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EVALUATION OF THE ENTOMOLOGICAL SITUATION IN THE SURVEILLANCE ZONE OF YAOUNDE MALARIA ERADICATION PILOT PROJECT (CAMEROON)

by

F.-X. Pajot and H. Bailly-Choumara
Service National d'Eradication du Paludisme
de la République fédérale du Cameroun

Office de la Recherche scientifique
et technique Outre-Mer (I.R.CAM)

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1. Introduction

The Yaoundé surveillance zone is a forest area, lying entirely within the department of Nyong and Sanaga, and now measuring some 72 km from west to east and 88 km from north to south across its greatest extent in either direction. It is roughly the shape of a circle, centred south of Yaoundé, the administrative capital of the Federal Republic of Cameroon. Its area is 3944 km^2 (see map).

Its climate is tropical, with two dry seasons (December to March and June to August) and two rainy seasons (wettest month October). The mean annual rainfall is 1700 mm.

Relative humidity is high all the year round, the mean annual minimum humidity being 60% and the mean maximum 98%.

The mean annual temperature is 24°C., with mean minimum temperature 19°C. and mean maximum 30°C.

As to phytogeography, most of the surveillance zone is in the Sterculiaceae semi-ombrophilous forest zone, a zone of dense, humid, semi-deciduous forest of medium height characterized by an abundance of Sterculiaceae (Letouzey, 1958). The Mbalmayo region, in the south, belongs more nearly to the Congolese semi-ombrophilous forest zone.

These forests in the surveillance zone have been much encroached upon by plantations and in many places have become degraded. In particular, tree-shaded cocoa plantations are prominent, a plant formation having many of the ecological features of a forest.

2. Malaria control measures up to February 1960

The Yaoundé zone, which in 1953 was 1899 km^2 in area and contained 201 villages, was divided into 109 groups of 150 houses each, and the 109 groups were put through five different insecticide treatment schedules. This was operation "confetti". Small hamlets were not treated because "the expense is not commensurate with the general usefulness of protecting them" (Choumara, 1962).

The first spraying cycle took place in the spring of 1954. In May to June 1955 a second cycle was completed. It was then decided that control operations should be reorganized, and since over half the houses had not been treated and the performance of the sprayers was very poor, the method used in operation "confetti" was criticised. It was accordingly decided that the entire zone should be treated with dieldrin.

In 1956 a joint WHO-UNICEF-France mission visited the project and recommended yearly treatment with dieldrin, together with a trial of chemoprophylaxis restricted to 20 000 people.

In 1957 there was a further reorganization of the Yaoundé zone. It was now to be fully covered (Memorandum on Cameroon Antimalaria Pilot Project, 1957) by treatment with residual insecticides: one part with DDT (2 g per m^2) twice a year, and the other with dieldrin (0.6 g per m^2) once a year. In each of these sectors about 10 000 people were to be given monthly antimalaria treatment.

Following the excellent results obtained and described in the communication of Livadas et al. at the Lisbon Congress in September 1958, it was decided that all spraying should be suspended in the pilot zone in February 1960, and a surveillance system came into operation on 1 March.

3. Entomological observations

At the beginning of 1959 R. Chastang, discussing the results of entomological work (Gariou, 1957, 1958; Livadas et al., 1958; Mouchet, 1958), was able to say that no larvae or imagos of A. funestus had been detected in the course of surveys since 1955.

Likewise, A. gambiae had not been found in the pilot zone since 1957, either at the adult or at the larval stage.

Larvae of A. moucheti were still collected during surveys of breeding places. But imagos had virtually ceased to be captured in houses in the pilot zone, at any rate during the period when the insecticides were definitely effective.

Similarly, imagos of A. nili had not been caught in the course of regular surveys in the pilot zone since 1955, but larvae continued to occur in most streams in the forest area.

Thus the two latter anopheles, usually regarded as secondary vectors, well adapted to forest conditions, still remained; but their small numbers and their exophily prevented their maintaining transmission by themselves after the disappearance of A. gambiae and A. funestus.

Larvae of A. gambiae were found again for the first time in November 1960, in large numbers, at Mbalmayo, a town in the south of the Yaoundé pilot zone, in the middle of a sector treated with dieldrin. Adults were also caught, but in very small numbers. These anopheles were resistant to dieldrin and susceptibility tests showed that they belonged to a pure strain of dieldrin-resistant homozygotes (Gariou & Mouchet, 1961).

At the same time increasingly marked local transmission made its appearance in this area, spread beyond it and was proved to be taking place at Yaoundé, where A. gambiae was found again in April 1961 for the first time since 1957.

This transmission accompanied by the occurrence of the major vector was strongly suspected in various places in the surveillance zone, and its existence was spectacularly demonstrated on the resumption of night-catching and dissection of salivary glands and midguts.

In the second quarter of 1961, 25 female A. gambiae were caught in the surveillance zone, 267 in the third quarter of the year and 767 in the fourth (Bailly-Choumara Report, 1962).

Since the normal chemotherapeutic measures associated with surveillance methods had not been sufficiently successful in preventing rapid deterioration of the epidemiological situation, a resumption of insecticide spraying was decided upon in March 1962.

What, once again, is the entomological situation now, six months later, in this part of South Cameroon? The following evaluation, which gives the results of surveys made in the second quarter of 1962, the short dry season, endeavours to answer that question.

Entomological evaluation of the present situation. Our results are divided into three main categories. Two of these relate to the pilot zone, or rather to the "surveillance" zone where malaria cases detected are being treated with drugs. Two areas are distinguished in the zone: one has received no insecticide treatment since the end of 1960; this is referred to in our tables as "Pilot zone, untreated". In the other, referred to as "Pilot zone, treated", treatment of huts with DDT was resumed on 1 March 1962. One-sixth of the zone has been treated at the time of writing.

A third area is referred to as "Outside the pilot zone"; it consists of land round the surveillance zone which had initially been treated in the same way as the surveillance zone but has had no new spraying since the end of 1959 and beginning of 1960.

During the second quarter of 1962, entomological investigations, as in the previous quarter, consisted chiefly of study of the biology of the anopheline vector A. gambiae and of that species' advance in the pilot zone.

Also, after the houses in certain communities in the pilot zone had been sprayed with insecticide, we investigated the fate of the anopheline population following that first treatment.

During this second quarter we caught 7227 adult A. gambiae: 5331 of them outside the pilot zone, 1889 inside the pilot zone in the untreated part, and seven in the part recently treated with DDT.

In the as yet untreated part of the pilot zone the data for this quarter showed a sharp increase in the number of anopheles caught above the previous quarter's figure - 1889 A. gambiae, 1859 of them female, in 230 man-hours of day-time capture and 792 man-hours of night capture, as against in the previous quarter 179 female A. gambiae in 378 man-hours of day-time capture in houses and 816 man-hours of night capture (Pajot Report, 1962).

Climatic conditions in April and May were more favourable to the anophelines than in the previous months. Total rainfall was 217.8 mm in the month of April, and 240 mm in May. June saw the return of the dry season, and the total rainfall for that month did not exceed 85 mm.

Diurnal anopheles density in houses outside the pilot zone: the average anopheles density in these houses was high; 11.7 female A. gambiae per room. The high figure is largely due to the very high density in houses in the village of Mkolmekok - 15.7 female A. gambiae per room in this quarter of the year. These results do not correspond with the general average in South Cameroon outside the pilot zone, they are distinctly higher. That is because, wishing to obtain the largest possible number of anophelines for the purpose of dissecting them or making them lay, we sent the catchers straight to houses near breeding places which we knew to be positive. In general - a point we have now verified once again - the distribution of anopheles in a locality is confined to a few huts near the breeding place.

Diurnal density of anophelines caught inside houses in the pilot zone in the area untreated or not yet treated: total average density was again fairly high; 1.8 female A. gambiae per room (caught with a tube) and 1.1 female A. gambiae per room (caught after spraying with pyrethrum). As in the area outside the pilot zone these figures are mainly due to the high densities found, in this case, in two villages: Ntouessong IV and Ntouessong V (in one hut near the breeding places we found 133 female A. gambiae), the anopheles density of the other villages being low.

Table 1 gives the total results of catches of anopheles at night on human bait in and outside houses outside the pilot zone: 1610 A. gambiae were caught ouside houses, and 945 inside. They were caught at three catching stations.

Note the very high degree of exophagy of A. gambiae: 61.9 bites per man-night out of doors, as against 5.6 indoors.

In regard to the other anopheles - caught exclusively in the village of Ebogo (forest) - note that they are strictly exophagous and that the most anthropophilic species is A. paludis with a biting rate of 34.4 bites per man-night. This is a distinctly higher rate than that found for the first quarter (24). The biting rate per man-night for A. hargreavesi rose from 8.1, in the previous quarter, to 28.2.

Study of the hourly distribution of attacks by A. gambiae in these night catches outside the pilot zone shows that the mosquitos bite all night long. There is no really slack period. Maximum anopheline aggressiveness out of doors does not occur at the same time as the maximum agressiveness indoors.

Further, the results for this quarter differ somewhat in general, in regard to aggressiveness, from those for the previous quarter. During the first quarter (the short dry season) the mosquitos' period of maximum activity between two and three o'clock was very marked. In the second quarter, the general curve is spread out more evenly over the whole night, while a sharp difference is found between agressiveness indoors, with maximum before midnight, and agressiveness out of doors, with maximum at the end of the night.

The table also gives the total numbers of anophelines caught at night in the part of the pilot zone which was not treated or had not yet been treated. Here again, note the pronounced exophagy of A. gambiae: 14.8 bites per man-night out of doors, against 3.3 indoors.

It appears from the hourly distribution of attacks by A. gambiae shown by these all-night catches in the pilot zone that the mosquitos bite all night long. There is no slack period. Anopheline aggressiveness is at its maximum between four and five o'clock, distinctly later than during the previous quarter.

TABLE 1. NIGHT CAPTURES ON HUMAN BAIT

1 April 1962 to 30 June 1962

Place of capture	Number caught	Species	Out of doors			Indoors		
			Total number of females	Total number of catchers	Bites per man-night	Total number of females	Total number of sleepers	Bites per man-night
Outside the pilot zone	6 out of doors	*A. gambiae*	1 610	52	61.9			
	7 indoors	*A. gambiae*				945	167	5.6
	2 out of doors	*A. hargreavesi*	268	19	28.2			
	1 indoors	*A. hargreavesi*				0	21	0
	2 out of doors	*A. moucheti*	34	19	3.5			
	1 indoors	*A. moucheti*				0	21	0
	2 out of doors	*A. paludis*	326	19	34.3			
	1 indoors	*A. paludis*				0	21	0
Pilot zone, untreated part or before treatment		*A. gambiae*	640	86	14.8	661	196	3.3
		A. paludis	3	9	0.6	1	10	0.1
Pilot zone, treated part	7 out of doors	*A. gambiae*	7	68	0.2			
	5 indoors	*A. gambiae*				0	84	0

N.B. Out of doors each catcher only works half the night.

Table 2 gives the results of dissections of female anopheles caught outside the pilot zone. We had the salivary glands and non-engorged midguts dissected.

TABLE 2. DISSECTIONS OF FEMALE ANOPHELES
(SALIVARY GLANDS AND MIDGUT)

1 April 1962 to 30 June 1962

Place of capture	Species	Salivary glands			Midgut		
		Number examined	+	Sporozoite rate	Number examined	+	Oocyst rate
Outside the pilot zone	A. gambiae	946	108	11.4	579	3	0.5
Pilot zone, untreated part or before treatment	A. gambiae	884	75	8.4	463	4	0.8
Pilot zone, treated part	A. gambiae	3	0	0	3	1	-

The sporozoite rate was 11.4 for 946 dissections, and the oocyst rate 0.5 for 579 midguts dissected. We found three infections of the midgut alone without infection of the corresponding salivary glands.

The high sporozoite rate of the anophelines in this area outside the pilot zone was chiefly due to Nkolmekok, a village about 10 km as the crow flies from the northern border of the pilot zone, where we found for the whole quarter a sporozoite rate of 14.2 in 596 dissections (the rate rose to 60 per cent. for 300 dissections in January 1962).

The table also shows the results of dissections of female anopheles caught inside the pilot zone in areas not treated or before treatment. In this case we found a total sporozoite rate of 8.4 in 884 dissections of salivary glands.

Note how high this figure is. It is mainly accounted for by the village of Ntouessong V, where we found for the whole quarter a sporozoite rate of 11.6 in 602 dissections. Note also the high rate in the anophelines caught at Nkolnda: 4.4 in 68 dissections.

Dissections of the few anopheles caught inside the pilot zone in the treated area were negative.

Since the detection of breeding places is very important in forest country and we wanted to study by this means the spread of A. gambiae in this area, we concentrated particularly on that point during the second quarter.

The number of breeding places containing A. gambiae found during this quarter was distinctly larger than the number found during the previous quarter (317 in 4136 man-hours of work, as against 35 in 934 man-hours of work during the previous quarter).

TABLE 3. LARVAE COLLECTED
1 April 1962 to 30 June 1962

Anopheles	Vectors		Non-vectors		Total man-hours of work
	A. gambiae	A. nili	A. coustani	A. obscurus	
Outside the pilot zone	139 breeding places	1 breeding place	-	-	1 770
Pilot zone, untreated part or before treatment	157 breeding places	-	4 breeding places	1 breeding place	1 856
Pilot zone treated part	21 breeding places	3 breeding places	2 breeding places	-	510

In towns (Yaoundé, Mbalmayo) the main breeding places are cultivation furrows, sand borrow-pits, and puddles, as well as in warehouses, saw-mills, slaughter-houses and quarries.

At Yaoundé (Pajot Report, 1962) 28 out of 62 breeding places consisted of stagnant puddles in the numerous cultivation furrows that occur at the bottom of most of the valleys in the town. It is market gardens, chiefly, that produce this type of breeding place. Of the others, one was found in a pit dug to obtain sand (these borrow-pits are permanent breeding places); three were formed by tracks left by cattle; 11 were due to ruts in roads and at the side of roads and badly maintained tracks or on the premises of undertakings employing a large number of vehicles; 11 were puddles immediately adjoining houses (usually inside courtyards); and eight were puddles at the edge of badly kept [illegible].

In villages the breeding places are often ruts left by lorries, and sand or earth borrow-pits made in the course of house building. Note that all the breeding places we have mentioned, both in towns and in villages, are without exception man-made and could in most cases be eliminated if elementary road making and repair were done at the village level.

These results are set out in Table 3 which shows, here again, that a certain number of A. gambiae breeding places continue to occur in the treated part of the pilot zone. We found breeding places still containing larvae two months after DDT spraying in neighbouring houses.

We determined the DDT susceptibility of anophelines caught inside the pilot zone before treatment using the method recommended by WHO. The tests showed that a 2 per cent. concentration of DDT kills all the anophelines.

We also tested a few adult anophelines developed from larvae caught in the treated area two months after treatment. All of the 11 adults tested with DDT were killed by a 2 per cent. concentration (mortality of the controls, nil).

Attempt to estimate the survival rate of A. gambiae in a village in the pilot zone.

We caught and dissected at Ntouessong, which had not yet been re-treated with insecticides a large enough number of A. gambiae for us to attempt the calculation of the daily survival rate of the anopheline population.

The results obtained by the parasitological laboratory for the village of Ntouessong are as follows:

	Examined	Positive all forms	P. falciparum gametocytes	Gametocyte index
May 1962	219	162	32	14.6
June 1962	248	181	46	18.5
Total	467	343	78	16.7

This average gametocyte index (x) of 16.7 per cent. may be accepted as valid since $\chi^2 = 1.25$, so that there is a probability of 0.26 (95 per cent. confidence limits) that the difference between the two months is of a purely chance nature.

Russian authors (Oganov & Rajevskj) have determined with a high degree of accuracy in the USSR the length of the extrinsic cycle of Plasmodium vivax as a function of the external temperature; we shall take the liberty of extrapolating these conclusions and applying them to Plasmodium falciparum in Africa, the extrinsic cycle of which is known to be 12 days at 25°C.

Since the average temperature in June was 23.1°C, it follows that the time taken for completion of the extrinsic cycle of Plasmodium falciparum during that period was 15 days (N = 15).

The gonotrophic cycle is known to be two days, i.e. an Anopheles bites once every two days (a = 1/2). The sporozoite rate (S) of the village is 11.7.

We assume that all the gametocytes were infective; there is a strong probability that this was in fact the case during the epidemic flare-up observed. Applying the various values given above to Macdonald's formula (1957)

$$S = \frac{ax \quad p^n}{ax - \log_e p}$$

we see that all the terms of the equation are known except "p", the anopheles' average daily survival rate.

It is difficult to deduce the value of "p" from this formula, and it would be simpler to draw graphs as Hamon, Choumara et al. (1958) have done, but it is possible to calculate the values of S for various values of "p".

Where p = 0.95, S = 0.287.

Where p = 0.90, S = 0.091.

The sporozoite rate at Ntouessong was 11.7, therefore S = 0.117; which gives us roughly p = 0.91.

A daily survival rate of 0.91 does not seem to us inconsistent, either with the climatic conditions or with the epidemic flare-up observed.

<u>The inoculation rate (h).</u> In this village we found an average of 11.1 bites per man per night, and a sporozoite rate (S) of 0.117.

Assuming anthropophily to be one (this was confirmed by all the precipitin tests made earlier in the pilot zone) and that the length of the gonotrophic cycle (a) is two days, the inoculation rate can be calculated by Macdonald's formula as follows:

$$h = mabs$$

$$m = \text{the anopheline density in relation to man}$$

$$= \frac{\text{number of bites per man per night}}{\text{length of gonotrophic cycle}}$$

$$= \frac{11.1}{a}$$

$$h = 11.1 \times 1 \times 0.117$$

$$= 1.3$$

This means that each person in this village (Ntouessong) is likely to receive more than one infective bite a day - a conclusion supported by the increase of the parasite rate in three months from under five to 73 per cent.

Conclusion

These high figures show that two and a half years after spraying stopped and three years after the apparent disappearance of A. gambiae in the pilot zone, this vector attained in the course of a few months the level and the infectivity that it had had before any insecticide treatment; in certain cases it is even possible to speak of a higher degree of infectivity, for example in the village of Nkolmekok already mentioned where we found at the beginning of the dry season a momentary sporozoite rate of 60 per cent. in 300 dissections, a rate never before observed in A. gambiae to our knowledge in Africa.

What, it may be asked, are the chief factors responsible for this reappearance: importation of a dieldrin-resistant strain of high vector efficacy; or residual populations of anopheles developing in "forgotten" localities that are difficult to get at, and subsequently spreading?

It is possible that the causes are multiple; none of the entomological data justify us in favouring one interpretation rather than another.

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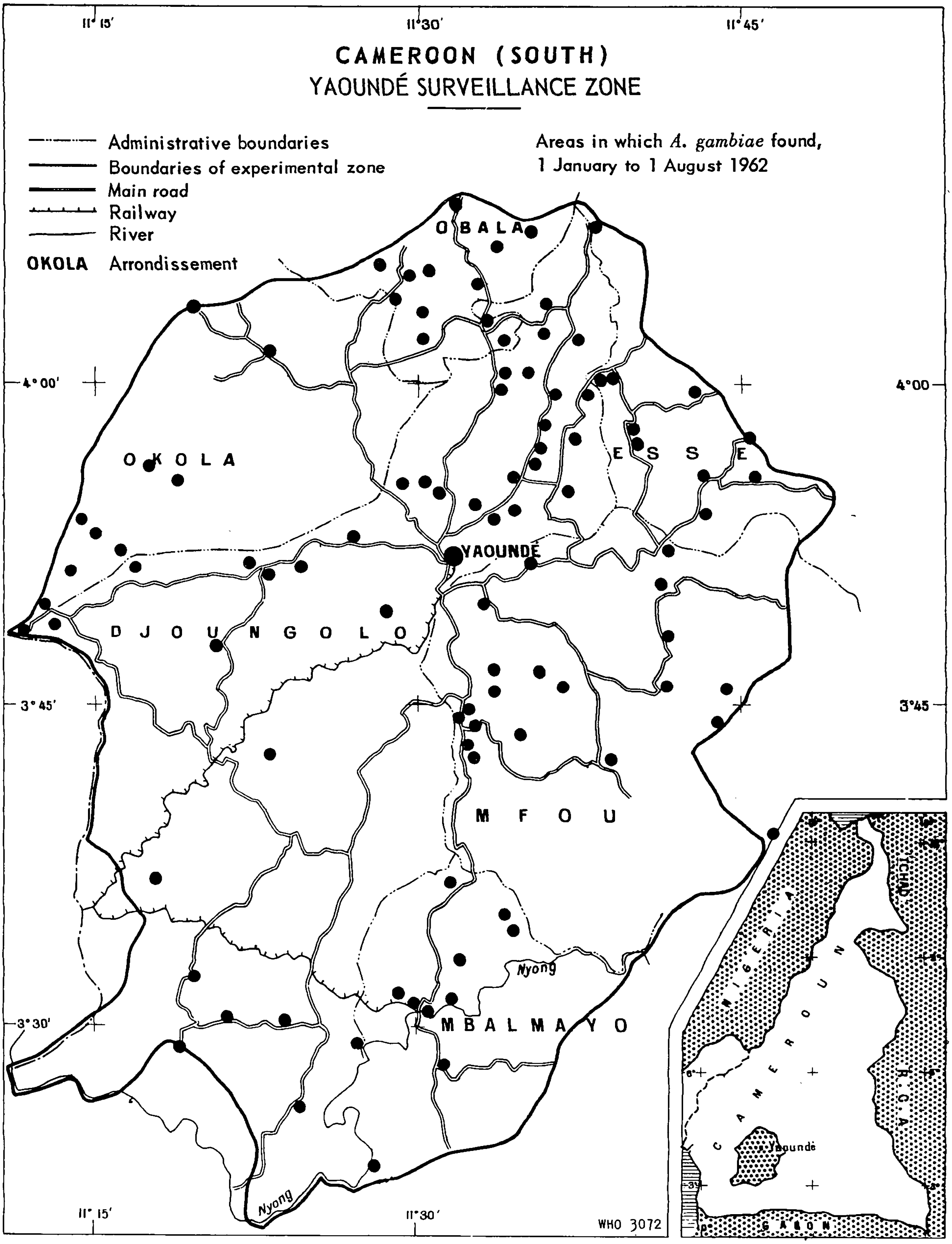
11° 15'
11°30'
11°45'
CAMEROON (SOUTH)
YAOUNDÉ SURVEILLANCE ZONE
Administrative boundaries
Boundaries of experimental zone
Main road
Railway
River
OKOLA Arrondissement
Areas in which A. gambiae found,
1 January to 1 August 1962
4°00'
3°45'
3°30'
OBALA
OKOLA
ESSE
YAOUNDÉ
DJOUNGOLO
MFOU
MBALMAYO
Nyong
NIGERIA
TCHAD
CAMEROUN
R.C.A.
Yaoundé
GABON
WHO 3072