Israel Journal of Entomology

Vol. XXIII(1989) pp. 95-97

THE COMBINATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* WITH A MONOMOLECULAR FILM

GILLIAN M. ROBERTS
Wolf son Mosquito Control Project, University of Southampton, Chilworth Manor, Southampton, S09 1XT, UK

ABSTRACT

The use of *B.t.i.* wilh a surface-active monomolecular film (monolayer) is a very safe combination of two mosquito control methods wilh minimal environmental impact For *B.t.i.*, the monolayer can be regarded as a formulation agent, itself with complementary insecticidal action. Monolayer kills mainly the mature larvae and pupae, and prevents oviposition of some species. *B.t.i.* rapidly kills the feeding larval instars. Laboratory experiments show that the monolayer spreads the *B.t.i.* over the water surface and also prevents the sinking of the crystal toxin by keeping the *B.t.i.* near the surface and therefore longer in the feeding zones of the larvae.

The application frequency is less than either larvicide alone, providing an economical method of control. As the mixture is self-spreading it can be applied from small plastic bottles, no special spray equipment being necessary. The larvicide is particularly useful in rural participation schemes as it is safe and easy to apply. Field tests have been carried out in Sri Lanka as part of an integrated control programme where only environmentally safe methods are used. Survival and multiplication of predators further delays the need for reapplication which chemical insecticides would have killed.

The air-water interface is important in all stages of the life of the mosquito. The surface tension of the water is used boih by the emerging adult mosquito and for the respiration of the larvae and pupae, also in many species eggs are laid on the water surface. By reducing the surface tension of the water surface these activities become difficult or impossible. Spraying the water surface with oil has been common practice for many years but this is pollutive and harmful to other aquatic life. Monomolecular films (monolayers) of high surface pressure spread better than oils and are non pollutive. They are invisible, biodegrade slowly and very few non-target organisms are affected. They reduce the surface tension of the water so that the breathing siphons and pupal trumpets become wetted (McMullen, 1977), while the emerging adult drowns on the surface and the egg laying adult becomes trapped. Anophelines are more vulnerable than culicines (Reiter and McMullen, 1978). Pupae and late larval instars die quicker than younger instars. The mortality is highest at night when the dissolved oxygen content of the water is reduced by the respiration of vegetation, causing rapid anoxia of the larvae and pupae (Reiter, 1978). If an overdose of a monolayer is applied it is incapable of forming multilayers; a reservoir of the bulk material, similar to a drop of oil, appears on the water surface, and feeds the monolayer as the monolayer biodegrades.

Bacillus thuringiensis var. *israelensis (B.t.i.)* is lethal to feeding larvae and is quick to kill (Margalit and Dean, 1985; Burges, 1982). However it tends to sink where it is sprayed and afler 24 hours it does not persist in the water environment (Margalit and Dean, 1985). Results from my experimental work with pond mud suggest that, rather than adhering to the mud, the toxin crystal is broken down by biodegrading organisms.

My initial investigations were carried out to see, if by combining *B.t.i.* with a monolayer, the *B.t.i.* could be prevented from sinking and therefore be available to the feeding larvae for extended periods. Results showed that the monolayer was capable of holding the *B.t.i.* for 24 hours. In addition, the reservoirs of monolayer floating on the surface stored the *B.t.i.* and slowly released it as the monolayer biodegraded and drew replacement molecules across the water surface from the reservoir. The two larvicides complemented each other in other ways. *B.t.i.* is most active against the younger instars and not against the nonfeeding 4th instars and pupae; the monolayer is less active against young instars, which rely more on cutaneous respiration than using the water surface, and lethal to the pupae and older larvae. Also, the self-spreading qualities of the monolayer take the *B.t.i.* to all parts of the water surface so careful spraying becomes unnecessary (Roberts and Burges, 1984).

Several monolayer materials were screened and the best combination appeared to be a monolayer which remains liquid at room temperature, combined with a *B.t.i.* primary powder. They mixed well, and to overcome the problem of the *B.t.i.* sinking in the monolayer liquid during storage, it was held in suspension by the addition of a gelling agent. Unfortunately, once mixed, the keeping qualities of the *B.t.i.* become unstable after 3 weeks at tropical temperatures. The dosage rates required are low, so it is advisable to mix small quantities at a time rather than to be left with a surplus. The dosage used in Sri Lanka consisted of 5% *B.t.i.* in monolayer, applied at the rate of 3 litres/hectare neat. This is equivalent to 150 grams of *B.t.i.*/hectare so is an economical use of *B.t.i.*. The monolayer dosage has been calculated to persist on clean water for 3 days to reduce the necessity for frequent spraying. In Sri Lanka, where the aim is to reduce the numbers of mosquitoes, spraying is carried out monthly.

The laboratory investigations have included experiments with different bottom substrates, persistence of activity and the distance the *B.t.i.* is carried by the monolayer. The test species of larvae used have been in the main *Aedes aegypti* and *Anopheles stephensi*. As the monolayer is slow acting, unless the oxygen content of the water has been reduced, the mortality readings taken after 24 hours are due to the *B.t.i.*. This proved to be useful when comparing monolayers to detect any loss of activity of the *B.t.i.*. The more soluble the monolayer the greater the loss of the activity of the *B.t.i.*.

Both these methods of control, one biological and one physical, are ideal for use in community action programmes in rural areas. They, unlike chemical pesticides, are safe for the villagers to apply, requiring neither sophisticated equipment nor special clothing. An accidental overdose is not harmful to the environment. Larviciding fits well into an integrated mosquito control programme and should be included in Government Health Programmes (Rafatjah, 1982). An increasing number of diverse methods are urgently needed to supplement residual spraying and anti-malarial drugs, both of which are experiencing increasing resistance. So many area authorities hesitate before the introduction of new ideas because they want to evaluate. In contrast, very little evaluation is carried out on existing mosquito control methods; residual spraying is often routine regardless of the results obtained. Spraying with chemical insecticides is expensive, requiring skilled labour and costly pesticides and is often harmful to natural predators of the mosquito.

Diversification is not necessarily difficult to organize. The work in Sri Lanka has been carried out by the Sarvodaya Movement, which happens to have a highly-organized community-participation scheme covering 8,000 rural villages. Integrated mosquito control methods have slotted well into their other activities which include pre-schools and health care. Not every country has communities organized on this scale but there are many which have primary health care schemes, which could assist. One of the most satisfying strategies is to start with a pilot operational area to iron out problems and to establish a methodology which can spread gradually. Additional methods, such as larvivorous fish, draining and water management, nets impregnated with permethrin (Lines, Myamba and Curtis, 1987) and insect repellant sticks should also be phased in. The benefit of self-help schemes offers enormous potential in the fight against malaria and other diseases carried by mosquitoes. The villagers would also benefit in times of racial conflict, when they are often out of reach of government assistance, as experienced in Sri Lanka, when they are forced through circumstance to fend for themselves.

REFERENCES

Burges, H.E. 1982. Control of insects by bacteria. *Parasitol.* 84:79–86.

Lines, J.D., Myamba, J. and Curtis, C.F. 1987. Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Med. Vet. Entomol.* 1:37–51.

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

McMullen, A.I., Reiter, P. and Phillips, M.C. 1977. Mode of action of insoluble monolayers on mosquito pupal respiration. *Nature* 267: No. 5608 244–245.

Rafatjah, H.A. 1982. Prospects and progress on IPM in world-wide malaria control. *Mosq. News* 42(4):491–497.

Reiter, P. and McMullen, A.I. 1978. The action of lecithin monolayers on mosquitoes. *Annals Trop. Med. Parasitol.* 72:163–176.

Reiter, P. 1978. The influence of dissolved oxygen content on the survival of submerged mosquito larvae. *Mosq. News* 38(3):334–337.

Roberts, G.M. and Burges, H.D. 1984. Combination of *Bacillus thuringiensis* var. *israelensis* and a surface active monolayer for mosquito control. *Brit. Crops Protn. Confr.* 1:287–292.