Some Environmental and Biological Factors Influencing the Activity of Entomopathogenic *Bacillus* on Mosquito Larvae in Brazil

RAGB Consoli, CJ Carvalho-Pinto */, MA Oliveira /**, BS Santos, MA Lamounier, RSA Alves***, CMB Silva***, L Rabinovitch ***

Centro de Pesquisas René Rachou - FIOCRUZ, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil *Departamento de Microbiologia e Parasitologia, UFSC, Florianópolis, SC **Departamento de Parasitologia, ICB/UFMG Belo Horizonte, MG ***Departamento de Bacteriologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

The influence of environmental and biological factors on the efficacy of Bacillus thuringiensis serovar israelensis *and* B. sphaericus *as mosquito larvicides are reviewed. The importance of strain dependence, cultivating media/methods, mosquito species/specificity, formulations and their relation to mosquito feeding habits, as well as temperature, solar exposure, larval density and concomitant presence of other aquatic organisms are addressed with reference to the present status of knowledge in Brazil.*

Key words: mosquito control - *Bacillus thuringiensis* s. *israelensis* - *Bacillus sphaericus* - environment - biological factors

Mosquitoes (Diptera: Culicidae) are considered the most important group of vectors of diseases, both in number of disease agents and in magnitude of health problems caused worldwide (Merritt et al. 1992). Many populations of mosquito vectors of diseases have developed resistance to synthetic organic insecticides, used mostly during the last half of this century. Thus interest in alternative strategies as well as in integrated control grew (WHO 1982, 1986). Among many methods and processes developed in recent years, bacterial insecticides have been the most successful and also the most widely researched. They are becoming increasingly important in mosquito control. Their specificity and harmlessness to the majority of organisms makes them compatible with current environmental concerns.

Environmental factors such as variability in climatic conditions (light, temperature), physical and chemical conditions present in the breeding sites, concomitant presence of predating and competing organisms may affect the availability and toxicity of bioinsecticides. Specific and strain/population features of agents and target, their formulations or physiological status are also important. Very few data on these topics are presently available in Brazil or in other Latin American countries.

Received 24 June 1994
Accepted 25 November 1994

The toxicity of bioinsecticides derived from *Bacillus thuringiensis* serovar *israelensis* (BTI) and *B. sphaericus* is directly dependent on the strain, cultivating media/methods (WHO 1987a, De Barjac 1989). Usually strain identification is done by biochemical, physiological and eletrophoretic methods (Zahner et al. 1989, De Barjac & Frachon 1990). Siegel et al. (1993), using cellular fatty acid analysis, identified four commercial brands (Acrobe, Bactimos, Teknar and Vectobac) as well as IPS 82 as being the same strain of BTI and identified Skeetal as a different one. New isolates and processes are continuosly tested. In Brazil, during the last five years, at CPRR/FIOCRUZ a total of 229 experiments envolving 193,000 *Aedes* larvae and 118 tests using 196,200 *Culex* larvae were performed. Zahner et al. (1990) described the isolation of BTI samples from soils in the Brazilian northeast and their toxicity against *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. fluviatilis*. Silva et al. (1991) and Rabinovitch et al. (1993) reported the development of new culturing processes for BTI and BS respectively, presenting both good toxicity and economic viability.

A large number of publications address the differential toxicity of BTI and BS for different mosquito species (Singer 1980, Davidson & Sweeney 1983, Mulla et al. 1984, 1985, WHO 1984, 1985, 1987 a, b, 1985, Lacey & Undeen 1986, Lacey et al. 1988, De Barjac 1989, Bowles et al. 1990, Priest 1992). Charles (1987) de-

scribed various ultrastructural events in the midgut of *An. Cx. quinquefasciatus* and *Ae. aegypti* following ingestion of BS spore/crystal complex. Additionally, Singh and Gill (1988) reported anomalies produced by BS in the CNS of *Cx. quinquefasciatus*. Mulla and Singh (1991) pointed out morphogenetic alterations in the same mosquito species produced by BTI. Most studies aimed to assess the efficacy of BTI and BS against different mosquito species were done with 3rd and 4th instar larvae, but susceptibility can be increased when younger larvae are employed (Mulla et al. 1984, WHO 1984, Lacey & Undeen 1986, Lago et al. 1991). Oliveira et al. (1992) testing three experimental of BTI and three commercial formulation (Vectobac, Teknar and Skeetal) on all four larval instars of *Cx. quinquefasciatus* and *Ae. fluviatilis,* observed, in most instances, an inverse proportional relation of toxicity to larval age. When BS (strain 2362) was employed, a similar effect seemed to occur (Oliveira, personal comunication).

The formulation and presentation of BTI and BS bioinsecticides, as well as their persistence in the water is directly related to their efficiency (Burges 1982, Lacey 1984, Fridlender et al. 1989, Matanmi et al. 1990, Karch et al. 1990, 1991, Lord 1991, Yousten et al. 1992, Nasci et al. 1994).

The knowledge of the particle size that are optimaly ingested by mosquito larvae in their natural habitats and the areas where they forage could greatly enhance the success of BTI and BS as particulate larvicides (Merrit et al. 1992). The same authors, considered most *Anopheles* as lentic planktonic swimmers, surface feeders with a collecting-filtering feeding mode that use microorganisms and detritus as the main food resource. *Culex* spp. and *Ae. aegypti* were regarded mainly as planktonic swimmers and divers: the former is filter-feeding on microorganims and detritus in the water column, the latter is mostly shredding on organic surfaces and sediments, ingesting microrganisms, detritus and parts of dead invertebrates. Additionally, Dahl et al. (1993) pointed out that *Cx. quinquefasciatus* larvae were able to select the size of the particles ingested and that *Ae. aegypti* usually ingested larger particles than the forrner species. Avissar et al. (1994) observed that shredding larvae ingest filamentous particles more easily then sphaerical ones, the reverse occuring with filter feeders. Starved larvae and larvae exposed to phagostimulants can also ingest much larger amounts of particles than otherwise (Dadd et al. 1982, Rashed & Mulla 1989). Very little is known of the feeding mode, feeding micro-habitat, food resources and prefered particle size of vectors and potential vectors in Brazil, especially of malaria vectors. Research of these topics should be very important in regard to ingestable biolarvicides.

Temperature is another important factor influencing the efficacy of BTI and BS insecticides. Substantial decreases in temperature are usually related to a reduced larvicidal activity. Wraight et al. (1981), Mulla et al. (1985, 1988), Becker et al. (1992), Tousignat et al. (1992) and Chui et al. (1993) reported a reduction of BTI toxicity proportional to the number of freezing and thawing cicles. Carvalho-Pinto and Consoli (1994a) tested BS strain 2362 against *Cx. quinquefasciatus* at temperatures of 15, 20 and 25°C and observed a reduction of LC 50 with increasing temperature. Since most research on this topic was done in temperate climates, data in tropical climates concerning local vectors should be desirable.

Solar or direct ultra-violet exposure can sometimes impair BTI and BS activity. De Barjac (1989), Ignoffo et al. (1981), Lacey (1984), Priest (1992), Becker et al. (1992) observed that BTI had its activity reduced 2.5 to 4.0 fold after 7 hr of solar exposition in Germany, when tested against *Cx. pipiens* and *Ae. aegypti*. When tested in Brazil, BS (strain 2362) also showed a progressively reduced activity against *Cx. quinquefasciatus* when exposed to 1, 2, 4, 6 and 8 hr of direct sunligth (Carvalho-Pinto & Consoli 1994a). In this case, as well, additional data obtained at tropical conditions should be useful.

Becker et al. (1992) and Chui et al. (1993) reported an inverted relation between larval density and efficacy of BTI against *Cx. pipiens* and *Ae. vexans*. Carvalho-Pinto and Consoli (1994a), using 10, 25, 50 or 100 *Cx. quinquefasciatus* fourth instar larvae in 150 ml samples, failed to observe significant diferences (a= 0.05) in the activity of BS (strain 2362) both after 24 or 48 hr.

The influence of the concomitant presence of other aquatic organisms, predators or others, in mosquito breeding places in relation to the efficacy of BTI or BS has been addressed in several publications. Becker et al. (1992) showed that the presence of the filter-feeder *Daphnia curvirostris* can reduce the quantity of BTI toxins in the water and consequently its efficacy. Marten et al. (1993), on the other hand, reported a synergic action of BTI with three genera of cyclopoid copepods (*Macrocyclops, Mesocyclops* and *Acantocyclops*). Orduz and Axtell (1991) observed the compatibility of BTI and BS with the fungal pathogen *Lagenidium giganteum* in control of mosquito larvae. Reyna et al. 1993, in Clark and Suarez 1993) also observed the synergic action of *Buenoa* sp. (Hemiptera: Notonectidae) and BTI against *Cx. quinquefasciatus*. The possibility of survival and reproduction of entomophatogenic bacteria inside other organisms has been considered. Snarski (1990) detected a few BTI spores in the faeces of the fish

Pimephales promelas after two weeks. *Belostoma micantulum* (Hemiptera: Belostomatidae) is a common predator in Brazilian water habitats, that may include mosquito breeding places (Consoli et al. 1989). While BS could be detected in the gut of this predator up to 10 days after its ingestion of infected larvae, no evidence of BS replication could be observed there (Carvalho-Pinto et al. 1993). The development of *B. micantulum*, its reproductive ability and number predated larvae was not substantially influenced by the ingestion of BS infected *Cx. quinquefasciatus* larvae (Carvalho-Pinto & Consoli 1994b).

The complex envirommental and biological factors influencing both bioinsecticides and their targets should be considered together in each instance to optimize mosquito control. New data, specially from tropical regions, where mosquitoes and mosquito-borne diseases are major problems, will be very necessary.

REFERENCES

AvissarYJ, Margalit J, Spielman A 1994. Incorporation of body components of diverse microorganisms by larval mosquitoes. *J Am Mosq Control Assoc 10:* 45-50.

Becker N, Zgomba M, Ludwig M, Petric D, Rettich F 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. *J Am Mosquito Control Assoc 8:* 285-289.

Bowles DE, Meisch MV, Wethersbee AA, Jones JW, Efird P, Bassi D 1990. Efficacy of *Bacillus sphaericus* formulations against *Psorophora columbiae* larvae in small rice plots. *J Am Mosq Control Assoc 6:* 631-634.

Burges HD 1982. Control of insects by bacteria. *Parasitology 84:* 79-117.

Carvalho-Pinto CJ, Rabinovitch L, Alves RSA, Silva CNB, Consoli RAGB 1993. Destino de *Bacillus sphaericus* 2362 após ingestão pelo Hemíptero predador *Belostoma micantulum* (Hemiptera:Belostomatidae). *XVII Congress Brasil Microbiol* - Santos, SP - Resumos, p.39.

Carvalho-Pinto CJ, Consoli RAGB 1994a. Eficácia de *Bacillus sphaericus* 2362 frente a alguns fatores ambientais. *IV Simpósio de Controle Biológico - SICONBIOL* - Gramado, RS - Anais, p. 131.

Carvalho-Pinto CJ, Consoli RAGB 1994b. Efeito de *Bacillus sphaericus* na biologia do predador *Belostoma micantulum* (Hemiptera:Belostomatidae). *IV Simpósio de Controle Biológico- SICONBIOL* - Gramado, RS - Anais, p. 319.

Charles JF 1987. Ultrastructural midgut events in Culicidae larvae fed with *Bacillus sphaericus* 2297 spore/crystal complex. *Ann Inst Pasteur / Microbiol 138:* 471-484.

Chui VWD, Koo CW, Lo WM, Qiu Xu-Jia 1993. Laboratory evaluation of Vectobac - 12 as and teflubenzuron against *Culex* and *Aedes* mosquito larvae under different physical conditions. *Environ Int 19*: 193-202 in *Entomol Abstracts* 25: *41* (2122-225), 1994.

Consoli RAGB, Pereira MH, Melo AL, Pereira LH 1989. *Belostoma micantulum* Stal, 1858 (Hemiptera: Belostomatidae) as a predator of larvae and pupae of *Aedes fluviatilis* (Diptera: Culicidae) in laboratory conditions. *Mem Inst Oswaldo Cruz 84*: 419-424.

Daad RH, Kleinjan JE, Merrill LD 1982. Phagostimulant effects of simple nutrients on larvae *Culex pipiens* (Diptera: Culicidae). *Ann Entomol Soc Am 75:* 605-612.

Dahl C, Sahlen G, Grawe J, Johannisson A, Amners H 1993. Differential particle uptake by larvae of three mosquito species (Diptera: Culicidae). *J Med Entomol 30:* 537-543.

Davidson EW, Sweeney AW 1983. Microbial control of vectors: A decade of progress. *J Med Entomol 20:* 235-247.

De Barjac H 1989. New facts and trends in bacteriological control of mosquitoes. *Mem Inst Oswaldo Cruz, 84* (Suppl. III): 101-105.

De Barjac H, Frachon E 1990 Classification of *Bacillus thuringiensis. Entomophaga 35:* 233-240.

Fridlender B, Keren-Zur M, Hofstein R, Bar E, Sandler N, Keynan A, Braun S 1989. The development of *Bacillus thuringiensis* and *Bacillus sphaericus* as biocontrol agents: From research to industrial production. *Mem Inst Oswaldo Cruz 84*(Suppl. III): 123-127.

Ignoffo CM, Garcia C, Kroha MJ, Fukuda T, Couch TL 1981. Laboratory tests to evaluate the potential efficacy of *Bacillus thuringiensis* var *israelensis* for use against mosquitoes. *Mosquito News 41:* 85-93.

Karch S, Monteny N, Jullien JL, Sinègre G, Coz J 1990. Control of *Culex pipiens* by *Bacillus sphaericus* and role of nontarget arthropods in its recycling. *J Am Mosq Control Assoc 6:* 47-54.

Karch S, Manzambi, ZA Salaum JJ 1991. Field trials with Vectolec (*Bacillus sphaericus*) and Vectobac (*Bacillus thuringiensis* (H-14)) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. *J Am Mosq Control Assoc 7:* 176-179.

Lacey LA 1984. Production and formulation of *Bacillus sphaericus. Mosquito News 44*(2 - Part I): 153-159.

Lacey LA, Undeen AH 1986. Microbial control of backflies and mosquitoes. *Ann Rev Entomol 31:* 265-296.

Lacey LA, Lacey CM, Peacock B, Thiery I 1988. Mosquito host range and field activity of *Bacillus sphaericus* isolate 2297 (serotype 25). *J Am Mosq Control Assoc 4:* 51-56.

Lago GM, Peres MD, Figueroa AM, Gonzalez FAC 1991. Resultados de las aplicaciones en pilotaje del biolarvicida *Bacillus sphaericus* 2362 en criaderos de mosquitos del municipio Santa Cruz del Norte (Provincia La Habana). *Rev Cubana Med Trop 43:* 39-44.

Lord JC 1991. Sustained release pellets for control of *Culex* larvae with *Bacillus sphaericus. J Am Mosq Control Assoc 7:* 560-564.

Marten GG, Che W, Bordes ES 1993. Compatibility of cyclopoid copepods with mosquito insecticides. *J Amer Mosq Control Assoc 9:* 150-154.

Matanmi BA, Federici BA, Mulla MS 1990. Fate and persistence of *Bacillus sphaericus* used as a mosquito larvicide in dairy wastewater lagoons. *J Am Mosq Control Assoc 6:* 384-389.

Merritt RW, Daad RH, Walker ME 1992. Feeding behaviour, natural food and nutritional relationships of larval mosquitoes. *Annu Rev Entomol 37:* 349-376.

Mulla MS, Darwazeh HA, Davidson EW, Dulmage HT, Singer S 1984. Larvicidal activity and field efficacy of *Bacillus sphaericus* strains against mosquito larvae and their safety to nontarget organisms. *Mosquito News 44*: 336-342.

Mulla MS, Darwazeh HA, Ede L, Kennedy B, Dulmage HT 1985. Efficacy and field evaluation of *Bacillus thuringiensis* (H-14) and *Bacillus sphaericus* against floodwater mosquitos in California. *J Am Mosq Control Assoc 1:* 310-315.

Mulla MS, Darwazeh HA, Titze NS 1988. Efficacy of *Bacillus sphaericus* 2362 formulations against floodwater mosquitos. *J Am Mosq Control Assoc 4:* 172-174.

Mulla SM, Singh N 1991. Delayed mortality and morphogenetic anomalies induced by the microbial control agent *Bacillus thuringiensis* ser.(H.14) in *Culex quinquefasciatus*. *J Amer Mosq Control Assoc 7:* 420-423.

Nasci RS, Wright GB, Willis FS 1994. Control of *Aedes albopictus* larvae using time-release larvicide formulations in Louisiana. *J Am Mosq Control Assoc 10:* 1-6.

Oliveira MA, Consoli RAGB, Rabinovitch L, Lamounier MA, Macêdo ME, Santos BS, Cavados CFG, Silva MHL 1992. Susceptibilidade dos diversos estádios larvais de *Culex (Culex) quinquefasciatus* Say 1823 e *Aedes (Ochlerotatus) fluviatilis* Lutz, 1904 (Diptera: Culicidae) a amostras de *Bacillus thuringiensis israelensis* (serotipo H - 14) Berliner, 1911. *IX Jornada Paul Parasit* - Ribeirão Preto, SP - Resumos, p. 47.

Orduz S, Axtell RC 1991.Compatibility of *Bacillus thuringiensis var. israelensis* with the fungal pathogen *Lagenidium giganteum* (Oomycetes: Lagenidales). *J Amer Mosq Control Assoc 7:* 188-193.

Priest FG 1992. Biological control of mosquitos and other biting flies by *Bacillus sphaericus* and *Bacillus thuringiensis* . *J Appl Bacteriol 72:* 357-369.

Rabinovitch L, Silva CNB, Alves RSA, Lamounier MA, Santos BS, Consoli RAGB 1993. Meio de cultivo proteico permite a rápida e elevada esporogênese do *Bacillus sphaericus* (BS) 2362 tóxico contra larvas de *Culex quinquefasciatus*. *XVII Congress Brasil Microbiol* Santos, SP - Resumos p. 47.

Rashed SS, Mulla MS 1989. Factors influencing ingestion of particulate materials by mosquito larvae (Diptera: Culicidae). *J Med Entomol 26:* 210-216.

Reyna M, Solis A, Gorrochotegui N, Rebollar EA 1993. Effect of Bactimos on the predatory capacity of *Buenoa* sp. upon *Culex quinquefasciatus* larvae. in GG Clark, & MF Suarez. Mosquito vector control and biology in Latin America - a Thrid Symposium. *J Amer Mosq Control Assoc 9:* 441-453.

Siegel JP, Smith AR, Maddox JV, Novak RJ 1993. Use of fatty acid analysis to characterize commercial brands of *Bacillus thuringiensis* var. *israelensis*. *J Amer Mosq Control Assoc 9:* 330-334.

Silva MHL, Magalhães MS, Sanches EG, Santos BS, Lamounier MA, Consoli RAGB, Rabinovitch L 1991. Desenvolvimento de processo para a produção industrial de bioinseticida a base de *Bacillus thuringiensis* var. *israelensis*. *I Feira e Congress Latino-Americano de Biotecnologia*, São Paulo, SP - Resumos p. 132.

Singer O 1980. *Bacillus sphaericus* for the control of mosquitoes. *Biotechnol Bioeng 22:* 1335-1355.

Singh GJP, Gill SS 1988. An electron microscope study of the toxic action of *Bacillus sphaericus* in *Culex quinquefasciatus* larvae. *J Invertebr Pathol 52:* 237-247.

Snarski VM 1990. Interaction between *Bacillus thuringiensis* subsp. *israelensis* and the Fathead Minnows, *Pimephales promelas* under laboratory conditions. *Appl Environ Microbiol 56:* 2618-2622.

Tabashnik BE 1994. Evolution of resistance to *Bacillus thuringiensis*. *Ann Rev Entomol 39:* 47-79

Tousignant ME, Boisvert JL, Chalifour A 1992. Reduction of mortality rates of *Bacillus thuringiensis* var. *israelensis* aqueous suspensions due to freezing and thawing. *J Amer Mosq Control Assoc 8:* 149-155.

WHO 1982. The role of biological agents in integrated vector control and the formulation of protocols for field testing of biological agents. Report of the sixth meeting of the scientific working group on biological control of vectors. WHO /TDR / VEC - SWG (6) / 82.3, 46 pp.

WHO 1984. Report of the seventh meeting of the scientific working group on biological control of vectors. TDR / BCV / SWG - 7 / 84.3, 33 pp.

WHO 1985. Informal consultation on the development of *Bacillus sphaericus* as a microbial larvicide. TDR / BVC / *Sphaericus* / 85.3, 24 pp.

WHO 1986. Resistance of vectors and reservoirs of disease to pesticides. Tenth report of the Expert Committee on Vector Biology and Control. Technical Report Series, 737, 87 pp.

WHO 1987a. Report of an informal consultation on the detection, isolation, identification and ecology of biocontrol agents of disease vectors. TDR/BVC/ICGE/87.3: 41 pp.

WHO 1987b. Report of an informal consultation on the role of molecular biology and genetic engineering in the development of biocontrol of disease vectors. TDR/BVC/MB-GE/87.3: 15 pp.

Wraight SP, Molloy D, Jamnback H, Mccoy P 1981. Effects of temperature and instar on the efficacy of *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* strain 1593 against *Aedes stimulans* larvae. *J Invertebr Pathol 38:* 78-87.

Yousten AA, Genthner FJ, Benfield EF 1992b. Fate of *Bacillus sphaericus* and *Bacillus thuringiensis* serovar *israelensis* in the aquatic environment. *J Am Mosq Control Assoc 8* : 143-148.

Zahner V, Momen H, Salles CA, Rabinovitch L 1989. A comparative study of enzime variation in *Bacillus cereus* and *Bacillus thuringiensis* . *J Appl Bacteriol 67:* 275-282.

Zahner V, Cavados CFG, Rabinovitch L, Santos BS, Lamounier MA & Consoli RAGB 1990. New samples of crystalliferous *Bacillus* isolated from brazilian soils. *II Simpósio de Controle Biológico - SICONBIOL* - Brasília, DF - Anais p. 91.