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NOTES ON THE BIONOMICS OF ANOPHELES CAMPESTRIS, REID, AND ON ITS DISAPPEARANCE FOLLOWING HOUSE-SPRAYING WITH RESIDUAL INSECTICIDES

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Summary.

Entomological surveys made in the malaria eradication pilot project area in Selangor prior to the start of house spraying revealed the presence of *Anopheles campestris* Reid, an important vector of malaria and filariasis in Malaya. It was mainly confined to a narrow coastal belt which extended beyond both the northern and southern boundaries of the experimental area. *A. campestris* is anthropophilic, it is also both endophagic and endophilic.

Following house spraying with DDT, this species disappeared from the project area, but it could still be demonstrated beyond the boundaries of the sprayed area. There is convincing evidence that *A. campestris* also disappeared from another area which has been regularly sprayed with dieldrin for malaria control purposes.

It is considered that house spraying with residual insecticides would be the most effective and most convenient method of malaria control in any area where *A. campestris* is the sole, or the more important vector of malaria. So far as any future malaria eradication programme is concerned, the response of this mosquito is extremely encouraging.

Introduction.

In February 1960 a malaria eradication pilot project was started by the Government of the Federation of Malaya, with the assistance of the World Health Organisation, in a part of the State of Selangor. The purpose of this pilot project was to investigate the possibility of malaria eradication in Malaya by means of the simultaneous spraying of houses with a residual insecticide (DDT) and the distribution of anti-malarial drugs. An area of approximately 500 square miles considered typical of the rural parts of western Malaya was chosen for the project. It stretches from the west coast, inland across the coastal plain, to the central spinal hill range.

Prior to the introduction of malaria eradication techniques into the area, a thorough entomological survey was made by a variety of methods so as to ascertain the distribution of the known malaria vectors and other anopheline mosquitos. Surveys were also made in localities contiguous with the project area.

During these preliminary surveys, *Anopheles campestris* Reid, (1962), which was previously known as the dark-winged form of *A. barbirostris* (Reid 1942) was commonly found along a coastal belt, which continued beyond both the northern and southern boundaries of the experimental area and also extended inland for a short distance along the Selangor River. Information available shows that this species is an important vector of human malaria in Malaya, (Hodgkin, 1956; Reid, 1962), although at times in the past its status as a vector has been in doubt because of confusion with other members of the *A. barbirostris* group, and also because it is usually found in association with other vectors such as *A. letifer* and *A. sundaicus*. *A. campestris* is also a vector of filariasis due to the periodic form of *Brugia malayi*, (Reid 1953).

The Study Area

The area chosen for the project has a coastline about twenty four miles in length, fringed by mangrove swamp. The coastline is broken by the estuary of the Selangor River. Generally the limits of the mangrove are the bunds built to protect cultivated areas from periodical inundation by the sea. Inland of the mangrove is the flat coastal plain which leads to the foothills of the central spinal hill range of Malaya. Apart from the fishing villages and small towns on the coast, there is a well-settled and highly cultivated coastal strip devoted to small-holdings (Malay kampongs) running the whole length of the study area; this also extends beyond both its northern and southern boundaries. This strip is centred about the coastal road which follows the main bund, it averages perhaps four miles in depth. One of the principal crops grown

within this coastal strip is the coconut palm. Except for this, the rest of the coastal plain is mostly devoted to rubber and oil-palm estates, but there are also some kampongs identical with those of the coast, except that as one proceeds inland the cultivation of coconuts becomes less frequent. Part of the northern boundary of the study area, inland of the coastal kampongs, is formed by the rice fields of Tanjong Karang. The cultivation of rice is not typical of this part of Malaya, and these fields are a relatively recent development.

Previous to the start of the project the only anti-malarial measures carried out within the study area were drug prophylaxis in some schools where there was evidence of much malaria and drainage and oiling of possible breeding places close to the larger villages. There is no evidence that residual insecticides had ever been used in the area for health purposes, although small amounts have been used for agriculture.

Survey Methods.

Coincident with the start of the entomological surveys in the study area a complete house survey was made. All houses were numbered and maps drawn to the scale 11 inches per mile (these were based on the existing land utilization maps). Each individual map covered an area of $2\frac{1}{2}$ miles (north to south) x $3\frac{1}{2}$ miles. Because of this it was possible to locate breeding and resting sites of mosquitos accurately, later these same maps were used to assess the efficiency of the spraying operations.

Throughout these investigations all larval surveys were made by teams of four to six men working under a supervisor, each survey usually occupying one entire working day. The supervisor was responsible for allocating work in the area under survey, and for producing sketch maps of the various places from which larvæ were collected. So far as was possible all '*barbirostris*' larvæ collected were hatched out in the laboratory, so as the make certain of the identification.

House searches were made by teams of two men, who together searched each house for ten minutes. Collections from cattle shelters were usually made with more than four men, who worked from dusk until at least 2230 hours. Night catches on human baits were made from either dusk to midnight or from dusk to dawn on a number of occasions. These catches were made by teams of three men, one team catching inside a house, another team catching outside in the open.

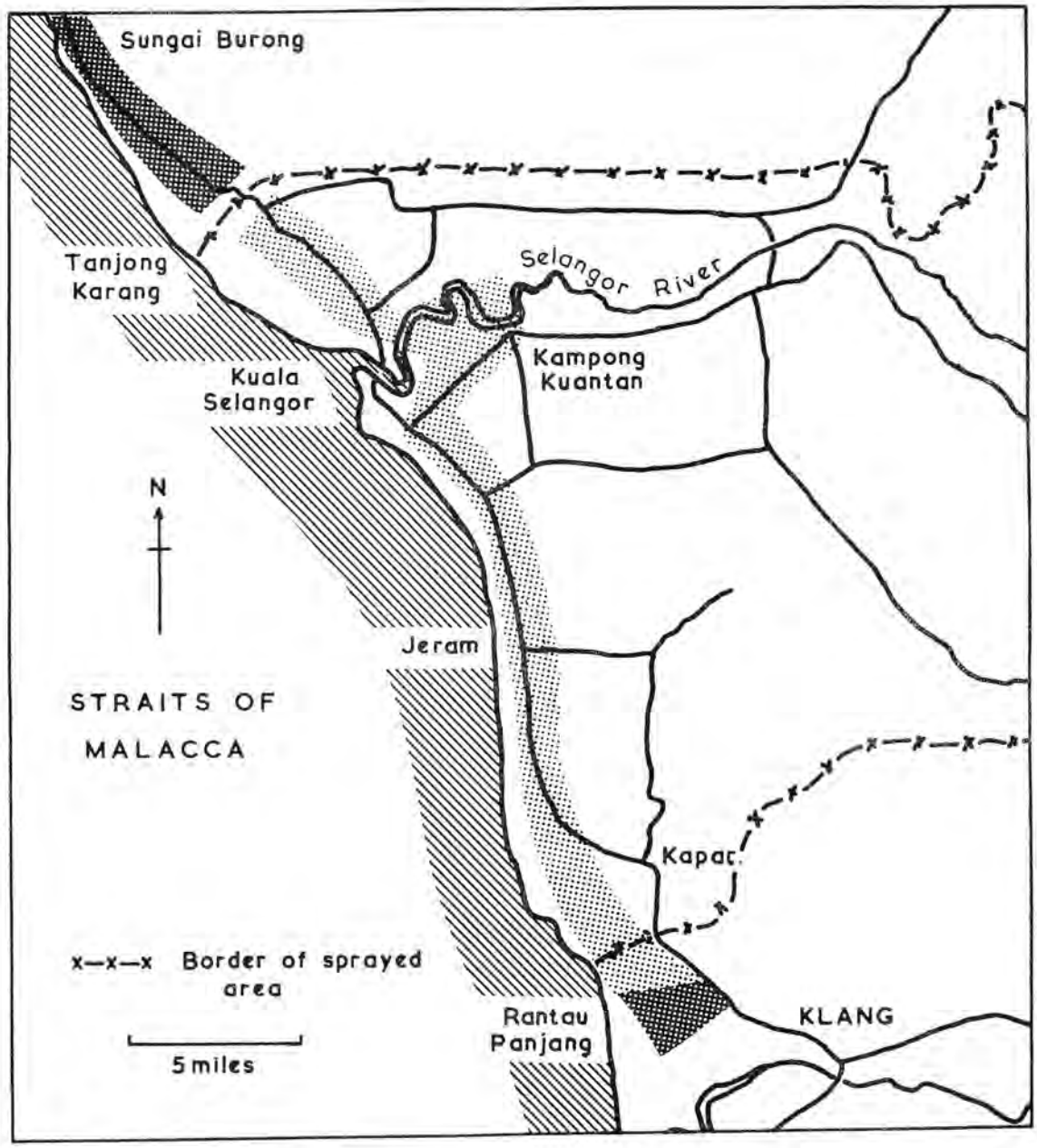
Pyrethrum knock-down catches were introduced in 1962 after it was found during work outside the sprayed area that this method of survey was more sensitive than simple house searches for resting mosquitos (Moorhouse and Wharton, to be published)

Findings before the start of spraying operations.

Breeding of *A. campestris* in the study area was found to be limited to those parts of the coastal plain where the soil is composed of stiff clays (recent marine alluviums) as previously described by Reid (1962). The larvæ were commonly in wells; ditches with standing water; and "borrow pits" between the coconut palms. Always the water was deep, with some vegetation, and with moderate shade such as is given by the coconut palm. The larvæ do not tolerate pollution of the water; because of this it was not usual to find breeding in the immediate vicinities of the villages, but breeding was widespread between the more scattered houses of the kampongs. Hodgkin (1940) recorded *A. campestris* from breeding places with salinities up to 15 per cent of sea water, but in these present investigations the larvæ have never been found in saline waters. Apart from places where pollution of the possible breeding sites occurred, or where the waters were demonstrably salty, breeding was found along the length of the whole coastal kampong strip. Breeding was not found in the rice fields at Tanjong Karang as was expected after Reid's (1962) observations in the Krian (State of Perak) rice fields. This absence is probably explained by the lack of sufficient shade trees at the edges of the fields.

Wharton (personal communication) found that the average number of eggs laid by the gravid female under laboratory conditions was high, as many as four hundred. Despite this, the density of fourth stage larvæ in individual pools in the study area was always very low, it seems that mortality during development must be extremely high. This is possibly due in part of the predacious habits of certain culicine larvæ which are found in the same habitats.

Studies on the behaviour of *A. campestris* show that it is probably the most endophagic and anthropohilic of all the Malayan anophelines. Man-biting catches conducted inside and outside houses demonstrate that unlike



Legend to map.

Map showing the distribution of *A. compestris* (strippled areas) in the malaria eradication pilot project area, and in its environs in the State of Selangor, Malaya, in 1960. The fine stippling shows those areas from which it has since diappeared following house spraying; the heavy stippling shows where it can still be found outside the sprayed area.

other Malayan anophelines, *campestris* bites more readily inside the house than outside. During investigations into the biting cycle of this mosquito, simultaneous indoor and outdoor man-biting catches were made from 1800 hours until 0600 hours the following morning, on six occasions at Sungei Burong. A total of 74 specimens were caught biting indoors and 17 outdoors, a ratio of 4.3: 1 in favour of indoor biting, (Moorhouse and Wharton, to be published). These same studies on the biting activity of campestris show that it is active throughout the whole night, but that there is a moderate peak of biting between 2300 and 0300 hours.

Reid (1961) first demonstrated the anthropophilism of *campestris* when he exposed man and calf simultaneously in net-traps placed about fifty yards apart. The total numbers of mosquitos caught produced a ratio of 3.4: 1 in favour of biting man as opposed to calf. During the man-biting catches mentioned above at Sungei Burong, various animal shelters were also searched at night but *A. campestris* was not found, although small numbers were found resting in nearly all the nearby houses visited at the same time. However, on other occasions during these present investigations a few specimens have been found in animal shelters during night catches. By means of serological tests, Reid and Weitz (1961) identified the origins of blood meals in 27 *campestris* caught resting outdoors during the daytime at Rantau Panjang. Of these 17 had fed on man and eight on monkey, (it was not possible to determine the origins of the other two feeds with accuracy). These findings differ from those of Wharton and Eyles (personal communication) who exposed man and monkeys (*Macaca irus*) in separate net-traps on the ground, and monkeys in the forest canopy for thirty nights. They obtained a ratio of slightly more than 11: 1 in favour of *A. campestris* biting man rather than monkey. Monkeys exposed in net-traps on the tree platform did not attract any *campestris*.

A. campestris is also endophilic or house-haunting. During the preliminary surveys daytime house searches for resting anophelines, and pyrethrum knock-down catches revealed that unlike the other Malayan anophelines, *campestris* frequently rests in the houses throughout the day. Generally they are found on mosquito nets or on clothes hanging on the walls in the more quiet parts of the house. Bonne-Webster and Swellengrebel (1953) are almost certainly referring to this particular species when they remark on *A. barbirostris* as often being found in houses in Malaya, resting on the walls and on mosquito nets. However, their mention in the same paragraph of large numbers also being found in cattle shelters, and to a low avidity for human blood must relate to other members of the *barbirostris* complex, possibly to *A. barbirostris sensu stricto*. All stages of ovarian development are to be found amongst those resting in the houses during the day, which suggests that some at least, may spend the greater part of the time between the blood-meal and egg-laying within the house.

An example of the frequency of house-resting during the daytime is given in table 1.

TABLE 1.

Showing the results of day-time house searches for *A. campestris* at Sungai Terap in the six weeks previous to the start of house spraying. Each house searched by two men for ten minutes.

Date	Number houses		Number of *campestris* found	
	searched	with resting *campestris*	Total	N'ber blood-fed
27.3.61	3	2	5	4
29.3.61	10	3	4	3
7.4.61	21	8	20	15
24.4.61	21	10	41	— *
8.5.61	24	8	14	6

* Information not available.

This gives the results of house searches made at Sungai Terap, a kampong on the coast road between Tanjong Karang and Kuala Selangor, in the six weeks prior to the start of spraying operations. It was probable that *campestris* was the only vector of malaria in the kampong. A further example is provided by a survey made in houses along the coast road between Tanjong Karang and Sungei Burong outside the experimental area in May 1962. In this locality *A. campestris* is the principal if not the sole vector of malaria. Twenty eight houses were searched, each by two men for approximately ten minutes between 1600 hours and 1800 hours. A total of 19 *A. campestris* were found in eight of the twenty eight houses visited; 15 of these had partially digested blood-meals. During these same surveys the only other anophelines caught were two specimens of *A. lesteri*, both found in the same house. *A. lesteri* is the most common anopheline of this coastal area.

Susceptibility to DDT.

Throughout these investigations it has not been possible to catch a sufficiently large number of adult *campestris* at any one time to undertake insecticide susceptibility tests. However, Wharton (1958) reared *campestris* in the laboratory from eggs laid by wild-caught females. He determined that the LC 50 of DDT for blood-fed adults reared in the laboratory was 1.3 per cent.

Relation to Malaria.

The relation of *campestris* to malaria has recently been discussed by Reid (1962), who shows that on the west coast of Malaya it must be considered an important vector. Within the project area it has usually been found along with either *A. sundaicus* or *A. letifer*, because of which it is not easy to assign any particular role to the species in this area. In 1960, parasite rates varying from 0% — 46% were found amongst children (aged 6-9 years) living in the coastal strip within the project area where *campestris* was a vector. It may be significant that the lower parasite rates were found in the more densely populated parts of the kampongs and the small villages, where generally this particular species is absent. But because distances are so small it is very difficult to draw conclusions. At Sungai Burong where *campestris* is considered to be the sole vector, epidemiological surveys made in 1962 show that there was a parasite rate of 16% amongst the Malay children and a spleen rate of 14.6%. Similar spleen rates are to be found in neighbouring kampongs along the road.

Reid (1962) summarised the dissection data from this species and records that an average sporozoite rate of 0.33% was obtained from almost 15,000 specimens dissected from localities on the western side of Malaya. In these present investigations 321 *campestris* have been dissected, one was found with oocysts.

House Spraying and its results.

The insecticide used in this project was DDT, supplied as a 25 percent emulsifiable concentrate. The spraymen used compression sprayers; they were trained to work with an insecticide concentration, spray pressure, width of swath and speed, such as to give a deposit on the wall of two grammes of technical DDT per square metre. All the internal walls of the houses, the outside porches, and the eaves were treated; also the undersides and the back of all pieces of furniture. Where the houses were raised on stilts, the underfloor area was sprayed along with the various supporting pillars. Spraying of animal shelters and ancilliary buildings was carried out only when they were attached to; or were less than ten feet from the house.

The first cycle of spraying started in March 1961, since when spraying has been repeated at intervals of six months. To the present four cycles are complete (August 1963). Few house-holders object to spraying, in fact, most welcome it. In each cycle more than 98 per cent. of the houses have been sprayed.

Following house-spraying *A. campestris* rapidly disappeared from the project area. At Sungei Terap which has previously been mentioned, spraying started on 17th May 1961 and was completed one week later. A daytime search for house-resting mosquitos was made on 10th June but no *campestris* were found. Because of this the whole locality was surveyed intensively during the next weeks, but without any success. Larval surveys were also made for *campestris*, but none were found. In an adjacent kampong (Ujong Permatang) a single adult was found resting in an unsprayed house three weeks after the start of spraying in the area, and at the same time larvæ were found nearby. Larvæ were also found near to Jeram three weeks after the completion of the spraying in the area. Surveys for both the larvæ and the adults were then made along the whole length of the coastal belt where this species

had formerly been present, but none were found.

Since this time frequent surveys have been made especially for this species throughout the project area. Methods used have been house-searches and pyrethrum knock-down collections during the day, larval surveys (with hatching out), night catches from both human and animal baits, and house searches at night; but the presence of *A. campestris* has not been demonstrated A summary of the surveys made is to be found in Table 2. Outside the sprayed area, to both the north and the south, *campestris* can still be found, as it has been at all times of year since these investigations began.

During these various surveys along the coastal strip the larvæ of *A. sinensis*, *A. lesteri*, *A. barbirostris*, *A. vagus*, *A. sundaicus*, *A. separatus*, and *A. letifer* have been found, as have the adults of *A. lesteri*, *A. sinensis*, *A. vagus*, *A. sundaicus*, *A. subpictus*, *A. separatus*, *A. letifer*, *A. tessellatus* and *A. kochi*.

A completely independant check for the presence or absence of *A. campestris* in the project and the contigious areas, was made by staff of the Institute for Medical Research, Kuala Lumpur in June 1962. This was after two complete cycles of spraying. The persons who made the surveys were fully conversant with the habits of this mosquito. Anopheline larvæ were collected from 109 likely sites along the coastal belt in which the species had previously been found, these collecting sites being centred on each milestone along the coast road. All '*barbirostris*' larvæ were bred out. The results agreed with the previous findings that although *campetris* can be demonstrated to both the north and south of the sprayed area, it is absent where spraying has been carried out.

Irritant effects of DDT.

The rapid disappearance of *A. campestris* after house spraying precluded the possibility of making observations on the irritant effects of DDT. However, the work of Reid and Wharton (1956) on trials of residual insecticides in window-trap huts is of considerable interest. These workers showed that DDT has the effect of driving an increased percentage of *campestris* out of the trap-huts into the window-traps before feeding, but that the effect is small. Previous to spraying the trap-huts with DDT 74.3%, (90/121) of *campestris* found in the window traps were blood-fed. In the first four weeks after spraying the hut, the percentage blood-fed in the trap dropped to 60.7% (31/51), in the second four weeks after spraying 72.2% (13/18) of those in the traps were blood-fed. If the lowered percentage of blood-fed mosquitoes in the window trap can be accepted as a measure of the irritability of the species to the insecticide then it would appear that *campestris* is mildly irritated by DDT. However the present findings that the species disappeared after widespread spraying suggests that this irritation is of no significance.

Spraying with dieldrin at Rantau Panjang (Klang).

In March 1957 house spraying was started by the health authorities in Rantau Panjang, a coastal kampong adjacent to the northern boundary of Klang, because of the high endemicity of malaria. The insecticide used was dieldrin (Dieldrex 15), applied with a "Four Oaks Sprayer" so as to give a deposit of 40 mgs dieldrin per square foot. A second cycle of spraying started at the beginning of May 1958, since then spraying has been repeated at approximately six monthly intervals up to the present time. During the first three years of the spraying only the internal walls of the houses were treated. But in November 1960 it was also decided to spray under the eaves, the outside porches, and where the house was raised on stilts, the under floor area; as was to be done in the malaria eradication pilot project a few miles to the north. This has been carried out in the subsequent cycles.

Although entomological records are not complete for this kampong, there can be no doubt that *A. campestris* was an important vector of malaria in Rantau Panjang prior to the start of spraying operations, but *A. sundaicus* was also present. Human-baited net traps were operated in the kampong in both 1951 and 1952. In 1951, these net-traps were operated in 166 nights. The anophelines caught included 1,222 *campestris* and 12 *A. sundaicus*, 750 of the *A. campestris* were dissected and one had a heavy sporozoite infection. (Reid, 1952). In the following year 1952, 1,525 *campestris* and 27 *A. sudaicus* were caught in the net-traps. Of the *campestris* 1,362 were dissected and 14 had sporozoite infections (Reid 1954). There are no records of the malaria parasite rate in the area at this time.

In March 1957, a parasite survey was made amongst the school children of the kampong; of 117 examined, 25 were found to be infected, a parasite rate of 12.3%. At the

TABLE 2.

Summary of Principal surveys for *Anopheles campestris* in the study area.

	Larval surveys		House-resting surveys by day and night							Cattle shelter catches		
	Number surveys made	Number surveys *campestris* found	Number houses searched		Houses with *campestris* resting		Total Number *campestris* found		Knock-down catches	Searched	With *campestris*	Total number caught
			Day	Night	Day	Night	Day	Night				
Before spraying 1960-61	15	12	248	16	52	3	79	4	—	5	4	12
After spraying 1961	18	1 * 1 **	412	2	1*	0	1*	0	—	6	0	0
1962	49	0	201	2	0	0	0	0	93	13	0	0
1963 until August	28	0	105	33	0	0	0	0	104	3	0	0

* three weeks after spraying at Ujong Permatang
** three weeks after spraying at Jeram

same time a pyrethrum knock-down catch for anophelines was made in twenty houses during the day and a single specimen of *A. campestris* was taken. After this the kampong was sprayed with dieldrin. The parasite survey and the knock-down catch were repeated in May 1958, at this time a parasite rate of 22.3% was found amongst the children, and once again a single specimen of *campestris* was caught in one of twenty houses searched. These surveys were again followed by house-spraying with dieldrin; the spraying has since been repeated every six months. Since the start of the regular spraying the parasite rate of the school children has shown a decline; 8.4% in 1959 (7 of 83 children examined), 6.5% in 1960, (7 of 107 examined) 0% in 1961, (0 of 125 examined and 2.8% in 1962 (3 of 104 examined). Fluctuations can be expected in the parasite rate of these children because of some of them live in unsprayed areas and also because both *A. campestris* and *A. sundaicus* are present in unsprayed areas immediately to the north of the kampong where malaria is endemic.

Wharton (personal communication) operated human-baited net-traps on many occasions in 1961 and 1962 in the same general localities at Rantau Panjang as Reid used in 1951 and 1952. In 1961 he recorded two specimens as belonging to the *A. barbirostris* group, the exact species is not known. In 1962 *A. campestris* was not caught in the net-traps. Larval surveys and pyrethrum knock-down catches were made in the kampong especially for *A. campestris* in November 1962, but none were found.

Conclusions.

Reid and Wharton (1956) remark that the factors involved in determining the degree of control of a vector mosquito which can be achieved by a residual insecticide may be summarised under three headings. These are, the susceptibility of the species to the insecticide; the frequency of contact with the insecticide, which as they remark depends upon the frequency of entry into the sprayed premises, which in turn depends on the mosquitos' habits; and finally, upon the duration of contact with the insecticide on each occasion of entry.

Although the Lc50 of DDT to this species is high (1.3%) Reid and Wharton, (1956) working with sprayed trap-huts demonstrated that during the first month after spraying with DDT at the rate of two grammes per square metre, an average mortality of 72 per cent. was obtained with *campestris*. In these experiments the trap-huts were emptied of all mosquitoes by 0700 hours in the morning. All living specimens were placed in cages so as to observe the mortality during the following twenty four hours. The period of contact with the insecticide is thus likely to have been shorter than under natural conditions. In the second month after spraying the trap-huts, the mortality over 24 hours dropped to an average of 32 per cent., and in the third and subsequent months the deposit failed to kill this species. Because of this Reid and Wharton came to the "disturbing conclusion" that so far as this particular species was concerned it was not very susceptible to DDT at the concentration used, (which is the same as is now being used in the malaria eradication pilot project), and that,

> "the kills (of many species) were too low, or lasted too short a time, for practical purposes. With species likely to make frequent contact with the insecticides these low kills might be sufficient but not with species having only occasional contact, i.e., all these investigated excepted *C. p. fatigans*."

These present findings, however, suggest that *A. campestris* has far more contact with residual insecticides than was previously realised. It is considered that it fulfils all the requirements for enabling highly successful control to be achieved by the use of residual insecticides, and that in any anti-malaria programme unless there are special reasons for avoiding the use of insecticides, then house-spraying is the method of choice as far as this species is concerned. So far as any future malaria eradication program is concerned, the response of this mosquito is extremely encouraging.

ACKNOWLEDGEMENTS.

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