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THE PMI VECTORLINK ANGOLA PROJECT

ANNUAL ENTOMOLOGY REPORT

MAY 2020–APRIL 2021

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Abt Associates | 6130 Executive Boulevard | Rockville, MD 20852
T. 301.347.5000

abtassociates.com

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Acronyms

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
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
PMI	(U.S.) President's Malaria Initiative
SOP	Standard operating procedures
WHO	World Health Organization

EXECUTIVE SUMMARY

In collaboration with the National Malaria Control Program (NMCP), the President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in Angola between May 2020 and April 2021 in seven provinces, including five PMI focus provinces (Cuanza Norte, Lunda Sul, Malanje, Uige, and Zaire) and two additional provinces (Huambo and Luanda) chosen based on existing insectary infrastructure and entomology capacity. Insecticide susceptibility tests were conducted in six provinces to inform NMCP decisions of insecticides for future insecticide-treated net (ITN) distribution campaigns. World Health Organization (WHO) susceptibility bioassays using *Anopheles gambiae* s.l. and *An. funestus* s.l. were conducted with deltamethrin, permethrin, and alpha-cypermethrin, with and without pre-exposure to the synergist piperonyl butoxide (PBO). The team also conducted bottle bioassays to determine the susceptibility status to chlorfenapyr at the various sites. Starting in November 2020, the project supported monthly community-based longitudinal monitoring of malaria vector densities and species composition in Huambo Province. U.S. Centers for Disease Control and Prevention (CDC) light trap (LT) collections were conducted monthly with regular support from VectorLink entomology technicians.

The project made considerable progress with infrastructure and personnel capacity development. Angola has never had a susceptible strain of *Anopheles,* therefore VectorLink/PMI processed and obtained authorizations from the MOH to import live eggs and arrangements were made to import a susceptible strain of *An. coluzzii* SUA strain from The University of the Witwatersrand in South Africa. Moreover, the existing insectary in Huambo was expanded; three converted shipping containers were installed to allow dedicated space for mosquito rearing, mosquito identification and processing, insecticide susceptibility tests, data entry, and equipment storage. Equipment and materials needed for molecular analysis to be conducted at the *Instituto Nacional de Investigação em Saúde* (INIS) in Luanda were procured, including a laboratory-specific freezer for mosquito sample storage and reagents required for sporozoite Enzyme-Linked Immunosorbent Assays (ELISAs) and polymerase chain reaction (PCR) for mosquito species identification. VectorLink also continued to build entomological skills within Angola, organizing a national training in Huambo which included VectorLink technicians, Elimination 8 (E8)/Mentor Initiative, and other national stakeholders as NMCP. The training focused on insecticide susceptibility tests, including WHO tube tests for susceptibility, and community-based surveillance using indoor CDC LT. VectorLink Senior Entomology Technicians led on-site, hands-on training for three mosquito brigade staff in each of seven provinces prior to insecticide susceptibility data collection during the rainy season. The District Health Information Software 2 (DHIS2)-based VectorLink Collect database was used in Angola for the first time in 2020 and, after a remote training, all entomological data was entered into VectorLink Collect and exported for data analysis to prepare the annual report.

Anopheles gambiae s.l. was the predominant species collected as larvae and reared into adult mosquito for susceptibility testing in Cuanza Norte, Lunda Sul, Malanje, Uíge, and Zaire, while *An. funestus* s.l. were reared from larvae in Luanda. Insecticide susceptibility tests showed that *An. gambiae* s.l. were resistant to all pyrethroid insecticides that were tested, while resistance of *An. funestus* s.l. to deltamethrin and alpha-cypermethrin was recorded in Luanda. Deltamethrin resistance was recorded in all six provinces tested, with mortality ranging from 21% in Malanje to 72% in Cuanza Norte. Pre-exposure to piperonyl butoxide (PBO) followed by deltamethrin produced a large increase in mortality in all sites, with susceptibility restored in Cuanza Norte and Luanda. Alpha-cypermethrin was tested in four sites and yielded a similar trend, with resistance present in all sites, with PBO pre-exposure resulting in a large increase in mortality. Permethrin resistance was present in all four provinces tested, with mortality ranging from 30% in Lunda Sul to 67% in Malanje. PBO pre-exposure resulted in an increase in mortality in all four sites, although mortality rates were below 90% in all sites and the increase in mortality was particularly small in Lunda Sul (only 51% mortality reached). The presence of pyrethroid resistance in all provinces tested indicates that the NMCP should consider alternative ITNs for future net distribution campaigns. Although susceptibility was not fully restored in several sites, the general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, particularly with deltamethrin or alpha-cypermethrin. A better option may be Interceptor G2 ITNs, as susceptibility to chlorfenapyr was recorded in all four sites tested.

In Huambo, *An. funestus* s.l. was the most common malaria vector species collected during the trapping period and the density was stable from November 2020 through February 2021 with between 1-2 *An. funestus* s.l. per trap. There was an increase in mean numbers collected in March and April 2021, reaching a mean of 3 *An. funestus* s.l. per trap in April. Longitudinal trapping over six months showed that there was a relatively low biting risk from malaria vector species between November and April. The entomological inoculation rate will be calculated in Year 3 following sporozoite ELISA testing of samples to determine *P. falciparum* infection rates.

Despite great effort over several months, susceptibility testing was not possible in Huambo. After not locating any viable *An. gambiae* s.l. or *An. funestus* larval sites, the team used double net traps to collect 53 adult *An. funestus* s.l., most of which were already blood-fed and were then successfully used for forced oviposition. However, all larvae had died after about 3-4 weeks. In the coming year (Year 3 of the project), the team will prioritize completion of susceptibility tests in all provinces, including Huambo, and continue to build the entomological skills of mosquito brigade members (morphological identification to the species level for primary malaria vector species, and practice of susceptibility testing).

1. INTRODUCTION

Malaria remains a serious public health problem in Angola despite sustained malaria control strategies. Malaria is endemic throughout the country and 100% of the population is at risk, but there is significant heterogeneity in transmission across the country. *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 (FY 2020 Angola Malaria Operational Plan). There are five *Anopheles* species responsible for transmission in the country: *An. 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 (Malaria Operational Plan, 2018).

The Angola NMCP strategy includes three vector control methods—insecticide-treated nets (ITNs), indoor residual spraying (IRS), and larviciding—and aims to cover 80% of the population at risk of malaria with at least one vector control and prevention measure. A survey in 2016 showed that 31% of households in Angola owned at least one ITN (2015–16 Demographic and Health Survey). By comparison, across all countries in sub-Saharan Africa in 2016, 80% of households owned at least one ITN[1]. PMI and the Global Fund have been key partners supporting procurement and distribution of pyrethroid ITNs. Angola took a major step in 2017 towards universal coverage of ITNs, conducting a three-phase nationwide mass campaign in 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 Kwanza Sul Province (use: access ratio 0.87), 51% in Cunene Province (0.69 use: access ratio) and 33.3% in Uige (0.77 use: access) (VectorWorks, 2019). The country is currently preparing for the next mass campaign which will begin in 2022.

From 2012 to 2016, the PMI AIRS project 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 Provincial de Saúde* (DPS). In 2014, PMI constructed and established an 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, Uige, and Zaire) where insecticide resistance was evaluated in 2015-2016. From 2016 to 2019, PMI did not support entomological monitoring activities in Angola.

PMI reestablished 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—one in Huambo Province and the other in Lunda Sul Province—to determine species composition, vector behavior, and vector susceptibility to different insecticides. In Year 2, 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. Between November 2020 and April 2021, the project conducted monthly community-based entomological surveillance in Huambo Province and insecticide resistance testing in seven provinces (Huambo, Kwanza Norte, Luanda, Lunda Sul, Malanje, Uige, and Zaire).

This report presents results from entomological monitoring conducted from November 2020 to April 2021, as well as key project contributions to country-level capacity building efforts.

1 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.

2. METHODOLOGY

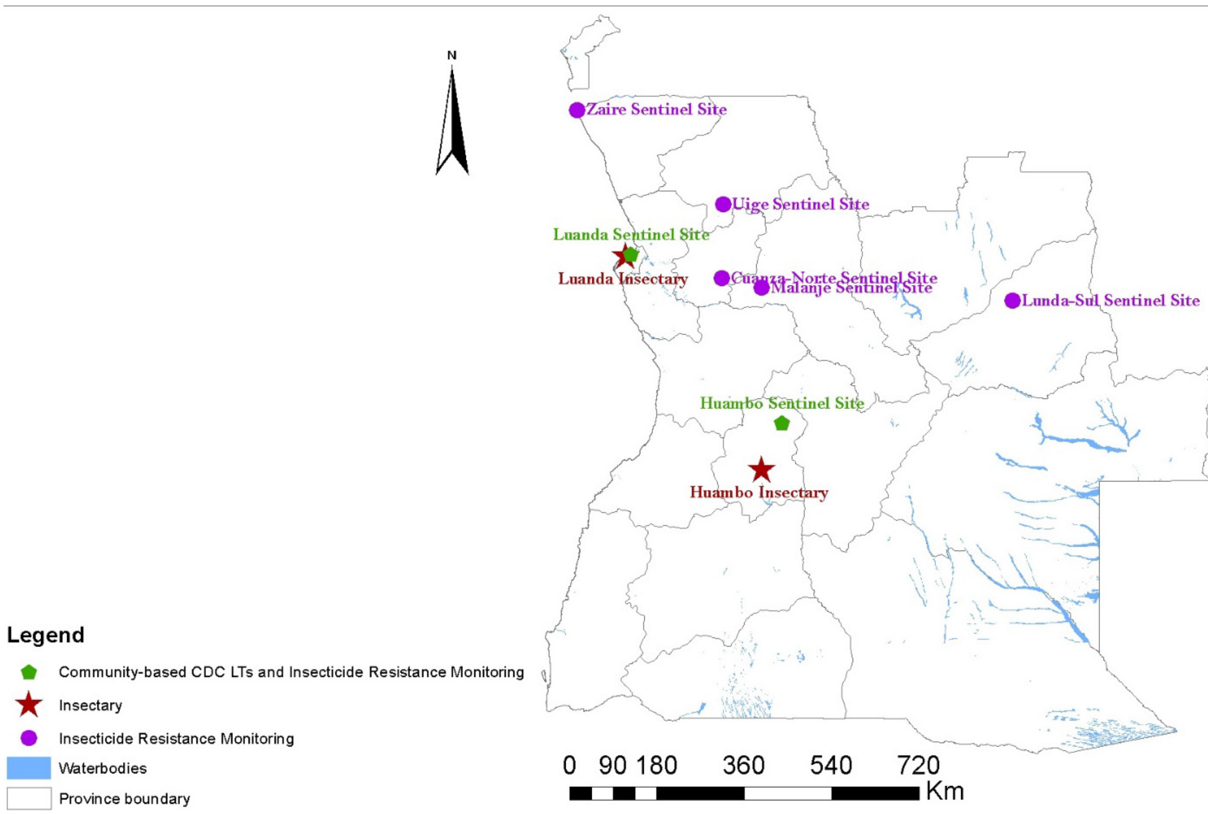
Details of VectorLink standard operating procedures used for WHO susceptibility tests, bottle bioassays, CDC light trap, Prokopack aspiration, and insectary rearing can be found at https://pmivectorlink.org/resources/tools-and-innovations/.

2.1 STUDY SITES

Between November 2020 and April 2021, insecticide resistance monitoring was conducted in sites across seven provinces—five PMI focus provinces (Cuanza Norte, Lunda Sul, Malanje, Uige, and Zaire) and two provinces (Huambo and 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 (November 2020 to April 2021) and conducted training of brigade staff as part of capacity development. Section 3 contains more training details.

Monthly vector bionomics monitoring was scheduled in two provinces (Figure 1 and Table 1). Monthly CDC LT collections were conducted in Huambo using community-based surveillance, with regular support from VectorLink entomology technicians. Huambo was chosen as the pilot site for this approach to engage entomology staff and the NMCP year-round, rather than for just a few months during the peak transmission season.

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



The activities were conducted according to the PMI VectorLink Angola Year 2 work plan (Table 1).

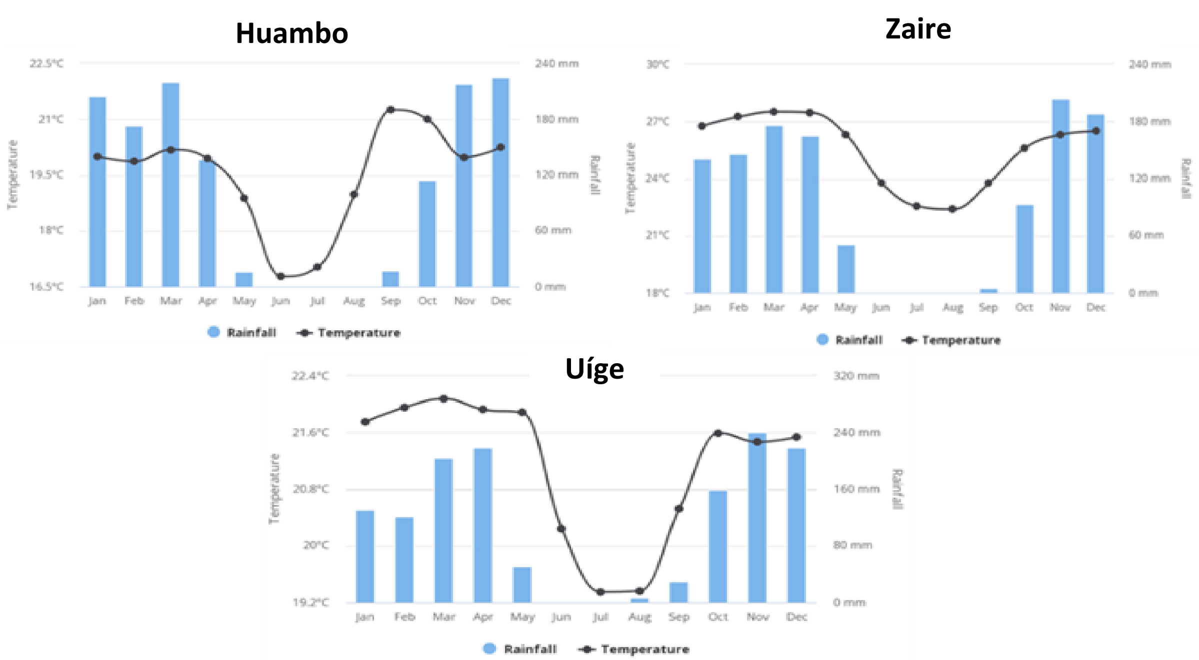
Table 1: Summary of PMI VectorLink Angola Entomological Activities (2020-2021)

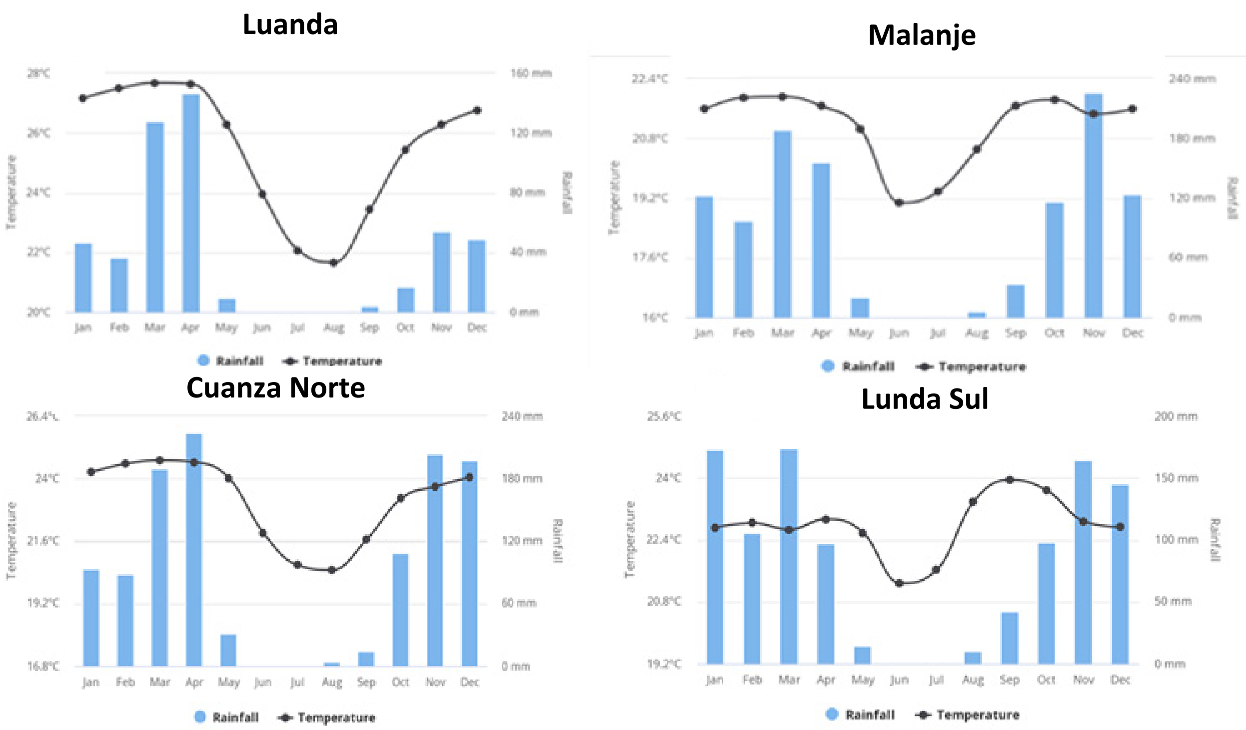
Activity	Purpose	Sites	Timeline	Frequency	Status
Insecticide Susceptibility Testing	To determine vector susceptibility to three pyrethroid insecticides with and without the synergist PBO, and to chlorfenapyr.	Lunda Sul, Cuanza Norte, Luanda, Malanje, Huambo, Zaire and Uíge.	November 2020-April 2021	Approximately two sites per month.	Completed in six out of seven provinces. No susceptibility data for Huambo.
Community Ento Surveillance: Monthly species composition, vector density, human biting rate and EIR	To gather more detailed longitudinal information on malaria vector dynamics and behavior.	Huambo Province (Ngandarinha Village) and Luanda Province (TBD).	November 2020-April 2021	Trapping in 60 houses per site per month	Completed in Huambo Province. Postponed in Luanda due to COVID-19 restrictions.
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.	Lunda Sul, Cuanza Norte, Luanda, Malanje, Zaire, Uíge and Huambo.	June-August 2021	Annually	Delayed due to COVID-19 travel restrictions and arrival of internationally procured laboratory supplies.

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

Figure 2 shows the mean rainfall and temperature for the seven sites over the reporting period.

Figure 2: Average Monthly Temperature and Rainfall (1991-2016) in Longitudinal Entomological Monitoring and Insecticide Susceptibility Sites





Source: Climatic Research Unit of University of East Anglia

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

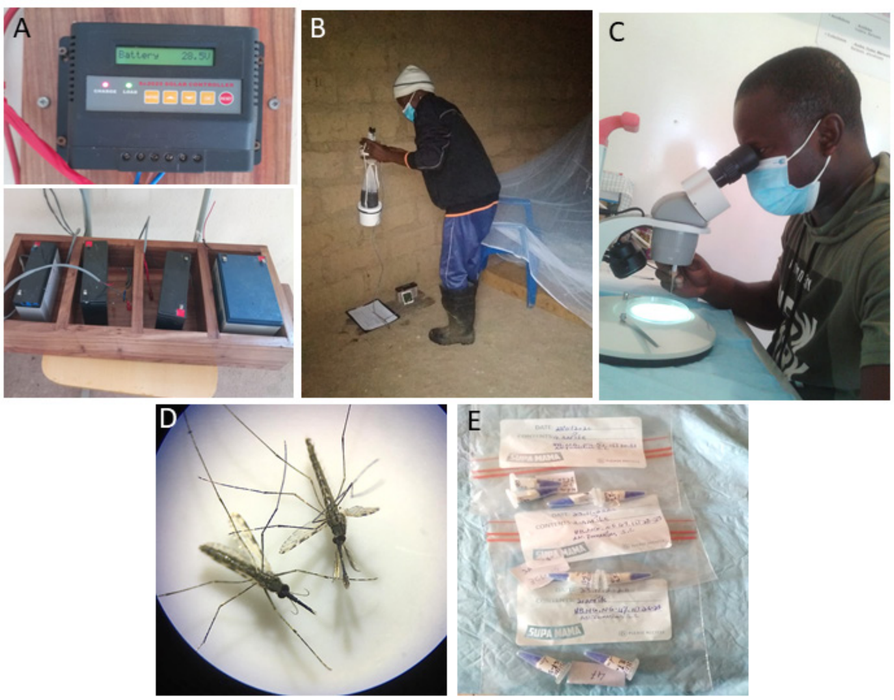
VectorLink Angola started a pilot of community-based entomological surveillance in Huambo Province in November 2020. The team planned to conduct a similar pilot in Luanda during the reporting period but had to postpone due to COVID-19 restrictions. The plan to conduct the pilot in Luanda during Year 3. In Huambo, the project recruited a motivated community member from Ngandarinha Village (a site where many *An. funestus* were captured by CDC LTs in 2016) and 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 collector 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 mosquito collector surveyed three different houses per night located within walking distance of their own house using CDC LTs. The light traps were hung indoors overnight (Figure 3), and collections were conducted for 20 nights per month for a total of 60 houses per month (same houses each month). 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 standard operating procedures (SOPs) and ensure that data forms were fully completed, and mosquito samples were correctly stored and labelled. Community collectors separated mosquito species by genus and VectorLink technicians subsequently conducted mosquito identification using the key of Gillies and Coetzee, 1987. Community collectors are being gradually trained so that they can carry out species identification in the near future. When setting CDC LTs, the community collector also gathered basic information through a 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. Samples were stored at -20°C in the freezer until further molecular laboratory analyses, to take place at INIS in Luanda. Data was recorded on paper forms by the community-based collectors before being updated by VectorLink technicians after mosquito identification and sending to the data clerk to enter into 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 per month
Larval collections	9:00 a.m. to 2:00 p.m.	Daily	As many larvae as possible from several locations

Figure 3: Photographs of Batteries Being Charged by Solar Panels in Ngandarinha Village, Huambo Province (A), Community Mosquito Collector Removing a CDC LT (B), VectorLink Technician Conducting Mosquito Identification in Huambo Province (C), *Anopheles* Mosquitoes from CDC LT (D), and *Anopheles* Mosquitoes from CDC LT Stored Individually for Molecular Analysis (E)



2.3 Larval Collections

From November 2020 to April 2021, VectorLink conducted susceptibility testing in six provinces (testing was not possible in Huambo Province; see Section 2.4). Larval collections were conducted daily by the provincial mosquito brigades in close coordination with a Senior VectorLink Entomology Technician in Cuanza Norte, Luanda, Lunda Sul, Malanje, Uíge, and Zaire (Figure 4). A sub-sample of *Anopheles* mosquitoes reared from larvae were identified morphologically (Gillies, M.T. & Coetzee, M., 1987) before testing to determine the species composition. After completion of insecticide testing, all mosquitoes were killed and morphologically identified to corroborate species.

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, Luanda, Lunda Sul, Malanje, Uíge, and Zaire) by Senior VectorLink Entomology Technicians.

Insecticides tested in 2020-2021 (in order of priority[2]) were:

- Deltamethrin 0.05% with and without pre-exposure to PBO 4%
- Alpha-cypermethrin 0.05% with and without pre-exposure to PBO 4%
- Chlorfenapyr 100µg/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 in makeshift insectaries (using portable humidifiers to improve adult rearing conditions) before conducting susceptibility testing with adult *An. gambiae* s.l. or adult *An. funestus* s.l. following VectorLink SOPs that are based on WHO procedures.

Figure 4: Photographs of *An. funestus* s.l. and *An. gambiae* s.l. Larval Collections (A, B, C), WHO Susceptibility Tests (D), Transfer of Mosquitoes During Susceptibility Tests (E), and Holding Tested Mosquitoes for 24 Hours at Controlled Temperature and Humidity (F)

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 (Figure 4). 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

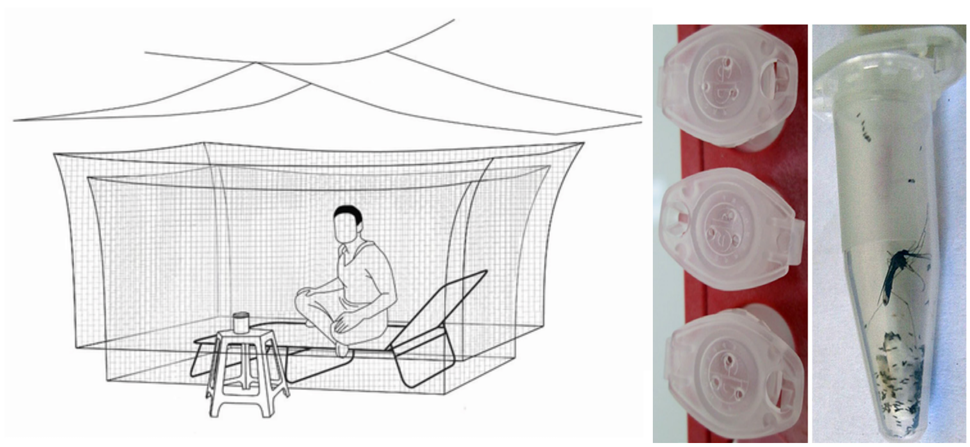
2 Order of priority was adopted based on difficulties collecting sufficient larvae in Lunda Sul and Cuanza Norte to test all insecticides.

24 hours post-exposure was the primary outcome measure. Four replicates of 25 *An. gambiae* s.l. or *An. funestus* s.l. were exposed to each insecticide.

CDC bottle bioassays were completed in four sites (Luanda, Malanje, Uíge, and Zaire) to determine the susceptibility status of *An. gambiae* s.l. or *An. funestus* s.l. to chlorfenapyr using a diagnostic dose of 100µg/bottle. Four replicates of 20-25 female *An. gambiae* s.l. or *An. funestus* s.l. were exposed for 60 minutes to chlorfenapyr 100ug/bottle. After 60 minutes of exposure, 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 total, following the 60-minute exposure. 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 according to the insecticide tested and site.

In Huambo, where the major vector species is *An. funestus*, adult mosquito collections were made using Prokopack aspiration and double-net traps to gather mosquitoes for forced oviposition followed by rearing of offspring for susceptibility tests. These trapping methods were used in January (10 double net, 10 Prokopack), March (18 double net, 10 Prokopack) and April (10 double net, 2 Prokopack) during peak numbers of *An. funestus*. Mechanical Prokopack aspiration was conducted between 5:30 and 7:00 a.m. by sweeping the aspirator close to the wall, ceiling, and furniture of the house for approximately 15 minutes per house (depending on size of the house) to collect resting mosquitoes. Double net traps were set up overnight; an inner net protected an adult volunteer while a second untreated larger net surrounded the first net, leaving a gap of about 30 cm above the ground to allow entry of mosquitoes between the nets (Figure 5). Mosquitoes, attracted to the odor plume emanating from all sides of the inner and outer nets, were aspirated individually early the following morning (between 5:30 and 6:00 a.m.) as they rested on the inner sides of the two nets. Blood-fed *An. funestus* adults were then taken back to the Huambo insectary and, after three days placed into individual Eppendorf tubes lined with a small piece of moist filter paper for forced oviposition (Figure 5). Eggs were subsequently washed into larval pans and larvae reared to adults. Larvae were fed with a combination of Tetramin tropical fish food and pond algae (based on feedback from University of the Witwatersrand).

Figure 5: Diagram of Double Net Trap (L) and Eppendorf Tubes Used for Forced Oviposition (R)



Sources: Tangena J-AA et al. (2015) double net trap diagram on left, and Nepomichene et al. Malar J (2017) 16:21 forced oviposition photos on right.

Despite great effort over several months, susceptibility testing was not possible in Huambo. As shown by CDC LT collections, the main malaria vector in the area is *An. funestus* s.l. and, despite considerable effort, there were no *An. gambiae* s.l. or *An. funestus* larval sites located in the area. Efforts were made to collect *An. funestus* s.l. adults using double net traps and Prokopack aspirators. Double net traps were found to be the most successful approach with 53 *An. funestus* s.l. collected, most of which were already blood-fed and were then used for forced oviposition. The forced oviposition method was successful, and most females laid eggs which were then washed into larval pans and reared. Larval growth was slow and, after about 3-4 weeks, all larvae had died and therefore susceptibility testing with adult *An. funestus* s.l. could not be completed. It was also not possible to find *An. gambiae* s.l. larvae in Luanda despite searches across a wide area and at different time points. Only *An. funestus* s.l. larvae were collected in Luanda and reared to adults for testing.

2.5 Laboratory Analysis

Molecular analysis and ELISAs will be conducted using samples collected in Year 1 from Huambo and Lunda Sul, and for samples collected from the seven provinces in Year 2. 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.

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 1 Samples					
Huambo	n/a	n/a	144	218	362
Lunda Sul	100	50	172	634	956
Subtotal Year 1	**100**	**50**	**316**	**852**	**1,318**
Year 2 Samples					
Cuanza Norte	100	50	n/a	n/a	150
Huambo	100	50	160 (20 per month)	1,600 (200 per month)	1,910
Luanda	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 2	**700**	**350**	**160**	**1,600**	**2,810**
Total Year 1 + Year 2	**800**	**400**	**476**	**2,452**	**4,128**

2.6 Data Management and Analysis

The DHIS2-based VectorLink Collect instance for entomological data management was used in Angola for the first time in 2020. VectorLink Monitoring and Evaluation Specialists trained and supported PSI 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 2021, 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:

- Human biting rate (HBR) = 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

Over the six-month trapping period (November 2020-April 2021), a total of 764 *Anopheles* were collected by indoor CDC LT (Figure 6). Four *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. coustani,* and *An. squamosus*) were collected, with the primary malaria vector species *An. funestus* s.l. (n=664, 87%) and *An. gambiae* s.l. (n=60, 8%) predominating. Double-net traps were used from January to April 2021 to collect sufficient *An. funestus* for susceptibility tests and 53 *An. funestus* s.l. were collected using this method (Figure 7). Indoor Prokopack collections were also conducted to collect *Anopheles* for susceptibility tests and yielded 8 *An. funestus* s.l. (Figure 8).

Figure 6: Species Composition of *Anopheles* Collected by Indoor CDC LTs in Ngandarinha Village, Huambo Province (November 2020-April 2021)

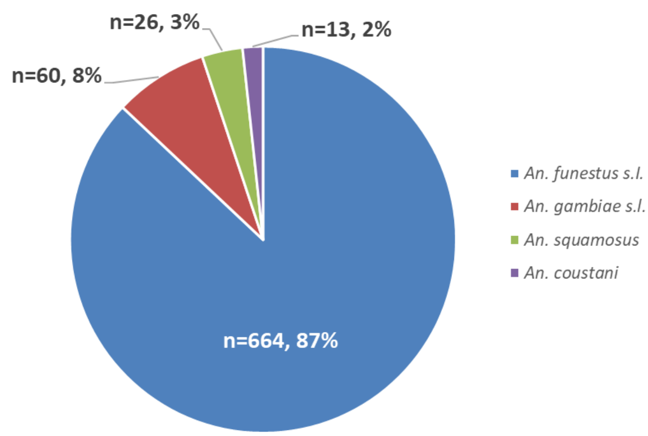


Figure 7: Species Composition of *Anopheles* Collected by Indoor Double Net Trap in Ngandarinha Village, Huambo Province (January-April 2021)

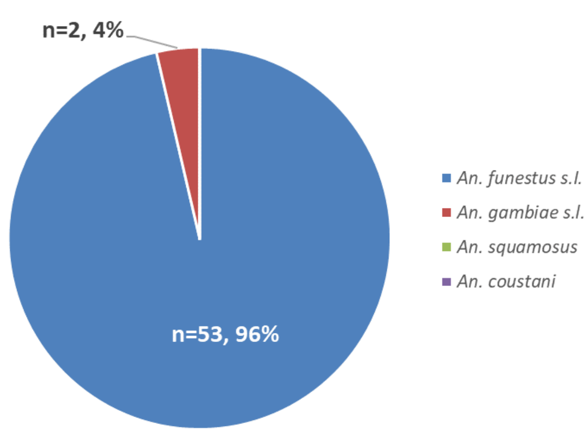
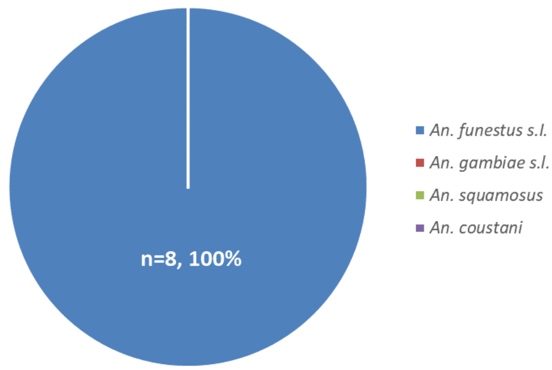


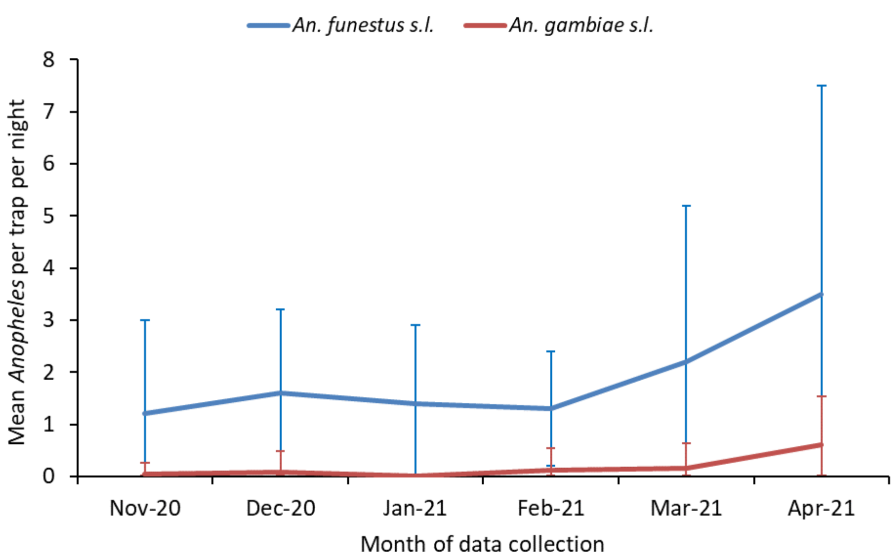
Figure 8: Species Composition of *Anopheles* Collected by Indoor Prokopack Aspiration in Ngandarinha Village, Huambo Province (January-April 2021)



3.2 Malaria Vector Human Biting Rates (by Indoor CDC LT)

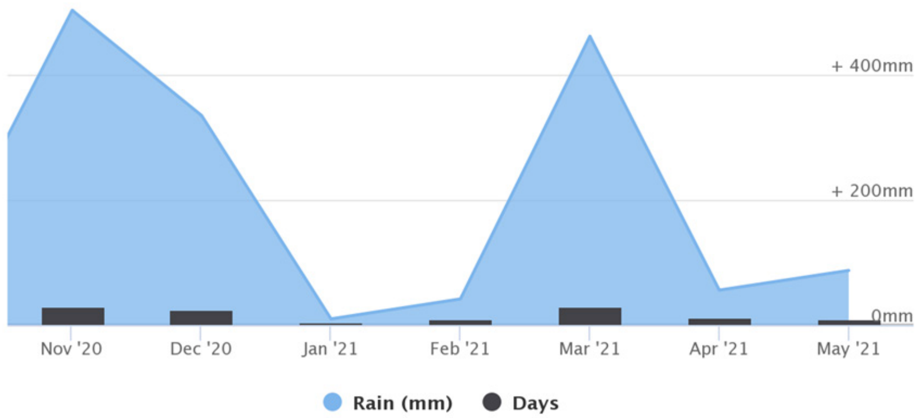
Figure 9 shows the mean number of primary malaria vector species (*An. funestus* s.l. and *An. gambiae* s.l.) collected per trap per night in Ngandarinha Village, Huambo Province between November 2020 and April 2021. *An. funestus* s.l. was the most common malaria vector species collected and the density was stable from November 2020 to February 2021 with about 1-2 *An. funestus* s.l. collected per trap. There was an increase in mean numbers collected in March and April, reaching a mean of 3 per trap in April. This may be linked to high rainfall during March (Figure 10).

Figure 9: Mean Number of *An. funestus* s.l. and *An. gambiae* s.l. Collected per Night by Indoor CDC LT in Ngandarinha Village, Huambo Province (November 2020-April 2021)



Note: 60 CDC LTs were used per month.

Figure 10: Rainfall (mm) and Number of Rain Days per Month in Huambo Province (November 2020-May 2021)



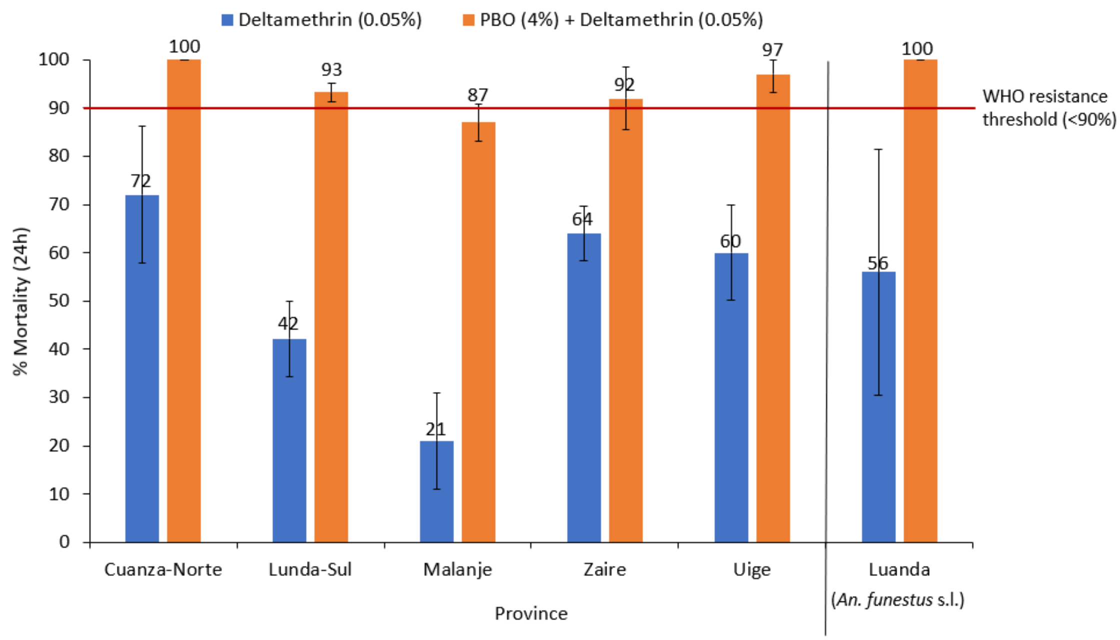
Source: https://www.worldweatheronline.com/huambo-weather-averages/huambo/ao.aspx

3.3 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 Cuanza Norte, Lunda Sul, Malanje, Uíge, and Zaire, while *An. funestus* s.l. were collected in Luanda. VectorLink Angola Senior Entomology Technicians conducted insecticide susceptibility testing with at least two pyrethroid insecticides with and without PBO synergist pre-exposure in six sites.

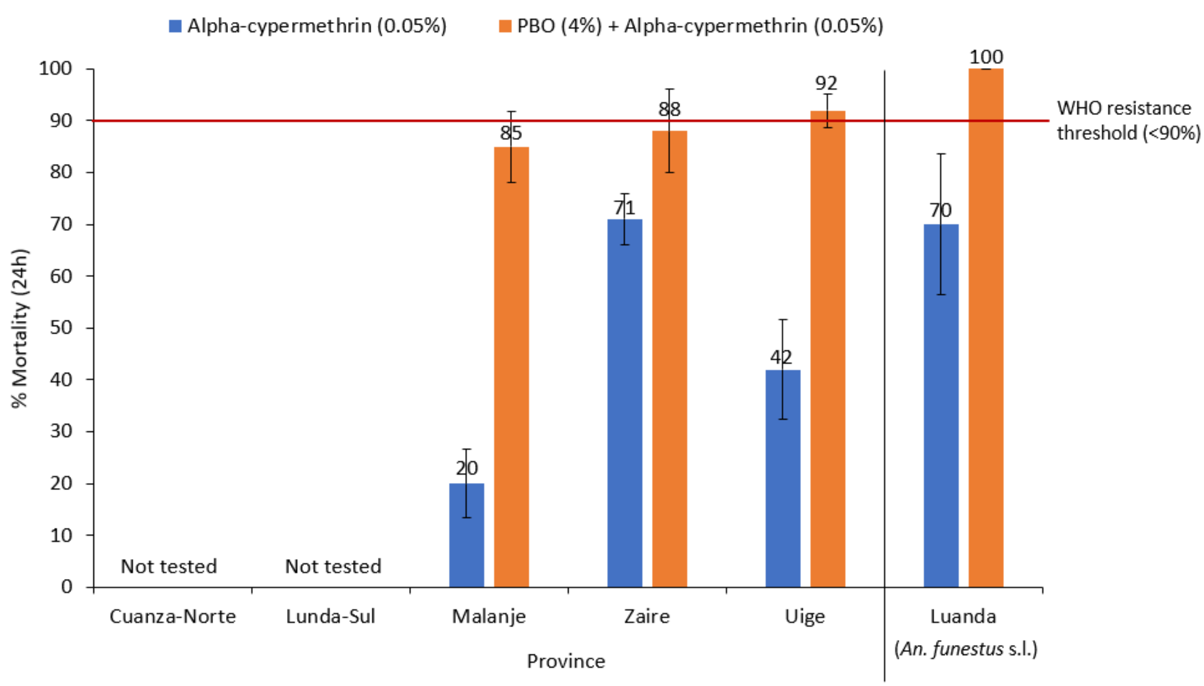
Deltamethrin (0.05%) was tested in all six sites and showed resistance in all sites, with mortality ranging from 21% in Malanje to 72% in Cuanza Norte (all sites below the 90% threshold for resistance) (Figure 11). Pre-exposure to PBO followed by deltamethrin produced a large increase in mortality in all sites, with susceptibility fully restored in Cuanza Norte and Luanda, while mortality increased to greater than 90% in Lunda Sul, Uige, and Zaire. Alpha-cypermethrin was tested in four sites and produced a similar trend, with resistance present in all sites, and with PBO pre-exposure resulting in a large increase in mortality. Susceptibility was fully restored in Luanda and mortality increased substantially in Malanje (from 20% to 85%) and Uige (42% to 92%) and to a lesser degree in Zaire (Figure 12). Permethrin resistance was present in all four sites tested, with mortality ranging from 30% in Lunda Sul up to 67% in Malanje. PBO pre-exposure resulted in an increase in mortality in all four sites, although mortality rates were below 90% in all sites and the absolute mortality was lowest in Lunda Sul (only 51% mortality reached) (Figure 13).

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



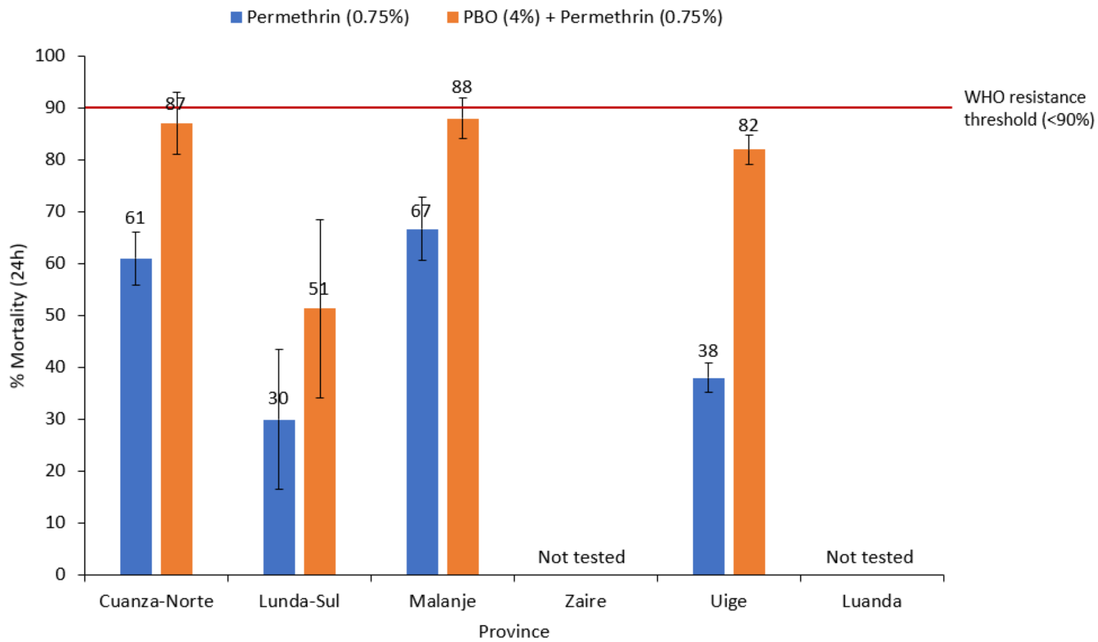
Note: Approximately 100 mosquitoes were tested per insecticide.

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



Note: Approximately 100 mosquitoes were tested per insecticide.

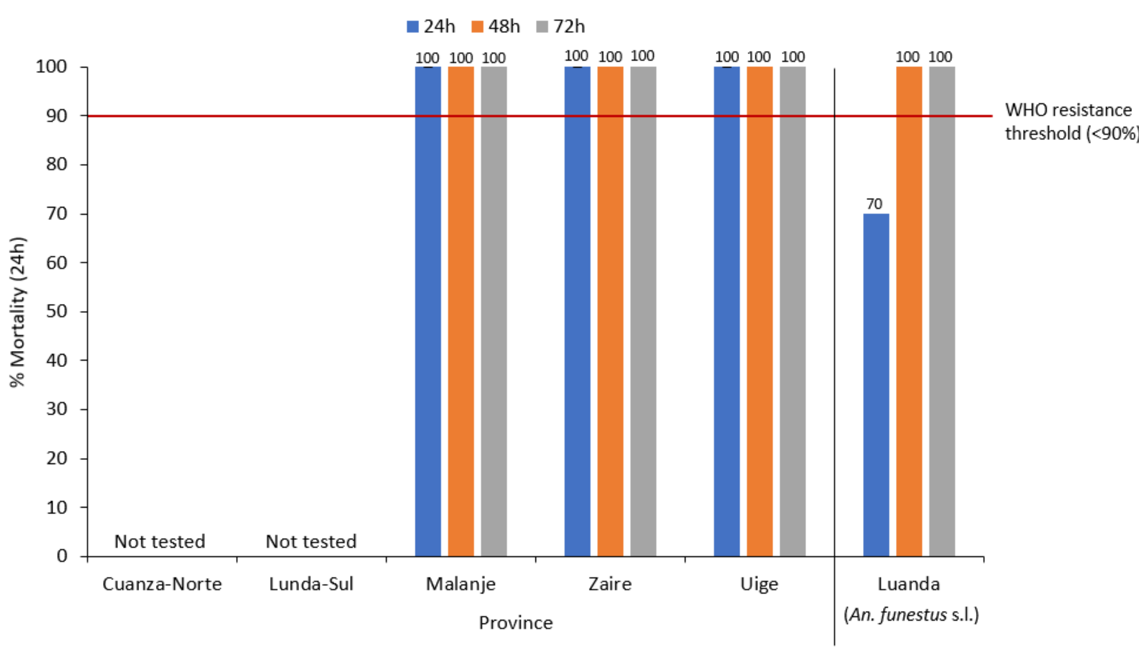
Figure 13: Percentage Mortality of *An. gambiae* s.l. in WHO Tube Tests with Permethrin (0.75%) and PBO (4%) + Permethrin (0.75%)



Note: Approximately 50-100 mosquitoes were tested per insecticide.

CDC bottle bioassays using the PMI VectorLink recommended dose of 100µg/bottle for chlorfenapyr produced 100% mortality in all four sites tested (Luanda, Malanje, Uíge, and Zaire) within 72 hours of exposure (Figure 14). This indicates that *An. gambiae* s.l. from Malanje, Uige, and Zaire, and *An. funestus* s.l. from Luanda are susceptible to chlorfenapyr.

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



Note: Approximately 100 mosquitoes were tested per insecticide.

Note that no susceptibility data is presented for Huambo as it was not possible to collect sufficient mosquitoes for testing.

The maps below (Figures 15-18) summarize the 2021 susceptibility data by province collected by VectorLink. PBO increased the mortality levels substantially in most sites but did not fully restore susceptibility. These results can help inform the NMCP on choice of ITNs for future mass distribution campaigns.

Figure 15: Map Summarizing Results of Deltamethrin Susceptibility Tests (With and Without PBO) by Province in Angola

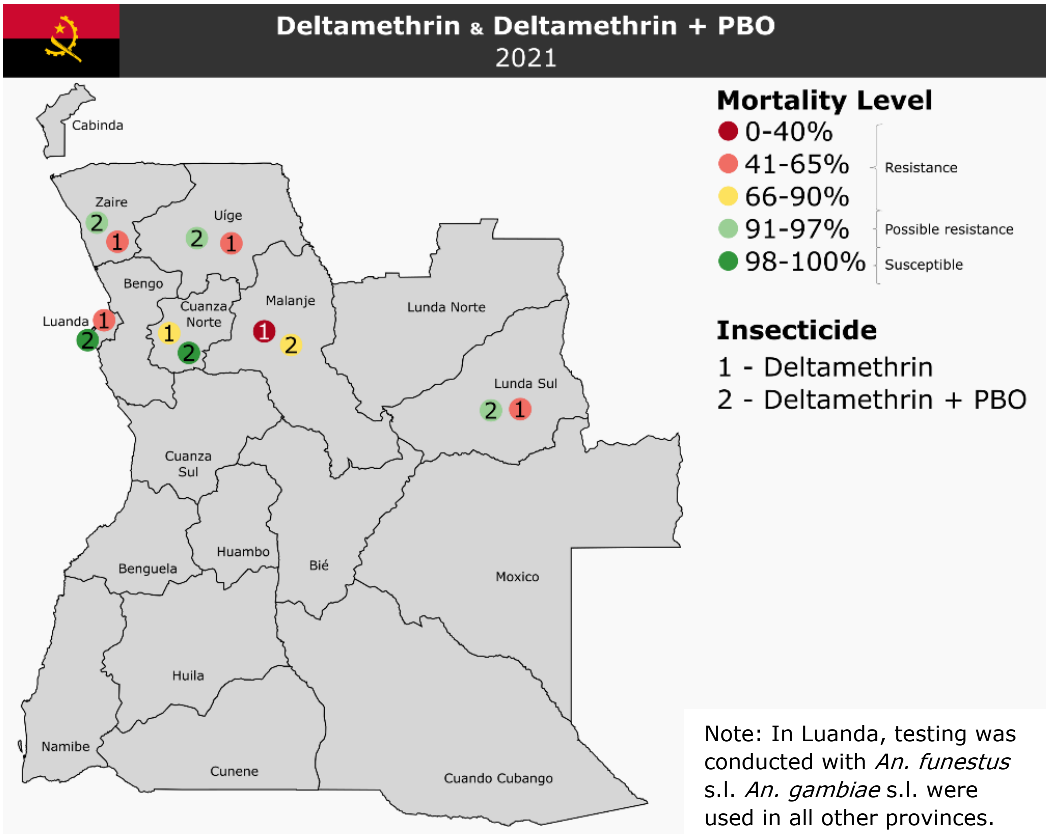


Figure 16: Map Summarizing Results of Alpha-Cypermethrin Susceptibility Tests (With and Without PBO) by Province in Angola

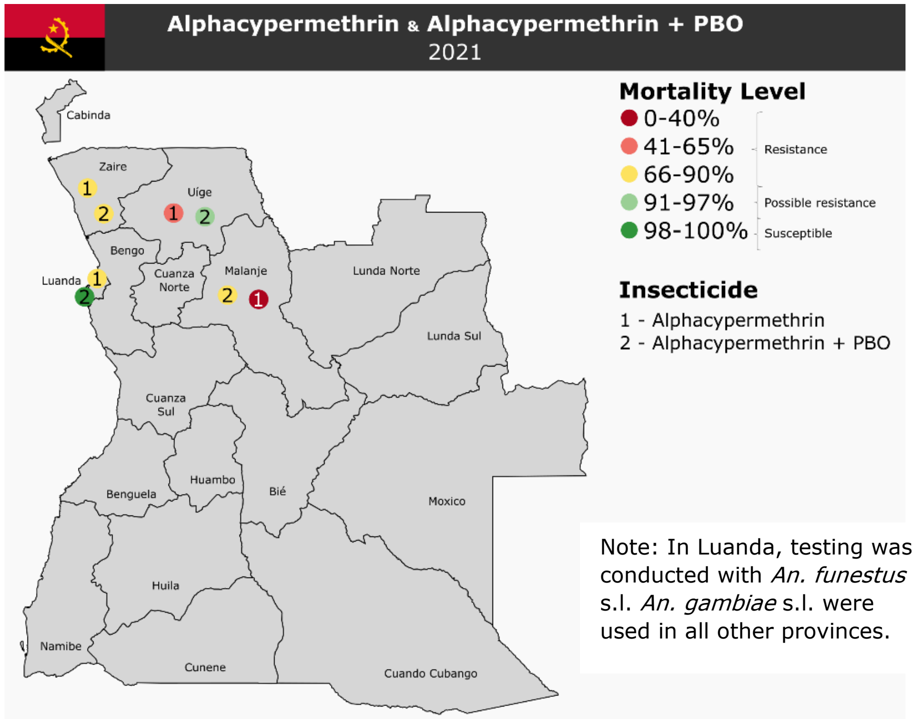


Figure 17: Map Summarizing Results of Permethrin Susceptibility Tests (With and Without PBO) by Province in Angola

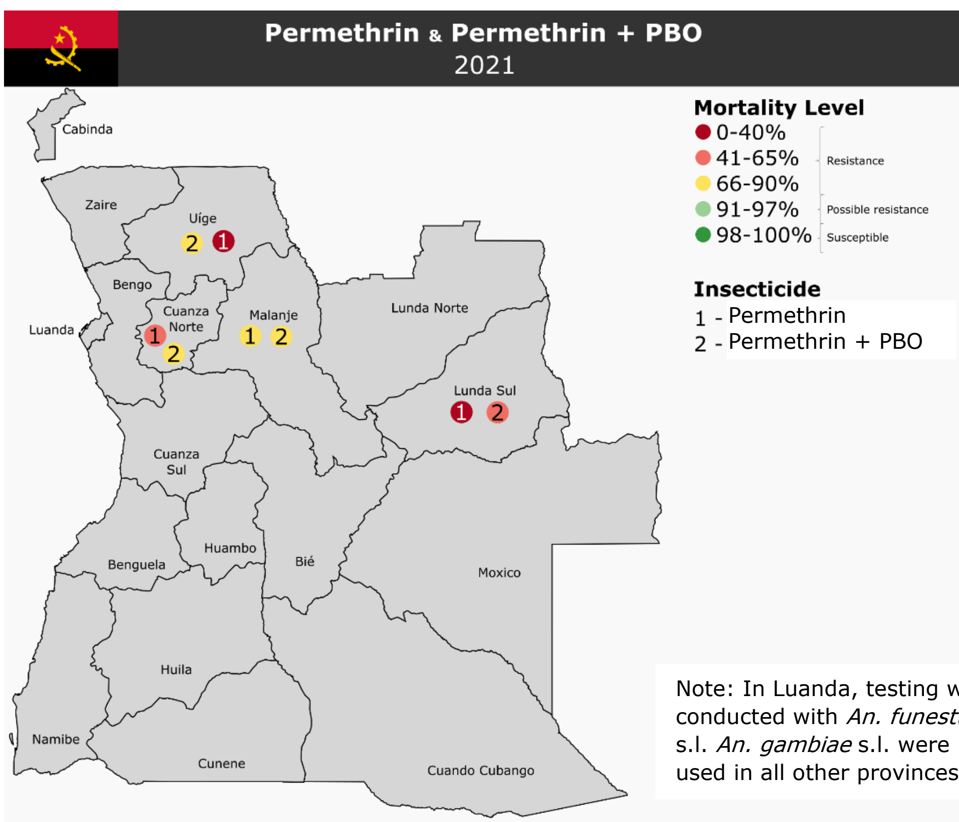
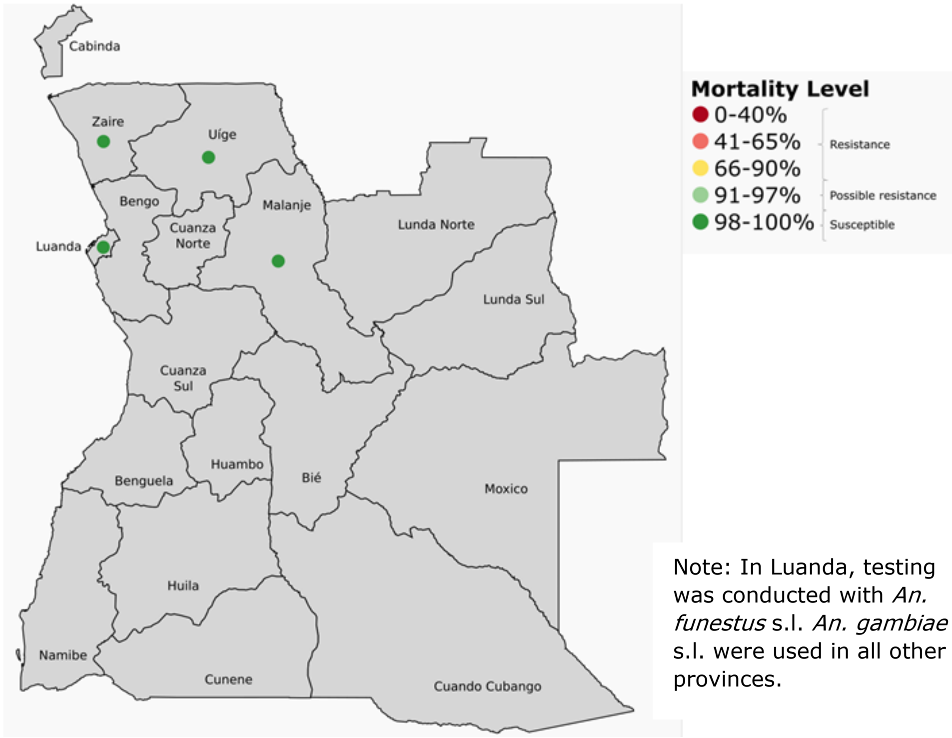


Figure 18: Map Summarizing Results of Chlorfenapyr Susceptibility Tests by Province in Angola



4. Capacity Building and Sustainability

The VectorLink Angola Entomology Coordinator and VectorLink Entomology Technicians (two full-time Senior and two Junior who participated during the peak season) continued to work closely with the NMCP, INSP/INIS, DPS (including mosquito brigades), and other partners to develop entomological capacity. In Year 2, the project focused on building basic provincial capacity related to insecticide susceptibility testing.

4.1 National-Level Entomological Training

VectorLink organized a national training in Huambo in October 2020 which included VectorLink technicians (from Luanda and Huambo), representatives from Global Fund/MENTOR Initiative, and other national stakeholders such as NMCP. The training was completed in one week and focused on insecticide susceptibility tests, including WHO tube tests for susceptibility as well as a new community-based surveillance using indoor CDC LT that started in November 2020. The training involved both classroom and practical sessions. VectorLink Senior Entomology Technicians led the training with support of NMCP. During the training, participants gained knowledge on which insecticides are prioritized for susceptibility testing and why; became familiar with project SOPs and WHO guidelines for susceptibility testing; and conducted WHO tube tests.

4.2 Provincial-Level Entomological Trainings

The VectorLink Senior Entomology Technicians conducted on-site, hands-on training for three mosquito brigade staff in each of seven provinces prior to insecticide susceptibility data collection during the rainy season (from November 2020-April 2021). 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 built provincial capacity for long-term sustainability. Mosquito brigade staff did not conduct susceptibility bioassays, but they did observe synergist bioassays being conducted by VectorLink Senior Entomology Technicians and bottle bioassays with chlorfenapyr. At the end of the training, all mosquito brigade attendees were able to 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.

4.3 Impact of COVID-19 on Other Planned Trainings

4.3.1 Laboratory Training

Laboratory analyses were not conducted during this workplan year due to COVID-19-related restrictions and delays in the arrival of laboratory equipment and supplies. A large laboratory specification freezer for mosquito sample storage and a fridge/freezer arrived in Luanda in June 2021 along with laboratory reagents needed for sporozoite ELISA and PCR for mosquito species identification. Laboratory work will be conducted in Year 3, with training due to start in July 2021 together with INIS support and guided by PMI/CDC.

4.3.2 VectorLink Regional Laboratory Training

VectorLink Angola planned to support one participant from INIS to participate in a regional advanced laboratory training to be organized by the VectorLink home office together with PMI and CRID Cameroon.

The training was expected to occur over 12 days and focus on PCR for species identification, resistance mechanisms, blood meal source, and ELISA/PCR for sporozoite infection detection. Due to COVID-19, this training was postponed.

4.3.3 VectorLink Regional Entomology Training

VectorLink Angola was due to support one participant from the NMCP or 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. Due to COVID-19, this training was postponed.

4.3.4 Short-Term Technical Assistance (STTA)

Three STTA trips planned during the reporting period did not occur due to COVID-19 travel restrictions. One was to support VectorLink entomology technicians and mosquito brigades on insecticide resistance testing and resistance mechanisms (PBO synergist tests) and ensure high quality data collection. The second was to provide technical assistance to VectorLink data clerks and entomology coordinator in the implementation of the VectorLink Collect. The third was for PMI to provide technical assistance in the form of a two-week training of INIS staff to establish molecular activities.

4.4 Vector Control Working Group Meetings

In collaboration with NMCP and other vector control partners, VectorLink Angola supported three vector control working group meetings (in February, April, and June) 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, INIS, GF/MENTOR, and USAID/PMI, and covered several important topics such as:

- Nomination of board members and working groups for specific topics.
- Revision and conclusion of the Angola Insecticide Resistance Management document.
- Revision and conclusion of the Angola Vector Control Management document.

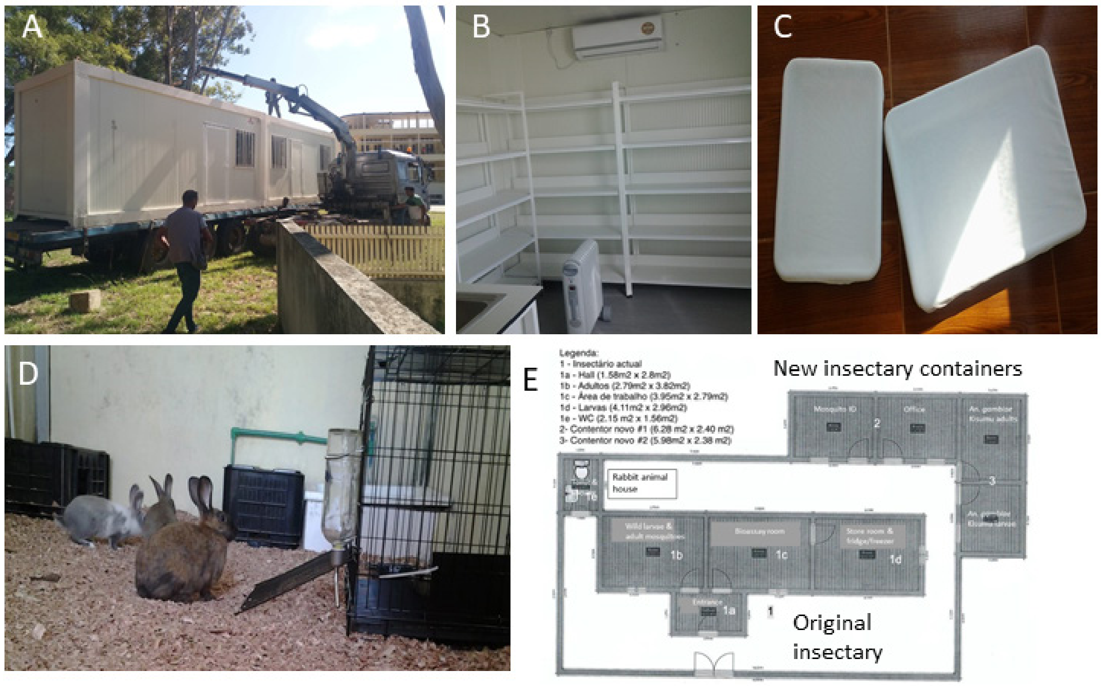
4.5 Support to GF/MENTOR

From late 2020 to early 2021, GF/MENTOR conducted entomological monitoring as well as IRS in Cuando Cubango Province. VectorLink provided support and technical assistance to the team—sharing the project's SOPs and data collection tools to harmonize across implementing partners. GF/MENTOR staff also attended training in Huambo (led by VectorLink technicians) on insecticide susceptibility testing. During the period of collection, GF/MENTOR also sent samples to Huambo for morphological identification confirmation by VectorLink entomological staff.

4.6 Insectaries

Angola has never had a characterized susceptible reference strain of *Anopheles* and did not have formal regulations in place to import. In February 2021, VectorLink Angola received written approval from the Ministry of Health to import a susceptible strain of *An. gambiae* mosquitoes. Arrangements have been made to import a susceptible strain of *An. coluzzii* SUA strain from The University of the Witwatersrand in South Africa. In preparation for rearing a susceptible laboratory strain, the existing infrastructure in Huambo was expanded by installing three converted shipping containers so that there is sufficient dedicated space for mosquito rearing, mosquito identification and processing, insecticide susceptibility tests, data entry, and equipment storage (Figure 19). There are separate rooms for rearing of the susceptible larvae and adult mosquitoes as well as rooms for sample processing, bioassay testing, and an animal rearing space for blood-feeding adult female mosquitoes. An insectary rearing manual has been developed based on MR4 insectary rearing protocols and VectorLink Angola Entomology Technicians have received remote guidance regarding maintaining an insectary colony.

Figure 19: Photographs of Converted Shipping Container Being Delivered (A), Larval Rearing Room in the New Insectary (B), Preparation of Covered Larval Trays for Rearing an Insectary Strain (C), Animal House for Rabbit Rearing (D), and Insectary Floor Plan Showing the Old and New Sections of the Huambo Insectary (E)



5. DISCUSSION

Insecticide susceptibility tests showed that pyrethroid resistance is widespread in Angola. In all five provinces, *An. gambiae* s.l. were resistant to the pyrethroid insecticides tested (deltamethrin, permethrin, and alpha-cypermethrin). Pyrethroid resistance was also recorded in Luanda with *An. funestus* s.l. when tested with deltamethrin and alpha-cypermethrin. The wide distribution of pyrethroid resistance in all evaluated sites provides the rationale for the NMCP and PMI to procure non-pyrethroid ITNs for future net distribution campaigns. In the future, 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.

One alternative ITN option to mitigate pyrethroid resistance is deployment of PBO synergist nets with deltamethrin, alpha-cypermethrin or permethrin. Synergist bioassays conducted by VectorLink showed that PBO pre-exposure increased mortality rates for deltamethrin, alpha-cypermethrin, and permethrin in all sites. The amount of the increase however varied drastically among sites and insecticide type, and rarely restored full susceptibility. Mortality increased the least for PBO followed by permethrin, with mortality less than 90% in all sites tested and as low as 51% in Lunda Sul. Although susceptibility was not fully restored in several sites, the general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, particularly with deltamethrin or alpha-cypermethrin. Dual active ingredient nets (dual AI) such as Interceptor G2 and Royal Guard are also an option. Interceptor G2 should be considered in Angola, as susceptibility to chlorfenapyr was recorded in all four sites tested with 100% mortality. However, the increased cost may be prohibitive. As part of the 2022 mass campaign, the Global Fund plans to support the NMCP through the procurement and distribution of PBO ITNs in Benguela and Cuanza Sul Provinces (the remainder of the country will receive standard pyrethroid ITNs). 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.

Longitudinal trapping in Ngandarinha Village in Huambo Province over six months showed that there is a relatively low biting risk from malaria vector species in this province between November and April, with a mean of between 1-3 female *An. funestus* s.l. captured per night per house. The entomological inoculation rate will be calculated in Year 3 following sporozoite ELISA testing of samples to determine *P. falciparum* infection rates. This is provisional data and there are plans to expand longitudinal monitoring to more sites to better understand malaria vector species dynamics in Angola.

Local capacity was strengthened through employment of two Entomology Coordinators, two Senior Entomology Technicians, two seasonal Junior Entomology Technicians, and by continuing to work closely with at least three mosquito brigade staff in each of the seven provinces. Through on-site, hands-on training, mosquito brigade staff (some of which are in their second year of working with the project) were able to collect *Anopheles* larvae and recognize key characteristics of *An. funestus* s.l. and *An. gambiae* s.l. adults for morphological identification. They also observed project SOPs for susceptibility testing in action. At the national level, representatives from the NMCP, INIS, and Global Fund/MENTOR Initiative gained more experience in insecticide susceptibility testing and were introduced to community-based surveillance. However, it was difficult to collect large numbers of larvae in any of the provinces—partly due to the unpredictable rainfall patterns and partly due to limited local knowledge on the location of productive larval sites. Therefore, in most provinces, only two of the three pyrethroids were tested and it was not possible to conduct any pyrethroid resistance intensity assays. COVID-19 restrictions resulted in the cancellation of project STTAs, as well as regional and national in-person trainings. Restrictions also delayed the start of longitudinal trapping in Luanda. Nevertheless, the team was able to make considerable progress through a combination of remote training and small-scale local training led by VectorLink Senior Technicians.

A new online entomology data system called VectorLink Collect was successfully implemented and utilized with local staff participating in remote training on data entry and management.

Based on the above, the following are key priorities for the project and its partners in Year 3:

- Expand community-based entomological surveillance to three provinces (Huambo, Luanda, and Lunda Norte) through training of community collectors and district brigade staff.
- Enhance the reach, depth, and regularity of capacity building efforts by developing content for an entomology module, which will be hosted on an eLearning platform currently being used for health workers in Angola.
- Work with the NMCP and other stakeholders to integrate VectorLink entomology data into the national DHIS2 system.
- Help establish molecular laboratory capacity in Angola to perform key entomological analysis, including polymerase chain reaction (PCR) for species identification and to determine resistance mechanisms, and enzyme-linked immunosorbent assays (ELISAs) to determine sporozoite infection rates.
- Complete susceptibility evaluation in Luanda with *An. gambiae* and in Huambo.
- Establish susceptible colony in country.