurrent with established mosquito trials. For a *Culicoides* spp. specific protocol, please refer to Appendix II.

By including both *Culicoides* biting midges and mosquitoes during field cage test evaluations (Figure 8), vector control professionals can determine if treatments effectively kill each vector that encounters the insecticidal droplets. This information is critical for determining the success of a vector control operation, especially if pre- and post-trapping data yields a different result. By knowing if a population is susceptible to the product used, program managers can

Figure 8. A) Obenauer's AMCA project team setting up an area for a field cage test evaluation (Brunswick, GA). B) Pritt's AMCA project team member preparing a tripod for field evaluation.



Images provided by Peter Obenauer and Kevin Pritts.

begin troubleshooting and modifying their program if other data suggest that control goals are not being met.

To conduct successful *Culicoides* spp. field evaluations, vector control professionals must use specialized collection methods and cage designs. Standard mosquito-testing equipment with larger mesh sizes, collection bags, and standard cage designs may allow escape, alter droplet exposure, or bias efficacy measurements. Verification of droplet deposition at the cage stand location with impingers, or a known susceptible strain of mosquitoes, may help elucidate if ULV droplets reached the target and serve as an effective positive control. Also the use of NoSeeUm netting (<0.6 mm or ~0.02 in mesh) in cage design is among the most critical factors for obtaining reliable results.

Before performing a field cage trial using a smaller mesh size capable of containing *Culicoides* spp., professionals should ensure that the mesh allows droplets to reach the target insects. As noted in Corbel's AMCA project, further work is needed to identify a standard mesh size for conducting cage bioassays on *Culicoides* spp. and adequately measuring the efficacy of the applications. Additionally, cage shape may significantly influence droplet trajectories and measured mortality. Cylindrical versus "pancake" cage designs may produce different results.

A modified FMEL CDC light trap collection system can improve collection and survival of live specimens needed for bioassays and field cage tests. To use this design as a live trapping method, vector control professionals need to replace the vial containing alcohol with a fine mesh material. Additionally, maintaining live *Culicoides* spp. for extended periods is operationally difficult. To help overcome this hurdle, researchers should process *Culicoides* spp. rapidly after collection, maintain insects in cooler temperatures, and minimize holding times to help ensure successful field trials.

Programs may also use field cage tests to begin investigating insecticide susceptibility and

possible indicators of resistance under real-world conditions.[73] If susceptibility in trials begins to decrease over time in field-collected populations of *Culicoides* spp., insecticide resistance may be suspected. As such, control programs should begin performing field cage tests as soon as possible and continue them regularly to identify any early signs of concern. For more information on conducting field cage trials to monitor for insecticide resistance, please refer to the AMCA's manual on field cage testing found in the AMCA's Training Center under the AMCA's Best Management Practices Manuals section.

At the time of writing this guidance, monitoring insecticide resistance in *Culicoides* spp. populations using other industry standard methods (i.e., CDC bottle bioassays) is challenging due to the lack of diagnostic doses and times for most common active ingredients and species of *Culicoides* biting midge. Nevertheless, previous research has published a diagnostic dose and time for *Culicoides furens* to permethrin.[74] Additionally, vector control professionals may use current diagnostic doses and times for different mosquito species as a starting point to determine diagnostic doses and times for *Culicoides* spp. as long as susceptible colonies or populations of the target species are available.[75]

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APPENDIX I- DRAFT DISEASE RESPONSE PLAN: OROPOUCHE FEVER

Draft disease response plan courtesy of Lee County Mosquito Control District. For more information regarding this plan, please contact Lee County Mosquito Control personnel at Leecountymcdfl.gov or the AMCA Technical Advisor. This response plan can serve as a beginning point for organizations to create a personalized plan for OROV. This protocol should be modified to fit the needs of the specific vector control program.

INTRODUCTION

Oropouche fever is an emerging zoonotic disease caused by Oropouche virus (OROV) and is historically vectored by *Culicoides* biting midges, specifically *Culicoides paraensis*. It was first isolated in Trinidad and Tobago in 1955 having two transmission cycles, sylvatic and urban. More recently, increasing cases of OROV infection in humans have been reported in South and Central America, Cuba, and Haiti. In 2024, 108 cases were recorded in 6 U.S. states and Caribbean territories and were the first recorded cases in the U.S.

With the recent expansion of OROV, experts are uncertain as to what vector is involved in this transmission. Some concerns are that the virus may have evolved to one transmission cycle (urban) and that mosquitoes may be the primary vector. Studies report that *Aedes* and *Culex* mosquitoes have been infected with OROV, but their vector competence is still in question.

During control operations, all label required personal protection equipment will be required. Insect repellent will be utilized to minimize exposure to infected mosquitoes and *Culicoides* biting midges. All efforts will be contingent upon label restrictions, weather conditions, availability of resources, arthropod management plans, and local, state, and federal laws.

POSITIVE HUMAN RESPONSE

Travel-Associated Cases

1. Analyze all known information concerning the case reports.
2. Evaluate the timeliness of the reports and calculate approximate infection zones where *Culicoides* biting midges and mosquitoes may become infected and initiate a locally acquired focus of infection.
3. Set adult biting fly traps (BGS, GAT or CDC) with *Culicoides* biting midge nets to check for the presence of adult *Culicoides* biting midges and mosquitoes at the residence and place of employment of the travel-associated case.
4. Ground adulticide (ULV) area if warranted.
5. Ground larvicide (LV truck) area if warranted.
6. Conduct LV residual insecticide barrier treatments if warranted.
7. If potential vectors are present, personnel will conduct a door-to-door inspection to at least a 325-meter radius from the residence and place of employment of the travel-associated case.
8. All potential vectors captured in adult biting fly traps will be preserved at -80° F.

Locally Acquired Case(s)

1. Analyze all known information concerning the case reports.

2. Evaluate the timeliness of the reports and calculate approximate infection dates and infection sites.

3. Day 1 and 2: Aerial and ground adulticide (ULV) at least a one square mile polygon around the suspected infection site alternating one ULV technique each day.

4. Day 3: Personnel will conduct a door-to-door inspection up to an 800-meter radius from the suspected site of infection. Conduct barrier treatments on foliage, tree holes, fences, and other potential *Culicoides* biting midge resting areas around suspected site of infection at least to 1 acre. Treat with residual insecticide with LV backpack sprayer. Reapply as needed according to label (approx. 4-6 weeks).

5. Day 3-4: Ground larvicide (LV truck) up to a one-mile radius around the suspected site of infection.

6. Day 5-7: Ground adulticide (ULV) at least a 2-mile radius around the suspected infection site; rotate product spray schedule.

7. Day 8: Set adult biting fly traps (BGS, GAT or CDC) with *Culicoides* biting midge nets to check for the presence of adult *Culicoides* biting midges and mosquitoes at the residence and place of employment at suspected site of infection.

8. Day 9: Adulticide by ground or air if warranted.

SURVEILLANCE

1. Maintain increased trap and larval monitoring until notified by public health department that the threat is alleviated.

2. Collected *Culicoides* biting midges and mosquitoes should be preserved and submitted to the state laboratory for virus confirmation.

PUBLIC OUTREACH

Once notified of a local case of Oropouche, the following actions should take place immediately:

1. Post bite prevention information and disease type information on social media and <organization's website>*. (see below for examples)

2. Initiate Public Service Announcement through local media to emphasize the necessity of protecting yourself from mosquitoes.

3. Coordinate messaging with appropriate health department.

Public Service Announcement (Example):

"To reduce the exposure to biting midges or mosquitoes and the diseases they could carry, <organization's name> asks that you practice the 5 D's of mosquito protection. Dress in long sleeves and pants if you plan on being outdoors when mosquitoes are active. Dawn and Dusk are the most active periods. Deet-based mosquito repellent is an effective repellent (always follow label instructions). Drain water from containers weekly; don't grow your own mosquitoes. For more information visit <website and/or email> or call <phone number>.

*Replace text highlighted in yellow with your organization's specific contact information.

APPENDIX II- FIELD CAGE TEST PROTOCOL FOR *CULICOIDES* BITING MIDGES AND MOSQUITOES

The following protocol can serve as a beginning point for organizations to perform field cage trials using Culicoides biting midges and mosquitoes simultaneously. This protocol should be modified to fit the specific needs of the program.

Field cage trials evaluate the effectiveness of insecticide products, application methods, and equipment used to manage vectors under real-world conditions.[73] For more information on conducting field cage trials, please refer to the AMCA's manual on field cage testing found in the AMCA's Training Center under the AMCA's Best Management Practices Manuals section.

1. Capture wild caught *Culicoides* spp. using modified CDC miniature light traps and baited with dry ice (CO_2). Similarly, collect mosquitoes using traps baited with dry ice for use in the trials for a side-by-side comparison of mortality with the *Culicoides* biting midges.

2. Bring back all wild caught insects to the laboratory, enumerate and transfer to plastic bug dorms, or similar meshed cage, and supply with 10% sucrose solution. Place solution on top of the cage, not inside, to prevent the insects from getting stuck in the moisture.

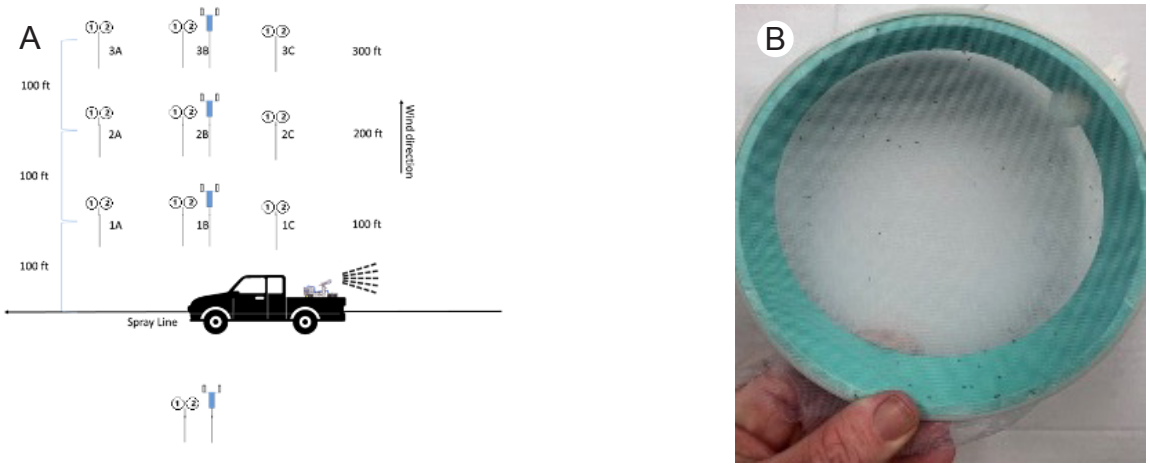
3. Conduct field trials in open areas where surrounding tree lines or vegetation will not likely impact the wind patterns. Ideally, use a field of 1000 feet x 1000 feet that is drivable on four sides. Minimally, a field of 600 feet x 600 feet that is drivable on two sides is necessary. It is best if there are multiple spray routes that the truck can take. Wind can be very unpredictable and plays a significant role in successful field trials. Therefore, setting up a field site that can be easily adjusted is crucial for a smooth trial.

4. Place 9 sampling stations in a grid-shaped pattern downwind from the release location (Figure 9). This design utilizes 3 stations at 100 ft from the vehicle's drive path, 3 stations at 200 ft, and 3 stations at 300 ft from the drive path. There should be 100 ft distance horizontally between each station as well. Two additional sampling stations should be set at least 100 ft upwind of the drive path to serve as a control.

5. Each station should have a "T"-shaped PVC pole, or tripod with one slide impinger on top, holding two 1in. Teflon-coated slides and two cages hanging from the cross arms. One cage should contain ~25 mosquitoes, and the other should contain ~25 *Culicoides* biting midges (Figure 9). Alternatively, *Culicoides* spp. and mosquitoes may be simultaneously contained within the same cage.

6. Place insects in test cages ~1 hr before the trial begins and hang on poles ~15 mins before the application commences. The truck should pass upwind of sampling locations, following standard operating procedures currently used in mosquito control. Run trials during a time that would be used during standard operations (e.g., at sunset).

Figure 9. A. Typical configuration for a field cage test. B. Cage used for field *Culicoides* spp. bioassays. NoSeeUm netting (<0.6 mm or ~0.02 in mesh) was attached to a PVC circular tube and secured with two zip ties. A 0.5 in entrance hole was drilled into the side of the tube and sealed with a cotton plug containing a 10% sucrose solution.



Images provided by Peter Obenauer.

7. Collect cages and slides 30 min after the spray application is completed and transport to the laboratory to monitor for mortality. Measure mosquito and *Culicoides* spp. mortality at 1 hr, 2 hr, 12 hr, and 24 hr intervals.

8. Collect the rotating slides along with the sample test cages. Transport them to the laboratory for proper reading. Record droplet size and density for each sampling station using available software.

9. Perform statistical analysis to determine if there are significant differences in the mortality of mosquitoes and *Culicoides* spp. when utilizing standard mosquito control protocols for ULV adulticide treatments.