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Ministry of Community Development,
Mother and Child Health

PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS Task Order 4)

ZAMBIA 2014
ENTOMOLOGY REPORT

Introduction

Malaria is a critical public health challenge in Africa. Malaria is endemic and transmission is stable with a seasonal peak from November to April in Zambia. Indoor residual spraying (IRS) is one of the key malaria control strategies of the Zambian National Malaria Control Centre (NMCC).

In 2014, the President's Malaria Initiative (PMI) provided financial and technical support to the NMCC and district health offices for IRS and entomological surveillance activities through the PMI AIRS project.

Entomological surveillance provides pertinent information for malaria vector control programs as it helps to monitor the response of malaria vectors to the vector control interventions, such as IRS and the distribution of LLINs. It is indeed a key component for IRS programming because it provides information on the impact of IRS on vector density, and vector behavior in IRS areas as compared to non-IRS areas. Entomological activities also help to assess the quality of the IRS operations, the decay rates of the insecticide applied and the susceptibility of vectors to insecticides recommended by the World Health Organization Pesticide Evaluation Scheme (WHOPES) for use in malaria vector control.

To effectively monitor and evaluate the impact of IRS, PMI through AIRS Zambia is supporting the NMCC to generate data on key entomological indicators that include:

- Malaria vector species composition
- Vector distribution and seasonality (vector density)
- Vector behavior
- Vector susceptibility and mechanisms of resistance
- Quality assurance of IRS and decay rate of insecticide applied
- Sporozoite rates, entomological inoculate rates (EIR) and parity rates

The major entomological activities performed during the period this report covers were:

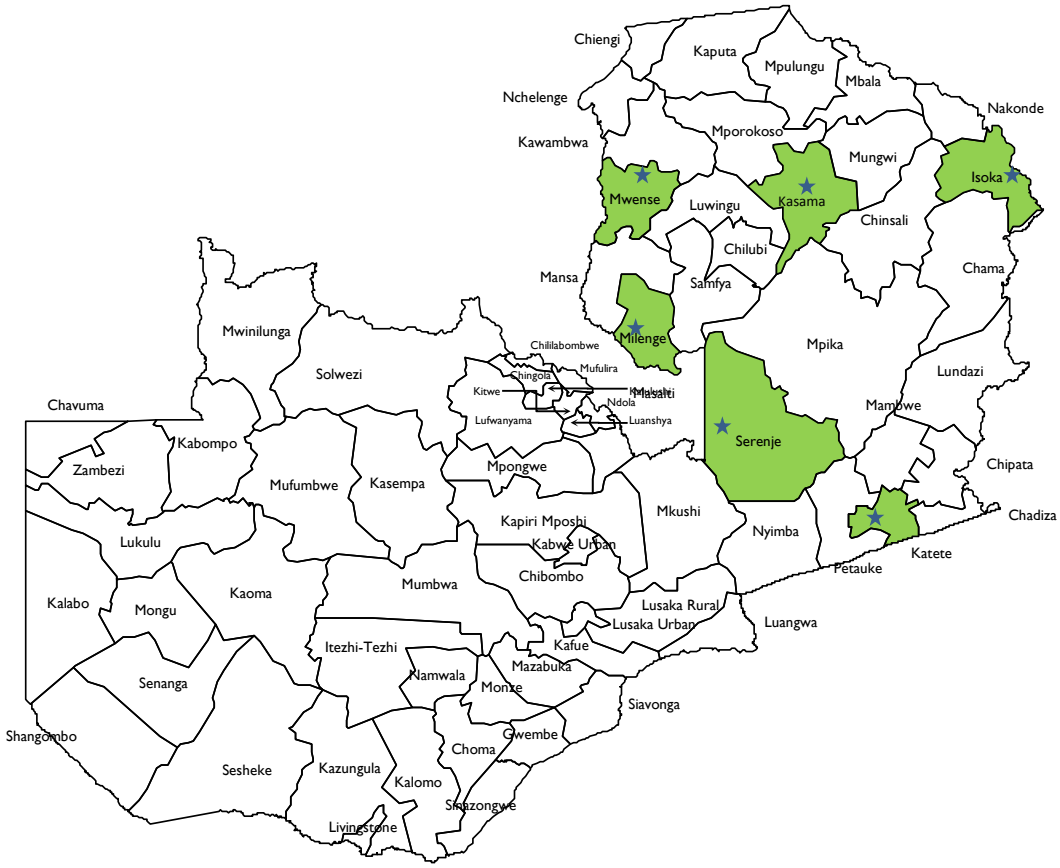
- Train the entomological coordinator, insectary technicians, and the Environmental Health Technician (EHT) from sentinel districts to collect mosquitoes, identify the mosquitoes collected and to perform the ovary dissection.
- Identify the malaria vector species found in the sentinel sites and determine their distribution.
- Determine the seasonal changes in vector density and behavior of malaria vectors in areas with IRS as compared to control areas.
- Assess the quality of the spraying and monitoring the decay rate of the insecticide applied.
- Determine the susceptibility level of the main malaria vectors in Zambia to all four classes of insecticides approved by WHOPES.
- Support the continued functionality of the national insectary and laboratory through the procurement of pre-fabricated structures, insectary equipment and salary support of insectary staff.
- Support the continued functionality of the IRMTWG to collate and interpret entomological data through bi-annual meetings.

1-Materials and Methods

1-1-Study sites

Zambia is situated in the Southern African region with a population of approximately 13 million. Zambia has a tropical climate with three seasons; a cool, dry winter (May–August), a hot, dry season (September–October), and a hot, rainy season (November– April). The annual rainfall varies from 600 to 1,100 mm across the country. The total size of Zambia is 752,612 km2. Entomological monitoring & evaluation was performed in six sentinel sites. In each district, one village with IRS was selected and another unsprayed village was selected as the control.

FIGURE 1: MAP OF DISTRICTS AND ENTOMOLOGICAL SENTINEL SITES



Entomological Sentinel Sites

1-2- Malaria Vector Density and Behavior Monitoring

Adult mosquito collection was done using Pyrethrum Spray Collection (PSC), CDC light traps and backpack aspiration of indoor resting mosquitoes.

1-21- Pyrethrum spray collection

PSC was used to sample indoor resting mosquitoes in the morning hours, between 4:00am and 7:00am in 16 houses for three mornings in each sentinel site (eight in treatments and eight in controls). Before PSC was performed, all occupants were asked to vacate the house without disturbing the resting mosquitoes. The eaves, windows, and other escape routes around the house were sprayed with the pyrethrum mixture (0.025 percent pyrethrum emulsifiable concentrate with 0.1 percent piperonyl butoxide in kerosene), using a small hand sprayer, followed by spraying of the walls and roof space inside the house. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected from the white sheets that was placed on the floor before spraying and sorted by species.

1-2-2- CDC light trap collection

CDC light traps were installed inside a total of eight houses (four in treatment and four in control) in each sentinel area for four consecutive nights. The CDC light trap was suspended in a bedroom 1.5 meters above the floor and about 50 cm from a human sleeping under an ITN on the foot side. Traps were set from 18:00pm until 6:00am to ensure that surveillance was conducted during the primary host-seeking period.

1-2-3 - Backpack aspiration

The CDC Backpack Aspirator was used to capture adult indoor resting mosquitoes that were used in insecticide susceptibility bioassays. Backpack aspiration was performed in 10 houses between 4:00am and 7:00am to minimize the chance that mosquitoes would leave the house before the catch is performed. Captured mosquitoes were stored in collection cups inside coolers and were provided access to cotton pads soaked with sugar water to keep them alive.

1-3- Susceptibility tests

F1 generation female malaria main vectors aged 2-5 days, reared from eggs of field-caught mosquitoes, were used for the susceptibility tests. The mosquitoes were exposed to diagnostic doses of various insecticides using insecticide-impregnated papers, as described by the WHO guideline. At least one insecticide from each class was tested in most of the sentinel sites. A sub-sample of dead and surviving mosquitoes was preserved for identification to the species level using PCR. All resistance study data will be added to the PMI database.

1-4- Quality assurance of IRS and insecticide decay rates

Cone bioassays were used to evaluate the quality of spraying and to monitor the decay rate of the insecticide after spraying. The first wall bioassay was conducted 24 hours after spraying in 10 houses per sentinel site. The test was carried out using known susceptible *An.*

gambiae s.s. Kisumu strain reared in the national insectary at the NMCC. About 10 females of *An. gambiae* s.s. Kisumu strain were introduced per cone and exposed for 30 minutes. For each house, the cones are put at 3 locations: 0.5 m, 1 m and 1.5 m above the ground. The number of mosquitoes knocked down after 30 min exposure and dead at the end of 24 hours holding period were registered. The cone bioassays were performed in all six sentinel sites.

2-Results

2.1 Vector Species Composition and Densities

Mosquito collections were carried out on a monthly basis from 6 sentinel sites using CDC light traps and PSC except in Kasama, Milenge, Mwense, Serenje in December and Isoka in January. *Anopheles* adult mosquitoes were identified to species using Gilles & Coetzee 1987 identification key.

2.1.1 Culicidae diversity before IRS campaign

Prior to the IRS campaign in September and October 2014 (dry season), AIRS collected baseline data using CDC light traps (indoor only) and PSC in all sentinel sites. A total of 1,278 mosquitoes were collected both from the control and spray sites. About 36% of mosquitoes (458) collected were Culicinae. The other 820 mosquitoes collected were identified as *Anopheles*. Forty-six *Anopheles* mosquitoes collected during this survey missed one or more structures needed to identify to species level and were not identified to species. From the total 774 *Anopheles* mosquitoes collected and identified to species, 718 (92.7%) were *An. funestus* s.l., 20 (2.6%) *An. gambiae* s.l., 36 (4.7%) were other *Anopheles* species (*An. tchekedii* (21), *An.s squamosus* (2), *An. tenebrosus* (4), *An. daudi* (3), *An. keniensis* (2), *An. smithii* (1), *An. pharoensis* (1), *An. parensis* (1), and *An. fuscivenosus* (1)). Table 1 below shows the culicidae diversity in the six sentinel sites before IRS started.

Table 1: Distribution by species of mosquitoes collected by PSC and CDC Light trap in September and October 2014 in the six sentinel sites

Districts	Villages	Status	CDC light trap				Pyrethrum Spray Catch				Total *Anopheles*		Total Culicinae mosquitoes		Total	
			An.funestus s.l.	*An.gambiae* s.l.	Others *Anopheles*	Culicinae	*An. funestus* s.l.	*An .gambiae* s.l.	Others *Anopheles*	Culicinae						
			n	n	n	n	n	n	n	n	n	%	n	%	n	%
Kasama	Kalonga	Sprayed	13	3	0	4					16	80	4	20	20	100
	Nandola	Control	2	1	2	0					5	100	0	0	5	100
Katete	Mbalani	Sprayed	0	0	0	145	0	0	0	75	0	0	220	100	220	100
	Robert	Control	0	0	0	21	0	0	0	4	0	0	25	100	25	100
Isoka	Nsalamba	Sprayed	3	2	3	66	2	0	1	40	11	9.4	106	90.6	117	100
	Chilanga	Control	0	0	0	3	6	0	0	57	6	9	60	91	66	100
Mwense	Shibesa	Sprayed	219	8	1	8	9	0	0	10	237	93	18	7	255	100
	Chebele	Control	107	6	0	2	5	0	0	5	118	94	7	6	125	100
Milenge	Lunga	Sprayed	212	0	24	10	133	0	5	7	374	96	17	4	391	100
	Niyambo	Control														
Serenje	Chibobo	Sprayed	-	-		-	1	0	0	1	1	50	1	50	2	100
	Chichi	Control	-	-	-	-	6	0	0	0	6	100	0	0	6	100
Total			556	20	30	259	162	0	6	199	774	63	458	37	1232	100

- PSC was not performed in September and October in Kasama and also at Nyiambo village in Milenge. CDC light trap collections were not performed in Serenje.

Table 2: Average density of *An. funestus* s.l. collected by CDC light trap collection per trap per night in the six sentinel sites in September-October 2014

				CDC light trap			
Month	Districts	Villages	Status	Number of traps	Number of night collection	Total *An. funestus* s.l	Average density per trap per night
September-October	Kasama	Kalonga	Sprayed	4	4	13	0.8
		Nandola	Control	4	4	2	0.1
	Katete	Mbalani	Sprayed	4	4	0	0.0
		Robert	Control	4	4	0	0.0
	Isoka	Nsalamba	Sprayed	4	4	3	0.2
		Chilanga	Control	4	4	0	0.0
	Mwense	Shibesa	Sprayed	4	4	219	13.7
		Chebele	Control	4	4	107	6.7
	Milenge	Lunga	Sprayed	4	4	212	13.3
		Niyambo	Control				
	Serenje	Chibobo	Sprayed			-	
		Chichi	Control			-	

The average density of *An. funestus* s.l. per trap in September-October was 13.7 per trap in Shibesa and 6.7 in Chebele and 13.3 in Lunga prior to the IRS campaign. The average density of *An. funestus* s.l. per trap in Kasama, Katete and Isoka districts was low, less than one mosquito per trap per night on average.

Table 3: Average density of *An. gambiae* s.l. collected by CDC light trap collection per trap per night in the six sentinel sites in September-October 2014

				CDC light trap			
Month	Districts	Villages	Status	Number of traps	Number of night collection	Total *An.gambiae* s.l.	Average density per trap
September-October	Kasama	Kalonga	Sprayed	4	4	3	0.2
		Nandola	Control	4	4	1	0.1
	Katete	Mbalani	Sprayed	4	4	0	0.0
		Robert	Control	4	4	0	0.0
	Isoka	Nsalamba	Sprayed	4	4	2	0.1
		Chilanga	Control	4	4	0	0.0
	Mwense	Shibesa	Sprayed	4	4	8	0.5
		Chebele	Control	4	4	6	0.4
	Milenge	Lunga	Sprayed	4	4	0	0.0
		Niyambo	Control				
	Serenje	Chibobo	Sprayed			-	
		Chichi	Control			-	

The average density of *An. gambiae* s.l. per trap per night in all districts and sentinel sites was low in September-October during the dry season.

Table 4: Average density of *An. funestus* s.l. collected by PSC per room in the six sentinel sites in September-October 2014

				Pyrethrum Spray Catch		
Month	Districts	Villages	Status	Number of room	Total number of *An.funestus* s.l	Average density per room
September-October	Kasama	Kalonga	Sprayed			
		Nandola	Control			
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed	8	2	0
		Chilanga	Control	8	6	1
	Mwense	Shibesa	Sprayed	8	9	1
		Chebele	Control	8	5	1
	Milenge	Lunga	Sprayed	8	133	17
		Niyambo	Control	8		0
	Serenje	Chibobo	Sprayed	8	1	0
		Chichi	Control	8	6	1

Before IRS, the average density of *An. funestus* s.l. per house in Lunga, was high in September-October, but in all other sites it was low.

2.1.2 Culicidae Diversity after the IRS Campaign

A total of 9,228 mosquitoes were collected from all six sentinel districts during four months from November to March. A total of 7,944 (86.1%) *Anopheles* were collected, 7,760 were identified to species, and 184 were lost their structures used for identification and were not identified to the species level. AIRS will reinforce supervision of entomological field collectors during mosquito sampling to ensure that mosquitoes are collected and properly preserved without losing their morphological structures in the future. A total of 4,430 (*An. funestus* s.l. and 668 *An. gambiae* s.l. were collected from both intervention and the control sites. 2,662 other anophelines were collected. The other anophelines species collected were *An. squamosus*, *An. brumipes*, *An. tenebrosus*, *An. distinctus*, *An. fuscivenosus*, *An. tchekedii*, *An. coustani, An. parensis, An. obscurus, An. smithii, An. keniensis, An. argenteolobatus, and An. pretoriensis*. Table 5 below shows the culicidae diversity per site during the post intervention season.

Table 5: Distribution of species of mosquitoes collected by PSC and CDC Light trap in November, December 2014 and January and March 2015 in the 6 sentinel sites

Districts	Villages	Status	CDC light trap				Pyrethrum Spray Catch				Total Anophelinae		Culicinae		Total	
			An.funestus s.l.	*An.gambiae* s.l.	Others Anophelinae	Culicinae	*An. funestus* s.l.	*An .gambiae* s.l.	Others Anophelinae.	Culicinae						
			N	n	n	n	n	n	n	n	n	%	n	%	n	%
Kasama	Kalonga	Sprayed	102	20	29	5	19	0	1	62	171	71.8	67	28.2	238	100.0
	Nandola	Control	18	1	6	16	15	3	3	3	46	70.8	19	29.2	65	100.0
Katete	Mbalani	Sprayed	5	2	4	297	0	1	0	161	12	2.6	458	97.4	470	100.0
	Robert	Control	6	7	2	62	14	1	0	31	30	24.4	93	75.6	123	100.0
Isoka	Nsalamba	Sprayed	73	73	117	178	19	4	4	40	290	57.1	218	42.9	508	100.0
	Chilanga	Control	4	10	7	7	7	0	1	41	29	37.7	48	62.3	77	100.0
Mwense	Shibesa	Sprayed	604	49	8	90	257	16	1	24	935	89.1	114	10.9	1049	100.0
	Chebele	Control	229	381	5	59	2	64	0	21	681	89.5	80	10.5	761	100.0
Milenge	Lunga	Sprayed	1272	14	379	12	245	4	7	10	1921	98.9	22	1.1	1943	100.0
	Niyambo	Control	1051	15	2081	153	210	1	4	4	3362	95.5	157	4.5	3519	100.0
Serenje	Chibobo	Sprayed	2	0	0	0	31	0	0	0	33	100.0	0	0.0	33	100.0
	Chichi	Control	119	0	3	1	126	2	0	7	250	96.9	8	3.1	258	100.0
Total			3485	572	2641	884	945	96	21	404	7760	85.8	1284	14.2	9044	100.0

Table 6: Average density of *An. funestus* s.l. per trap in sites under IRS and in control sites in November and December 2014, January and March 2015

Months	Districts	Villages	Status	Number of traps	Number of night collection	Total *An. funestus* s.l.	Average density per trap
November	Kasama	Kalonga	Sprayed	4	4	9	0.6
		Nandola	Control	4	4	18	1.1
	Katete	Mbalani	Sprayed	4	4	0	0.0
		Robert	Control	4	4	0	0.0
	Isoka	Nsalamba	Sprayed	4	4	0	0.0
		Chilanga	Control	4	4	0	0.0
	Mwense	Shibesa	Sprayed	4	4	258	16.1
		Chebele	Control	4	4	82	5.1
	Milenge	Lunga	Sprayed	4	4	200	12.5
		Niyambo	Control	4	4	257	16.1
	Serenje	chibobo	Sprayed				
		Chichi	Control	4	4	4	0.3
December	Kasama	Kalonga	Sprayed				
		Nandola	Control				
	Katete	Mbalani	Sprayed	4	4	0	0.0
		Robert	Control	4	4	0	0.0
	Isoka	Nsalamba	Sprayed	4	4	10	0.6
		Chilanga	Control	4	4	1	0.1
	Mwense	Shibesa	Sprayed				
		Chebele	Control				
	Milenge	Lunga	Sprayed				
		Niyambo	Control				
	Serenje	chibobo	Sprayed				
		Chichi	Control				
January	Kasama	Kalonga	Sprayed	4	4	9	0.6
		Nandola	Control				
	Katete	Mbalani	Sprayed	4	4	3	0.2
		Robert	Control	4	4	2	0.1
	Isoka	Nsalamba	Sprayed				
		Chilanga	Control				
	Mwense	Shibesa	Sprayed	4	4	89	5.6
		Chebele	Control	4	4	62	3.9
	Milenge	Lunga	Sprayed	4	4	20	1.3
		Niyambo	Control	4	4	38	2.4
	Serenje	chibobo	Sprayed	4	4	0	0.0

		Chichi	Control	4	4	7	0.4
March	Kasama	Kalonga	Sprayed	4	4	84	5.3
		Nandola	Control	4	4	0	0.0
	Katete	Mbalani	Sprayed	4	4	2	0.1
		Robert	Control	4	4	4	0.3
	Isoka	Nsalamba	Sprayed	4	4	63	3.9
		Chilanga	Control	4	4	3	0.2
	Mwense	Shibesa	Sprayed	4	4	257	16.1
		Chebele	Control	4	4	85	5.3
	Milenge	Lunga	Sprayed	4	4	1052	65.8
		Niyambo	Control	4	4	756	47.3
	Serenje	chibobo	Sprayed	4	4	2	0.1
		Chichi	Control	4	4	108	6.8
Total	Kasama	Kalonga	Sprayed	12	4	102	2.1
		Nandola	Control	8	4	18	0.6
	Katete	Mbalani	Sprayed	16	4	5	0.1
		Robert	Control	16	4	6	0.1
	Isoka	Nsalamba	Sprayed	12	4	73	1.5
		Chilanga	Control	12	4	4	0.1
	Mwense	Shibesa	Sprayed	12	4	604	12.6
		Chebele	Control	12	4	229	4.8
	Milenge	Lunga	Sprayed	12	4	1272	26.5
		Niyambo	Control	12	4	1051	21.9
	Serenje	chibobo	Sprayed	8	4	2	0.1
		Chichi	Control	12	4	119	2.5

The average density of *An. funestus* s.l. per trap in sprayed sites varied between 0.1 *An. funestus* s.l. per trap and 26.5 *An. funestus* s.l. per trap-night. In control sites, the average density was between 0.1 and 21.9. The mean average density for sprayed site was 7 *An. funestus* s.l. per trap versus 5 *An. funestus* s.l. per trap for control sites. A relatively higher density was also observed in most of the areas selected for IRS than the control during the pre-intervention season.

Table 7: Average density of *An. gambiae* s.l. per trap in sites under IRS and in control sites in November and December 2014, January and March 2015

Months	Districts	Villages	Status	Number of traps	Number of night collection	Total *An. gambiae* s.l.	Average density per trap
November	Kasama	Kalonga	Sprayed	4	4	2	0.1
		Nandola	Control	4	4	0	0.0
	Katete	Mbalani	Sprayed	4	4	0	0.0
		Robert	Control	4	4	0	0.0
	Isoka	Nsalamba	Sprayed	4	4	0	0.0
		Chilanga	Control	4	4	0	0.0
	Mwense	Shibesa	Sprayed	4	4	16	1.0
		Chebele	Control	4	4	22	1.4
	Milenge	Lunga	Sprayed	4	4	0	0.0
		Niyambo	Control	4	4	0	0.0
	Serenje	Chibobo	Sprayed				
		Chichi	Control	4	4	0	0.0
December	Kasama	Kalonga	Sprayed				
		Nandola	Control				
	Katete	Mbalani	Sprayed	4	4	0	0.0
		Robert	Control	4	4	0	0.0
	Isoka	Nsalamba	Sprayed	4	4	4	0.3
		Chilanga	Control	4	4	0	0.0
	Mwense	Shibesa	Sprayed				
		Chebele	Control				
	Milenge	Lunga	Sprayed				
		Niyambo	Control				
	Serenje	Chibobo	Sprayed				
		Chichi	Control				
January	Kasama	Kalonga	Sprayed	4	4	0	0.0
		Nandola	Control				
	Katete	Mbalani	Sprayed	4	4	1	0.1
		Robert	Control	4	4	2	0.1
	Isoka	Nsalamba	Sprayed				
		Chilanga	Control				
	Mwense	Shibesa	Sprayed	4	4	10	0.6
		Chebele	Control	4	4	33	2.1
	Milenge	Lunga	Sprayed	4	4	0	0.0
		Niyambo	Control	4	4	0	0.0
	Serenje	Chibobo	Sprayed	4	4	0	0.0

		Chichi	Control	4	4	0	0.0
March	Kasama	Kalonga	Sprayed	4	4	18	1.1
		Nandola	Control	4	4	1	0.1
	Katete	Mbalani	Sprayed	4	4	1	0.1
		Robert	Control	4	4	5	0.3
	Isoka	Nsalamba	Sprayed	4	4	69	4.3
		Chilanga	Control	4	4	10	0.6
	Mwense	Shibesa	Sprayed	4	4	23	1.4
		Chebele	Control	4	4	326	20.4
	Milenge	Lunga	Sprayed	4	4	14	0.9
		Niyambo	Control	4	4	15	0.9
	Serenje	Chibobo	Sprayed	4	4	0	0.0
		Chichi	Control	4	4	0	0.0
Total	Kasama	Kalonga	Sprayed	12	4	20	0.4
		Nandola	Control	8	4	1	0.0
	Katete	Mbalani	Sprayed	16	4	2	0.0
		Robert	Control	16	4	7	0.1
	Isoka	Nsalamba	Sprayed	12	4	73	1.5
		Chilanga	Control	12	4	10	0.2
	Mwense	Shibesa	Sprayed	12	4	49	1.0
		Chebele	Control	12	4	381	7.9
	Milenge	Lunga	Sprayed	12	4	14	0.3
		Niyambo	Control	12	4	15	0.3
	Serenje	Chibobo	Sprayed	8	4	0	0.0
		Chichi	Control	12	4	0	0.0

There was relatively higher density of *An. gambiae* s.l. at the Mwense sites than the other sentinel sites. The density was higher in Chebele control site as compared to Shibesa (sprayed site), with 7.9 and 1 *An. gambiae* s.l. per trap per night, respectively. Almost similar density of *An. gambiae* s.l. was observed during the pre-intervention season using the CDC light trap collections.

Table 8: Average density of *An. funestus* s.l. in sites under IRS and in control sites in November and December 2014, January and March 2015

				Pyrethrum Spray Catch		
Month	Districts	Villages	Status	Number of room	Total number of *An. funestus* s.l.	Average density per room
November	Kasama	Kalonga	Sprayed	8	8	1
		Nandola	Control	8	9	1
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed	8	0	0
		Chilanga	Control	8	0	0
	Mwense	Shibesa	Sprayed	8	98	12
		Chebele	Control	8	0	0
	Milenge	Lunga	Sprayed	8	142	18
		Niyambo	Control	8	19	2
	Serenje	Chibobo	Sprayed	8	0	0
		Chichi	Control	8	11	1
December	Kasama	Kalonga	Sprayed			
		Nandola	Control			
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed	8	11	1
		Chilanga	Control	8	0	0
	Mwense	Shibesa	Sprayed			
		Chebele	Control			
	Milenge	Lunga	Sprayed			
		Niyambo	Control			
	Serenje	Chibobo	Sprayed			
		Chichi	Control			
January	Kasama	Kalonga	Sprayed	8	2	0
		Nandola	Control			
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed			
		Chilanga	Control			
	Mwense	Shibesa	Sprayed			
		Chebele	Control			
	Milenge	Lunga	Sprayed	8	15	2
		Niyambo	Control	8	172	22
	Serenje	Chibobo	Sprayed	8	2	0

		Chichi	Control	8	39	5
March	Kasama	Kalonga	Sprayed	8	9	1
		Nandola	Control	8	6	1
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	14	2
	Isoka	Nsalamba	Sprayed	8	8	1
		Chilanga	Control	8	7	1
	Mwense	Shibesa	Sprayed	8	159	20
		Chebele	Control	8	2	0
	Milenge	Lunga	Sprayed	8	88	11
		Niyambo	Control	8	19	2
	Serenje	Chibobo	Sprayed	8	29	4
		Chichi	Control	8	76	10
Total	Kasama	Kalonga	Sprayed	24	19	
		Nandola	Control	16	15	
	Katete	Mbalani	Sprayed	32	0	0
		Robert	Control	32	14	0
	Isoka	Nsalamba	Sprayed	24	19	1
		Chilanga	Control	24	7	0
	Mwense	Shibesa	Sprayed	16	257	16
		Chebele	Control	16	2	0
	Milenge	Lunga	Sprayed	24	245	10
		Niyambo	Control	24	210	9
	Serenje	Chibobo	Sprayed	24	31	1
		Chichi	Control	24	126	5

Table 9: Average density of *An. gambiae* s.l. in sites under IRS and in control sites in November, December 2014, January and March 2015

				Pyrethrum Spray Catch		
Month	Districts	Villages	Status	Number of room	Total number of *An. gambiae* s.l.	Average density per room
November	Kasama	Kalonga	Sprayed	8	0	0
		Nandola	Control	8	0	0
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed	8	0	0
		Chilanga	Control	8	0	0
	Mwense	Shibesa	Sprayed	8	0	0

		Chebele	Control	8	30	4
	Milenge	Lunga	Sprayed	8	0	0
		Niyambo	Control	8	0	0
	Serenje	Chibobo	Sprayed	8	0	0
		Chichi	Control	8	0	0
December	Kasama	Kalonga	Sprayed			
		Nandola	Control			
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed	8	2	0
		Chilanga	Control	8	0	0
	Mwense	Shibesa	Sprayed			
		Chebele	Control			
	Milenge	Lunga	Sprayed			
		Niyambo	Control			
	Serenje	Chibobo	Sprayed			
		Chichi	Control			
January	Kasama	Kalonga	Sprayed	8	0	0
		Nandola	Control	8	0	
	Katete	Mbalani	Sprayed	8	0	0
		Robert	Control	8	0	0
	Isoka	Nsalamba	Sprayed			
		Chilanga	Control			
	Mwense	Shibesa	Sprayed	8	0	
		Chebele	Control		0	
	Milenge	Lunga	Sprayed	8	0	0
		Niyambo	Control	8	0	0
	Serenje	Chibobo	Sprayed	8	0	0
		Chichi	Control	8	2	0
March	Kasama	Kalonga	Sprayed	8	0	0
		Nandola	Control	8	3	0
	Katete	Mbalani	Sprayed	8	1	0
		Robert	Control	8	1	0
	Isoka	Nsalamba	Sprayed	8	2	0
		Chilanga	Control	8	0	0
	Mwense	Shibesa	Sprayed	8	16	2
		Chebele	Control	8	34	4
	Milenge	Lunga	Sprayed	8	4	1
		Niyambo	Control	8	1	0
	Serenje	Chibobo	Sprayed	8	0	0
		Chichi	Control	8	0	0

Total	Kasama	Kalonga	Sprayed	24	0	0
		Nandola	Control	16	3	0.2
	Katete	Mbalani	Sprayed	32	1	0
		Robert	Control	32	1	0
	Isoka	Nsalamba	Sprayed	24	4	0
		Chilanga	Control	24	0	0
	Mwense	Shibesa	Sprayed	16	16	1
		Chebele	Control	16	64	4
	Milenge	Lunga	Sprayed	24	4	0
		Niyambo	Control	24	1	0
	Serenje	Chibobo	Sprayed	24	0	0
		Chichi	Control	24	2	0

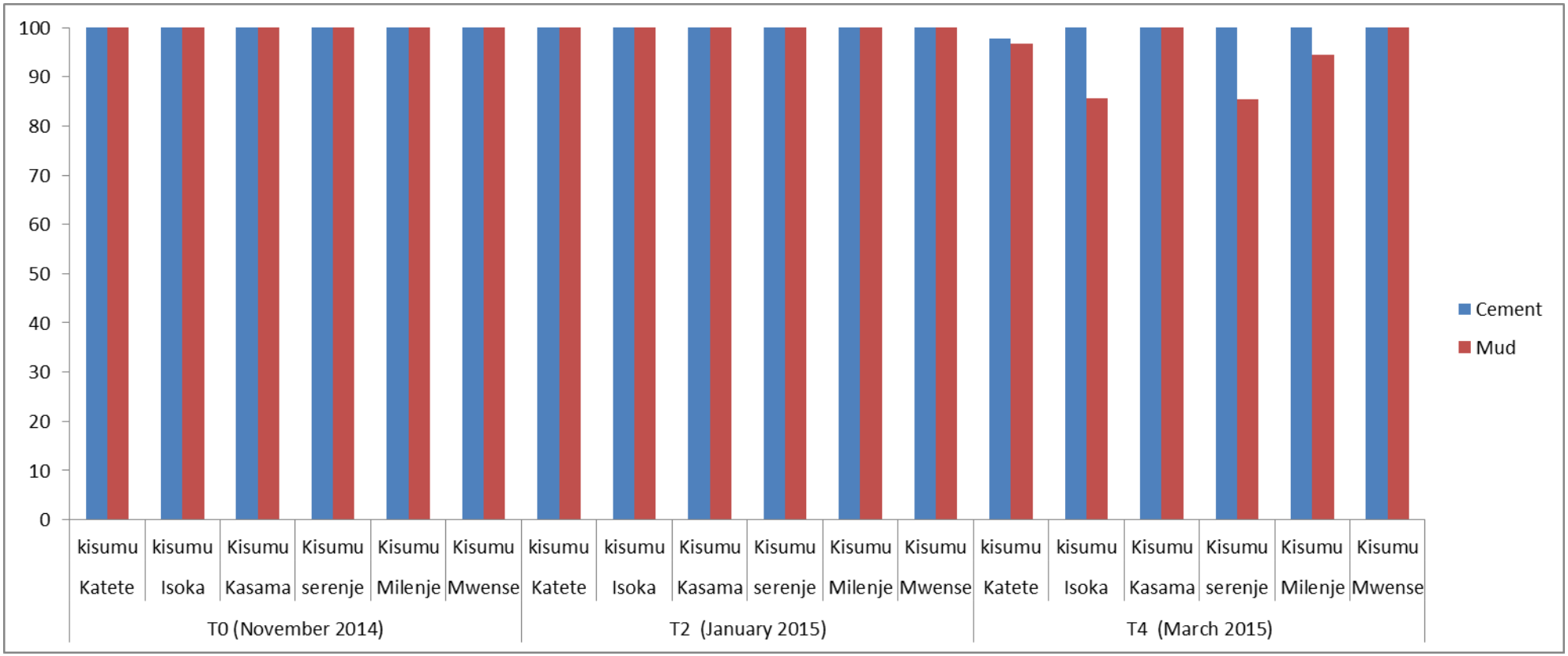
The mean average density of *An. funestus* s.l. and *An. gambiae* s.l. in sites under IRS was respectively 4.0 and 0.2 vectors per room, against 2.8 and 0.5 vectors per room in the control. A relatively, higher density of *An. gambiae* s.l. was observed in Mwense sentinel sites. Similar to the observations from the CDC light trap collections, a higher density of *An. gambiae* s.l. was observed in Mwense sentinel sites, with a higher density in the control village (4 *An. gambiae* s.l. per room) than the sprayed village (1 *An. gambiae* s.l. per room). At the pre-intervention season no *An. gambiae* s.l. mosquitoes were found in both sites.

2.2 Quality Assurance or IRS and Insecticide Decay Rate

Cone bioassays were conducted in 36 sprayed houses in the six districts. In each district, 2 types of houses were selected, 3 mud structures and 3 cement plastered structures. Two unsprayed structures, 1 mud and 1 cement, were picked as controls in each site.

The initial cone bioassay test was conducted 24 hours after houses were spayed. Subsequent tests were done in January and March two and four months after IRS campaign, to determine the decay rate of insecticide applied on the walls. The number of mosquitoes knocked down was recorded 30 min and 60 min after exposure and mortality recorded after 24 hours holding period. For the controls, the percentage of mosquitoes dead at the end of holding period was less than 5% for all the tests and correction formula was not used. All susceptible mosquitoes exposed to the treated walls were killed in November and January showing that the spray operators applied the insecticide required to kill malaria vectors. Two months after IRS campaign, the mortality rate of the susceptible strain was 100% on both mud and cement walls. In March 2015, four months after IRS campaign, the insecticide applied continued to be effective and the WHO minimum criteria of effectiveness was meet for both mud and cement walls (more than 80% of susceptible mosquitoes exposed were killed).

Figure 2: Percent mortality of susceptible strain after 30-minutes exposure to Pirimiphos-methyl CS (Organophospahte) via WHO Bioassay cone at T0 (24 hours after spraying in November), T2 (January two months after the spraying), and T4 (March four months after spraying)



2.3 Malaria Vector Susceptibility Using WHO Tube Tests

In 2014, adult indoor resting malaria vectors were collected using CDC backpack aspirators from 15 districts. The adult mosquitoes were kept in the insectary, eggs collected and the F1s progeny were used for susceptibility tests. At least one insecticide from each class was tested in most sites. Tables 5, 6, 7, 8 and 9, 10 and 11 below show the mortality rates of *An. funestus* s.l. and *An. gambiae* s.l. for each province and each site after their exposure to the diagnostic insecticide concentration for one hour and after a 24 hour holding period.

An. funestus s.l. and *An. gambiae* s.l. were susceptible to pirimiphos-methyl. A 100% mortality rate was recorded at all sites in 2014. However, the number of mosquitoes tested from most sites was lower than the number recommended by WHO, which is 100 mosquitoes per test and 50 for the control. Additional tests will be performed during the rainy season in 2015, when *An. funestus* s.l. and *An. gambiae* s.l. vectors are available relatively in large numbers.

Deltamethrin was tested at all sites in 2014. The results showed a high resistance of *An. funestus* s.l. to deltamethrin except in Mkushi district where the mortality rate was 100% in March 2014. Resistance to deltamethrin is suspected in Mungwi, Samfya and Isoka (Kampumbu site) where the test mortality rate was 96%, 91.9% and 92%, respectively. Similar results were obtained with permethrin. Resistance of *An. funestus* s.l. to permethrin was observed at all sites except in Kasama and Isoka districts where the mortality rates were 100% and 99.1%, respectively. *An. funestus* s.l. was resistant to bendiocarb at all sites except in Mansa (Mwa Nguni site) where resistance is suspected (the mortality was 97.5%, and according to WHO criteria this has been regarded as possible resistance). *An. funestus* s.l. was susceptible to DDT at all sites.

An. gambiae s.l. is resistant to deltamethrin and permethrin at all sites in 2014. The mosquitoes that survived will be assayed to determine the presence for Kdr mutation. The results also showed resistance of *An. gambiae* s.l. to DDT in all sites except at Kaweme site in Kawambwa district (98%) and at Kateshi site in Mansa district (99.5%) where *An. gambiae* s.l. is susceptible to DDT. A resistance of *An. gambiae* s.l. to DDT is also suspected at Talayi site in Milenge district (92%), at Chipota site in Kawambwa district (91%), and in Mwense district (Kashiba (92%), Mambilima (96%) and Mwa Nguni (97%) sites).

Most of the LLINs used for malaria control are impregnated with permethrin and deltamethrin. The resistance of *An. funestus* s.l., a major malaria vector in Zambia to those insecticides is a big concern. The use of an insecticide from other classes for IRS is helpful for vector resistance management in Zambia.

An. gambiae s.l. is susceptible to bendiocarb (carbamate) in Samfya, Milenge (Chipe, lunga and Talayi sites) and Mwense (Chongo and lubunda sites) districts. Both *An. gambiae* s.l. and *An. funestus* s.l. were susceptible to pirimiphos-methyl in all the sites where the tests were conducted.

Table 10: Susceptibility status of *An. funestus* s.l. in Central and Eastern Province in 2014

Province	District	DDT 4%		Deltamethrin 0.05%		Bendiocarb 0.1%		Pirimiphos-Methyl 0.25%		Permethrin 0.75%	
		14-Jan-Apr		14-Jan-Apr		14-Jan-Apr		14-Jan-Apr		14-Jan-Apr	
		N	%M	n	%M	n	%M	n	%M	n	%M
Central	Mkushi	37	100	54	100	27	81.5	34	100	50	42
	Serenje (chibobo)	57	100	30	60	45	78	25	100	25	36
	Serenje (chipundu)			25	68	50	84	11	100	30	60
Eastern	Katete			44	22.7			12	100		

Table 11: Susceptibility status of *An. gambiae* s.l. in Central and Copperbelt province in 2014

Province	District	DDT 4%		Deltamethrin 0.05%		Pirimiphos-Methyl 0.25%	
		14-Jan-Mar		14-Jan-Mar		14-Jan-Mar	
		n	%M	n	%M	n	%M
Central	Serenje (chibobo)	25	86	25	51	50	100
	Serenje (chipundu)	25	82	25	49.1	139	100
Copperbelt	Masaiti (Chishibar)					10	100
	Masaiti (Kafukany)					8	100
	Masaiti (Shimutey)					68	100
	Masaiti (Chishibambwe					25	100
	Masaiti (Kafukanya)					33	100

Table 12: Susceptibility status of *An. funestus* s.l. in Northern Province in 2014

Province	District	DDT 4%		Deltamethrin 0.05%				Bendiocarb 0.1%		Pirimiphos methyl 0.25%				Permethrin 0.75%			
		14-Feb-April		14-Feb-April		14—Apr		14-Feb-April		14-Feb		14--April		14-Feb		14-Apr	
		n	%M	n	%M	n	%M	n	%M	n	%M	n	%M	n	% M	n	%M
Northern	Kasama	40	100	92	9.7	104	34	99	78	65	100	38	100	103	77	113	100
	Mungwi			127	96			156	91	99	100						

Table 13: Susceptibility status of *An. gambiae* s.l. in Northern and Muchinga province in 2014

Province	District	DDT 4%		Bendiocarb 0.1%		Pirimiphos-Methyl 0.25%	
		14-Mar-Apr		14-Mar-Apr		14-Mar-Apr	
		n	%M	n	%M	n	%M
Northern	Kasama					62	100
	Mbala	40	32.5			97	100
	Mpulungu	80	23.8			69	100
	Mungwi			80	100	100	100
Muchinga	Chinsali					34	100
	Isoka					89	100

Table 14: Susceptibility status of *An. funestus* s.l. in Luapula province in 2014

Province	District	DDT 4%		Deltamethrin 0.05%		Bendiocarb 0.1%		Pirimiphos-Methyl 0.25%		Permethrin 0.75%	
		14-Feb-Mar		14-Feb-Mar		14-Feb-Mar		14-Feb-Mar		14-Feb-Mar	
		N	%M	n	%M	n	%M	n	%M	n	%M
Luapula	Chiengi (Mwabu kasenge)	25	100	76	53.9	69	88.4	40	100	45	23
	Chiengi (Mwengeswa)	25	100	74	48.9	64	76	89	100	69	27
	Kawambwa (Chipota)	30	100	44	72	25	77	25	100		
	Kawambwa (Kaweme)	40	100	25	64	23	55	82	100		
	Mansa (Kateshi)	50	100	70	60			25	100	25	44
	Mansa (Mwa Nguni)	50	100	50	56	80	97.5	128	100	25	36
	Mansa (Nsenama)					50	92.5	50	100		
	Milenge (Chipe)	75	100	92	58	100	75	107	100		
	Milenge (Katena)	30	100	50	76	100	89	88	100		
	Milenge (Lunga)	25	100	31	93.5	29	89.7	75	100		
	Milenge (Talayi)	28	100	100	67	60	80	80	100		
	Mwense (chongo)	72	100	93	90.3	45	84	15	100	72	21

	Mwense (Kashiba)	25	100	58	67.2	73	68.5	90	100	50	32
	Mwense (Lubunda)	87	100	100	69	97	64.9	97	100		
	Mwense (Mambilima)	25	100	100	73	100	75	66	100		
	Samfya (chilumba)	25	100					53	100		
	Samfya (kantashya)	50	100	124	91.9	37	50	120	100	100	61
	Samfya (Maximo)	48	100	100	83	60	85	46	100	100	67

Table 15: Susceptibility status of *An. gambiae* s.l. in Luapula province in 2014

Province	**District**	**DDT 4%**		**Deltamethrin 0.05%**		**Bendiocarb 0.1%**		**Pirimiphos-Methyl 0.25%**		**Permethrin 0.75%**	
		14-Feb-March		**14-Feb-March**		**14-Feb-March**		**14-Feb-March**		**14-Feb-March**	
		n	**%M**	**n**	**%M**	**n**	**%M**	**n**	**%M**	**n**	**%M**
Luapula	Chiengi (Mwabu Kasenge)	25	15.5	75	41	25	92	25	100	25	38.4
	Chiengi(Mwengeswa)	16	18.8	100	65	95	96.8	109	100	82	14.6
	Samfya (Chilumba)			60	43.3			25	100		

	Samfya (Kantashya)	50	92	25	87.3	25	100	100	100	19	57.9
	Samfya (Maximo)	100	83	49	69.3	100	98	50	100	50	65.4
	Milenge (Katena)	100	88	69	69.6	100	97.2	50	100	24	54.2
	Milenge (Chipe)	100	86	47	29.8	50	100	100	100		
	Milenge (Lunga)	37	70.3	50	71	25	100	128	100		
	Milenge (Talayi)	52	92	100	50.8	25	100	82	100		
	Kawambwa (Chipota)	25	91	32	47	25	84	25	100		
	Kawambwa (Kaweme)	25	98	38	59	52	99	81	100		
	Mwense (Chongo)	100	87	97	35.1	24	100	108	100	72	20.7
	Mwense (Kashiba)	50	92	65	63	85	80	125	100	30	50
	Mwense (Lubumda)	60	90	44	72.5	100	98	100	100		
	Mwense (Mambilima)	100	96	40	70	51	82.3	72	100		
	Mansa (Kateshi)	60	99.5	55	84.4			75	100	40	35
	Mansa (Mwa Nguni)	50	97	25	68	30	90	75	100	95	18.5
	Mansa (Nsenama)					27	88.9	25	100		

Table 16: Susceptibility status of *An. funestus* s.l. in Muchinga province in 2014

Province	Districts	DDT 4%		Deltamethrin 0.05 %		Bendiocarb 0.1%		Pirimiphos methyl 0.25%		Permethrin 0.75%	
		14-Apr		14-Apr		14-Apr		14-Apr		14-Apr	
		n	%M	n	%M	n	%M	n	%M	n	%M
Muchinga	Chinsali	32	100	52	58	45	67	46	100	23	83
	Isoka (Kampumbu)	50	100	46	92	42	80	42	100		
	Isoka (Malekani)	77	100	154	90	160	76	118	100	102	99.1

2.4 Training

AIRS conducted a series of in-class and practical workshops to prepare cadres of district-level Environmental Health Officers and Human Landing Collectors to manage entomological data collection in the sentinel sites. In addition, the AIRS Benin Technical Manager traveled from Benin to train the Insectary Technicians, 3 Environmental Health Officers and the entomological coordinator on the identification of adult *Anopheles* mosquitoes to species using Gilles & Coetzee 1987 identification key, the ovary dissection for parity determination in February.

2.5 Entomology Laboratory

PMI purchased and set up a modern prefab insectary at the National Malaria Control Centre. Through AIRS, PMI plans to furnish the insectary to facilitate its usage.

2.6 Challenges

- Lack of adequate skills in the field activities and in areas such as in morphological identification. Efforts have been made to improve skills through trainings.
- Poor compliance by some household owners
- Budget restrictions – prophylaxis for HLC