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CONTROL OF MOSQUITOES WITH *BACULLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND LARVIVOROUS FISH, *TILAPIA NILOTICA*, IN RICE FIELDS IN LIBERIA, WEST AFRICA.

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ABSTRACT

In most regions in Liberia farmers are engaged in agricultural production, especially in production of ricc which is the staple food in Liberia. Since larvae of *Anopheles gambiae* commonly breed in rice fields, this biotope significantly contributes to maintenance of population of this vector in the country. A considerable number of farmers arc also engaged in rearing fish in ponds and in cultivated rice fields. Since malaria is hyperendemic in most areas of Liberia, it was desirable to find out an effective, safe and economical method of control of *Anopheles* larvae breeding in rice fields and to evaluate the role of fish as a biological control agent of anopheline mosquitoes.

Two control agents, *Bacillus thuringiensis* var. *israelensis* and a larvivorous fish *Tilapia nilotica* have been used in control of *Anopheles gambiae* and other mosquitoes breeding in rice fields in the Suakoko area. An experimental rice field with 12 plots was built near the Central Agricultural Research Institute in Suakoko. On the first three plots *B.t.i.* was used alone. On the second three plots *T. nilotica* was used alone and on the third three plots *B.t.i.* was used in combination with T. *nilotica.* The last three plots were untreated, used as a control.

Effective doses *of B.t.i.* and population density of fish have been established and intervals *of B.t.i.* applications for this environment and region determined. Results on effects of each control agent alone and in their combination will be presented.

INTRODUCTION

Vector-borne diseases are often linked to poor socio-economic conditions but somecan be associated with projects aimed at economic development (Mather, 1983). Thus construction of dams may lead to an increase in the prevalence of malaria, schistosomiasis, filariasis and other vector-borne parasitic and viral diseases. In most regions of Liberia farmers are engaged in agricultural production, especially the production of rice which is the country's staple food.

Before the past two and half decades agricultural practices were that of upland rice cultivation and cash crop production. However, these decades have experienced the shift from these farming methods to swamp rice cultivation. In order to maximize rice production, experts and external donor agencies from developed countries have recommended irrigation rice cultivation over that of the traditional method of swamp cultivation in many developing countries.

Since larvae of *Anopheles gambiae,* malaria vector in Africa, breed in rice fields, this biotope may significantly contribute to the maintenance of populations of this vector in parts of the country where large scale irrigations related to farming are carried out. Since most areas in Liberia arc hyperendemic for malaria, rice fields obviously contribute to the intensity of transmission.

The current economic situation in this country does not provide resources for use of conventional malarial control based on chemical insecticides. Moreover, the emergence and spread of insecticidc resistance in many vector species, the concern with environmental hazards, and the high cost of new chemical insecticides make it apparent that vector control can no longer be dependent only on the use of chemicals. Therefore it is desirable to find alternative economic, safe and effective methods of mosquito control.

For these and many other reasons, increasing attention has been directed toward natural enemies of disease vectors such as parasites, pathogens and predators. In this study we chose to use *Bacillus thuringiensis* var. *israelensis (B.t.i.)* and a larvivorous fish, *Tilapia nilotica*, in a combination explained in this study. *Bacillus thuringiensis* serotype H-14 is an excellent candidate for biological control of larval mosquitoes (Goldberg and Margalit, 1977) and has been evaluated in trials under different climatic conditions in North America (Garcia and Des Rochers, 1979; Sebastien and Brust, 1981; Dames et al., 1981; Wraight et al., 1982). This promising biological control agent has also been evaluated in studies on rice fields (Sandoski et al., 1985; McLaughlin and Billodeaux, 1983; Lacey and Inman, 1985). However, very few trials have been done with this agent in West Africa.

Undoubtedly a lot has been done with fish as biological control agents for mosquitoes. The fish that has long been used for this purpose is *Gambusia affinis affinis*. In recent times the quest for suitable substitutes for *Gambusia affinis affinis* on mosquitoes has focused attention on the cichlid genus *Tilapia* (Legner and Medved, 1973). *Tilapia nilotica*, a fish used mostly in ponds by local farmers in Liberia, is believed to be larvivorous but its impact on mosquitoes has not been assessed in rice fields in West Africa.

MATERIALS AND METHODS

Study area

The study was conducted in Central Liberia at the Central Agricultural Research Institution (CARI) at Suakoko located about 192 kilometers inland from Monrovia, the capital city of Liberia. The climate is tropical with two seasons of wet (May–October) and dry (November–April)

Rice field

The experimental rice field was built in a natural swamp area that was supplied with water from a swamp above. The area was divided into 12 plots to suit our study needs. Each plot had a dimension of 10 meters wide and 15 meters long (150 sq. meters). The plots were separated from each other by mud bands that were 1 meter in width and 10 meters in length. After the plot construction was completed, the entire experimental field had two side drainage canals and a central irrigation canal. Each of these canals was 93.4 meters long and 1 meter wide. The 12 plots covered a total land surface area of about 1800 square meter. In addition to the stream flowing from the swamp above, two big wells were dug to serve as additional water sources in case of critical times during the dry season.

Cultivation

When the plots were being prepared, a 90 days variety of rice (BG90-2) was planted in the nursery. Depending on the water situation in the nursery beds and plots, irrigation was limited to a few hours daily. In all of our trials the BG90-2 variety was used. Rice was transplanted from nursery beds to plots after 21 days. Before transplanting all plots were thoroughly drained and subsequently flooded after the transplant. One day after flooding all plots were treated with Teknar HP-D (Zoecon Corporation, Dallas, Texas), a formulation of *B.t.i.* Twenty four hours after plot treatment all plots were sampled for the presence of mosquito larvae. When it was sure that no larvae were present in any of the plots, systematic sampling began on day zero. Day zero was the day after treatment.

Intervention

B.t.i. Application and fish stocking

Results from our laboratory and field trials with Teknar HP-D (*B.t.i.*, potency of 3000 AA units per milligram) led us to choose 15 grams of this formulation as the dosage to use on each plot (150 sq. meters). This amount is equivalent to about 14.53 ml of this liquid formulation. Although we demonstrated in one of our trials that half of this amount (7.5 ml) was just as effective we continued to use 15 ml to treat each plot. The 15 ml of *B.t.i.* liquid was mixed with water in a hand sprayer (Birchmeier Senior) and manually applied over the entire surface of each plot that was to be treated. Just before treatment started, the 12 plots were divided into four groups of 3 plots. Group A was designated only for fish stocking, Group B for both *B.t.i.* and fish stocking, Group C only for *B.t.i.* treatment and Group D was used as control plots. In this trial Group C plots were first treated with *B.t.i.* 15 days after flooding. Subsequent *B.t.i.* applications were then carried out at 6 day intervals thoughout the length of the rice growth. Group B was first treated with *B.t.i.* 21 days after flooding and subsequent treatments followed every 12 days until harvest.

Those plots that were to be stocked with fish (Group A & Group B) had dug in them trenches around the rice in plot areas that were 0.61 meter deep and 1 meter wide. These trenches were dug in order to prevent the fish from dying in the event the plots were drained of water accidentally. Group A plots were each seeded with 300 fingerlings of *Tilapia nilotica* 9 days after flooding and Group B plots were seeded with the same number of fingerlings 21 days after flooding.

Mosquito larval sampling

Sampling once started on day zero continued every 3rd day until rice was harvested. A hybrobiological net with a diameter of 20 centimeters and a handle one meter long was the sampling tool in this study. Dipping for our study was not satisfactory, since larval distribution may be somewhat spotty, a certain number of larvae and especially pupae may escape and it is impossible to measure the surface covered by a single dip or series of dips (Cambourance, 1939). During sampling, the net was thrust down about 10 centimeters in the water in the rice plots and a sweep of one meter was swiftly performed. Five of these sweeps made up one sample from a surface area of 1 sq. meter (20 cm × 100 cm × 5).

Absolute density is the measure of the number of organisms in a defined area. By adding the attribute of area to the estimate it becomes possible to make comparisons among populations from different areas and quantify population levels (Steward and Miura, 1985). Moreover, absolute density can be determined with exactness (counting the entire population) or by estimate (counting a known proportion of the population), depending upon the intensity of the study. Since one of the aims of our study was to determine the proportion of a particular mosquito species, we chose to count a known proportion of the population in our study plots. To carry out this method we decided to use a quadrant device just described above, which sampled a surface area of 1 sq. meter in five random sweeps (Trpis, 1960; Service, 1971).

Since mosquito larvae are not uniformly distributed in any breeding site, surface areas of our plots were divided into four microbiotopes in order to get the true picture of the most important breeding sites in the rice fields. This also gave us the opportunity to make comparisons between plots. The four microbiotopes are summarized as follows:

MICROBIOTOPE I — area covering the center canal of the rice field (surface area of 93.4 sq. meters)

MICROBIOTOPE II — area within the plot surrounding the rice plants, with water and no vegetation (surface area 37.6 sq. meters)

MICROBIOTOPE III — the surface area of the plot covered by the rice plants (area 104 sq. meters)

MICROBIOTOPE IV — surface area of the two side canals (93.4 sq. meters each).

In each one of these microbiotopes, a total of five random sweeps were made thus constituting a single sample from 1 sq. meter of a microbiotope. The content in the sampling net was carefully emptied in a wide mouth rearing pan half filled with clean water. All larvae present in a sample were laboriously collected using small rubber bulb pipettes and were preserved in 75% ethyl alcohol in screw top vials. Samples were taken to the laboratory for counting and proper identification (using keys by Henry M. Gelfand, *The Anopheline Mosquitoes of Liberia,* 1954 and Botha De Meilion's *The Anophelini of the Ethiopian Geographical Region,* 1968). In addition to mosquito larval sampling, other experimental data which will not be treated in this paper were collected.

RESULTS AND DISCUSSION

The effectiveness of Teknar HP-D and the impact of *Tilapia nilotica* on mosquito larvae are presented in the graphs of 1–3. In order to clearly see the impact of the two control agents in this study we have only reported data on the 3rd and 4th mosquito larval instars. This is not to say that these agent are not effective against 1st and 2nd larval instars. It is simply because the residual effect of *B.t.i.* wears away after 24(+) hours thus the newly hatched 1st instar larvae are bound to be present when sampling is carried after this period. Therefore if we just look at total larval numbers on a given day we will be misled.

Figure 1 shows the relationship between the control plots and plots that were stocked with fish and treated every 12 days with *B.t.i.* There is clearly a significant difference between the larval population in these two groups of plots. The early high peak in *B.t.i.* & Fish curve comes before the date of treatment with *B.t.i.* and stocking of fish in these plots. Figure 2 and Figure 3 show results of control plots vs *B.t.i.* plots and control plots vs fish plots respectively. In the former the plots were first treated with *B.t.i.* 15 days after flooding the newly transplanted rice. The peak which occurs at day 27 on the *B.t.i.* curve is not unusual. During our study we observed that mosquito larvae, particularly *Anopheles,* reached 3rd instar stage within 6 days after eggs hatched. The peak is the result of largely 3rd larval instars that were collected in these set of plots 6 days after the previous treatment with *B.t.i.* Moreover, this date lies within the period of rice growth when larval populations in the rice fields are high. In the latter plots with fish and no other intervention, the fish curve in this graph is rather smoother than that of *B.t.i.* in the former graph. This smoothness could be attributed to the continuous predatory effect of the fish.

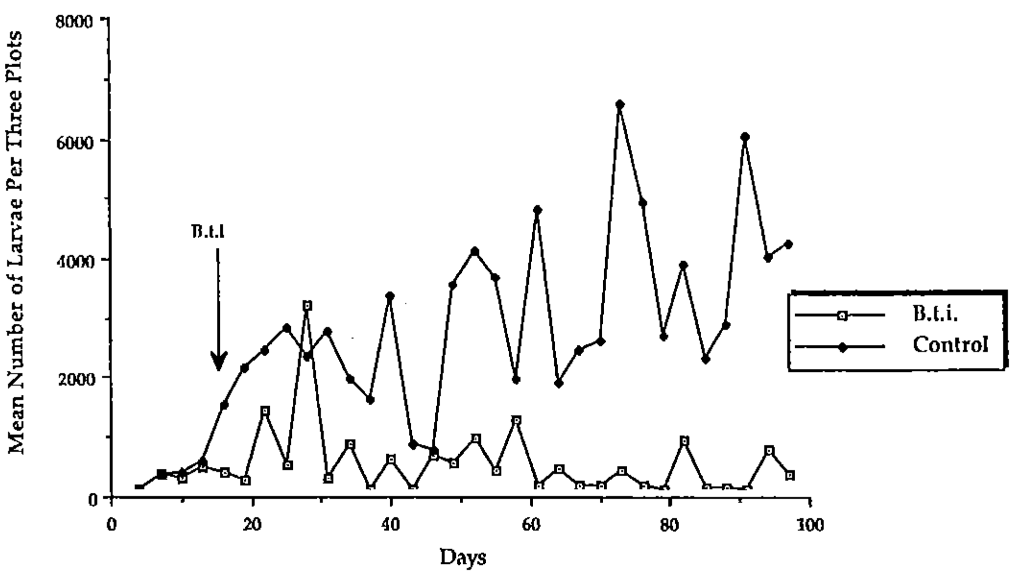

Fig 1. Population dynamics of mosquito larvae in three plots treated with *B.t.i.* and three untreated (control) plots.

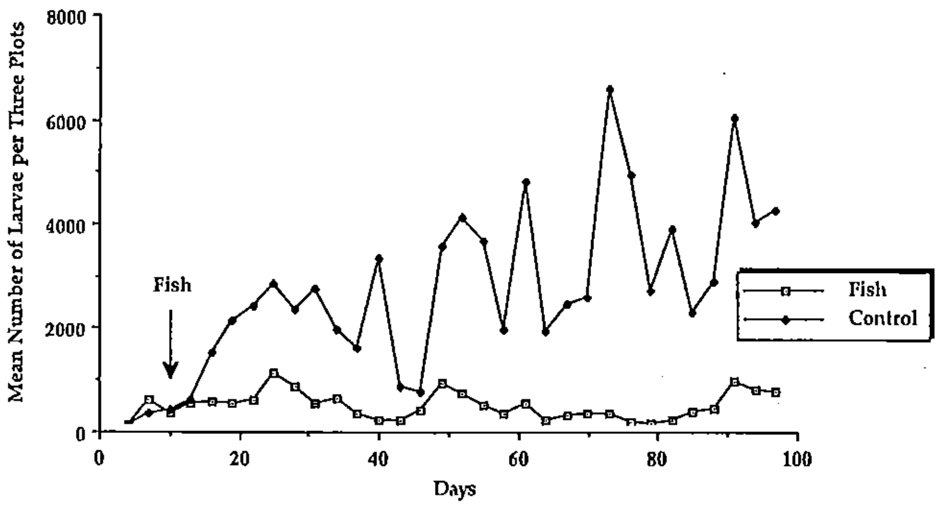

Fig 2. Population dynamics of mosquito larvae in three plots stocked with *Tilapia nilotica* and three control plots.

The interpretation of the data from our study indicated that Teknar HP-D penetrated the rice and was effective against mosquito larvae throughout the rice growth period. This study and others (Sandoski et al., 1985, Lacey an Inman, 1985, McLaughlin and Billodeaux, 1983), to name a few, affirm the potential of *B.t.i.* as an effective and practical means of controlling mosquito vectors breeding in rice. It also indicated that *Tilapia nilotica* could be very useful not only as food and

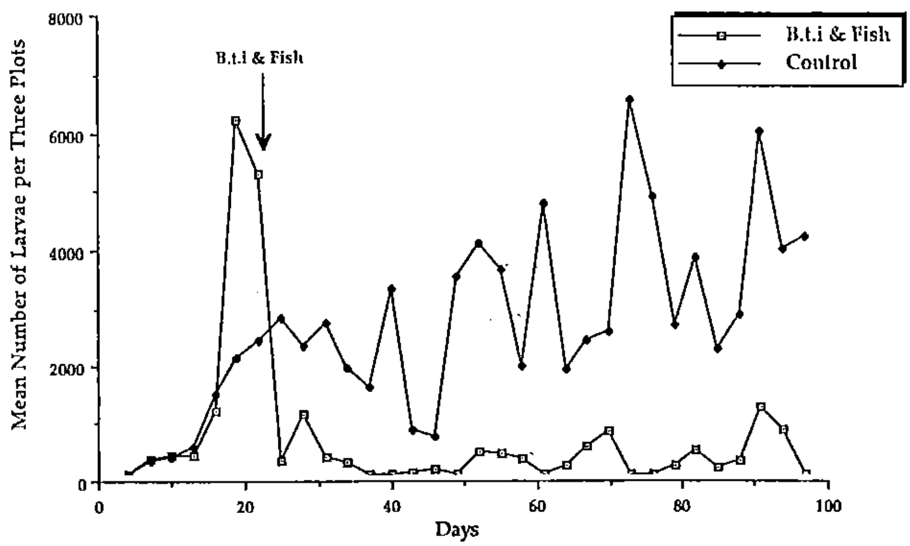

Fig 3. Population dynamics of mosquito larvae in three plots stocked with *Tilapia nilotica* and treated with *B.t.i.* and control plots.

protein source in tropical countries where malnutrition is a problem, but also a potential factor in the suppression of malaria in these areas.

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REFERENCES

Cambourance, F.J. 1939. A method for determining the larval *Anopheles* population and distribution in rice fields and other breeding places. *Rivist de Malariologia* 18:17–22.

Dame, D.A., Savage, K.E., Melsh, M.V. and Oldacre S.L. 1981. Assessment of industrial formulation of *Bacillus thuringiensis* var. *israelensis*. *Mosq. News* 41:540–546.

Garcia, R. and Des Rochers, R. 1979. Toxicity of *Bacillus thuringiensis* var *israelensis* to some California mosquitoes under different conditions. *Mosq. News* 39:541–544.

Goldberg, L.J. and Margalit J. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitatus, Aedes aegypt* and *Culex pipiens*. *Mosq. News* 37:355–358.

Lacey, L.A. and Inman, A. 1985. Efficacy of granular formulation of *Bacillus thuringiensis* (H-14) for the control of *Anopheles* larvae in rice fields. *J. Am. Mosq. Control Assoc.* vol 1.

Legner, E.F. and Medved, R.A. 1973. Predation of mosquito and chironomid midges in ponds by *Tilapia zillii* (Gervais) and *T. mossambica* (Peters). Proc. California Mosq. Control Assoc. 41:119–121.

Margalit, J. and Dean, D. 1985. The story of *Bacillus thuringiensis* var. *israelensis*. *J. Am. Mosq. Control Assoc.* 1:1–7.

Mather, T.H. 1983. Environmental Management for Vector Control (FAO publication).

McLaughlin, R.E. and Billodeaux, J. 1983. Effectiveness of *Bacillus thuringiensis* var. *israelensis* against *Psorophora columbiae* breeding in rice fields. *Mosq. News* 43:30–33.

Russel, P.F., West, L.S. and Manwell, R.D. 1946. Practical Malariology. Philadelphia, Saunders.

Sandoski, C.A., Yates, M.M., Olson, J.K. and Melsch, M.V. 1985. Evaluation of Beecomist-Applied *Bacillus thuringiensis* (H-14) against *Anopheles quadrimaculatus* larvae in rice fields. *J. Am. Mosq. Control Assoc.* 1:316–319.

Sebastien, R.J. and Brust R.A. 1981. An evaluation of two formulations of *Bacillus thuringiensis* var. *israelensis* for larval mosquito control in sod-lined simulated pool. *Mosq. News* 41:508–512.

Service, M.W. 1971. Studies in sampling larval populations of the *Anopheles gambiae* complex. *Bull. Wld. Hlth. Org.* 45:169–180.

Steward, R.J. and Miura, T. 1985. Density and population growth of mosquito fish (*Gambusia affinis*) in rice fields *J. Am. Mosq. Assoc.* 1:8–14.

Trpis, M. 1960. Mosquitoes of Rice Fields and Possibilities of Their Control, Biological Works 6/3. Slovak Academy of Science, Bratislava.

Wraight, S.P., Molloy, D. and McCoy, P. 1982. A comparison of laboratory and field test of *Bacillus sphaericus* strain 1953 and *Bacillus thuringiensis* var. *israelensis* against *Aedes stimulans* larvae (Diptera: Culicidae). *Can. Entomol.* 114:55–61.