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A FIELD ATTEMPT TO ASSESS THE MATING COMPETITIVENESS OF STERILE MALES PRODUCED BY CROSSING TWO MEMBER SPECIES OF THE ANOPHELES GAMBIAE COMPLEX

by

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General considerations

Laboratory cage experiments carried out in London with sterile males produced by crossing various member species of the Anopheles gambiae complex had shown conclusively that in quite low proportion to normal males they succeeded in mating with females and that these females laid infertile eggs (WHO Technical Report Series No. 268, 1964; Davidson, 1964; Davidson et al., 1967; Davidson, 1969a and 1969b). It was further shown that these females even when subsequently forcibly mated with normal males continued to lay sterile eggs (Bryan, 1968). The latest laboratory experiments (Davidson, 1969b) made use of those crosses which produced a high proportion of sterile male offspring and very few females (which are known to be fertile), viz, crosses between the males of species A and B and the females of A. melas and A. merus. First instar larvae from such crosses were added in varying proportion to breeding bowls containing normal first instar larvae and the mixed larvae reared together to the adult stage. The adults were then caged together and the number of females subsequently laying sterile eggs recorded. At the time of these experiments were being carried out, the likelihood of a field trial of the technique in the village of Pala near Bobo-Dioulasso, Upper Volta, was known. Thus a colony of species A from this village was used in London to cross with A. melas and the F_1 sterile male tested against the Pala species A strain. It was noted in the course of these experiments that this cross, Pala species A male by A. melas female, consistently gave high proportions of female offspring, and that the number of females laying sterile eggs from cages containing mixed normal and sterile males was considerably less then when a cross between the male of a species B strain from Kano, Nigeria, and female A. melas was used against the same species A strain from Pala. This last cross between Kano species B male and A. melas female resulted in far fewer females in the F_1 generation. It was thus decided to use this cross in the field trial in Upper Volta even though it was realized that a cross between two species was being used to attempt to control a third species. Hybrid vigour was considered to be an important characteristic of the sterile male and perhaps it would prove sufficient to overcome the natural mating barrier between species just as successfully as it had in cages. This then was the prime purpose of the field trial to find out if these sterile males would succeed in mating under natural field conditions with the females of a natural population.

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The existence of crosses producing almost nothing but sterile males suggested a simple way of introducing these sterile males into a natural population, namely by addition, at the stage where the F_1 eggs are about to hatch, into the few, permanent, identified, breeding places remaining in the dry season. The larvae destined to produce sterile males would then be expected to develop alongside the natural larvae under the same breeding conditions and the adult sterile males would be expected to emerge at the same time and at the same place as the normal males and females. It was with this idea in mind that computer calculations were made by Cuellar (1969a) of the most favourable ratio of sterile-male-producing eggs to normal eggs required to produce eradication in a comparatively short space of time. This ratio was indicated to be 2:1 (allowing for the fact that only half the hybrid eggs hatch) and if maintained over a period of approximately nine weeks' eradication would be expected, provided that the sterile males were equally good at mating and that no female lived longer than nine weeks. These calculations took into account that the mortality occurring between the egg stage and adult emergence under field conditions must be exceedingly high to maintain a static population (more than 98%) and that the continuous addition of the sterile-male-producing eggs would ensure a continuing high, aquatic-stage mortality.

The successful application of this method in the field would then depend on knowing all the breeding places and on having some method of estimating the natural population size. On a limited scale this would mean a small number of breeding places and in practice would indicate that the method should be applied in the dry season, preferably at the beginning, so that introductions of sterile males would be into a declining population.

A visit to the village of Pala in Upper Volta in October 1967 had shown these conditions of a small number of well-defined breeding places at the beginning of a dry season lasting four to five months to be present but in October 1968 when the field trial was initiated conditions were very different. Unusually late rains continuing into the beginning of November resulted in the presence of numerous, ill-defined, breeding places and it was not until the end of that month that conditions approached those existing in October 1967. Attempts made in October 1968 to estimate first instar larval population sizes by the mark-release-recapture method using larvae stained with Giemsa were of limited success. Laboratory trials with this stain had shown the blue coloration produced was persistent, easily recognized and had little adverse effect on the larvae. However, the number of small well-defined pools in which the method would have been expected to yield results of some accuracy were few and certainly not representative of the majority of breeding places. In fact much of the breeding was at the edges and in quiet back-waters of small rivers and irrigation ditches surrounding the village. Sample dips from different breeding sites near the town of Bobo-Dioulasso, in the village of Pala itself and in the two control villages, Koro and Borodougou, made in the first half of October 1968, showed the presence, usually, of low proportions of advanced larval stages and very small proportions of pupae, confirming a high mortality in the aquatic stages (Table I). Only 1% of more than 6000 aquatic stages counted were pupae. Not all the breeding places were the same in this respect, however. Temporary rain-water pools at Kua for example showed higher proportions of late larval and pupal stages. In Pala itself later, in November, when a rapid drying-up of breeding places was occurring, one pool was sampled over the eight days before it dried up completely and showed very heavy breeding, 80% of the material counted being in the fourth instar and pupal stages. This represented a concentration of breeding from a quite extensive river into a pool rapidly decreasing in size and to which little in the way of new individuals was being added.

To gain some idea of the survival of the sterile-male-producing larvae of the hybrid generation in a natural breeding place 700 of them were added in various larval stages over several days (all those available at the time) to a large pool near Bobo-Dioulasso estimated to contain some 21 000 first instar larvae by the mark-release-recapture technique. Samples of larvae and pupae were taken on successive days after this introduction and were reared to the adult stage in the laboratory. Emerging males were then dissected to see how many were sterile. Only one sterile male was recovered out of 86 dissected. The rough way in which

this experiment was carried out did not enable any accurate predictions to be made of expected proportions of sterile to normal males emerging from this breeding place but the detection of only one sterile male, after 700 were introduced, does indicate an exceedingly high mortality.

As well as being presented with a larger number of breeding places than originally expected, many of them ill-defined, and being faced with the impossibility of assessing larval population sizes with any degree of accuracy, logistic considerations also had to be taken into account in planning the field trial. According to Cuellar (1969a) 2 000 000 hybrid eggs a day would be required to control a daily emerging population of 10 000 males and females while the release of 300 000 adult sterile males would achieve the same object against the same population size, albeit in a slightly longer time, viz, 13 as compared with nine weeks (Cuellar, 1969b). With the time and facilities available it was decided that there would be more likelihood of achieving the required number of adult sterile males than of the eggs and thus the F_1 generation was reared in the laboratory to the pupal stage under ideal conditions and with only a moderate aquatic-stage mortality. The pupae were then to be taken to the village of Pala and there released.

The project area

The village of Pala near the town of Bobo-Dioulasso in Upper Volta was selected as the site for sterile-male releases because of its small size, its relative isolation and its proximity to the laboratory and insectary facilities of the Entomology Section of the Centre Muraz (Office de la Recherche Scientifique et Technique Outre-Mer). These laboratories also serve as a WHO International Reference Centre under Dr J. Hamon, who acts as the principal investigator. In addition, the village is well known entomologically and has served as a site for numerous investigations by French research workers in the past. Close to it are two other similarly isolated villages, Koro and Borodougou, which served as controls.

Actual distances "as the crow flies" between these villages and the town of Bobo-Dioulasso are:

Bobo-Dioulasso centre to Pala	7 km
Pala to Koro	4 km
Koro to Borodougou	4 km
Pala to Borodougou	7 km

It was considered important that the village in which the release of sterile males was to be made was sufficiently isolated that little or no immigration of mosquitos from surrounding areas could occur, that, in fact, any effect of sterile-male mating with local natural females would not be masked by a large immigration of fertilized females from outside the treated area. A survey of the village of Pala and its surroundings did reveal that virtually all breeding was in the immediate vicinity of habitations and the immediate surrounding cultivated land, but at a distance of 2 km to the east and west were two permanent rivers which might have been sources of additional breeding. To establish whether mosquitos emerging from these rivers could reach the village, mosquitos reared in the insectary at Bobo-Dioulasso and marked with a daylight fluorescing pigment "Dayglo" Fire-Orange were released from the rivers at points nearest to the village. The mosquitos used were from eggs derived from the natural population of Pala. Pupae were separated into lots of 50 and adults both male and female allowed to emerge overnight in half-pint unwaxed paper cups with netting tops. The exact number alive in each cup was determined before marking. Marking was done, on the afternoon of the day following emergence, by laying the cups on their sides and puffing twice from a plastic bottle with a quarter-inch diameter tube attached to its cap, and containing the fine red dust. The adults were then released towards dusk. Releases were made between 15 October and 24 October. A total of 8444 males and females was released mostly from the river between Pala and Koro. Only one mosquito was recovered in Pala and this on 4 November. It was a female, still well marked and full-gorged when caught resting inside a house. It subsequently laid eggs which hatched and on dissection was found to have laid three times.

Between 5 and 9 November the insecticide Abate was applied to the two rivers and their associated pools. A waterfall on the river east of Pala was used as a source for an application of 1 ppm for 30 minutes and evidence of its efficiency was given by the disappearance of Simulium larvae for a distance of 1-1/2 km from the point of application. Other places were treated at a rate of 560 mg of active product per 10 m^2. This "insecticidal barrier" was a further insurance of the isolation of the village of Pala.

Pala is a small village of some 500 inhabitants housed in mud-walled and mud-ceilinged dwellings mostly joined on to one another (Figs. 2 and 3). The total number of rooms in the village was estimated to be in the region of 250. A small permanent river arising from underground springs to the west of the village flows close to the north-east border of the village and this, along with a few permanent pools to the north and south-east, forms the main breeding area of A. gambiae in the dry season. In the wet season an additional temporary small river runs to the south of the village to join the permanent one south-east of the village and in addition numerous small rain-water pools occur all around the village. All showed A. gambiae breeding in the rainy season. Fig. 1 shows the village and the surrounding area and pinpoints the actual sites where sterile-male releases were made. To the north of the village a small mud-walled, thatch-roofed house served as a field laboratory where female A. gambiae caught in houses in the village (and in the control villages) were kept for oviposition.

It is almost certain that the A. gambiae population in Pala is entirely composed of species A. More than 40 identifications have been made from this village over the past 10 years. Species B was found in 1958 and again in 1965 and 1966 but since then only species A has been identified - this includes 18 identifications made just prior to the present field trial. Five identifications of species A were made from the control village of Borodougou in the present investigation.

The control village of Borodougou resembled Pala in many ways with permanent water in evidence close to the village in the dry season. Koro differed in being on top of a small hill with permanent water less in evidence and some distance from the village.

Colonies and cross-production

The two species of the A. gambiae complex used to produce sterile males were species B and A. melas. The species B strain was a dieldrin-resistant one originating from Kano, Nigeria, and started as a colony in the Ross Institute in London in 1958. The A. melas strain had its origins near Harbel, Liberia, was started as a colony in the Harbel Institute for Medical Research prior to 1962, and was subcultured in the Ross Institute in London in 1962.

Both strains were sent to Bobo-Dioulasso from London by air in the egg stage. Little difficulty was experienced in starting the species B colony in the insectaries of the Centre Muraz but considerable difficulty was experienced in establishing A. melas. Though numerous consignments of this species were dispatched from as early as January 1968 it was only in July that a self-perpetuating colony was eventually started from a comparatively small number of individuals, presumably through some process of selection of a few mosquitos adapted to the particular conditions of the Centre Muraz insectaries. It was not until the middle of October, however, that the A. melas production was high enough to start crossing to produce sterile males.

The high chlorine content of the town water supply ruled out its use for larval rearing. In its place distilled water with sufficient sea-salt added to give 20% sea water (7 g per litre) was used for rearing A. melas and river water from the river east of Pala, north of the point of introduction of the insecticide, Abate, was used for rearing the species B colony and the cross. Rearing was carried out in rectangular enamel containers of various sizes with strict attention to particular densities for particular surface areas, especially in the case of A. melas. Here one larva per 1.5 in^2 was aimed at. Hybrid larvae were reared in higher density at something of the order of one per in^2.

For the first 24 hours of larval life a small quantity of spinach-juice, produced by pounding spinach leaves, was given and thereafter finely ground Farex (a proprietary cereal baby-food) was sprinkled on to the water surface in greater and greater quantity and at more frequent intervals as the larvae grew in size. The aim was to add more food as soon as, or shortly after, previous food had been removed from the water surface. A change of water when the larvae reached the fourth instar proved advantageous in the case of A. melas but was not usually necessary with species B or the cross. Pupation usually started on the eighth day after the hatching of the eggs.

Pupae were collected by hand and counted into lots of 600 and each lot allowed to emerge into a 30-cm^3 cage. Ten per cent. sucrose solution was provided in each cage and changed every two days. The feeding of the females was on immobilized rabbits laid stomach downwards on the top of the cage. Rabbits were the only suitable small mammals readily available but were on the whole unsatisfactory, being subject to a high handling mortality and being excessively expensive. The species B colony readily laid eggs on damp filter-paper but the A. melas colony and the cross oviposited better on free water in glass dishes lined with filter-paper to prevent the stranding and desiccation of the eggs. All eggs were kept on damp filter-paper for one day before being introduced into the larval rearing containers.

The capacity of the insectary was such that approximately 3000 A. melas and 2600 species B pupae could be produced each day. Of the 3000 A. melas pupae 600 were used for colony maintenance while the other 2400 were set aside for emergence and for the isolation of approximately 1200 virgin females (removed within about 12 hours of emergence) for the cross. Of the 2600 species B pupae 200 were used for colony maintenance while the other 2400 were used as the source of approximately 1200 males for the cross. The cross between species B male and A. melas female was then made in cages of 300 males and 300 females. Thus at any one time seven cages of A. melas, three cages of species B and 28 cages of crosses were in existence producing an average of something like 6000 A. melas, 4000 species B and 40 000 hybrid eggs per day. Approximately 300 enamel containers with an average surface area of 500 in^2 were needed to rear these aquatic stages. As on average less than 50% of the hybrid eggs hatched (those destined to become sterile males; only very few females normally resulted from this cross), a maximum yield of hybrid pupae of something of the order of 15 000 per day was expected, allowing for some aquatic stage mortality. In fact this number was never reached; the maximum was 12 000 on one day and the average at the height of production was only about half of this figure.

For some unknown reason the percentage of females in the hybrid generation produced from the cross species B male by A. melas female fluctuated from a very low level to quite significant proportions (as high as 25%). This gave cause for concern as these hybrid females are capable of producing offspring if mated by normal males. In terms of the present experiment they could have mated with the natural species A. melas present in the village of Pala. The result of such in-mating would be in fact the production of a triple hybrid generation, the sex ratio of which would be expected to be normal but the males of which would again be sterile. Such a triple hybrid generation was purposely produced in the laboratory by caging hybrid females with males of an existing self-perpetuating colony of species A derived from the village of Pala and maintained over many generations by the Centre Muraz staff. The offspring did in fact show a normal sex ratio with all the males sterile, but the females remained fertile and could be again successfully crossed to species A males.

Of equal concern was the possibility that these hybrid females in the field would "take up" sterile males which might otherwise mate with the females of the natural species A population. Evidence was found that this was in fact occurring. Fortunately the eggs of hybrid females could be distinguished from species A eggs. They did in fact resemble those of the mother species, viz, A. melas, having a broad deck and no significant gap between the dorsal edge of the float and the frill of the deck. A. gambiae species A eggs usually have a narrow, waisted deck and a considerable gap laterally between float and frill. Some of the eggs laid

by females caught in houses in Pala after sterile-male releases were made were of the hybrid type. None hatched, however, and it is concluded that the mating natural species A male by hybrid female never took place but that matings between the sterile males and hybrid females did occur occasionally.

Considerable time and effort had been devoted in London to an investigation of the reason for the appearance of females in the hybrid generation. A number of single families of both parent strains (species B and A. melas) were raised and crossed individually in an attempt to find two families which when crossed would consistently give very high proportions of male offspring. Initial apparent success in this search was followed by a later "reversion" to the state of inexplicable appearance of high female ratios and the problem remains unsolved.

The species B strain from Kano used in these investigations had been selected in the past for the marker mutant Black-diamond (Mason, 1967). This appears to be a single, autosomal, dominant gene and is expressed as a dark, diamond-shaped, patch of pigment on the larval thorax. In the early larval instars it can be seen in both sexes. In the fourth larval instar, however, it is only expressed in the female; it disappears with larval growth in the male. Being dominant it is also expressed in females of the hybrid generation. Thus its presence in fourth stage larvae of the hybrid generation indicated the presence of females and a constant watch was kept for it. Where it was present in considerable numbers larvae with it were removed. Unfortunately the parent species B strain was not pure for the marker gene and a proportion of the females in both parent and hybrid generations were unmarked. Regular sampling of the sex ratio of hybrid generation was done in addition to the searching for marked larvae and the fluctuation in the percentage of females is given in Table II. Regular dissections of hybrid males were also carried out to confirm sterility and ensure that no mistakes had been made in the crossing procedure. No evidence of any mistake was ever found.

Hybrid pupal releases

Hybrid pupae were collected by hand and separated into lots estimated by experience to be between 500 and 1000. Counting as such was not done but an indirect measurement of quantity was used. A glass funnel 16 cm in diameter was graduated by pouring in lots of 100 pupae in an excess of water and running off the water until the pupae formed a continuous, uninterrupted film when at rest. If no shadow was cast and the water run off very slowly an indication of the "end-point" was given when "overcrowding" would cause sudden violent activity among the pupae. When graduation was completed the unknown quantities could be measured with a high degree of accuracy (v. infra).

For the transportation of the pupae from the laboratory to the village of Pala, a distance of 9 km by road, batches were filtered through gauze squares which were then laid on absorbent cotton-wool in 9-cm-diameter, plastic Petri-dishes. Water was added from a pipette to float out the pupae into a single layer, after which the excess water was again removed with the pipette. Each container was labelled with the estimated number of pupae. The pupae were released in the field merely by removing the gauze from the Petri-dish and floating off the pupae (Fig. 5).

Throughout the period of pupal releases a check was kept on the accuracy of the funnel method of estimating pupal numbers, the sex ratio of emerging adults and the mortality occurring due to handling and transportation by allowing some of the batches of pupae to produce adults from artificial containers, usually glass Kilner jars placed in different positions in the village of Pala. These containers were collected on the day following adult emergence, taken back to the laboratory in Bobo-Dioulasso and there the contents were carefully sorted and counted into pupal skins, dead pupae and dead adults. A sample of the pupal skins was then sexed by examination of the terminalia under a binocular dissecting microscope.

This proved a very simple and rapid method of checking the sex ratio. The total pupal skins sexed in this way were 18 120 and the percentage female was just over 7%. The mortality determined from these jar counts averaged 10% of 56 651 individuals counted and this latter number approached very closely the estimated number of 54 450 determined by funnel measurement.

The method and site of pupal release in the village of Pala were of necessity varied according to the quite dramatic climatic changes which occurred during the period from the end of October 1968 until the beginning of January 1969. Initially, on logical grounds, it was decided that the ideal release sites would be those breeding places showing heavy larval densities with high proportions of advanced stages, this in itself indicating lack of predators. Four such places were found around the village (A, L, M and N in Fig. 1; L and M are depicted in Figs. 4 and 5) and hybrid pupae were released in these for about the first month along with sample jars of pupae immersed in water at H, O and P (see Fig. 1) to give indications of mortalities (v. supra). Masses of pupal skins were recovered from the natural breeding places indicating good adult emergence. From 24 November, however, a marked change in climate occurred with night-time air temperatures falling as low as 12°C. At the same time the natural breeding places used as points of release dried up; the last rainfall of the year was on 3 November. On the first of these cold nights emergences from various types of artificial containers were tried both in empty houses in the village (B and F in Fig. 1) and immersed in the remaining water of the river at H and a remaining pool at P (see Fig. 1). Considerable mortality occurred in all the containers in all the sites but particularly in aluminium pots where the heat exchange had been greatest. Clay pots and glass jars were more satisfactory but if immersed in water outside it was essential that the water-level inside and outside the container was the same. Where heat exchange had been great a prolongation of the pupal stage by several hours was evident and containers still with live pupae in them were left for a further day. All containers both inside houses and immersed in water had to be protected from predators, in particular goats, sheep, chickens and large lizards of the genus Varanus. This was done by covering them with wire chicken-netting, but this did not prevent the entry of small frogs into those containers immersed in the river and in pools.

By the end of November all that was left in the way of water in and around the village was the river, which incidentally was polluted in parts by the washing of clothes and cooking utensils by the villagers, and permanent pools to the north and south-east. Light A. gambiae breeding was evident in some of these pools and as the water temperature remained above 20°C it was decided to release pupae in them though some risk of loss through predators (frogs and fish) was inevitable. Thus releases were made at Q, R, S and T (see Fig. 1) from 29 November to 5 December. Pupal skins could be found in these pools after release but never in the large quantities found in the earlier releases. Because of this and because of the relative remoteness of most of the pools from the village it was eventually decided to release again from glass Kilner jars housed in a bed of straw (Fig. 7) inside two large mosquito cages (50 cm^3) placed at point J on the north-east side of the village on the edge of the river. The cages were large enough to hold six to nine jars and the feet of the cages were immersed in water containers as a protection against ant invasion. The mosquito-netting top of each cage was replaced by wire chicken-netting, the holes in which were large enough to allow escape of the sterile males while forming a protection against predators. All further releases until 3 January 1969 were made from these cages. Direct estimates of mortalities and sex ratios were made from sample jars on each day. This was considered to be the most satisfactory method of release under the prevailing climatic circumstances.

Post-release observations

Two kinds of hand-catch were made at regular intervals after sterile-male releases started in the village of Pala: of mosquitos resting inside houses and of those resting outside in specially constructed shelters. Two types of the latter were tried: the conventional pit-shelter of the Murihead-Thomson type (Fig. 6) and oil-drums let into the banks of the rivers. The latter type yielded very little; most of the outside-resting mosquitos were

derived from two Muirhead-Thomson pit-shelters situated on two sides of the village (see Fig. 1). In the control villages only house-catches were made. House-catches yielded both males and females. Pit-shelter catches gave mostly males and only an occasional female.

/Light traps were also tried both inside and outside houses but yielded only the occasional male and female A. gambiae./

Female A. gambiae caught were tubed in glass or plastic containers with netting tops through which water was added to a depth of 0.5 cm for oviposition. These tubes with females both from Pala and from the control villages were kept in the "field laboratory" at Pala adequately protected against ant invasion and covered to maintain a high humidity. Any females not developing eggs were dissected to find out whether they were fertilized or not and whether nulliparous or parous. Those dying in the gravid state without having oviposited were also dissected to see if they had spermatozoa in the spermatheca. Ovipositions were classified as normal in size and floating, small and sunken. They were kept for several days if they did not hatch. Samples of all but the sunken eggs (which were difficult to identify) were examined whether they hatched or not to see if they were species A or hybrid. Females from Pala which oviposited were released in the "field laboratory" and the ovipositions from these females which hatched were returned to breeding places in the village.

Male A. gambiae were dissected and classified from the appearance of the testes as sterile or normal. The difference was very marked and could be seen under the high power of the binocular dissecting microscope after rupture of the testes with a needle. The normal showed the unwinding coils of mature spermatozoa. The abnormal showed the discrete spermatocytes (round cells) and immature spermatozoa.

Results

Table II sets out the detailed results in treated and control villages before and, in periods mainly of one week, after the release of sterile males. An unfortunate accidental insecticidal contamination of a part of the insectary in which the hybrid generation was being reared resulted in only very small releases in the first period from 30 October to 9 November. Peak production of the order of 7000 pupae a day occurred over the period 24 November to 14 December. After this a decline occurred associated with the effect of the cooler weather lengthening the gonotrophic cycle of the adult female and the larval aquatic cycle. Releases ceased on 3 January 1969 when approximately 1000 larvae remaining in the rearing bowls were released in the permanent water remaining in Pala.

Mortalities, as estimated from sample containers, started off at the low level of 5% but increased to around 15% at the period of peak production which, as will be remembered, coincided with the advent of adverse weather conditions and the drying-up of the main breeding sites. A gradual improvement in mortalities in the latter part of December is attributed to the change in method of release to that from glass jars bedded in straw in large cages.

The percentage of females in the hybrid generation exceeded 10% at the beginning of significant releases but declined rapidly to negligible levels for the remainder of the release period.

That the sterile males were departing successfully from the release points and mixing in significant proportion with the natural A. gambiae population is shown by their capture both resting in houses and in the outside shelters. In fact from the middle of November onwards, about 75% of the males caught were sterile ones; the proportion in outside shelters slightly exceeded that in houses.

Between mid-September and the end of October the number of female A. gambiae caught per room in the village of Pala averaged 6.5. An average of 9.9 females per room in September had declined to an average of 5.2 in October. The control village of Koro showed the slightly higher figure of 7.2 in the month of October while Borodougou, where most of the control collections were made, showed the significantly higher average of 14.9 in October. This latter control village showed densities consistently higher than Koro and Pala. When sterile-male releases started on a small scale the density in Pala was of the order of seven female A. gambiae per room but this fell sharply in the middle of November to a figure of two per room and thereafter declined steadily until at the end of December no females were recovered by the hand-catching method. Apart from two caught on 7 January no further females were captured in this village though searches continued until 13 January. Control catches declined markedly until the end of November but then showed a temporary increase in the first half of December followed by a fall again in December but even on 3 January nearly two females per room could still be found in Borodougou.

A total of 1804 female A. gambiae was tubed from the village of Pala before sterile-male releases were made and from the control villages of Koro and Borodougou over the whole period of the experiment. Seventy per cent. of them laid eggs, exactly the same proportion as those tubed from Pala (numbering 502) after releases started. Of the latter, 23% died in the gravid state without laying; 4% of these appeared to be without spermatozoa in the spermatheca. Seven per cent. did not develop eggs; three-quarters of these were nulliparous and mostly without spermatozoa; a single parous female without spermatozoa could have been a hybrid or Pala female mated with a sterile male. In the control villages over the same period of sterile-male releases slightly higher percentages of females did not develop eggs or died in the gravid state without laying. Again most of the non-developers were nulliparous and a small percentage of the dead-gravids had no spermatozoa. All the parous non-developers were positive for spermatozoa. Details of these observations are given in Table III.

Of 1245 layings from female A. gambiae caught in Pala before sterile-male releases and from the two control villages throughout the experiment, 1214 hatched; 22 of the 31 which did not hatch were considered as abnormal layings; only two of these abnormal layings came from Pala. After sterile-male releases the percentage of abnormal layings was about the same in both Pala and the control villages (in the region of 5%). The total percentage of normal layings which did not hatch was 5.88 from female A. gambiae caught in Pala in the two-and-a-half months over which sterile males were released. In the same period in the control villages only 1.35% of normal layings did not hatch. However, 2.52% of the normal layings from Pala proved to be from hybrid females and only 3.36% from species A females.

Discussion

An attempt has been made in Table IV to simplify the detailed results of Table II, to calculate an approximate total daily emergence of natural male and female A. gambiae in the village of Pala from the number of females caught per room and to relate these figures to the number of sterile males thought to be required from Cuellar's (1969b) estimation of 300 000 sterile males to eradicate a daily emerging population of 10 000 males and females in a period of 13 weeks. The figure for the actual number of sterile males released makes a generous allowance for mortality before and at emergence and for the emergence of some females.

It can be seen from these calculations that only from the second week in November did the number of sterile males released exceed the estimated number required, though it must be emphasized that many of the basic figures used in the calculations are to some extent "inspired guesses". The proportion of normal species A ovipositions which did not hatch in the village of Pala, though definitely exceeding that in the control villages, did not show any definite sign of increase as the sterile males released approached and exceeded the numbers estimated to be required, however. The conclusion is reached, therefore, that the sterile males were not mating on any significant scale with the natural species A females. The sterile males were certainly present in high proportion as judged from their capture both in houses and in outside

resting places and did mate with their own females as witnessed by the number of hybrid ovipositions which did not hatch. Nine out of 21 normal-looking ovipositions which did not hatch proved to be from hybrid females.

Other factors may also have contributed to this failure to produce significant numbers of sterile ovipositions. Low night-time temperatures may have affected the behaviour of the introduced sterile males which, it may be remembered, were reared under artificial conditions on artificial food. Marked fluctuations in temperature were recorded in the enamel containers in which they were raised, from as low as 17°C to as high as 34°C on the same day. Fluctuations in natural breeding places were less. The lowest of the few water temperatures recorded was 19°C and the highest 24°C. Morning visits to release sites also showed the presence of emerged sterile males resting very close to and often still inside pupal containers, just above the water.

A further factor, already referred to, may have been the use of a hybrid between two species to compete with a third. If some form of barrier exists which prevents the sibling species of the A. gambiae complex from crossing under natural conditions, and all available evidence points to the existence of such a barrier (Paterson, 1967; Ramsdale, 1967), then the barrier between the elements of two species and a third must be equally, if not more, effective. The fact remains, however, that such barriers are not effective under cage conditions and it was the primary purpose of this field trial to find out whether the same successful results attained in the laboratory cage would be achieved under natural conditions allowing a natural mating behaviour pattern. The answer from this trial would appear to be that a small amount of mating does occur between sterile male and natural female but that the proportion of sterile males required to produce any marked reduction in population size would be much higher than the calculations made by Cuellar (1969a and 1969b) had indicated. These calculations assumed an equal mating ability of sterile and normal male, as had been indicated from laboratory cage experiments.

TABLE I. THE PERCENTAGE DISTRIBUTION OF AQUATIC STAGES IN BREEDING PLACES IN THE VICINITY OF BOBO-DIOULASSO IN OCTOBER AND NOVEMBER, 1968

Larval instars	Locality and dates							
	Bobo-Dioulasso town	Bobo-Dioulasso outskirts	Kua	Pâla	Koro	Borodougou	Total	Pala "M"
	9-10/10	11-15/10	3-5/10	8-19/10	7-14/10	7-11/10	3-19/10	20-27/11
I	39 (96)	66 (1264)	50 (195)	47 (747)	58 (111)	70 (1412)	61 (3825)	<1 (5)
II	20 (50)	21 (407)	13 (50)	21 (322)	21 (41)	21 (429)	20 (1299)	2 (39)
III	22 (55)	9 (164)	14 (55)	18 (275)	15 (29)	5 (105)	11 (683)	18 (328)
IV	18 (44)	3 (56)	18 (70)	14 (211)	6 (11)	3 (59)	7 (451)	62 (1085)
Pupae	1 (3)	1 (14)	5 (22)	<1 (5)	0 (0)	1 (16)	1 (60)	18 (312)

The numbers in parentheses are the actual numbers on which the percentages are based.

TABLE II. DETAILED RESULTS OF HAPPENINGS IN THE VILLAGE OF PALA AND IN TWO CONTROL VILLAGES BEFORE AND AFTER THE RELEASE OF STERILE MALES IN PALA

	Period										
	Before sterile-male release	After sterile-male release									
	28/8-29/10	30/10-9/11	10-16/11	17-23/11	24-30/11	1-7/12	8-14/12	15-21/12	22-28/12	29/12-3/1	4-13/1
PALA											
No. of pupae released	0	5 463	22 730	34 900	51 670	52 580	49 580	39 320	22 060	17 510	0
Mortality %			5(1803)	8(10 175)	10(8727)	15(6633)	16(8339)	12(7779)	12(6910)	9(6285)	
Females %	6(1866)	1(218)	11(1284)	11(9223)	5(3620)	5(1076)	1(2217)	3(1738)	3(1200)	<1(1100)	
% sterile males in houses		0(12)		38(24)	44(32)	80(25)	82(67)	55(22)	85(19)	91(32)	62(42)
% sterile males in outside shelters				94(28)	88(88)	89(27)	100(16)	50(2)	100(4)	50(2)	50(6)
No. of rooms searched	113	24		44	100	53	176	58	52	52	115
No. of females per room	3.3	6.8		1.9	1.3	0.9	0.4	0.3	0.1	0	0.02
No. of females ovipositing	585	96		61	89	35	60	10	4	0	2
No. of ovipositions hatching	581	92		49	82	29	53	9	3	0	2
Non-hatchers:											
Normal - species A	2	1		3	3	2	3	0	0	0	0
Normal - hybrid	0	0		4	2	1	2	0	0	0	0
Abnormal	2	3		5	2	3	2	1	1	0	0
CONTROLS											
No. of rooms searched	64	5	13	44	29	15	8	29		14	
No. of females per room	11.3	20.4	4.5	1.6	1.5	4.3	5.8	2.1		1.8	
No. of females ovipositing	364	54	40	41	33	39	27	41		21	
No. of ovipositions hatching	357	45	37	39	32	37	27	39		20	
Non-hatchers:											
Normal - species A	3	0	0	1	0	2	0	0		1	
Abnormal	4	9	3	1	1	0	0	2		0	

The numbers in parentheses are the actual numbers on which the percentages are based.

TABLE III. THE PERCENTAGE OF FEMALE A. GAMBIAE OVIPOSITING IN PALA AND THE CONTROL VILLAGES AND THE CONDITION OF THOSE NOT OVIPOSITING

	No. of females tubed	% oviposition	Non-developers					Dead and gravid		
			% not developing eggs	% nulliparous and sperm negative	% nulliparous and sperm positive	% parous and sperm negative	% parous and sperm positive	% dying gravid without laying	% sperm negative	% sperm positive
PALA after sterile-male release	502	70(349)	7(36)	65	9	3	23	23(117)	4	96
CONTROLS (over the same period)	616	65(398)	10(61)	69	13	0	18	25(157)	3	97

TABLE IV. PREDICTED AND ACTUAL HAPPENINGS IN THE VILLAGE OF PALA AND IN THE CONTROL VILLAGES AFTER THE RELEASE OF STERILE MALES IN PALA

Period	Females per room per day	Calculated daily emergence[a] (♂ + ♀)	Sterile males required per day[b]	Actual sterile males emerged per day[c]	% of males captured which were sterile[d]	Pala normal species A layings which did not hatch	Control normal layings which did not hatch
10-16/11	5	2 000	60 000	2 600	-	-	0/40
17-23/11	2	800	24 000	4 000	68	3/61	1/41
24-30/11	1.3	520	15 600	5 900	76	3/89	0/33
1-7/12	1	400	12 000	6 000	85	2/35	2/39
8-14/12	0.4	160	4 800	5 700	86	3/60	0/27
15-21/12	0.3	120	3 600	4 500	54	0/10	0/41
22-28/12	0.1	80	2 400	2 500	87	0/4	-
29/12-3/1	0	?	?	2 300	89	-	0/21
4/1-13/1[e]	0.02	8	240	?	61	0/2	-

[a] No. of females per room caught by hand is estimated to be 50% of those present and these represent 1/4 of the total allowing for 75% exophily. It is assumed that there are 250 rooms in Pala.

'p' is reckoned as 0.9, the number of ovipositions taken as 3 and the time for these as 10 days.

Thus 5 per room = $\frac{5 \times 2 \times 4 \times 250}{10}$ = 1000 females
= 2000 males + females per day

[b] Based on Cuellar (1969b) who calculates that 300 000 sterile males per day will eradicate a population of 10 000 emerging adults of both sexes per day in 13 weeks.

[c] Calculated from daily average for that period less 20% for mortality (varied from 5-16%) and hybrid females (these varied from less than 1% to 11% but most of the time were less than 5%).

[d] Captured both in houses and outside shelters.

[e] Last pupal release was on 3/1/69 but about 1000 larvae were also released and would be expected to produce a few males over the following days.

SUMMARY

1. Sterile males produced by crossing two member species of the A. gambiae complex have been released into a natural population of species A of this complex in a small isolated village near Bobo-Dioulasso, Upper Volta.

2. The two species crossed to produce sterile males were species B and A. melas and the male of the former crossed with the female of the latter produced an F_1 generation almost entirely composed of sterile males.

3. Releases were made in the pupal stage from artificial containers or by introduction into natural breeding places.

4. Significant releases were made over a period of about two months into a declining natural population though for the first month it is doubtful whether the numbers were adequate.

5. A high proportion (some 75%) of males caught resting in houses and in outside shelters after releases started were shown to be sterile.

6. Only a very small percentage (3.36%) of ovipositions from females caught in the village where sterile male releases were made were considered to be normal ovipositions, from natural females, which did not hatch. In control villages 1.35% of normal-looking ovipositions did not hatch.

7. While recognizing that factors such as the adverse climate conditions, the artificial conditions in which the sterile males were reared up to the pupal stage and possibly the inadequate numbers of sterile males released over part of the time may have contributed to the failure to produce a higher proportion of sterile ovipositions, it is generally concluded that while the sterile males are highly competitive in their mating behaviour with normal males in the confines of laboratory cages they are not so under the free natural conditions of the field. Additionally, the use of a cross between two species to control a third may have been a further barrier to sterile male and natural female mating.

ACKNOWLEDGEMENTS

This project could not have been carried out without the full co-operation of the Laboratoire d'Entomologie, Centre Muraz, Bobo-Dioulasso. This was unstintingly given by Monsieur Jacques Hamon and his staff both in providing facilities for rearing the large numbers of mosquitos involved, in providing ample technical assistance and in giving the benefit of their long experience of local conditions.

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FIG. 1 PALA VILLAGE AND SURROUNDINGS

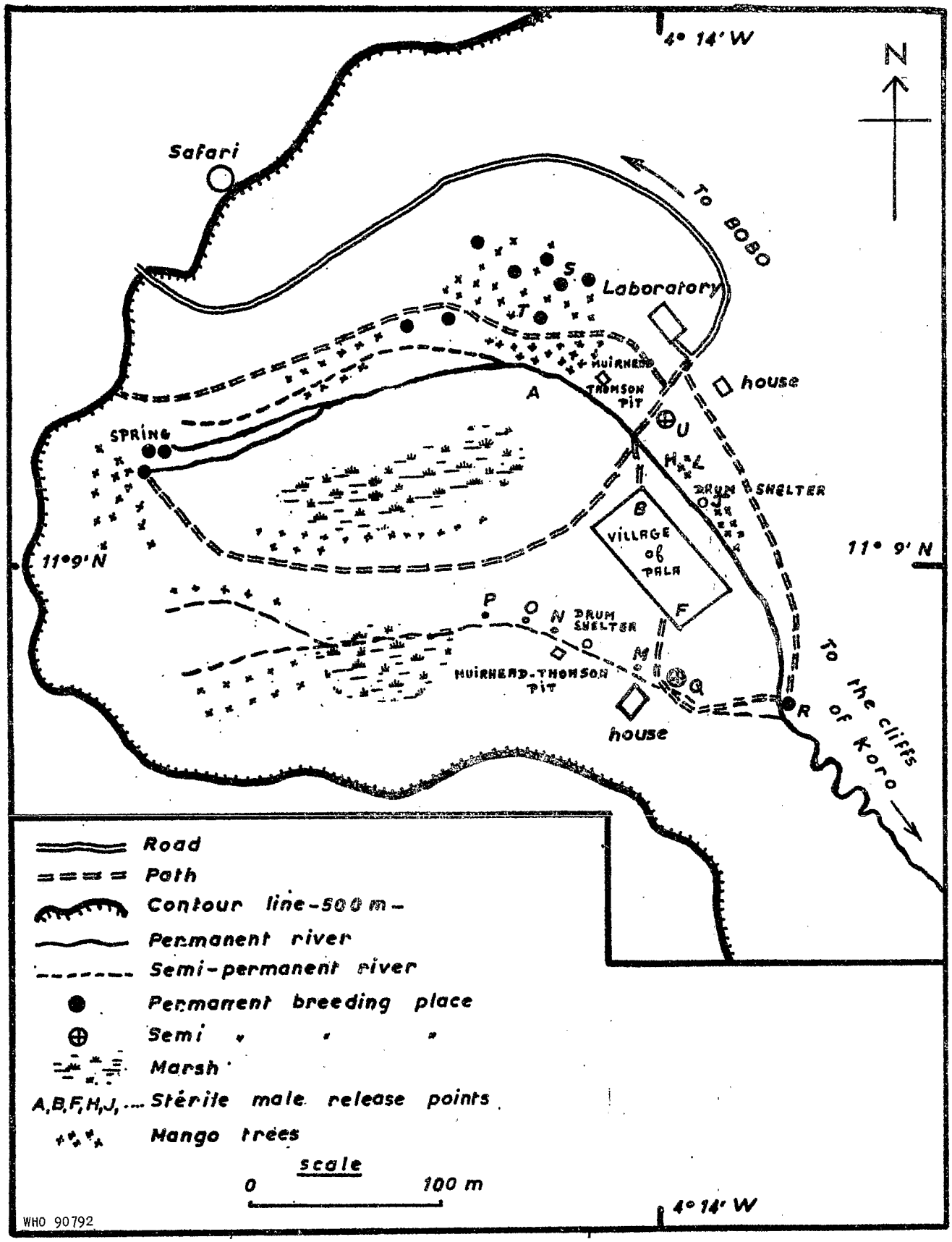


FIG. 2. PALA VILLAGE

WHO 90793

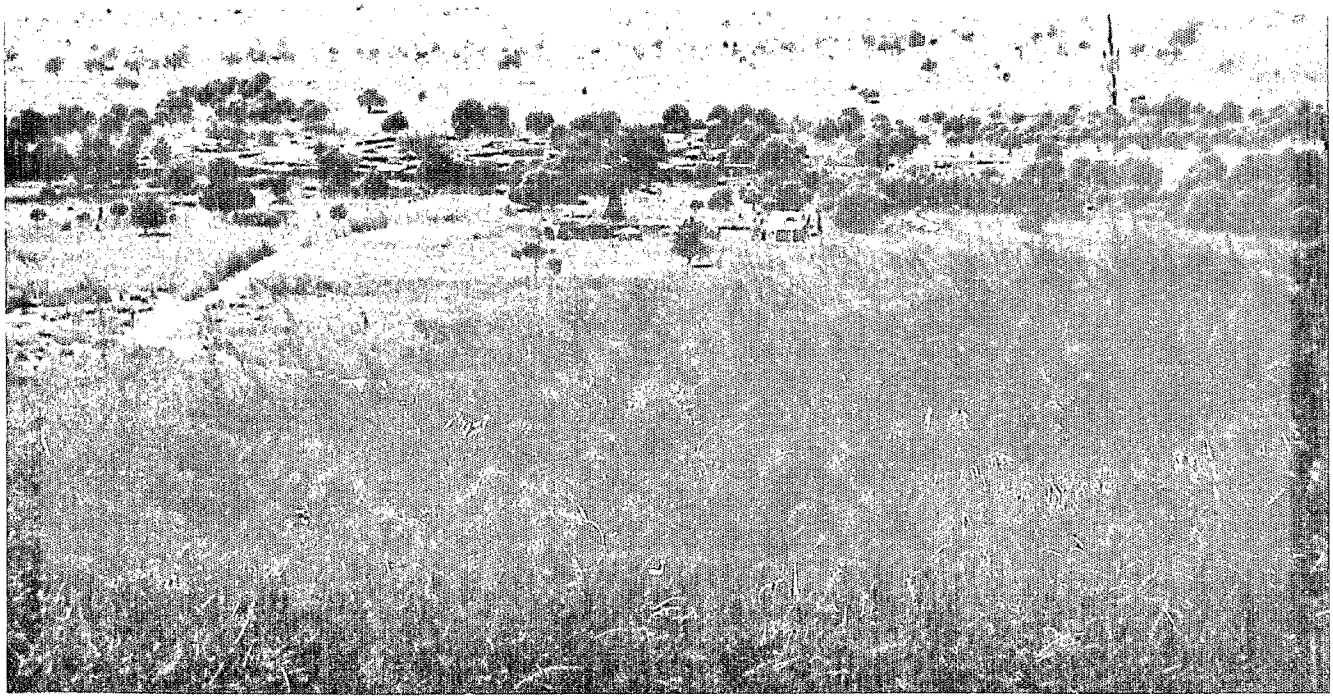


FIG. 3. INSIDE THE VILLAGE OF PALA

WHO 90794

FIG. 4. BREEDING SITE L (ACTUAL SIZE ABOUT 0.5 m IN DIAMETER)

WHO 90795

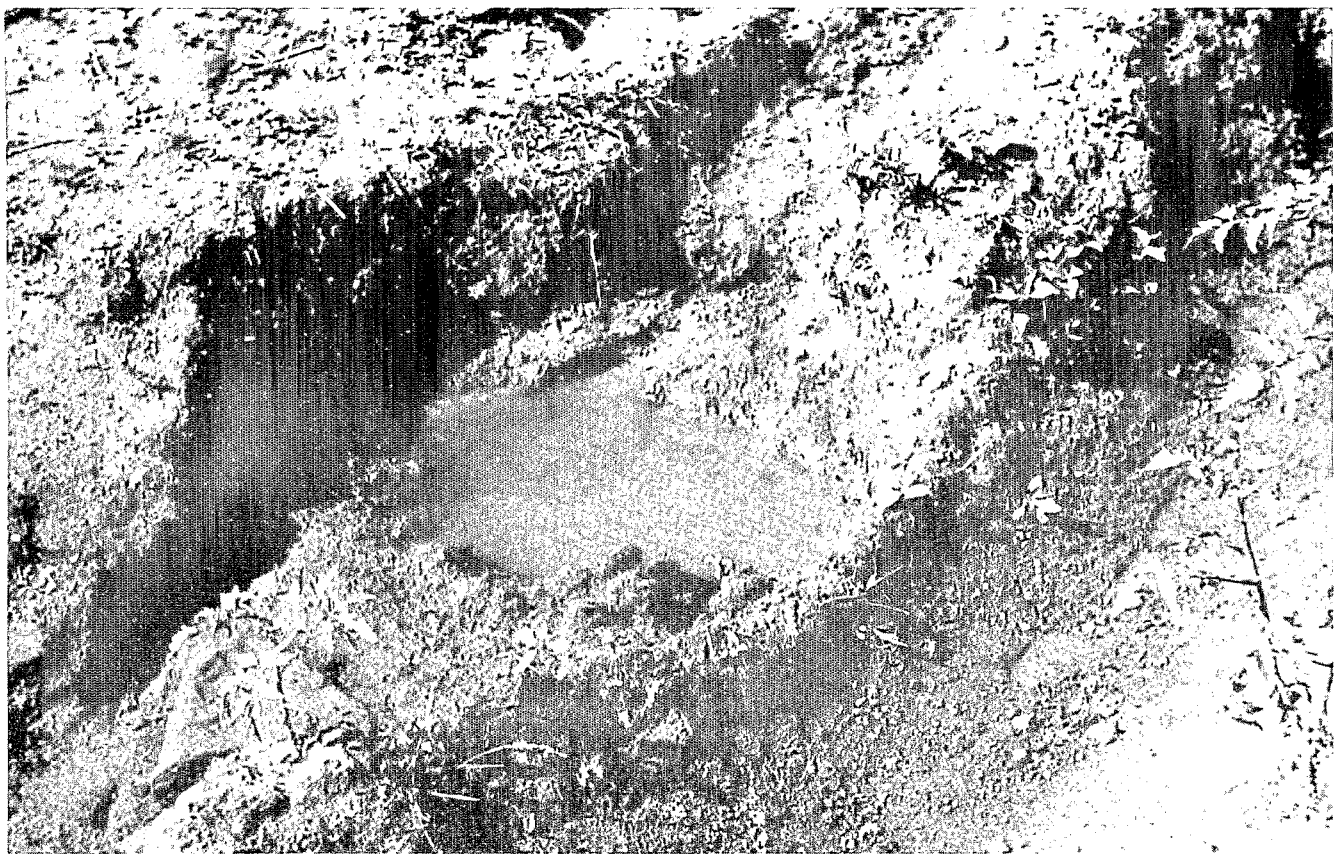


FIG. 5. RELEASING PUPAE OF STERILE MALES AT BREEDING SITE M.

WHO 90796

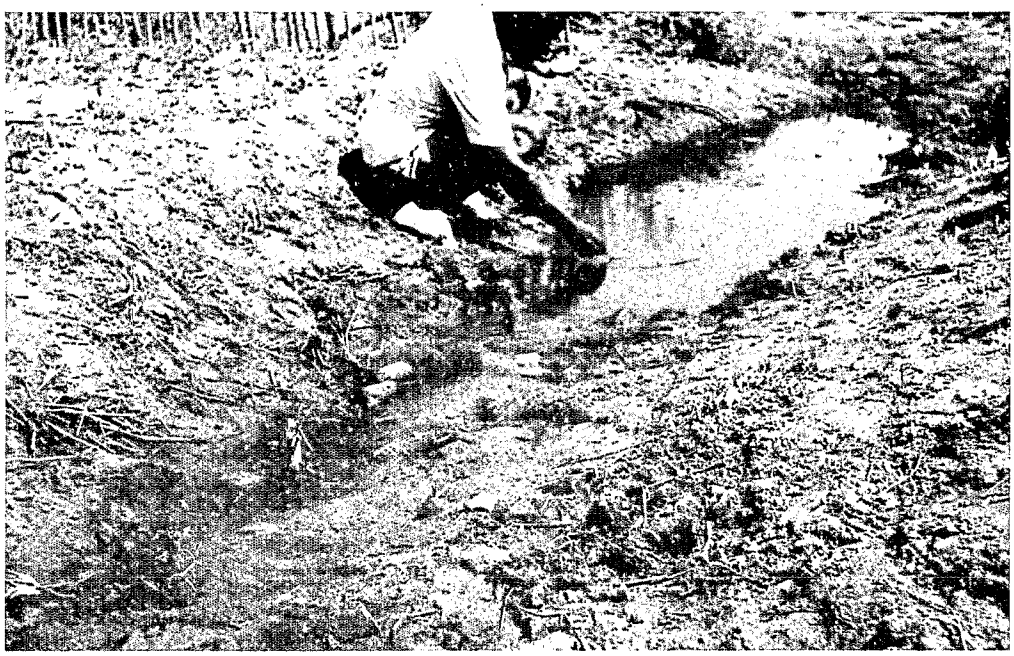
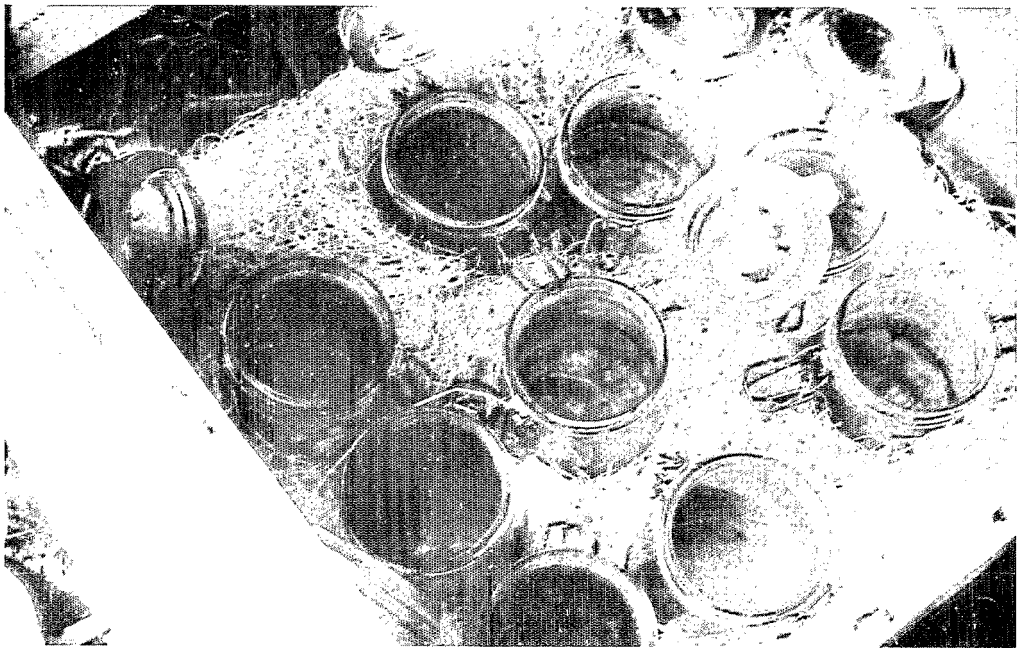


FIG. 6. SEARCHING FOR OUTSIDE-RESTING MOSQUITOS IN A MUIRHEAD-THOMSON PIT

WHO 90797

FIG. 7. KILNER JARS BEDDED IN STRAW AND CONTAINING PUPAE OF STERILE MALES

WHO 90798



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WHO/VBC/69.149 Corr. 1
WHO/MAL/69.687 Corr. 1

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CORRIGENDA

Page 6, line 18 : 250 to read 311
Page 9, line 6 : 10% to read 5%
Page 10, line 6 : A. melas to read A males

Under References :

Cuellar, C.B. (1969a) In press to read Bull. Wld Hlth Org., 40, 205-212
Cuellar, C.B. (1969b) In press to read Bull. Wld Hlth Org., 40, 213-219
Davidson, G. (1969) In press to read Bull. Wld Hlth Org., 40, 221-228

Under Acknowledgements read: This project could not have been carried out without the full co-operation of the Director of the Centre Muraz, Bobo Dioulasso. Our special thanks go to Monsieur Jacques Hamon and his staff for providing facilities for rearing the large numbers of mosquitos involved, for providing ample technical assistance and for giving the benefit of their long experience of local conditions.

New Table IV (recalculated from the increase in the number of rooms in Pala from 250 to 300)

TABLE IV : PREDICTED AND ACTUAL HAPPENINGS IN THE VILLAGE OF PALA AND IN THE CONTROL VILLAGES AFTER THE RELEASE OF STERILE MALES IN PALA

Period	Females per room per day	(A) Calculated daily emergence (♂ + ♀)	(B) Sterile males required per day	(C) Actual sterile males emerged per day	(D) % of males captured which were sterile	Pala normal species A layings which did not hatch	Control normal layings which did not hatch
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17-23/11	2	960	28 800	4 000	68	3/61	1/41
24-30/11	1.3	624	18 720	5 900	76	3/89	0/33
1- 7/12	1	480	14 400	6 000	85	2/35	2/39
8-14/12	0.4	192	5 760	5 700	86	3/60	0/27
15-21/12	0.3	144	4 320	4 500	54	0/10	0/41
22-28/12	0.1	48	1 440	2 500	87	0/4	-
29/12-3/1	0	?	?	2 300	89	-	0/21
(E) 4/1-13/1	0.02	10	300	?	61	0/2	-

(A) No. of females per room caught by hand is estimated to be 50% of those present and these represent 1/4 of the total allowing for 75% exophily. It is assumed that there are 300 rooms in Pala.

'p' is reckoned as 0.9, the number of ovipositions taken as 3 and the time for these as 10 days.

Thus 5 per room = $\frac{5 \times 2 \times 4 \times 300}{10}$ = 1200 females

= 2400 males + females per day

(B) Based on Cuellar (1969b) who calculates that 300 000 sterile males per day will eradicate a population of 10 000 emerging adults of both sexes per day in 13 weeks.

(C) Calculated from daily average for that period less 20% for mortality (varied from 5-16%) and hybrid females (these varied from less than 1% to 11% but most of the time were less than 5%).

(D) Captured both in houses and outside shelters.

(E) Last pupal release was on 3/1/69 but about 1000 larvae were also released and would be expected to produce a few males over the following days.