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THE PMI VECTORLINK ANGOLA PROJECT

ANNUAL ENTOMOLOGY REPORT

MAY 2022–APRIL 2023

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

AI	Active ingredient
CDC	(U.S.) Centers for Disease Control and Prevention
DHIS2	District Health Information Software 2
DPS	*Direcções Provincial de Saúde* (Provincial Health Directorate)
ELISA	Enzyme-Linked Immunosorbent Assay
ICCT	*Instituto de Controlo e Combate a Tripanossomíases* (Institute for the Control and Combat of Trypanosomiasis
INIS	*Instituto Nacional de Investigação em Saúde* (National Institute of Health Research)
ITN	Insecticide-treated net
IRS	Indoor residual spraying
Kdr	Knock-down resistance
LT	Light trap
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PCR	Polymerase chain reaction
PMI	(U.S.) President's Malaria Initiative
PSI	Population Services International
SOP	Standard operating procedures
STTA	Short-term technical assistance
WHO	World Health Organization

EXECUTIVE SUMMARY

In collaboration with the National Malaria Control Program (NMCP), the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in Angola between May 2022 and April 2023 in seven provinces, including five PMI-focus provinces (Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire) and two additional non-PMI-focus provinces (Huambo and Luanda) selected based on existing insectary infrastructure and entomology capacity. Six collection efforts (months) of vector bionomics monitoring were conducted over the reporting period in Huambo and Luanda to determine species composition and malaria transmission intensity, using indoor U.S. Centers for Disease Control and Prevention (CDC) light traps (LTs). For each collection month and site, 60 CDC LT collections were completed. Mosquitoes collected were morphologically identified to the species level and a proportion of the known malaria vectors *Anopheles funestus* s.l. and *An. gambiae* s.l. were sent to the molecular laboratory at the *Instituto Nacional de Investigação em Saúde* (INIS) for species identification and sporozoite infection assays.

Insecticide susceptibility tests were conducted in six provinces (Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire) to inform insecticide-treated net (ITN) procurement decisions made by the NMCP, PMI, and other partners. World Health Organization (WHO) susceptibility bioassays targeting *An. gambiae* s.l./*An. funestus* s.l. were conducted with the pyrethroids (deltamethrin, alpha-cypermethrin, and permethrin) with and without pre-exposure to the synergist piperonyl butoxide (PBO), while CDC bottle bioassays were used to determine the susceptibility to the pyrrole insecticide chlorfenapyr at the various sites. Due to challenges collecting sufficient quantities of larvae, most provinces were only able to test two of the three pyrethroids. In Luanda, insecticide susceptibility testing was done for *An. funestus* s.l. collected using Prokopack and CDC LTs collections indoors and outdoors. In all provinces, a proportion of pyrethroid resistant and susceptible samples were sent to INIS for species PCR analysis and *kdr* analysis.

Anopheles funestus s.l. was the most abundant vector collected through CDC LTs in Huambo and Luanda provinces combined (99.3%) with a very low overall proportion of *An. gambiae* s.l. (0.5%). *Anopheles funestus* s.l. monthly densities in Huambo ranged from 0.3 to 4.5 vectors/trap/night with the lowest density observed in June 2022 and peak density observed in February 2023. In Luanda, monthly densities ranged from 10.7 to 49.7 vectors/trap/night with the lowest density in June 2022 and the peak density in January 2023. *Anopheles gambiae* s.l. densities were generally low in both Huambo (range 0-0.3; peak density in February 2023) and Luanda (range 0.02-0.08; peak density observed in May 2022). There was relatively very low risk of biting from *An. gambiae* s.l. in both provinces; for *An. funestus* s.l., a high risk of biting was observed in Luanda and a moderate risk in Huambo. The entomological inoculation rate will be calculated in late 2023 following sporozoite analysis of samples to determine *Plasmodium falciparum* infection rates.

There was widespread pyrethroid resistance in five provinces (Cuanza-Norte, Lunda-Sul, Malanje, Uíge and Zaire) with confirmed resistance to two or more of the pyrethroid insecticides tested (deltamethrin, alpha-cypermethrin, and permethrin). All vectors were fully susceptible to chlorfenapyr at all sites tested. Due to the widespread distribution of pyrethroid resistance, the recommendation to the NMCP and partners supporting ITN campaigns is for distribution of ITNs containing PBO or dual active ingredient nets in lieu of pyrethroid-only nets in these provinces.

During the reporting period, VectorLink also collaborated with the NMCP to establish the country's first two susceptible *An. gambiae* colonies, which will help support the sustainability of entomological capacity in Angola. After troubleshooting and achieving optimal rearing conditions (temperature and humidity), *An. coluzzii* eggs received from Witwatersrand University and susceptible *An. gambiae* s.s. Kisumu eggs received from CDC Atlanta were received and have been successfully reared at the *Instituto de Controlo e Combate a Tripanossomíases*

(ICCT) in Luanda. As of June 2023, the *An. coluzzii* colony has reached the F11 generation and the *An. gambiae* s.s. colony is on F10 generation.

Local capacity was strengthened through a variety of measures, including training and working closely with at least three mosquito brigade staff in each of the seven provinces. Short-term technical assistance (STTA) was provided by the Vectorlink Zambia Entomology Technical Manager in July 2022 and February 2023. The objectives of these STTAs were to provide support on laboratory workflow, ensure molecular sample processing protocols were followed, observe susceptibility testing in Malanje and to check on maintenance procedures of two susceptible colonies. One representative from Angola also participated in the project-wide training on data interpretation and visualization using VectorLink Collect, the project's District Health Information Software 2 (DHIS2)- based database. VectorLink Angola worked closely through 2022-23 with the NMCP, WHO, and other partners to incorporate the entomology modules into Angola's national DHIS2. This initiative will ultimately allow the NMCP to view all entomology data collected in Angola from all partners in a single database, enabling comprehensive evidence-based vector control decision making.

1. INTRODUCTION

Malaria remains a serious public health problem in Angola despite sustained malaria control strategies. Malaria is endemic throughout the country and is the principal cause of morbidity and mortality. Although the entire population is at risk, there is significant heterogeneity in transmission intensity across the country. Historically, the northeast provinces of Cabinda, Cuanza-Norte, Lunda-Norte, Lunda-Sul, Malanje, and Uíge are hyperendemic, the central and coastal provinces of Benguela, Bie, Cuanza-Sul, Huambo, Luanda, Moxico, and Zaire are mesoendemic with stable transmission; while the four southern provinces bordering Namibia have highly seasonal transmission and are prone to epidemics[1]. *Plasmodium falciparum* is the primary malaria parasite in Angola and accounts for an estimated 87% of malaria cases. The other three *Plasmodium* species known to cause human malaria—*P. vivax*, *P. malariae*, and *P. ovale*—are also present and cause about 7%, 3%, and 3% of reported malaria cases, respectively[2]. There are five *Anopheles* species responsible for transmission in the country: *Anopheles gambiae* s.s., *An. funestus*, *An. melas* (in coastal areas), *An. arabiensis*, and *An. pharoensis* (in southern, unstable mesoendemic areas). In addition, *An. rufipes* and *An. coustani* have been identified as secondary vectors in Huambo and Zaire provinces[1].

Angola's National Malaria Strategic Plan 2021-2025 outlines a four-pronged vector control strategy which aims to protect at least 80% of the population at risk by 2025 using 1) insecticide-treated nets (ITNs), 2) indoor residual spraying (IRS), 3) prevention of malaria in pregnancy, and 4) larviciding. The Demographic and Health Survey (2015-2016, Angola) showed that 31% of households in Angola owned at least one ITN[3]. By comparison, across all countries in sub-Saharan Africa in 2016, 80% of households owned at least one ITN[4]. PMI and the Global Fund have been key partners supporting procurement and distribution of ITNs. Angola took a major step in 2017 towards universal coverage of ITNs, conducting a three-phase nationwide mass campaign from 2017–2018, which resulted in the distribution of 6,693,503 ITNs to 2,379,943 registered household across 13 provinces. Following the campaign, a survey conducted in 2019 showed that 55.4% had access to a net in Cuanza-Sul Province (use: access ratio 0.87), 51% in Cunene Province (0.69 use: access ratio) and 33.3% in Uíge (0.77 use: access)[5]. The most recent mass ITN campaign started in April 2022 and the first phase ended in November 2022. PMI supported distribution in Cuanza-Norte, Lunda-Norte, Lunda-Sul, Malanje, Uíge, and Zaire, while the Global Fund is supporting Benguela and Cuanza-Sul. Distribution in 13 municipalities in Uige, Zaire, and Lunda-Norte provinces was not done during Phase 1 due to higher than originally estimated population and insufficiency of nets. However, PBO nets arrived in Angola in May 2023 and are currently being distributed to these areas (June-August 2023). In the remaining provinces, MOH will be procuring the nets, however this procurement has been delayed.

To ensure the mass campaign began on time, PMI supported the procurement of standard ITNs in 2019. VectorLink Angola susceptibility results obtained in 2020 recommended a shift to PBO nets, after procurement for the mass campaign had already been initiated. However, PMI coordinated with Global Fund, which was able to adjust their procurement to PBO nets for the select municipalities in Benguela and Cuanza-Sul. Moreover, PMI has procured exclusively PBO nets for continuous distribution activities in 2023 in PMI-focus provinces. These nets are expected to arrive in August 2023.

[1] U.S. President's Malaria Initiative Angola Malaria Operational Plan FY 2022. Retrieved from: www.pmi.gov.
[2] Malaria National Strategic Plan 2021-2025
[3] Instituto Nacional de Estatística - INE/Angola, Ministério da Saúde - MINSA/Angola, Ministério do Planeamento e do Desenvolvimento Territorial (MINPLAN) and ICF. 2017. Inquérito de Indicadores Múltiplos e de Saúde em Angola 2015-2016. Luanda, Angola e Rockville, Maryland, EUA: INE, MINSA, MINPLAN and ICF
[4] Koenker H, Arnold F, Ba F, Cisse M, Diouf L, Eckert E, et al. Assessing whether universal coverage with insecticide-treated nets has been achieved: is the right indicator being used? Malar J. 2018;17:355.
[5] VectorWorks 2019

From 2012 to 2016, the PMI Africa Indoor Residual Spraying Program conducted longitudinal entomological surveillance activities in Angola, collecting data on key entomological indicators from three provinces (Cunene, Huambo, and Malanje), in collaboration with the *Direcções Provinciais de Saúde* (DPS). In 2014, PMI established a container insectary in Huambo, the first in Angola since the end of the civil war. PMI also supported the training of technicians on basic malaria entomology techniques as well as on-the-job training and mentorship to continuously improve their skills. PMI also supported training of provincial and municipal health authorities from nine provinces representing three malaria transmission zones (Benguela, Cunene, Huambo, Huila, Luanda, Malanje, Namibe, Uíge, and Zaire) where insecticide resistance was evaluated in 2015-2016. From 2016 to 2019, PMI did not support entomological monitoring activities in Angola.

PMI re-established support to entomological activities through the VectorLink project starting in 2019. Between January-March 2020, VectorLink conducted one month of entomological surveillance in two sites—Huambo and Lunda-Sul provinces—to determine species composition, vector behavior, and vector susceptibility to different insecticides. In Year 2 (November 2020 and April 2021), the project continued to build entomological capacity within the NMCP/National Department of Public Health, *Instituto Nacional de Investigação em Saúde* (INIS), and DPS mosquito brigades in each province and conducted monthly community-based entomological surveillance in Huambo Province and insecticide resistance testing in six provinces (Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire).

In Year 3 (May 2021-April 2022), VectorLink continued monthly community-based entomological surveillance in Huambo Province and piloted this activity in Luanda in July 2021 and Lunda-Norte in December 2021. Insecticide resistance testing was conducted in seven provinces (Cuanza-Norte, Huambo, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire). The team also oversaw the set-up of an additional insectary container at INIS in Luanda, designed to support a local susceptible *An. gambiae* colony. Over the course of Year 3, VectorLink received three batches of eggs of susceptible *An. gambiae* mosquitoes and tried to rear the eggs to adults, but were unsuccessful due to issues with temperature and humidity controls, as well as unintentional effect of fumigation around the facility where the insectary was located.

In Year 4 (May 2022-April 2023), VectorLink conducted six months of community-based entomological surveillance in two provinces, Huambo and Luanda. Insecticide resistance testing was conducted in six provinces (Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire). The team also oversaw the set-up of two rooms at ICCT in Luanda, designed to support two local susceptible colonies: *An. coluzzii* and *An. gambiae* s.s. Kisumu. During the reporting period, VectorLink received one batches of eggs of *An. coluzzii* from Witwatersrand University in South Africa in January 2023 and *An. gambiae* s.s. Kisumu strain from CDC (Atlanta, USA) in February 2023. These attempts were successful and Angola now has two susceptible colonies fully established. As of June 2023, the *An. coluzzii* colony has reached the F11 generation and the *An. gambiae* s.s. colony is on F10 generation at ICCT in Luanda.

The rationale for selecting the above sites for PMI-supported entomological surveillance between 2019-2023 varied by province. Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire are PMI-focus provinces in Angola, while Huambo and Luanda were selected based on existing entomological infrastructure/capacity (including insectary containers) and trained staff based in these two provinces. Luanda was also a logical choice given the laboratory capacity at INIS and presence of ICCT there.

This report presents results from entomological monitoring conducted from May 2022 to February 2023 for community-based vector surveillance and October 2022 to May 2023 for insecticide resistance testing, as well as key project contributions to country-level capacity strengthening efforts.

2. METHODOLOGY

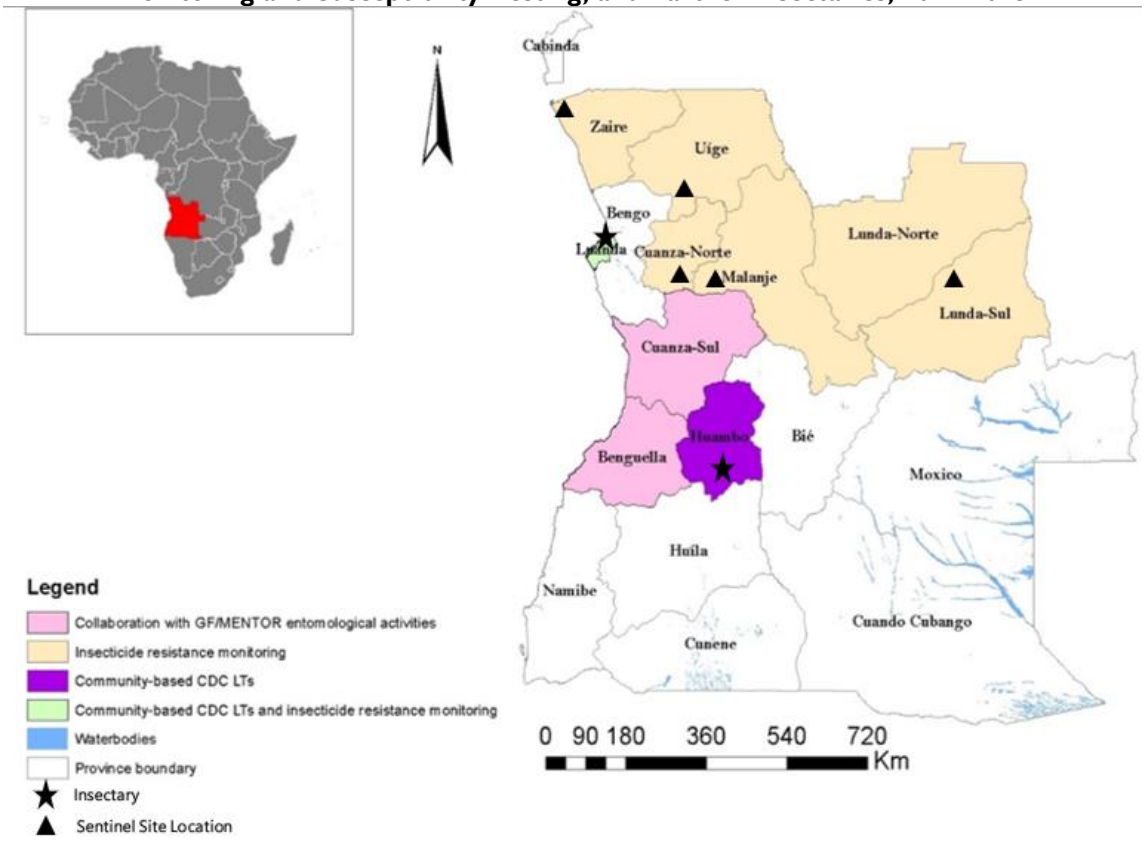
Details of VectorLink standard operating procedures (SOPs) used for WHO susceptibility tests, bottle bioassays, CDC light traps (LTs), and insectary rearing can be found online[6].

2.1 STUDY SITES

From October 2022 to May 2023, insecticide resistance monitoring was conducted in sites across six provinces—five PMI-focus provinces (Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire) and one province (Luanda) chosen based on existing insectary infrastructure and entomology expertise. Insecticide susceptibility data was collected primarily to inform ITN procurement decisions made by the NMCP and partners. VectorLink Angola worked together with NMCP mosquito brigades in each province to collect insecticide susceptibility data during peak mosquito abundance months (October 2022 to May 2023) and conducted training of brigade staff as part of capacity development. Section 3 contains more training details.

Between May 2022 and February 2023, vector bionomics monitoring by community-based surveillance using CDC LTs was conducted for a total of six months in two provinces (Huambo and Luanda). The trained community staff were regularly supported by VectorLink entomology technicians. Figure 1 shows the locations of the sites and Table 1 provides a summary of all activities conducted over the reporting period.

Figure 1: Map Showing Location of Planned VectorLink Angola Sentinel Sites for Entomological Monitoring and Susceptibility Testing, and Partner Insectaries, 2022-2023



[6] https://pmivectorlink.org/ resources/tools-and-innovations/.

The activities were conducted according to the PMI VectorLink Angola Year 4 work plan (Table 1) and the indicators covered are in line with the PMI technical guidance[7].

Table 1: Summary of PMI VectorLink Angola Entomological Activities (2022-2023)

Activity	Purpose	Sites	Timeline	Frequency	Status
Insecticide Susceptibility Testing	To determine vector susceptibility to three pyrethroid insecticides with and without the synergist PBO, and to chlorfenapyr.	Cuanza-Norte, Luanda, Lunda-Norte, Lunda-Sul, Malanje, Zaire, and Uíge.	October 2022-May 2023	About two sites per month (during peak season).	Completed in six out of seven target provinces. Testing in Lunda Norte was not completed due to time constraints and will be conducted by the Health for All project.
Community Ento Surveillance: Species composition, vector density, human biting rate and EIR	To gather more detailed longitudinal information on malaria vector dynamics and behavior.	Huambo (Ngandarinha Village) and Luanda (Mulundu Village).	May, June, July, and November 2022, and January and February 2023	Trapping in a target of 60 houses per site per collection month	Completed in Huambo and Luanda provinces for all six collections monthly. Collections were done in 60 houses per site per collection month.
Molecular assays	To identify mosquito species of the *An. gambiae* s.l. and *An. funestus* s.l. species complex, and determine mechanisms of pyrethroid resistance (*kdr*), and *P. falciparum* sporozoite rates.	Cuanza-Norte, Huambo, Luanda, Lunda-Norte, Lunda-Sul, Malanje, Uíge, and Zaire.	May 2022-April 2023	Throughout the year	Sample selection, dissection and processing occurred for Year 3 of the project.

Note: *kdr*=knockdown resistance, PBO=piperonyl butoxide, PCR=polymerase chain reaction.

2.2 Indoor CDC LT (Community-Based Longitudinal Surveillance Pilot)

In November 2020, VectorLink Angola started a pilot of community-based entomological surveillance in Huambo Province. In July 2021, the team started to conduct similar pilot in Luanda Province. In both locations, the project recruited a community member from Ngandarinha and Mulundu villages, respectively, and after training, provided them with CDC LTs, batteries, solar panels for charging batteries, a magnifying glass, and other entomology supplies needed to preserve and store mosquito samples. The community collectors and local mosquito brigade staff received basic training on mosquito collection and basic morphological identification to genus level. VectorLink technicians collected GPS coordinates of 60 houses, labelled house doors, and subsequently the community collector surveyed three different houses per night located within walking distance of their own house using CDC LTs. The CDC LTs were hung indoors overnight, and collections were conducted for 20 nights per month for a maximum of 60 houses per month (same houses each month). During the current reporting period, the initial setup was maintained, and collections were carried out simultaneously in both Huambo and Luanda provinces between May 2022 and February 2023. Table 2 below shows the sampling frequency and timing of all indoor CDC LT collections.

VectorLink technicians provided close supervision and continuous training, traveling to the field regularly to reinforce the project SOPs and ensure that data forms were fully completed, and mosquito samples were correctly stored and labelled (Figure 2). Community collectors separated mosquito specimens by genus and VectorLink technicians subsequently conducted mosquito species identification using the key of Coetzee 2020[8]. Community collectors are being gradually trained so that they can carry out morphological species identification in the future. When setting CDC LTs, the community collector also gathered basic information through a

[7] PMI Technical Guidance FY 2022

[8] Coetzee, M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). Malar J 19, 70 (2020)

questionnaire administered to the head of the household regarding the number of ITNs in the household, number of people that slept/did not sleep under a net the previous night, and physical inspection of ITN labels to record the brands of ITN present. Once collected and identified to species, mosquito samples were stored at -20°C in the freezer until molecular analyses, to take place at INIS in Luanda. Data was recorded on paper forms by the community collectors before being updated by VectorLink technicians after mosquito identification and sent to the data clerk for data entry in VectorLink Collect.

Table 2: Summary of Collection Methods

Collection method	Time	Frequency	Sample
Indoor CDC LT	6:00 p.m. to 6:00 a.m.	3 houses per day, 20 days a month	60 houses every collection month
Larval collections	9:00 a.m. to 2:00 p.m.	Daily	As many larvae as possible from several locations

Figure 2: Photograph of the Solar Panel Charging System for CDC LTs in Mulundu Village, Huambo Province (A), Community Collector Demonstrating Retrieval of Mosquitoes from a CDC LT as VectorLink Technician looks on (B), *Anopheles* Mosquitoes from CDC LT Stored Individually for Molecular Analysis (C)

2.3 Larval and Adult Collections

From October 2022 to May 2023, VectorLink conducted susceptibility testing in six provinces. Larval collections were conducted daily by the provincial mosquito brigades in close coordination with a Senior VectorLink Entomology Technician in Cuanza-Norte Lunda-Sul, Malanje, Uíge and Zaire (Figure 3). In Luanda, adult collections of *An. funestus* s.l. were conducted daily by VectorLink Entomology Technical

Manager, Senior Entomology Technician and Community Collector through a mix of Brogdon method and CDC LTs outdoors and indoors. A sub-sample of *Anopheles* mosquitoes reared from larvae/adults were identified morphologically (Coetzee, 2020) before testing to try and ensure the target vector species were used. After completion of insecticide testing, all mosquitoes were killed and morphologically identified to corroborate initial species identification.

2.4 Insecticide Susceptibility Testing, Synergist Assays, and CDC Bottle Bioassay Testing

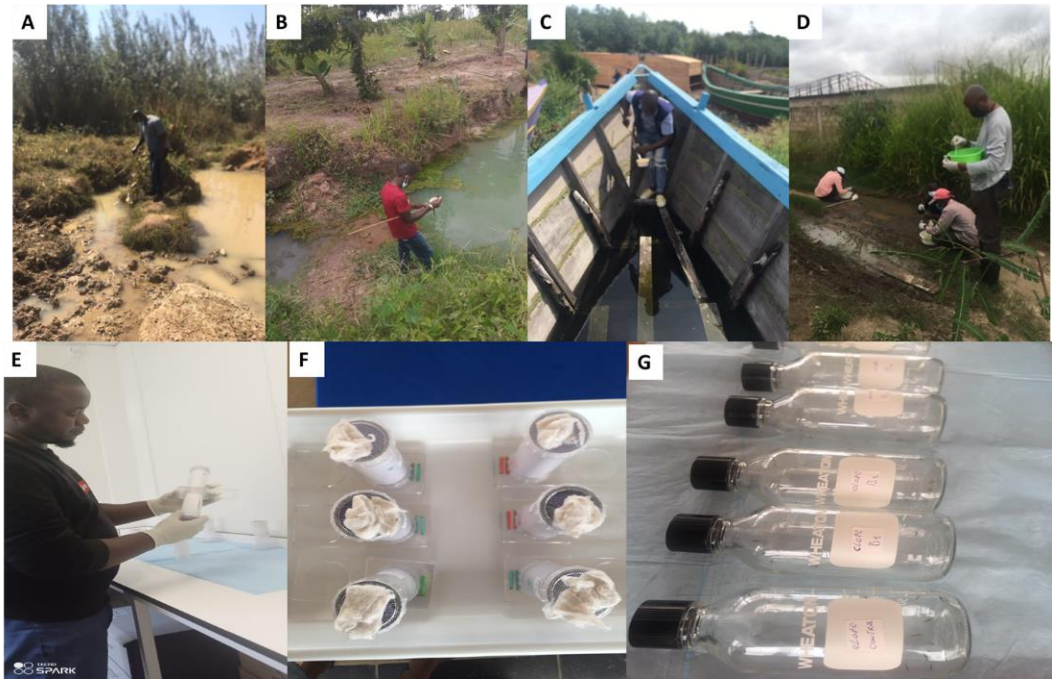
Insecticide susceptibility and synergist bioassay were conducted in the six study sites (Cuanza-Norte, Lunda-Sul, Luanda, Malanje, Uíge and Zaire) by Senior VectorLink Entomology Technicians and Entomology Technical Manager.

Insecticides tested in 2022-2023 (in order of priority):

- Deltamethrin 0.05% with and without pre-exposure to PBO 4%
- Alpha-cypermethrin 0.05% with and without pre-exposure to PBO 4%
- Chlorfenapyr 100 µg/bottle (tested in CDC bottle bioassays)
- Permethrin 0.75% with and without pre-exposure to PBO 4%

Mosquitoes were collected from the field as larvae and reared to adults before conducting susceptibility testing with adult *An. gambiae* s.l. (*An. funestus* s.l. for Luanda) following VectorLink SOPs that are based on WHO procedures. Figure 3 shows larval collection sites in some provinces and performance of susceptibility assays.

Figure 3: Photographs of *An. funestus* s.l. and *An. gambiae* s.l. Larval Collections (A, B, C, D), WHO Susceptibility Tests (E), Holding Tested Mosquitoes for 24 Hours at Controlled Temperature and Humidity (F), CDC Bottle Assays (G)



WHO susceptibility tests were conducted for deltamethrin, alpha-cypermethrin, and permethrin with and without pre-exposure to 4% PBO papers for 60 minutes followed by transfer to insecticide-treated papers for 60 minutes. All insecticide-treated filter papers were purchased at the diagnostic concentration from the WHO

collaborating center, Universiti Sains Malaysia. Exposure tests were always accompanied by negative control tests in which mosquitoes were exposed to filter papers impregnated with silicone oil, with additional control tests of pre-exposure to 4% PBO papers followed by silicone oil papers for synergist tests. Mortality at 24 hours post-exposure was the primary outcome measure. Four replicates of 25 *An. gambiae* s.l./*An. funestus* s.l. were exposed to each insecticide.

CDC bottle bioassays were completed in six sites (Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire) to determine the susceptibility status of *An. gambiae* s.l./*An. funestus* s.l. to chlorfenapyr using a diagnostic dose of 100 µg/bottle. Four replicates of 20-25 female *An. gambiae* s.l./*An. funestus* s.l. were exposed for 60 minutes to chlorfenapyr 100 µg/bottle. After exposure period, the mosquitoes were removed from the bottle, transferred to paper cups, and supplied with a sugar solution. Mortality was recorded every 24 hours for 72 hours (about 3 days) total. After completion of mortality recording, mosquitoes were stored individually in Eppendorf tubes in a freezer for future molecular analysis. Each tube was labelled with key information such as date of test, site, mosquito species, insecticide tested and whether the mosquito was susceptible or resistant. Eppendorf tubes were placed in Ziploc bags and labelled according to the insecticide tested and site.

In Luanda Province, where the major vector species is *An. funestus* s.l., Prokopack aspiration and CDC LT indoors and outdoors were set-up as per CDI Vectorlink SOP from the end of March 2023 to early May 2023 to collect adult mosquitoes for susceptibility tests. Prokopack aspirations were conducted early in the morning by sweeping the aspirator close to the wall, ceiling, and furniture for approximately 15 minutes per house (depending on size of the house) to collect resting mosquitoes. Blood-fed *An. funestus* s.l. adults were then taken back to the Luanda insectary for maintenance until numbers were enough to start susceptibility testing. Larvae were fed with a combination of Tetramin tropical fish food and pond algae (based on feedback from University of the Witwatersrand). Adults were fed with a 10% sugar solution twice a day.

Figure 4: Photographs of VectorLink Entomology Technicians Selecting Wild Adult *An. funestus* s.l. Collected using Prokopack Aspirators and CDC LTs in Mulundu Village, Luanda Province from March-May 2023

2.5 LABORATORY ANALYSIS

Molecular analysis of entomological samples collected under VectorLink began in June 2022, following optimization and training on molecular analysis protocols for malaria vectors provided through a STTA from a Senior CDC Entomologist who visited Angola in March 2022 and a virtual training held in September 2022. A pre-determined number of mosquito samples from monthly collections and from susceptibility tests will be tested by PCR to determine species composition (see Table 3). The proportion of *An. gambiae* s.l. and *An. funestus* s.l. tested will vary by site depending on species composition.

Dissection and processing of samples collected in Year 3 is in progress and is expected to be completed in September 2023. Samples from Year 4 of the project will be processed from May to October 2023. An amendment to the Year 3 Annual Entomology Report sharing results from the laboratory analysis is expected in late 2023. An amendment to this report will be drafted once those samples are processed and analyzed (late 2023/early 2024).

Table 3: Minimum Number of Samples for Laboratory Analysis at INIS, Luanda

Sentinel Site	WHO Pyrethroid Susceptibility Test Species ID	WHO Pyrethroid Susceptibility *kdr* Frequency (L1014F/L)	CDC LT Species ID	CDC LT Sporozoite ELISA	TOTAL
Year 3 Samples (planned) – collected between May 2021-April 2022					
Huambo	100	50	160 (20 per month)	1,600 (200 per month)	**1,910**
Luanda	100*	50*	160 (20 per month)	1,600 (200 per month)	**1,910**
Lunda Norte	100	50	160 (20 per month)	1,600 (200 per month)	**1,910**
Cuanza Norte	100	50	n/a	n/a	**150**
Lunda Sul	100	50	n/a	n/a	**150**
Malanje	100	50	n/a	n/a	**150**
Uige	100	50	n/a	n/a	**150**
Zaire	100	50	n/a	n/a	**150**
Subtotal Year 3	**800**	**400**	**480**	**4,800**	**6,480**
Year 4 Samples (planned) – collected between May 2022-May 2023					
Huambo	n/a	n/a	120 (20 per collection effort, 6 collection efforts)	1,200 (200 per collection effort, 6 collection efforts)	**1,320**
Luanda	100	50	120 (20 per collection effort, 6 collection efforts)	1,200 (200 per collection effort, 6 collection efforts)	**1,470**
Lunda Norte	100	50	n/a	n/a	**150**
Cuanza Norte	100	50	n/a	n/a	**150**
Lunda Sul	100	50	n/a	n/a	**150**
Malanje	100	50	n/a	n/a	**150**
Uige	100	50	n/a	n/a	**150**
Zaire	100	50	n/a	n/a	**150**
Subtotal Year 4	**700**	**350**	**240**	**2,400**	**3,690**

*Samples collected in Luanda for Year 3 were identified as *An. azevedoi* and therefore will not undergo lab analysis.

2.6 DATA MANAGEMENT AND ANALYSIS

The DHIS2-based VectorLink Collect instance for entomological data management has been used in Angola since 2020. VectorLink Monitoring and Evaluation Specialists trained and supported VectorLink Angola entomologists, technicians, and data clerks remotely on updated data workflows—including field paper collections, technical reviews, data entry, data cleaning, and analytics—to support the generation and use of high-quality entomological data. All entomological data collected in Angola in this reporting period was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. In 2022/2023, the NMCP, INIS, and PMI will have ongoing access to these results dashboards to support timely decision-making.

The following formula was used to calculate entomological indicators:

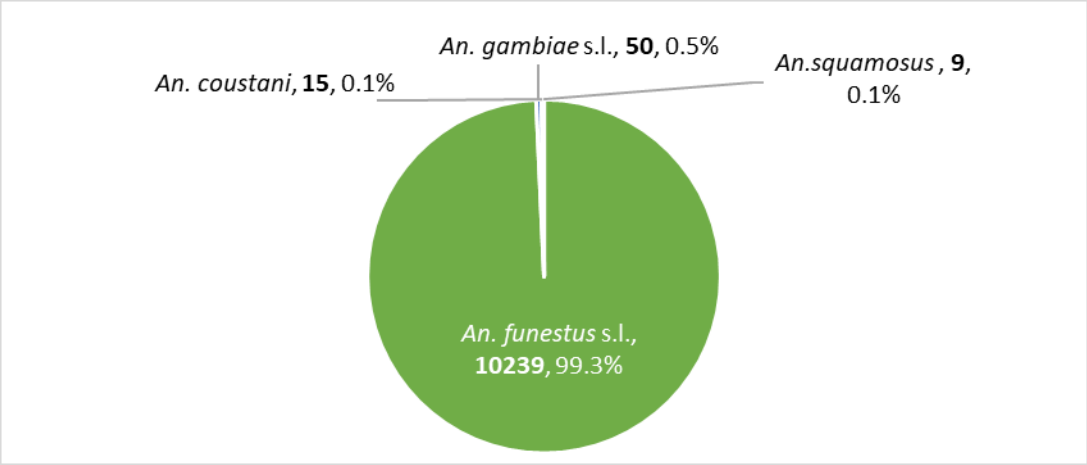
- Vector density (number/trap/night) = total number of *Anopheles* species collected by CDC LT during a specific period / total number of trap-nights.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION IN HUAMBO AND LUANDA

From May 2022 to April 2023, a total of 10,313 *Anopheles* mosquitoes were collected using indoor CDC LTs in two sentinel sites, consisting of 99.3% *An. funestus* s.l., 0.5% *An. gambiae* s.l., 0.1% *An. coustani*, and 0.1% *An. squamosus* (Figure 5).

Figure 5: Total Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Huambo and Luanda Sentinel Sites (May 2022–April 2023)



Anopheles funestus s.l. were more abundant than *An. gambiae* s.l. in Huambo (91.9% versus 4.7%) and Luanda (99.8% versus 0.2%) (Figures 6 and 7). Other species identified in Huambo were *An. coustani* and *An. squamosus*.

Figure 6: Total Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Ngandarinha, Huambo Province (May 2022-April 2023)

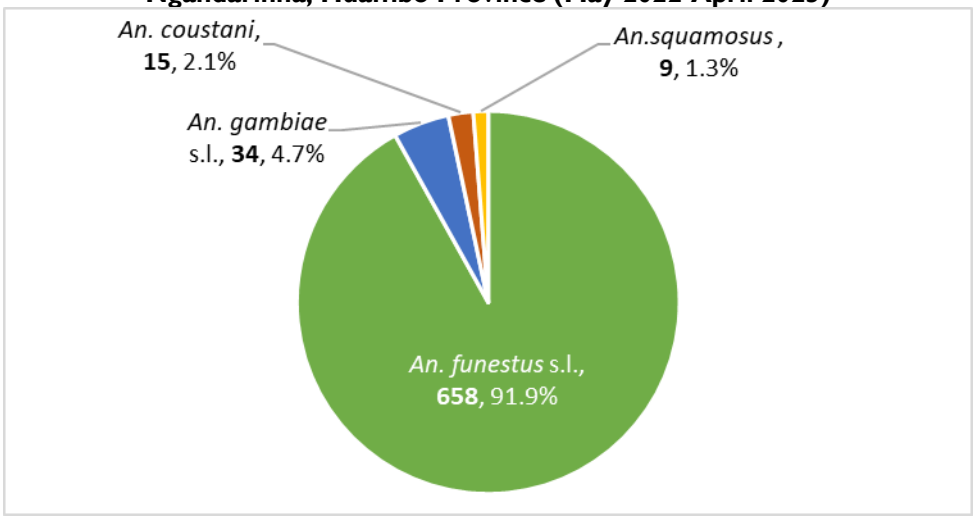
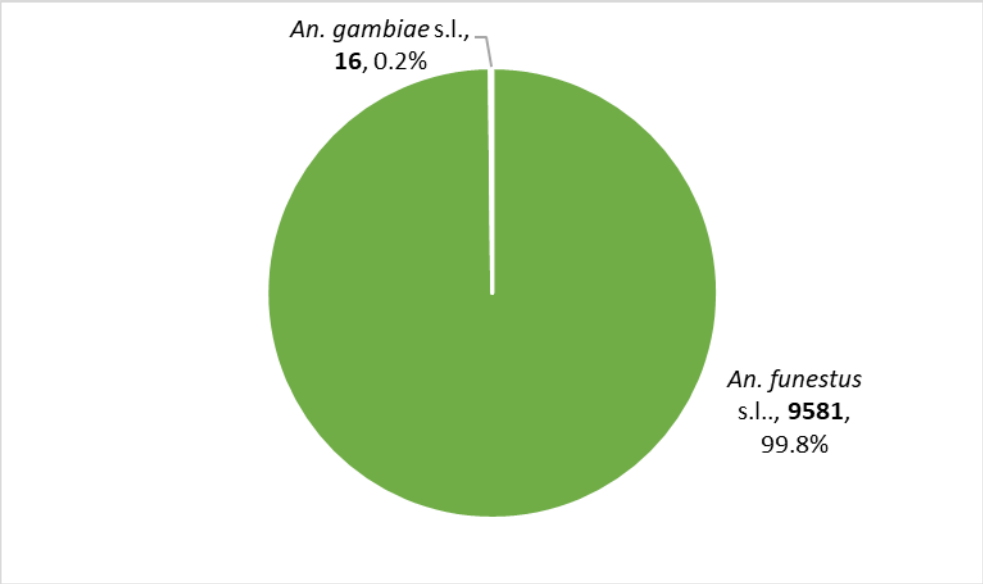


Figure 7: Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Mulundu Village, Luanda Province (May 2022-April 2023)[9]



3.2 Malaria Vector Density (by Indoor CDC LT)

Collections were completed as planned in Huambo and Luanda, with 60 trap nights per collection month for six months (May, June, July, and November 2022, January and February 2023) at each site, for a total of 360 trap nights total over the reporting period. The total numbers of vectors collected during each monthly collection effort by site are provided in Annex A.

Figure 8 shows the density (mean number of primary malaria vector species *An. funestus* s.l. and *An. gambiae* s.l.) collected per trap per night in Ngandarinha Village, Huambo Province and in Mulundu Village, Luanda Province between May 2022 to February 2023 with an overlay of monthly rainfall covering the reporting period.

Overall, *An. funestus* s.l. was the malaria vector species with the highest density in both provinces. The total number of *An. funestus* s.l. collected in Luanda was about 10 times more than Huambo and the overall density (number/trap/night) was about 8 times higher in Luanda than Huambo.

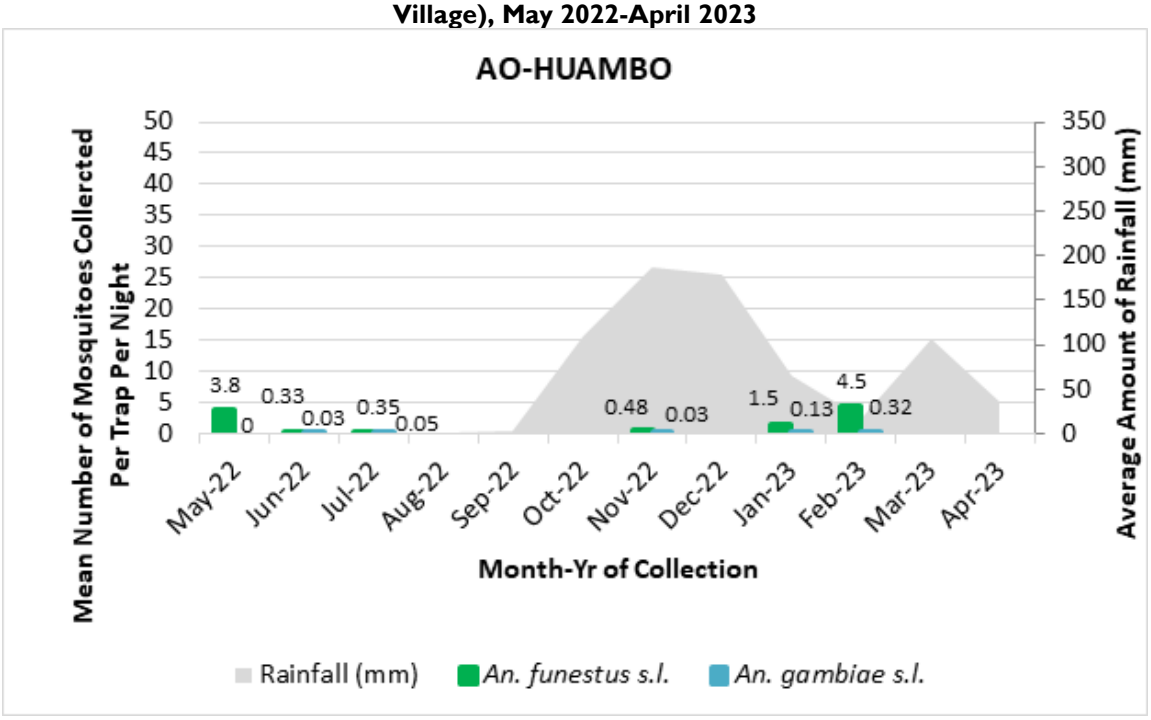
In Huambo, monthly *An. funestus* s.l. densities were between 0-1 vector/trap/night from June to July 2022 with a rise in density between November 2022 and February 2023, reaching a peak of 4.5 vectors/trap/night in February 2023. This may be linked to high rainfall in the preceding months (October to December 2022).

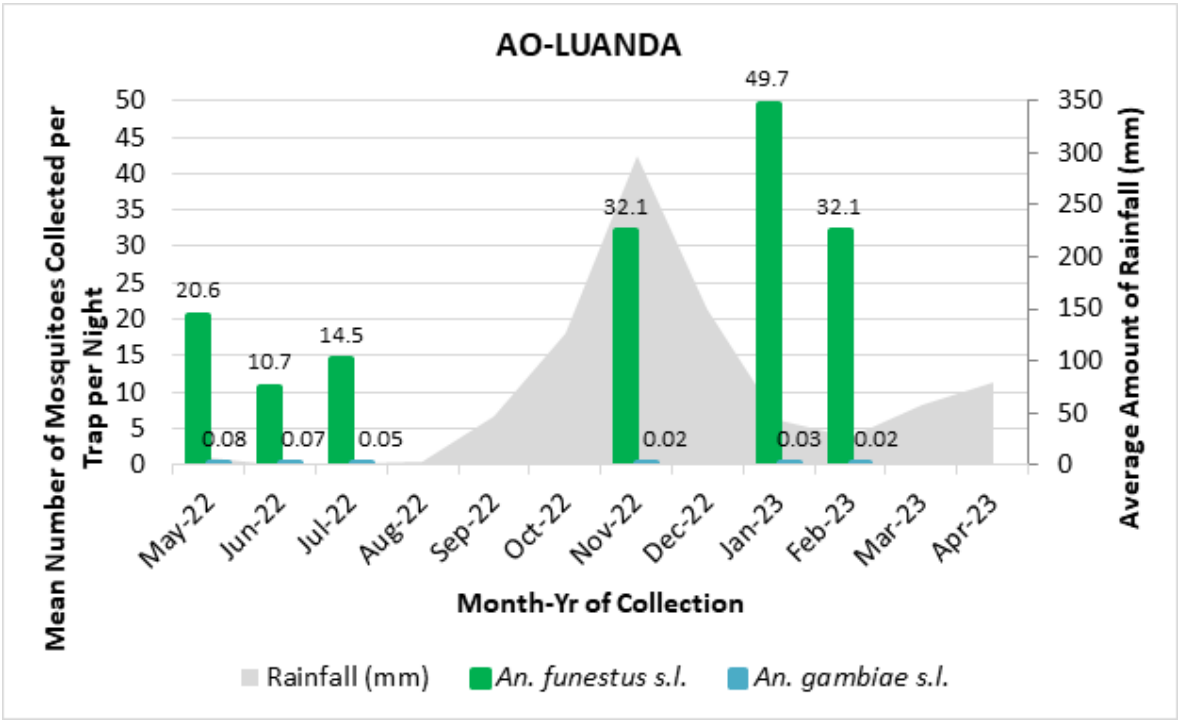
In Luanda, monthly densities of *An. funestus* s.l. were above 10 vectors/trap/night throughout the collection period with a peak density of 49.7 vectors/trap/night in January 2023. The high density may also be linked to the high rainfall in the preceding months of October to December 2022. The lowest monthly density (10.7 vectors/trap/night) was in June 2022.

Anopheles gambiae s.l. monthly densities were generally very low in both provinces (less than 1 vector/trap/night), with the highest density of 0.32 vectors occurring in February 2023 in Huambo Province.

[9] Samples collected in Luanda for Year 3 were identified as *An. azevedoi*. It is possible that this is also the case for Year 4, and will become clear when laboratory analysis of Year 4 samples is done.

Figure 8: Mean Number of *An. funestus* s.l. and *An. gambiae* s.l. Collected Per Night by Indoor CDC LT and Monthly Rainfall in Huambo Province (Ngandarinha Village) and Luanda Province (Mulundu Village), May 2022-April 2023





Note: 60 CDC LT collections were done in each site per collection month.

Source of Rainfall Data: https://www.worldweatheronline.com/huambo-weather-averages/huambo/ao.aspx

3.3 Abdominal Stage of Collected Mosquitoes from May 2022-April 2023

Most vectors collected by indoor CDC LTs from May 2022 to April 2023 in Huambo and Luanda were unfed: 90.1% *An. funestus* s.l. (n=10,239) and 96% *An. gambiae* s.l. (n=50). A small percentage were fed (8.9% *An. funestus* s.l. and 4% *An. gambiae* s.l.) and very few were either gravid or half gravid (1% *An. funestus* s.l. and no *An. gambiae* s.l.) (Figures 9 and 10).

Figure 9: Abdominal Stage of *An. funestus* s.l. Mosquitoes Collected by Indoor CDC LT in Huambo and Luanda Province (May 2022-April 2023)

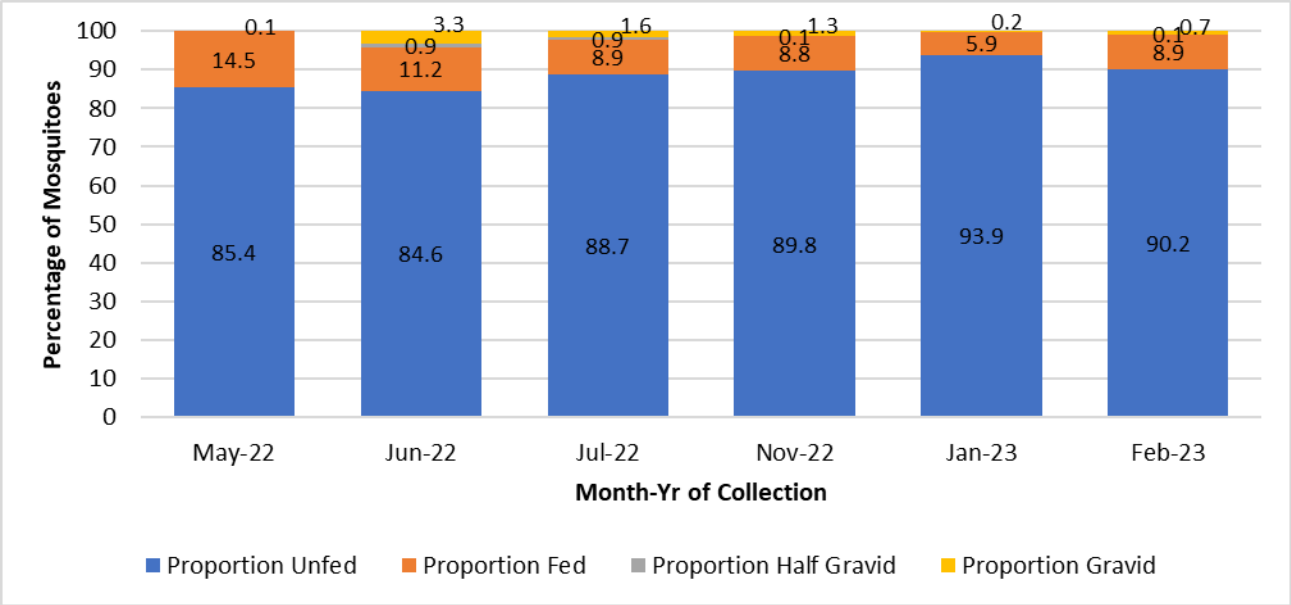
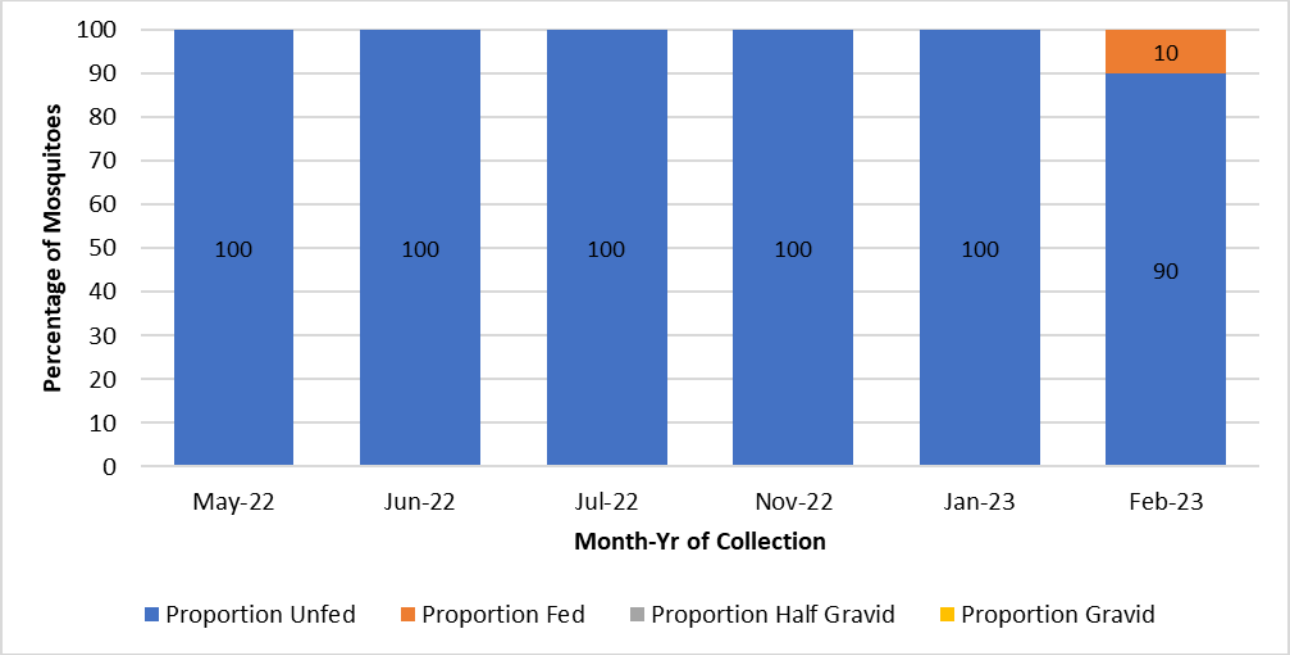


Figure 10: Abdominal Stage of *An. gambiae* s.l. Mosquitoes Collected by Indoor CDC LT in Huambo and Luanda Provinces (May 2022-April 2023)

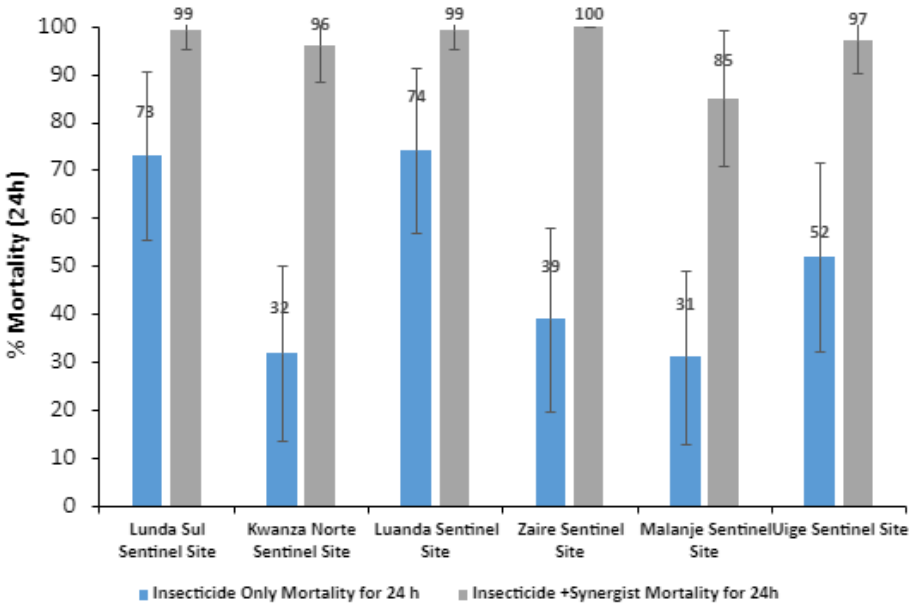


3.4 Insecticide Susceptibility, PBO Synergist, and CDC Bottle Assay Results

Insecticide susceptibility testing was conducted with *An. gambiae* s.l. that were collected as larvae in five sites Cuanza-Norte, Lunda-Sul, Malanje, Uíge and Zaire. In Luanda, insecticide susceptibility testing was conducted with *An. funestus* s.l. that were collected as adult mosquitoes of unknown age through a mix of Prokopack aspiration and CDC LT collections, both indoors and outdoors. VectorLink Angola Entomology Technicians conducted insecticide susceptibility testing with at least two pyrethroid insecticides with and without PBO synergist pre-exposure in each site. Insecticide resistance status was based on the standard WHO criteria: <90% mortality (confirmed resistance), 90-97% mortality (probable resistance), and ≥98% mortality (susceptible).

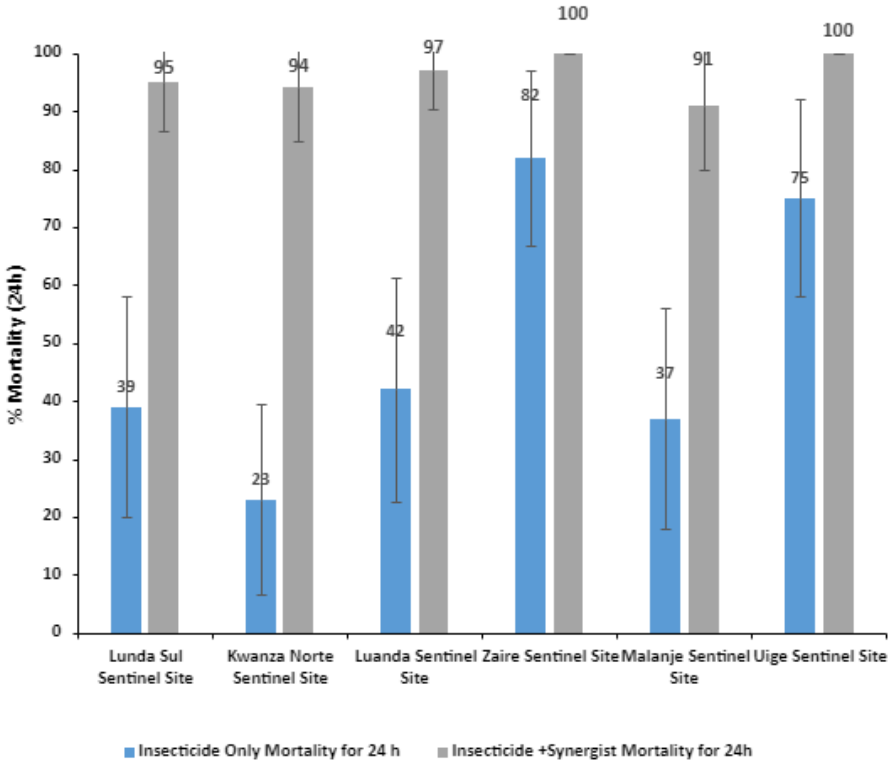
Deltamethrin (0.05%) was tested in all six sites and there was confirmed resistance in all sites (Cuanza-Norte, Lunda-Sul, Luanda, Malanje, Uíge, and Zaire) with mortality ranging from 31% in Malanje to 74% in Luanda (Figure 11). Pre-exposure to PBO followed by deltamethrin exposure resulted in susceptibility being fully restored in Lunda-Sul, Luanda, and Zaire and in large increases in absolute mortality in the other provinces, though susceptibility wasn't fully restored. Alpha-cypermethrin was tested in all six sites and produced a similar trend, with confirmed resistance in all sites (Cuanza-Norte, Lunda-Sul, Luanda, Malanje, Uíge and Zaire) mortality ranging from 23% in Cuanza-Norte to 82% in Zaire. PBO pre-exposure resulted in full restoration of susceptibility in Uíge and Zaire, and absolute mortality increased with partial restoration of susceptibility, in the other provinces (Figure 12). Permethrin susceptibility tests were performed in five provinces (Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire). Permethrin resistance was observed in all provinces tested with mortality ranging from 9% (in Lunda-Sul) to 28% (in Uige). PBO pre-exposure resulted in small increases in absolute mortality, with no restoration of susceptibility in any of the provinces tested (Figure 13).

Figure 11: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. (Luanda Province Only) Using WHO Tube Tests with Deltamethrin (0.05%) and PBO (4%) + Deltamethrin (0.05%)



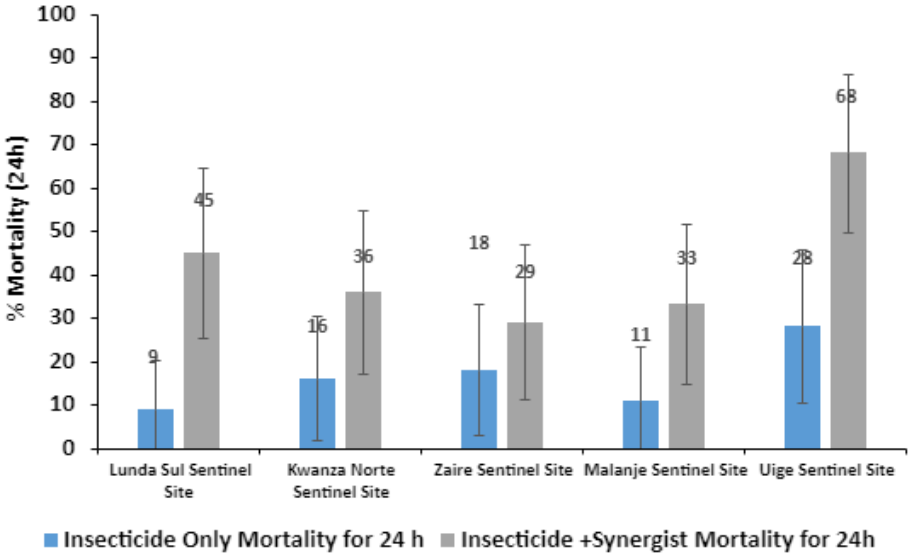
Note: Approximately 100 mosquitoes were tested per insecticide. The error bars represent 95% confidence intervals.

Figure 12: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. (Luanda Province Only) Using WHO Tube Tests with Alpha-cypermethrin (0.05%) and PBO (4%) + Alpha-cypermethrin (0.05%)



Note: Approximately 100 mosquitoes were tested per insecticide. The error bars represent 95% confidence intervals.

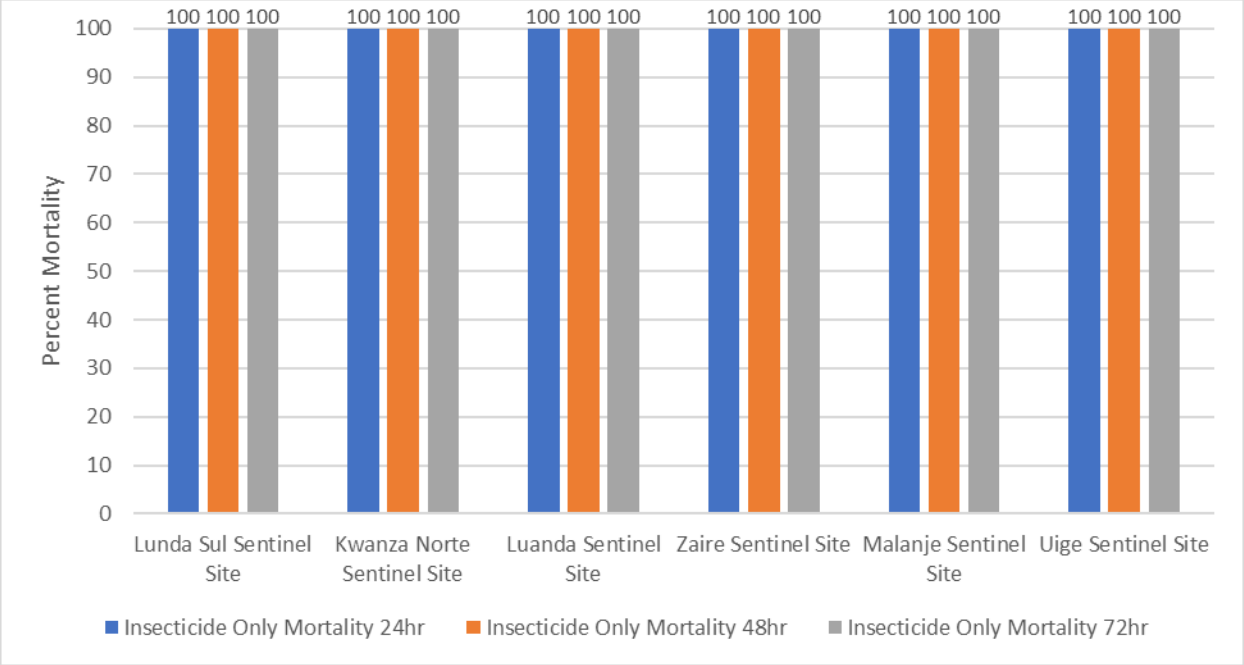
Figure 13: Percentage Mortality of *An. gambiae* s.l. in WHO Tube Tests with Permethrin (0.75%) and PBO (4%) + Permethrin (0.75%)



Note: Approximately 50-100 mosquitoes were tested per insecticide. The error bars represent 95% confidence intervals.

CDC bottle bioassays using the PMI VectorLink recommended dose of 100 µg/bottle for chlorfenapyr produced 100% mortality in the six sites tested (Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire) within 24 hours of exposure (Figure 14), indicating full susceptibility to chlorfenapyr.

Figure 14: Percentage Mortality of *An. gambiae* s.l. and *An. funestus* s.l. (Luanda Province Only) Using Bottle Bioassays with Chlorfenapyr (100 µg/bottle) 24, 48, and 72 Hours after Exposure



Note: Approximately 100 mosquitoes were tested per insecticide.

4. Capacity Strengthening and Sustainability

The VectorLink Angola Entomology Technical Manager, Entomology Coordinators (Field and Lab) and VectorLink Entomology Technicians continued to work closely with the NMCP, INSP/INIS, DPS (including mosquito brigades), ICCT, and other in-country partners to strengthen entomological capacity. In Year 4, the project focused on building community-based entomological surveillance capacity, and basic provincial-level capacity related to insecticide susceptibility testing and larval detection and monitoring.

4.1 Provincial-Level Entomological Trainings

The VectorLink Senior Entomology Technicians conducted on-site, hands-on training for three mosquito brigade staff in each of the six provinces prior to insecticide susceptibility testing (October 2022-May 2023). The training focused on methods for collection of mosquito larvae and adults, including collection of geolocation data for each site using GPS, basic morphological identification to genus level, insecticide susceptibility tests, and proper sample handling and storage. This helped to ensure the quality of work in the field through adherence to standard protocols and strengthened provincial capacity for long-term sustainability. Mosquito brigade staff did not conduct susceptibility bioassays, but they observed susceptibility bioassays being conducted by VectorLink Senior Entomology Technicians. At the end of the training, all mosquito brigade attendees were able to identify habitats, collect *Anopheles* larvae, recognize key characteristics of *An. funestus* s.l. and *An. gambiae* s.l. adults, and were familiar with project SOPs for susceptibility testing. Since 2019, the project has trained over 30 mosquito brigade members, 28 of which were still engaged with project activities this year.

In addition, NMCP staff participated and supervised in staff training and field work in all provinces at different times during the life of the project.

4.2 VectorLink Regional Trainings

VectorLink Angola sponsored participants at two project trainings during Year 4. The first took place in June 2022 and focused on strengthening the capacity of VectorLink M&E staff to analyze, visualize, and interpret entomology data using VectorLink Collect, the project's DHIS2-based database. The approach was designed so that VectorLink staff could cascade these skills and tools to NMCP staff, to aid them in vector control decision-making. In August 2022, VectorLink Angola supported two participants, one from the NMCP and one VectorLink Angola staff, to attend a six-day regional entomology training in Dakar, Senegal. The emphasis for this training was to build capacity of NMCPs in entomology best practices. Both trainings furthered the skills of Angolan entomology experts.

4.3 Short-Term Technical Assistance

Two STTA trips occurred during the reporting period to provide support to the in-country VectorLink team. Each was approximately two weeks in length. In July 2022, the Vectorlink Zambia Entomology Technical Manager Mohamed Bayoh provided support to the team on the laboratory workflow at INIS and ensured molecular sample processing protocols were followed. In February 2023, Dr. Bayoh returned to participate in susceptibility testing in Malanje and observed the maintenance procedures at ICCT of the two susceptible colonies *An. coluzzii* obtained from Witwatersrand University South Africa and *An. gambiae* s.s. Kisumu obtained from CDC (Atlanta, USA).

4.4 VECTOR CONTROL WORKING GROUP MEETINGS

In collaboration with NMCP and other vector control partners, VectorLink Angola supported four vector control working group meetings (in October and December 2022 and March and April 2023) to facilitate review of country-level entomological data and inform vector control decision-making. Meetings were held through a combination of in-person and remote participation by stakeholders including the NMCP, ICCT, INIS, MENTOR, the Clinton Health Access Initiative, and USAID/PMI, and covered several key topics such as:

- Draft, revision, and discussion of Midterm Review of National Malaria Strategic Plan 2021-2025
- Draft, revision, and discussion on definition of entomological indicators for integration into national DHIS2 Entomology Modules
- Updates and presentations from different stakeholders on the progress of entomological activities in country and challenges and next steps.

4.5 COLLABORATION WITH GLOBAL FUND/MENTOR

In 2022-2023, Global Fund/MENTOR started entomological surveillance and insecticide resistance activities in Benguela and Cuanza-Sul provinces, in addition to entomology activities associated with IRS activities in Cuando-Cubango and Cunene. VectorLink and Global Fund/MENTOR met regularly to discuss progress of activities, results, challenges faced in the field as well as possible solutions and next steps. Continued harmonization of entomology SOPs (field and laboratory methods and procedures) and data collection continued throughout the reporting period.

4.6 INSECTARIES

After experiencing challenges with the establishment of a susceptible colony throughout 2022, VectorLink investigated several parameters such as temperature, humidity, and water on wild mosquito species to determine the optimal conditions to support the emergence of eggs and rearing of larvae to pupae stage. The team was able to start and establish the first two susceptible colonies in the country—*An. coluzzii* from the Vector Reference Lab at the University of Witwatersrand in South Africa (eggs received in late January 2023) and *An. gambiae* s.s. (Kisumu strain) from CDC based in Atlanta, USA (eggs received in February 2023). Both colonies are housed and maintained at the ICCT insectary in Luanda. As of June 2023, the *An. coluzzii* colony has reached the F11 generation and the *An. gambiae* s.s. colony is on F10 generation.

Figure 15: Photograph of Vectorlink Angola Entomology Team Members Tending to *Anopheles* Larvae of Susceptible Colonies at ICCT in Luanda (March 2023)

5. Discussion

Anopheles funestus s.l. was the most abundant vector collected from CDC LTs inside houses in both Huambo and Luanda provinces combined (99.3%) and constituted 91.9% of vectors collected in Huambo and 99.8% of those collected in Luanda over the six months of surveillance conducted. Very few *An. gambiae* s.l. were collected in the two provinces. There was a relatively low biting rate (<1 host seeking vector/trap/night) from *An. funestus* s.l. vectors in Huambo during the non-peak period (between June 2022 to July 2022), with peaks occurring between January to February 2023. Overall, 89% of *An. funestus* s.l. and 98% *An. gambiae* s.l. were unfed. Based on the monthly trends, the proportion of unfed *An. funestus* s.l. fell below 90% in the months of May, June, July, and November 2022 and for *An. gambiae* s.l. it was at least 90% in all months that collections were done. The origin of blood meal among the few fed mosquitoes will be identified through PCR later in 2023, with the support of INIS, the key in-country partner conducting laboratory analyses of samples.

Insecticide susceptibility tests showed that pyrethroid resistance is widespread in Angola. There was confirmed resistance to all pyrethroid insecticides (deltamethrin, alpha-cypermethrin, and permethrin) in all six provinces tested for insecticide susceptibility during the reporting period. Synergist bioassays with PBO pre-exposure substantially increased mortality rates among deltamethrin and alpha-cypermethrin, but not permethrin resistant vectors in all sites tested. Susceptibility was either fully or partially restored in most instances. The degree of absolute mortality increase, however, varied among sites and insecticide type. The highest increase was observed in Cuanza-Norte for PBO+alpha-cypermethrin (from mortality of 23% to 94%). Susceptibility increased for PBO+deltamethrin exposures from 31% to 100%, from 23% to 95% for PBO+alpha-cypermethrin and from 9% to 45% for PBO+permethrin. In the future and if feasible, it would be useful to determine the pyrethroid resistance intensity as WHO states that when resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely[10]. There was full susceptibility to chlorfenapyr in all six provinces tested: Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire.

The wide distribution of pyrethroid resistance feeds into the rationale for the NMCP, PMI, and other partners to procure non-pyrethroid ITNs for future net distribution campaigns. One alternative ITN option to mitigate pyrethroid resistance is deployment of PBO synergist nets with deltamethrin, alpha-cypermethrin, or permethrin. Although susceptibility was not fully restored in several sites, the absolute increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, particularly with deltamethrin and alpha-cypermethrin, than standard pyrethroid-only nets. Dual active ingredient (AI) nets are also an option. Interceptor G2 should be considered in Angola, as susceptibility to chlorfenapyr was recorded in all six sites tested with 100% mortality. The increased cost for procuring new types of nets is a potential barrier to this approach; however, the country has started to transition to these new types of nets with support of Global Fund that distributed PBO nets to some municipalities in Benguela and Cuanza-Sul as part of the ongoing mass campaign. PMI also procured PBO nets to cover 13 municipalities that did not receive nets during the main phase of ITN mass campaign, as discussed above. It will be important to monitor the durability of PBO nets in Angola to help inform the NMCP and stakeholders regarding choice of ITNs for future mass campaigns.

After experiencing challenges with the establishment of a susceptible colony, VectorLink investigated several parameters such as temperature, humidity, and water on wild mosquitoes species to determine the optimal conditions to support the emergence of eggs and rearing of larvae to pupae stage and was able to start and establish the first two susceptible colonies in the country—*An. coluzzii* from the Vector Reference Lab at the

[10] Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO.

University of Witwatersrand in South Africa and *An. gambiae* s.s. (Kisumu strain) from CDC—at the ICCT Insectary in Luanda.

Challenges encountered during this work plan year included limited numbers of larvae in all provinces, which prevented some sites from testing all three pyrethroids. The limited larvae, coupled with unpredictable rainfall, did not allow the team to conduct any pyrethroid resistance intensity assays.

Local capacity was strengthened throughout the reporting period in several ways. These included collaboration at least three mosquito brigade staff in each of the seven provinces (the majority of which have worked with the project since its inception or for multiple years) and regular participation in field activities by NMCP staff. In-country entomological capacity was strengthening through in-person, hands-on training provided to VectorLink Angola, INIS, and ICCT staff during two STTAs by the VectorLink Zambia Entomology Technical Manager and one by a Senior CDC Entomologist focused on establishing molecular analysis capacity at INIS. One member of the VectorLink Angola team attended a regional training on entomology data visualization and interpretation using the DHIS2-based VectorLink Collect database. Participation in this training has been useful as VectorLink supports the NMCP with the ongoing process of importation and incorporation of VectorLink entomology modules into Angola's national DHIS2 system, with the overall goal of a harmonized database for all entomological data collected in Angola which can be harnessed by the NMCP for strategic and programmatic vector control decision making.

ANNEX A: NUMBER OF VECTORS COLLECTED USING CDC LTS IN HUAMBO AND LUANDA PROVINCES (MAY 2022-FEBRUARY 2023)

Site	Month	*An. funestus* s.l.	*An. gambiae* s.l.	*An. coustani*	*An. squamosus*	Total
Huambo	May 2022	230	0	2	2	**234**
	June 2022	20	2	0	1	**23**
	July 2022	21	3	2	0	**26**
	November 2022	29	2	1	2	**34**
	January 2023	91	8	2	2	**103**
	February 2023	267	19	8	2	**296**
Luanda	May 2022	1,237	5	0	0	**1,242**
	June 2022	642	4	0	0	**646**
	July 2022	871	3	0	0	**874**
	November 2022	1,924	1	0	0	**1,925**
	January 2023	2,980	2	0	0	**2,982**
	February 2023	1,927	1	0	0	**1,928**
Total		**10,239**	**50**	**15**	**9**	**10,313**