EFFECT OF DDT ON SURVIVAL AND BLOOD FEEDING SUCCESS OF *ANOPHELES ARABIENSIS* IN NORTHERN KWAZULU, REPUBLIC OF SOUTH AFRICA

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ABSTRACT. The effect of house spraying with DDT on blood-feeding and resting behavior of *An. arabiensis* in Natal Province, Republic of South Africa, was investigated. Indoor resting occurred in both control (unsprayed) and replastered (DDT on walls covered due to replastering) huts, but was minimal in fully DDT-sprayed huts. The percentage of bloodfed mosquitoes was >50% in both control and replastered huts, but in the latter huts there was a reduction in the percentage of gravid and an increase in the percentage of unfed mosquitoes. Large numbers were collected in exit traps irrespective of the DDT status of the huts. The percentage of catch that was bloodfed was lowest in exit traps fitted on DDT-sprayed houses. The percentage survival of bloodfed mosquitoes caught in exit traps exceeded 72% for all 3 hut types. The survival of unfed mosquitoes was, however, markedly lower. Susceptibility tests showed no physiological tolerance to DDT by the wild *An. arabiensis* population. Bioassays using colonized *An. arabiensis* showed the DDT deposits on hut walls to effect 100% kill with 15 minutes of exposure.

INTRODUCTION

The efficient control of vector species is central to the containment of malaria. The development of low cost residual insecticides after World War II revolutionized rural malaria control (Davidson 1982). The use of intra-domiciliary insecticides for malaria vector control has, however, met with variable success, depending on logistical and social factors, finance, vector behavior and the development of insecticide resistance (Fontaine 1983). It still, however, remains an important component of malaria control in some rural areas (Gratz 1985). The assessment of the efficiency of this measure in an area is essential to planning an effective malaria control strategy.

Three species of the *Anopheles gambiae* Giles complex, *An. quadriannulatus* Theobald, *An. arabiensis* Patton and *An. merus* Dönitz occur in Kwazulu (le Sueur and Sharp 1988), an area experiencing seasonal malaria transmission (Sharp et al. 1988). The malaria case rate has increased over the past 2 years, in part due to chloroquine resistance in *Plasmodium falciparum* Welch (Freese et al. 1988).

This study investigated intra-domiciliary DDT vector control in the malaria area of northern Kwazulu, Republic of South Africa.

MATERIALS AND METHODS

Specimens for the study were collected at Mamfene (36° 16′ E, 27° 23′ S), Ubombo district during the period January 1986 to April 1988. Exit traps (Muirhead-Thomson 1947) were used and emptied before 0800 h each day. Indoor resting catches were made by space spraying with a 4% pyrethrum and paraffin solution using a Rega pump.

After capture, the mosquitoes to be used for susceptibility testing were bloodfed, placed individually in breeding tubes to oviposit and held in an insectary (25 ± 1°C, 75 ± 5% RH). The breeding tubes were 25-ml plastic bottles with damp cotton wool covered by filter paper on the bottom and with gauze tops. Individual family broods were reared. Bioassay tests (World Health Organization 1975) were performed using 1- to 4-day-old adult females.

After removal from the exit traps, the mosquitoes were stored in collecting cups in an insulated container covered with damp muslin cloth. Mortality counts were done periodically until 12–16 h after collection (0800 h), and the gonotrophic status of both the surviving and dead mosquitoes was scored.

Species identification of the *An. gambiae s.l.* was by polytene chromosome analysis (Coluzzi 1968, Hunt 1973, Green and Hunt 1980) and isozyme electrophoresis (Mahon et al. 1976; Miles 1978, 1979).

Contact bioassays (World Health Organization 1975) were done on the DDT-sprayed walls of houses using 2- to 5-day-old *An. arabiensis* from a colony strain (KANB) that originated from Kanyemba, Zimbabwe (15°40′S, 30°20′E). A modification to the technique was in the use of shorter exposure times as well as the standard 1-h exposure.

RESULTS

Seventy-nine *An. arabiensis* were identified by chromosome analysis, 65 caught by exit trap

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and 14 by indoor resting collections. Sixty-five were identified by both chromosomes and electrophoresis. All 65 specimens identified by chromosomes had the following rf values: superoxide dimutase (SOD, E. C. 1.15.1.1.) 100/100; aspartate aminotransferase (AAT, E.C.2.6.1.1.) 100/100; and octogonal dehydrogenase (ODH, E.C. 1.1.1.73) 95/95. A further 559 specimens were identified by electrophoresis (Table 1). Identification of indoor resting and exit trap caught *An. gambiae s.l.* showed >96% to be *An. arabiensis* with very low numbers of *An. quadriannulatus* present (Table 1).

Both exit trap and indoor resting catches were successfully used to collect *An. gambiae s.l.* (Tables 2 and 3). The mean number caught per hut was higher in exit trap collections than in the indoor resting collections, but this difference was not significantly different ($t = 1.608$, $P = 0.108$). *Anopheles gambiae s.l.* were caught leaving huts irrespective of the presence or absence of DDT (Table 2). There was, however, an inverse relationship between the presence of DDT and the numbers of *An. gambiae s.l.* caught, with the highest numbers per hut being caught in control huts. The range in numbers caught was high irrespective of hut status, and pairwise comparisons with a Mann-Whitney test showed no significant difference between catches from huts of differing DDT status ($P > 0.5$).

Indoor resting occurred mainly in replastered and control huts (Table 3). Only 7 *An. gambiae s.l.* were caught resting indoors in a total of 79 DDT-sprayed huts, and 6 of these were caught in one hut. The Mann-Whitney test showed no significant difference between catches from control and replastered huts ($P > 0.7$), but in both cases these data were significantly different to that from the DDT-sprayed huts ($P < 0.003$).

A greater percentage of the *An. gambiae s.l.* caught leaving huts (Table 4) were unfed, followed by freshly fed and lastly a low number of gravid mosquitoes. There was an inverse relationship between the percentage of freshly bloodfed mosquitoes and the presence of DDT. The highest percentage of freshly bloodfed specimens were caught leaving control huts, while an increased proportion of unfed mosquitoes left the DDT-sprayed huts (Table 4). The percentage of gravid females caught in all huts was extremely low. Chi-square tests (2 × 3) of heterogeneity showed that the gonotrophic status of *An. gambiae s.l.* from the different hut categories were significantly different ($P < 0.001$).

The majority of *An. gambiae s.l.* caught resting indoors were freshly bloodfed, followed by unfeds, with the lowest numbers being in the gravid state (Table 5). The percentage bloodfed was high irrespective of the DDT status of the hut. The percentage gravid was higher than the percentage unfed in both the DDT-sprayed and the control huts. Only 6 *An. gambiae s.l.* were caught in the DDT-sprayed huts. In the replastered huts the percentage unfed was greater than the percentage gravid. The Mann-Whitney test showed no significant difference between the total numbers of *An. gambiae s.l.* caught in the control and replastered huts ($P > 0.7$), but a Chi-square test of heterogeneity showed the gonotrophic status of the mosquitoes from the 2 hut types to be significantly different ($P < 0.005$). Data collected from the DDT-sprayed huts were excluded from statistical analysis due to the low numbers caught ($n = 6$).

Survival of exit trap caught mosquitoes was highest in the control huts, less in the replas-

Table 1. Identification of *Anopheles gambiae s.l.* by electrophoresis from exit traps and indoor resting collections.

Trap	*An. arabiensis*	*An. quadriannulatus*
Exit trap	301	12
Indoor resting	236	10
Total	537	22

Table 2. Exit trap caught *Anopheles gambiae s.l.* in control, replastered and DDT sprayed huts.

	Control	Replastered	DDT	Total
No. huts	60	150	155	365
No. *An. gambiae s.l.*	252	512	312	1076
$\bar{x} \pm$ SE/hut	4.2 ± 1.3	3.4 ± 0.7	2.0 ± 0.5	2.94 ± 0.40
Range	0–59	0–86	0–68	0–86

Table 3. Indoor resting *Anopheles gambiae s.l.* in control, replastered and DDT sprayed huts.

	Control	Replastered	DDT	Total
No. huts	23	57	79	159
No. *An. gambiae s.l.*	68	213	7	288
$\bar{x} \pm$ SE per hut	2.9 ± 1.0	3.7 ± 1.5	0.1 ± 0.1	1.9 ± 0.5
Range	0–17	0–69	0–6	0–69

Table 4. Gonotrophic status of *Anopheles gambiae s.l.* from exit traps.

	% bloodfed	% gravid	% unfed	No. *An. gambiae s.l.*	No. huts
DDT	32.6	0.3	67.1	310	153
Replastered	41.9	5.5	52.5	489	146
Control	65.5	2.5	32.0	237	58
Total	44.7	3.4	51.9	1,036	357

Table 5. Gonotrophic status of indoor resting *Anopheles gambiae s.l.*

	% bloodfed	% gravid	% unfed	No. *An. gambiae s.l.*	No. huts
DDT	66.6	33.3	0.0	6	78
Replastered	61.5	13.8	24.6	195	53
Control	52.0	36.0	12.0	50	19
Total	61.0	18.9	20.0	251	150

tered huts and lowest in DDT-sprayed huts (Table 6). Overall survival was marginally increased (<7%) 8–12 months after DDT application in relation to 1–3 months after spraying. Overall survival in control huts was 16–18.6% higher than in replastered huts and approximately 40% higher than in DDT huts. The percentage of mosquitoes bloodfed was highest in the absence of DDT (86.6%), markedly reduced in the replastered huts (53.8 and 54.6%) and lowest in the DDT-sprayed huts (31.5 and 26.2%). Survival of bloodfed mosquitoes was high (>72%) irrespective of the DDT status of the hut, whereas survival of unfed mosquitoes was low, even in the control huts (<55%).

Twenty-five and 17 colonized female *An. arabiensis* were used, respectively, in 0.5- and 1.0-h control bioassays in non-DDT-sprayed huts, as part of the 3-month post-DDT spraying bioassays, and 20 and 36, respectively, in 10- and 30-min bioassays as part of the 9-month post-spraying bioassays. There was no mortality in any of the control bioassays. Five (n = 54), 10 (n = 53) and 30 min (n = 67) of contact on 3-month-old DDT was sufficient for 100% kill in all cases. In the case of 9-month-old DDT, 5 min of contact only effected 26% mortality (n = 19); however, 15 min or more (n = 54) of contact effected 100% mortality.

Families raised from 21 individually identified *An. arabiensis* totaling 339 individual mosquitoes were tested for DDT susceptibility. These mosquitoes and samples of colonized *An. arabiensis* were respectively exposed to 5 standard concentrations of DDT for 1-h and the 24-h mortality plotted on logarithmic probability paper to construct dosage mortality regressions (Fig. 1). Neither regression indicates insecticide resistance in the populations concerned at a discriminating dosage of 4.0% DDT. The figures indicate that the wild population (Fig. 1) had increased vigor tolerance relative to the colonized population. In the colonized population, 100% mortality was obtained at a concentration of 2.0% DDT, whereas in the wild population, this occurred at a 4.0% DDT concentration.

DISCUSSION

The diagnostic allozymes of chromosomally identified *An. arabiensis* correlated well with the results of Miles (1979) and Marchand and Mnzava (1985) for specimens from East Africa. In this study the specimens showed no obvious polymorphism for the AAT and SOD isoenzyme system, and all ODH rf values were monomorphic (95/95). These data validate the use of the Miles (1979) key for the electrophoretic identification of *An. arabiensis* from this area.

Anopheles arabiensis and *An. quadriannulatus* were caught both resting indoors and leaving huts in the study area. However, *An. arabiensis* was the dominant species, accounting for >96% of the identified specimens. As a consequence, these data are considered to reflect the behavior of *An. arabiensis.*

Overall, a higher number were caught leaving huts than resting indoors. However, a direct relationship did exist between the numbers caught leaving and the level of DDT. These data indicate the expected, with the highest numbers being caught in the control hut, a reduced number in the replastered huts where the underside of the roof had exposed DDT and the lowest number in the fully DDT-sprayed huts. Extremely low numbers were found resting in DDT-sprayed huts: of 79 huts investigated, only 2 were positive. Indoor resting occurred com-

Table 6. Percentage survival and percentage bloodfed of *Anopheles gambiae s.l.* from exit traps in relation to DDT status of hut.

Status of hut	Number		% bloodfed	% survival		
	Huts	Mosquitoes		Bloodfed	Unfed	Overall
DDT	49	222	31.5	72.9	30.9	44.1
Replastered[a]	13	91	53.8	93.9	38.1	68.1
Control	15	82	86.6	88.7	54.6	84.1
DDT	6	65	26.2	88.2	37.5	50.8
Replastered[b]	21	119	54.6	93.8	44.4	71.4
Control	14	82	86.6	95.8	54.5	90.2

[a] 1–3 months after DDT application.
[b] 8–12 months after spraying.

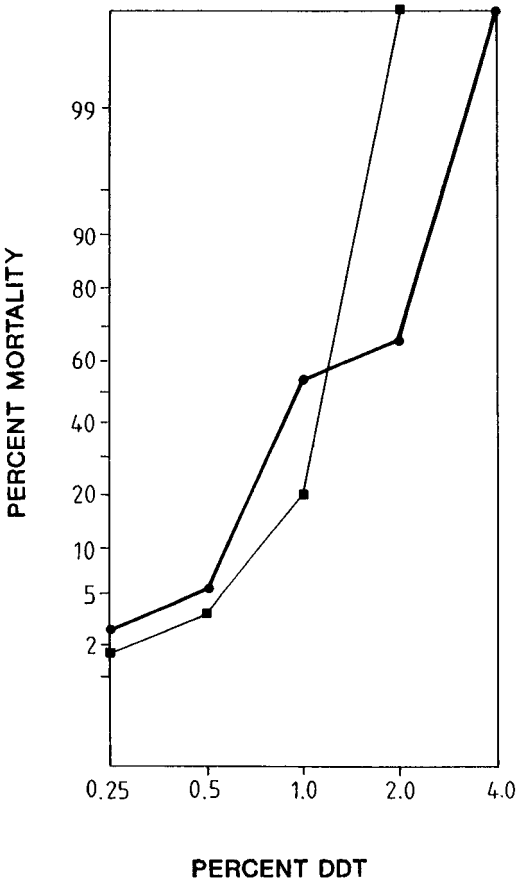

Fig. 1. DDT dosage mortality regression of colonized *Anopheles arabiensis* and wild caught *An. arabiensis*. ■—■ = colonized *An. arabiensis*; ●—● = wild caught *An. arabiensis*.

monly in both replastered and control huts. The indications from this are that the mosquitoes were resting on the DDT-free walls of the replastered huts. Had they been mainly resting on the underside of the roof, then comparable results would have been expected from both the DDT and replastered huts; the lack of physiological tolerance to DDT further precludes roof resting. These data are in contrast to that of Brun (1973) from Burkina Faso where in excess of 70% of the indoor resting *An. gambiae s.l.* were on the roof.

The fed:gravid ratio varied tremendously between trap types and within trap types depending on the DDT status of the hut. The indoor resting ratios from control (1.4:1) and replastered (4.5:1) huts were similar to those found for *An. arabiensis* resting in DDT-sprayed huts (3.7:1) and outdoors (2.2:1) (Haridi 1972) and by Shelley (1973) for indoor resting collections in control huts (3.9:1). However, in the replastered huts there was an increase in the bloodfed component of the ratio. This ratio was markedly different in the exit trap caught mosquitoes, with a marked increase in the percentage bloodfed (control huts = 25.8:1 and replastered huts = 7.9:1) and particularly so in the case of DDT-sprayed huts (101:1). From these data it is clear that a high percentage of bloodfed mosquitoes were leaving the huts and not resting indoors. This tendency was increased in the fully DDT-sprayed huts and could be related to the irritational effect of DDT (Muirhead-Thomson 1960).

The survival of mosquitoes caught leaving huts 1–3 months after DDT spraying was not markedly different from that 8–12 months after application. Survival varied depending on the gonotrophic status of the mosquito and in relation to the DDT status of the hut. The percentage of bloodfed mosquitoes showed an inverse relationship to the level of DDT and suggests that the presence of DDT hampered the taking of a bloodmeal. The percentage survival of exit trap caught bloodfed mosquitoes from DDT-sprayed huts (72.9%, 1–3 months after application, and 88.2%, 8–12 months after spraying) compares well with similarly treated data collected by Haridi (1972) who found an 88% survival. The increased survival of engorged females is further in agreement with the findings of Trapido (1954). The survival of engorged *An. arabiensis* in this study were far higher than that

found by Mpofu et al. (1988). They recorded 4% survival 3 months after DDT spraying and 30% survival at 8 months post-spraying, in contrast to the 72.9 and 88.2% found in this study. The reason for the higher survival recorded in this study can in part be attributed to the method of calculation of percentage survival and the mosquitoes used. In this study, only exit trap caught mosquitoes were used in this calculation, whereas Mpofu et al. (1988) used both exit trap and hut floor mortalities in their calculation. The mosquitoes used by Mpofu et al. (1988) were derived from a colony strain, whereas in this study, wild mosquitoes were used. Susceptibility tests done on both material from the wild population and colony-derived material showed the wild population to have increased DDT vigor tolerance relative to the laboratory colony material. Overall, survival of exit trap catches (all gonotrophic stages) in the study by Haridi (1972) was 94% and far higher than the 44.1 and 50.8% of this study. It is, however, expected that the proportion leaving and surviving will vary considerably according to the dosage, coverage, formulation, nature of the wall surface and behavior of a particular species (Muirhead-Thomson 1950, Hadaway 1951, Wilkinson 1952, Haridi 1972). The percentage mortality in this study is considered to be exaggerated as a result of high mortality in the unfed component of the exit trap catches. Unfed mosquitoes collected in exit traps from control huts only showed a 54.6 and 54.5% survival. Overall, survival of exit trap caught *An. arabiensis* showed an inverse relationship to the presence of DDT, with the lowest survival (44.1%) from the DDT-sprayed huts 1–3 months after spraying. If the mortalities of the unfed component of the exit trap catches are corrected using Abbott's formula, bearing in mind the limitation of using this correction with such a high mortality in the control, mortality of unfed mosquitoes caught by exit traps on replastered and DDT huts, respectively, was as low as 30.2 and 43.4%.

Bioassays in DDT-sprayed huts indicate minimal loss of effectiveness of the DDT deposits over time in killing *An. arabiensis*. In fact, 8–12 months after application an exposure time of 15 min was sufficient to effect 100% mortality. Dose mortality curves further showed no marked increased vigor tolerance or physiological resistance to DDT in the wild *An. arabiensis* relative to WHO standards, with 100% mortality occurring at a discriminating dosage of 4% DDT. Overall, a higher percentage of *An. arabiensis* were leaving huts than were resting inside. The mean number caught leaving replastered huts compared to control huts was reduced by 19% and in DDT huts by 52%. Assuming random entry of huts, this reduction in exit trap catch is taken to represent the numbers killed indoors. The findings of this study have implications for the malaria control program. Transmission was occurring in the study area, and during 1987 there were 709 malaria cases and during the period January to June 1988, 595 cases. The inverse relationship between the percentage bloodfed and presence of DDT in a hut clearly indicates behavioral avoidance. This could result in an increase in biting of man outdoors. The replastering of the inner walls of huts by the inhabitants for social and practical reasons is a further impediment to control of *An. arabiensis* by house spraying with DDT; the percentage of bloodfed *An. arabiensis* caught leaving these huts and surviving was high. Surveys in the area subjected to the DDT vector control program showed that the percentage of huts replastered varied from 1.7 to 72% at different localities and times. Surveys in the study area found 25.7% of huts to be replastered in April/May 1981 and 46.6% in April/May 1982. The exit trap survival data indicate that from replastered huts there is a 20–24% greater survival of mosquitoes than from the DDT huts. Overall, this figure is expected to be in excess of 50% should the differential kill in replastered and DDT huts prior to capture in the exit trap be taken into account. A reduction in the replastering of huts should therefore result in more efficient vector control. K. Newberry (personal communication) found that replastering of huts was to a large extent carried out to combat biting by bed bugs and that their control by insecticidal spraying has led to a significant decrease in the replastering rate in the districts of Ubombo and Ingwavuma.

The data presented indicate that effective control of *An. arabiensis* will not be possible under the current control strategy. To increase the effect of vector control in the study area would require an integrated approach using more than just the annual application of DDT or preferably changing to an alternative insecticide with a reduced irritant effect on the vector species.

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REFERENCES CITED

Brun, L. O. 1973. Contribution a l'etude biologique et ecologique des vecteurs majeurs de paludisme en Afrique de l'Ouest. ORSTOM C36(1):223 p.

Coluzzi, M. 1968. Cromosomi politenici delle cellule nutrici ovariche nel complesso gambiae genere *Anopheles*. Parassitologia 10:179–183.

Davidson, G. 1982. Developments in malaria vector control. Br. Med. Bul. 38:201–206.

Fontaine, R. E. 1983. The use of residual insecticides for the control of adult mosquitoes, pp. 49–81. *In:* M. Laird and J. Miles (eds.), Integrated mosquito control methodologies. Vol. 1. Academic Press, London.

Freese, J. A., B. L. Sharp, S. M. Ngxongo and M. B. Markus. 1988. *In vitro* confirmation of chloroquine-resistant *Plasmodium falciparum* malaria in Kwazulu. S. Afr. Med. J. 78:576–578.

Gratz, N. R. 1985. The future of vector biology and control in the World Health Organization. J. Am. Mosq. Control Assoc. 1:273–278.

Green, C. A. and R. H. Hunt. 1980. Interpretation of variation in ovarian polytene chromosomes of *Anopheles funestus* Giles, *Anopheles parensis* Giles and *Anopheles aruni*. Genetica 51:187–195.

Hadaway, A. B. 1951. Observations on mosquito behaviour in native huts. Bull. Entomol. Res. 41:63–78.

Haridi, A. M. 1972. Partial exophily of *Anopheles gambiae* species B in the Khashm Elgirba area in eastern Sudan. Bull. W. H. O. 46:39–46.

Hunt, R. H. 1973. A cytological technique for the study of *Anopheles gambiae* complex. Parassitologia 15:137–139.

le Sueur, D. and B. L. Sharp. 1988. The breeding requirements of three members of the *Anopheles gambiae* complex in the endemic malaria area of Natal, South Africa. Bull. Entomol. Res. 78:549–560.

Mahon, R. J., C. A. Green, and R. H. Hunt. 1976. Diagnostic allozymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera: Culicidae). Bull. Entomol. Res. 66:25–31.

Marchand, R. P. and A. E. P. Mnzava. 1985. A field test of a biochemical key to identify members of the *Anopheles gambiae* group of species in northeast Tanzania. J. Trop. Med. Hyg. 88:205–210.

Miles, S. J. 1978. Enzyme variation in the *Anopheles gambiae* Giles group of species (Diptera: Culicidae). Bull. Entomol. Res. 68:85–96.

Miles, S. J. 1979. A biochemical key to adult members of the *Anopheles gambiae* group of species (Diptera: Culicidae). J. Med. Entomol. 15:297–299.

Mpofu, S. M., P. Taylor, and J. Govere. 1988. An evaluation of the residual lifespan of DDT in malaria control. J. Am. Mosq. Control Assoc. 4:529–535.

Muirhead-Thomson, R. J. 1947. The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *Anopheles melas* in West Africa. Bull. Entomol. Res. 38:449–464.

Muirhead-Thomson, R. C. 1950. DDT and Gammexane as residual insecticides against *Anopheles gambiae* in African houses. Trans. R. Soc. Trop. Med. Hyg. 43:401.

Muirhead-Thomson, R. C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. W. H. O. 22:721–734.

Sharp, B. L., S. Ngxongo, M. J. Botha, F. Ridl and D. le Sueur. 1988. An analysis of 10 years of retrospective malaria data from the Kwazulu areas of Natal. S. Afr. J. Sci. 84:102–106.

Shelley, A. J. 1973. Observations on the behaviour of *Anopheles gambiae* sp. B in the Kambole village in the Zambezi valley, Zambia. Ann. Trop. Med. Hyg. 67:237–248.

Trapido, H. 1954. Recent experiments on possible resistance to DDT by *Anopheles albimanus* in Panama. Bull. W. H. O. 11:885–889.

World Health Organization. 1975. Manual on practical entomology in malaria. World Health Organization, Geneva.

Wilkinson, P. R. 1952. Distribution and fate of *Anopheles gambiae* and *Anopheles funestus* in two different types of huts treated with DDT and BHC in Uganda. Bull. Entomol. Res. 42:45–54.