Israel Journal of Entomology

Vol. XXIII(1989) pp. 69-76

POTENTIAL OF *BACILLUS THURINGIENSIS VAR ISRAELENSIS* FOR MOSQUITO MALARIA VECTOR CONTROL IN KENYA

T.K. MUKIAMA AND J.H.P. KAHINDI
Department of Botany, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

ABSTRACT

The main vectors of malaria in Kenya are *Anopheles arabiensis, An. gambiae* and *An. funestus.* In the Mwea Rice Irrigation Scheme of Central Kenya, Art *arabiensis is* the principal vector, accounting for over 83% of the total anopheline population. Larval breeding takes place from late March to mid-December. The population peaks correspond to the rainy seasons in April-May and October- November. The flooding phase of the rice cycle in August, an otherwise dry season, is the link that enables continuous breeding for nine months. The use of biological larvicides that are not only innocuous to other aquatic organisms but also environmentally safe is a desirable component of any futureintegTated control stralegy Bacillus thuringie*nsis var (B.t.i.) has* already been shown to be a promising agent of riceland mosquito control in Kenya. Laboratory tests have indicated that the LC 50's *for An. arabiensis* and An. *gambiae* are 1.86×10^{-6} and 2.05×10^{-6}B.t.i./ml respectively. Limited field testing with commercial preparations was commendably effective, but the larvicidal activity of the suspensions had limited persistence. It is suggested that further field testing with *B.t.i.* and more screening for local strains of *B.t.* should be encouraged in the future.

INTRODUCTION

Malaria and other mosquito-bome diseases continue to exact a heavy toll on manpower and productivity in many areas of Africa. Major endemic areas are more or less associated with patterns of human settlement and endeavour. In recently established irrigation schemes, nearly all crops requiring irrigation are known to be associated with large populations of mosquitoes. In the past, control strategies have relied heavily on the use of residual insecticides which though effective, have raised great concern due to environmental pollution and the development of resistant strains of insects. Behavioural polymorphism in some species, particularly the *Anopheles gambiae* Giles complex of malaria vectors, has further diminished the reliability of residual spraying. Faced with such problems, attention has been directed at the identification of other methods of control; and since no single method has demonstrated total effectiveness, the need for an integrated control approach has been universally accepted. In this regard, the use of biological control agents for larviciding should form an important component of any integrated approach to mosquito control.

Bacillus thuringiensis var. *israelensis* de Barjac *(B.t.i.)* and *B. sphaericus* Neide are spore-forming bacteria which produce toxins that are highly lethal to mosquito larvae, but are otherwise innocuous to most nontarget aquatic organisms and vertebrates. In Kenya, major vectors of malaria breed in most lowland areas, particularly in rice irrigation schemes. *B.t.i.* therefore should be a promising choice for a larvicide, and preliminary trials have yielded encouraging results.

MALARIA VECTOR MOSQUITOES OF KENYA

The principal vectors of malaria in Kenya belong to the An. *gambiae* complex, and include *An.*

arabiensis Patton, *An. gambiae* and *An. merus* Donitz (White, 1974; Highton et al., 1979; and Mosha and Petrarca, 1983). The other major vector is *An. funestus* Giles (Gillies and de Meillon, 1968). Recently, *An. pharoensis* Theobald of Mwea Irrigation Scheme was implicated as a vector (Mukiama and Mwangi, 1989b).

IRRIGATED RICE FARMING AND MOSQUITO BREEDING

Recent studies (1984–1985) in the Mwea Irrigation Scheme of Central Kenya showed that *An. arabiensis* comprised 83.9% of the total anopheline population, while *An. pharoensis* accounted for 15.7%. The other anophelines present were *An. funestus*, *An. pretoriensis* Theobald, *An. maculipalpis* Giles and *An. coustani* Laveran, which all made up 0.4% of the total.

Investigations of malaria infectivity by an enzyme-linked immunosorbent assay (ELISA) for *Plasmodium falciparum*, and by manual dissections gave sporozoite rates of 1.2% and 0.55% respectively for *An. arabiensis*, 1.3% and 0.68% respectively for *An. pharoensis*, and 1.6% and 1.25% respectively for *An. funestus*.

Bloodmeal analysis of *An. arabiensis* females showed a higher preference for feeding on bovids (51.9%) than humans (28.1%).

Although mosquito breeding in Mwea is to a limited extent directly dependent on rainfall, the development of the rice irrigation farms is largely responsible (the meteorological data for Mwea is shown in Table 1). The flat, waterlogged rice fields offer ideal larval breeding habitats, while the flooding phase of the rice cultivation cycle in (the otherwise dry month of) August links up the two peak breeding periods during the rainy seasons of April–June and October–November. This linkage results in continuous breeding for up to 9 months per year (Mukiama and Mwangi, 1989a).

Fig. 1 shows the seasonal fluctuations in larval numbers at two village sites, given as percentage of the total collections made.

Table 2 shows the total numbers and percentages of the different larval instars and pupae collected. It shows that the majority of the immature population consists of first and second instars, and that pupal productivity is less than 1%. Details are given by Mukiama and Mwangi (1989a).

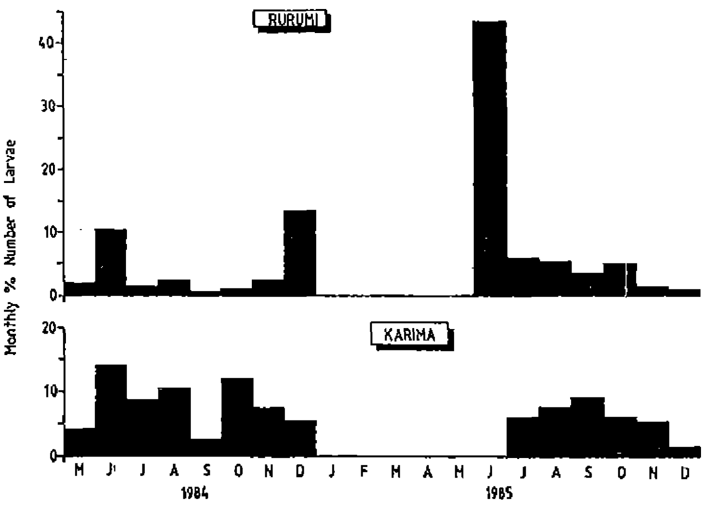

Fig. 1. Seasonal fluctuations in relative numbers of *An. arabiensis* larvae collected at two village sites in Mwea Irrigation Scheme during 1984 and 1985.

TABLE 1
Meteorological data for Mwea 1984–1986

Month/Year	Mean relative humidity (%)	Total rainfall (m)	Temperature (°C) Mean max.	Temperature (°C) Mean min.	Ricefield condition
1984					
Jan	48.5	5.8	29.9	14.4	NF
Feb	54.4	0*	31.6	14.3	NF
Mar	45.8	11.4	31.8	17.3	NF
Apr	56.8	144.1	30.9	18.9	PF
May	53.5	8.9	29.1	18.2	F
Jun	56.6	3.9	–	16.2	F
Jul	61.5	4.6	–	16.5	F
Aug	61.0	3.7	–	16.4	F
Sep	51.0	19.8	–	16.7	F
Oct	61.9	219.6	–	17.7	F
Nov	68.6	147.5	–	16.9	F
Dec	62.4	42.3	26.4	15.5	PF
1985					
Jan	52.7	10.6	29.4	14.5	NF
Feb	48.9	68.5	30.2	17.2	NF
Mar	53.6	69.0	31.0	16.9	NF
Apr	66.1	475.5*	27.2	16.6	PF
May	67.5	54.3	26.1	17.5	F
Jun	66.8	0.9	24.9	15.9	F
Jul	62.0	2.4	24.7	15.8	F
Aug	56.0	6.0	24.9	15.7	F
Sep	51.9	1.5	29.0	17.0	F
Oct	53.8	47.2	29.2	17.0	F
Nov	67.8	136.4	27.4	17.4	F
Dec	60.5	33.2	28.0	14.9	F
1986					
Jan.	43.6	3.2	30.9	14.0*	NF
Feb	37.9*	0*	32.8*	14.9	NF
Mar	52.2	90.3	30.9	16.7	NF
Apr	61.1	291.8	28.4	17.6	PF
May	68.5	105.8	26.7	17.1	F
Jun	68.9*	15.5	23.7	15.6	F
Jul	61.4	0*	24.4	14.5	F
Aug	55.2	5.8	26.7	14.1	F
Sep	49.3	17.6	28.1	15.6	F
Oct	51.6	52.0	30.0	16.9	F
Nov	68.1	224.1	26.9	16.8	F
Dec	65.7	151.1	26.5	15.7	PF
Mean	57.6	68.7	28.3	16.2	

Note: F – Flooded; PF – Partially Flooded; NF – Not Flooded; * – Indicates range.

TABLE 2
Numbers and percentages of larval instars and pupae of *An. arabiensis* collected in Mwea between May 1984 and December 1985

Village	1st instar	2nd instar	3rd instar	4th instar	Pupae	Total
Rurumi	895	1210	431	234	14	2784
Karima	452	671	446	287	24	1880
Total	1347	1881	877	521	38	4664
%	28.9	40.3	18.8	11.2	0.8	100

Fig. 2 shows the combined mean monthly numbers of adult mosquitoes collected resting indoors by CDC light traps and pyrethrum spray sheet collections. Both collections were highly correlated (r = 0.906), and their combined means are shown against the rainfall profile of Mwea. The degree of correlation between the mosquito numbers and the monthly rainfall measurements was very low (r = 0.03), suggesting that other factors must be responsible for the adult mosquito population fluctuations recorded.

Fig. 2 also shows the time of the year when the fields are artificially flooded in preparation for planting a new rice crop. It is apparent that high mosquito populations correspond to months when the rice fields are flooded, i.e. during the rainy seasons and their aftermath, and the flooding phase of the rice cycle. It is also clear that the mosquito population is at its lowest during the dry season (January–March) when no artificial flooding is attempted since no rice crop is planted.

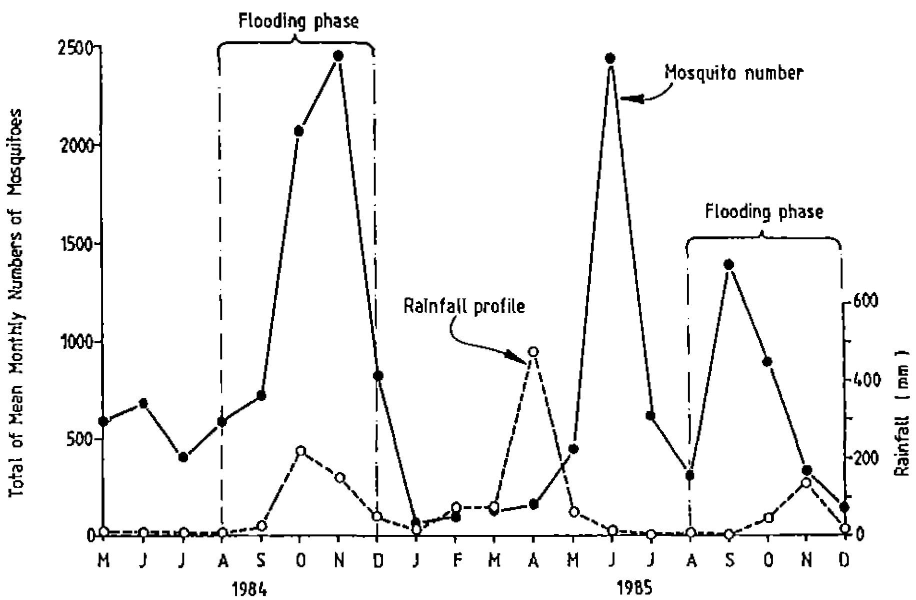

Fig. 2. The combined total mean monthly numbers of *An. arabiensis* per house per month caught in Mwea against the rainfall profile and the irrigation flooding phase.

B.T.I. TRIALS IN KENYA

In Kenya, two *B.t.i.* preparations from industrial and laboratory sources were recently tested for their efficacy. A wettable powder of *B.t.i.* (Roger Bellon, S.A. Lot R 15378) was bioassayed in the laboratory against, among others, larvae of *An. arabiensis* and *An. gambiae*. The tests involved 25 4th instar larvae of each species, with controls, and replicated 4 times for 4 days. The basic methodology was adopted from the Scientific Working Group on Biological Control of Vectors of Diseases (TDR/BVC-SWG (3) 79.3), with minor modifications. The LC 50's obtained after 24 hours were 1.86×10^{-6} mg *B.t.i.*/ml for *An. arabiensis* and 2.05×10^{-6} mg *B.t.i.*/ml for *An. gambiae* (Figs. 3 and 4).

Subsequent tests revealed that percentage mortality of the two species decreased per unit time with lowering of the concentration of *B.t.i.* (Figs. 5 and 6).

Two formulations of *B.t.i.*, Teknar and Vectobac, were later used for small scale field tests against four species of mosquitoes. Aqueous suspensions gave good results when applied at dosages of 0.125, 0.25 and 0.5 kg per hectare of *B.t.i.* Larval mortalities of *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say were not significantly different, although both were significantly different from those of *An. arabiensis* and *An. gambiae*. The anopheline larvae were less susceptible to *B.t.i.* than the culicine larvae.

Another important field observation was that the persistence of *B.t.i.* was rather limited. Larval mortality was considerably reduced when exposed to water from treated pools seven days later.

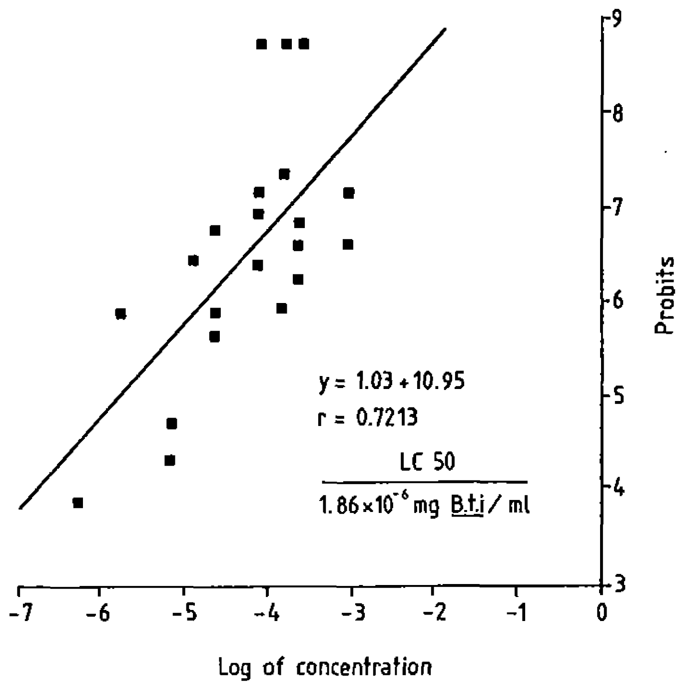

Fig. 3. The effect of different concentrations of *B.t.i.* on 4th instar larvae of *An. arabiensis* after 24 hours.

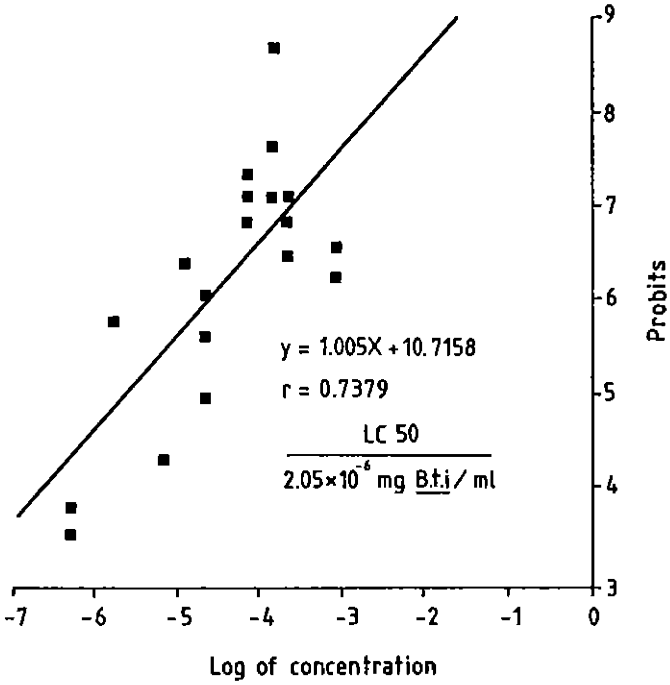

Fig. 4. The effect of different concentrations of *B.t.i.* on 4th instar larvae of *An. gambiae* s.s. after 24 hours.

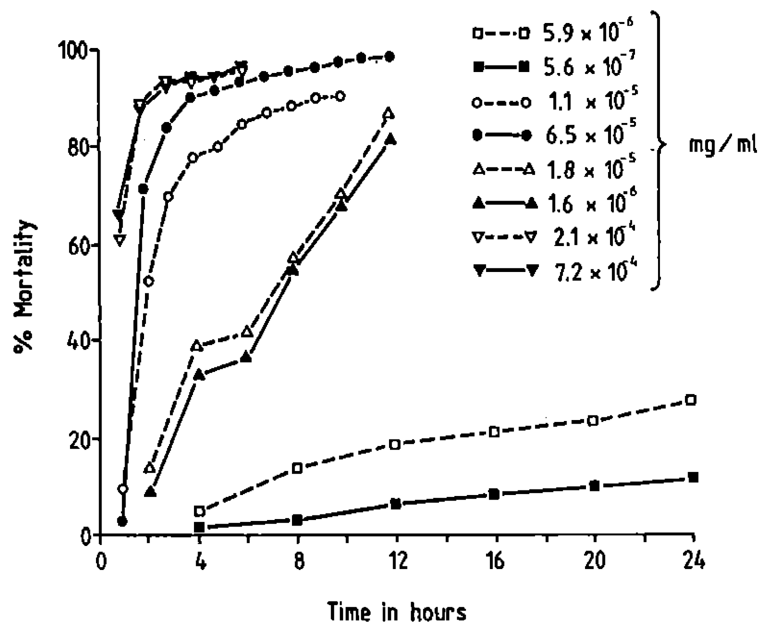

Fig. 5. The effect of different concentrations of *B.t.i.* on mortality of 4th instar *An. arabiensis* per unit time.

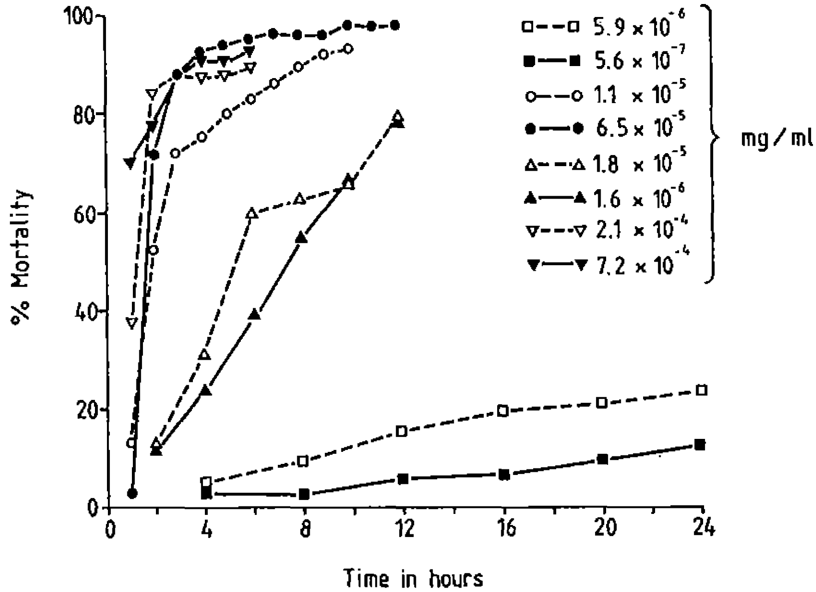

Fig. 6. The effect of different concentrations of *B.t.i.* on mortality of 4th instar *An. gambie* s.s. per unit time.

B.T.I. AS A COMPONENT OF MALARIA VECTOR CONTROL

As already suggested, any anopheline vector control programme in Kenyan rice fields should be an integrated one, incorporating climatic factors, the rice cycle, environmental management practices, insecticide spraying, the sterile insect technique (SIT) and other genetic measures, and biological means. In Mwea, control measures could be initiated during the rice growing phase from August to November. All major and subsidiary canals should be cleared up as much as possible to ensure uninterrupted water flow. Other operations should include weeding the fields and building levees alongside the canals to prevent seepage and overflow. Any remaining stagnant water bodies should then be larvicided with formulations of *B.t.i.*, possibly in combination with monomolecular organic surface films (Levy et al., 1984; Perich et al., 1987). From December to the end of February, intensive pyrethrum spraying should be launched and sustained. This, together with the prevailing dry weather conditions should considerably suppress the native population. From March, large numbers of sterile male *An. arabiensis* may be released and sustained to further suppress the noxious population genetically, particularly in view of the exophilic nature of *An. arabiensis*. This should be sustained up to the end of the short rainy season in December. To suppress the larval population, *B.t.i.* larviciding should again be initiated at the onset of the long rainy season in April and sustained throughout until the beginning of the dry season in January, or until all stagnant water bodies are drained or have dried up.

DISCUSSION

The control of *An. arabiensis*, and indeed all mosquito vectors may be directed at the breeding of vectors and thus reducing the vector population, or maybe directed against adults, whereby the general objective is similarly an adequate reduction in the vector population. A comprehensive review of available methods of mosquito vector control, with special emphasis on malaria vectors is available (WHO, 1982). Specific control measures against mosquitoes breeding in rice fields using orthodox techniques is adequately covered by Surtees (1970).

Chemical larviciding has been the main control method directed against immature stages of mosquitoes for a long time. However, recent advances in the development of *B.t.i.* formulations with improved toxicity and enhanced field persistence (Margalit and Dean, 1985), or incorporating MSF's (Levy et al., 1984; Perich et al., 1987), will definitely increase the role of microbial larviciding in future control applications.

The volume of information on *B.t.i.*, including its future potential, is gradually increasing (Ignoffo et al., 1981; Sebastian and Brust, 1981; Margalit et al., 1983; Margalit and Dean, 1985). In Kenya, local small scale trials have not only indicated a promising future for bacterial control of larval malaria vectors, but have also raised fundamental questions with regard to the search for local isolates of *B. thuringiensis* or closely related strains. It is conceivable that such isolates might have transferable characteristics that might enhance the toxicity, field persistence or the general performance of *B.t.* formulations in current use.

REFERENCES

Gillies, M.T. and de Meillon, B. 1968. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical region). 2nd edition. South African Institute of Medical Research, No. 54.

Highton, R.B., Bryan, J.H., Boreham, P.F.C. and Chandler, J.A. 1979. Studies of the sibling species *Anopheles gambiae* Giles and *Anopheles arabiensis* Patton (Diptera:Culicidae) in the Kisumu area, Kenya. *Bull. Entomol. Res.* 69:43–53.

Ignoffo, C.M., Garcia, C., Kroha, M.J., Fukuda, T. and Couch, T.L. 1981. Laboratory tests to evaluate the potential efficacy of *B.t.i.* for use against mosquitoes. *Mosq. News* 41:85–93.

Levy, R., Powell, C.M., Hertlein, B.C. and Miller, B.C. Jr. 1984. Efficacy of Arosurf MSF (Monomolecular Surface Film) base formulations of *Bacillus thuringiensis* var. *israelensis* against mixed populations of mosquito larvae and pupae: Bioassay and preliminary field evaluations. *Mosq. News* 44:357–543.

Margalit, J. and Dean, D. 1985. The story of *B.t.i. J. Am. Mosq. Control Assoc.* 1:1–7.

Margalit, J., Zomer, E., Erel, Z. and Barak, Z. 1983. Development and application of *B.t.i.* (H-14) as an effective biological control agent against mosquitoes in Israel. *Biotechnol.* 1983.

Mosha, F.W. and Petrarca, V. 1983. Ecological studies on *An. gambiae* complex sibling species on the Kenya Coast. *Trans. Royal Soc. Trop. Med. Hyg.* 77:344–345.

Mukiama, T.K. and Mwangi, R.W. 1989a. Field studies of larval *Anopheles arabiensis* Patton of Mwea Irrigation Scheme, Kenya. *Insect Sci. Appl.* 10:55–62.

Mukiama, T.K. and Mwangi, R.W. 1989b. Seasonal population changes and malaria transmission potential of *Anopheles pharoensis* and the minor anophelines in Mwea Irrigation Scheme, Kenya. *Acta Trop.* 46:181–189.

Perich, M.J., Rogers, J.T. and Boobar, L.R. 1987. Efficacy of Arosurf MSF and formulations of *Bacillus thuringiensis* var. *israelensis* against *Anopheles albimanus*: Laboratory bioassay. *J. Am. Mosq. Cont. Assoc.* 3:485–488.

Sebastian, R.J. and Brust, R.A. 1981. An evaluation of 2 formulations of *B.t.i.* for larval control in sod-lined simulated pools. *Mosq. News* 41:508–511.

Surtees, G. 1970. Control of mosquitoes breeding in ricefields. *J. Trop. Med. Hyg.* 74:255–259.

White, G.B. 1974. *Anopheles gambiae* complex and disease transmission in Africa. *Trans. Royal Soc. Trop. Med. Hyg.* 68:275–301.