PRODUCTION AND FORMULATION OF *BACILLUS SPHAERICUS*

LAWRENCE A. LACEY[1]
Insects Affecting Man and Animals Research Laboratory, Agricultural Research Service, USDA, Gainesville, FL 32604

ABSTRACT. Literature on the production and formulation of isolates of *Bacillus sphaericus* that are highly larvicidal for mosquitoes is reviewed with recommendations for future research. Both large and small scale fermentation is possible using a variety of raw materials or synthetic media. Amino acids are the preferred nitrogen source and the bacterium requires the vitamins biotin and thiamine for growth and Ca^{++} and Mn^{++} for sporulation. It is highly aerobic and grows well between 25° and 40°C with a neutral pH, but sporulation and toxin production may be inhibited above 35°C.

Formulation of *B. sphaericus* will enable easier storage, mixing and application as well as improve stability, efficacy and residual activity. In addition to wettable powders, optimal utilization of this bacterium will require the development of liquid, granular and sustained release formulations.

The desire to minimize environmental impact of insecticides as well as combat insecticide resistance has increased the utilization of microbial insecticides in North America and abroad. A number of organisms are commercially produced in a variety of formulations; however, with the exception of *Bacillus thuringiensis* var. *israelensis* de Barjac (H-14), they are used for the control of arthropod pests of crops, stored products and forests. The production and formulation of *Bacillus thuringiensis*, one of the most commonly employed microbial agents for the control of lepidopterous pests, has been an ongoing venture since the 1950's (Couch and Ross 1980). With this background, the commercial development of several formulations of *B. thuringiensis* (H-14) for the control of mosquito and black fly larvae rapidly followed registration of 2 wettable powders and a flowable concentrate in the United States. Although initially available products were efficacious against mosquitoes in open water, they were less useful where breeding sites were covered by thick vegetation, in deep, water polluted habitats, and where residual control was desired (Lacey 1984). Currently, granular formulations, slow release briquettes, highly dispersable and more concentrated flowable formulations are available to attain the desired level of control in a variety of habitats.

Another efficacious microbial control agent of mosquitoes is *Bacillus sphaericus* Neide. Although it has a narrower spectrum of activity than *B. thuringiensis* (H-14), it appears to be more active against larvae of certain genera of culicine species (Hertlein et al. 1981, Lacey and Singer 1982, Singer 1982); its spores and toxins may persist for a considerable length of time in the environment (Hertlein et al. 1979, Mulligan et al. 1980); it recycles under certain conditions (Urbina et al. 1983, Abstract, Am. Soc. Microbiol.); and it offers the additional advantage of not affecting several species of predaceous mosquitoes (Lacey 1983).

Unfortunately, there are no currently available commercially produced formulations of *B. sphaericus*. Primary powders grown in government and university laboratories and previously produced by Stauffer Chemical Company and Abbott Laboratories constitute the majority of experimental materials.

FERMENTATION

Bacillus sphaericus may be grown on a variety of synthetic media and raw materials. Amino acids are apparently the optimal carbon and nitrogen source. Although *B. sphaericus* may be grown with glutamic acid as the sole source of amino acid, growth and toxin yield are improved considerably with the addition of lysine, methionine, isoleucine and valine in synthetic media (Singer 1981a, 1982). The bacterium requires the vitamins biotin and thiamine for growth and Ca^{++} and Mn^{++} for sporulation (Singer et al. 1966, Myers and Yousten 1978). It does not grow well with starch and several other carbohydrates as carbon sources but may respond nutritionally to pyruvate acetate and several Kreb's cycle intermediates (Singer 1981a). *Bacillus sphaericus* is highly aerobic; larger spores and greater larvicidal activity result from increased aeration (Gibb 1983); however sporulation, but not toxin production, is inhibited when pure oxygen is substituted for air (Yousten et al. 1984). Although the bacterium grows well at temperatures between 25 and 40°C, sporulation and toxin production in the 1593 isolate are inhibited at temperatures above 35°C. When a neutral pH is maintained in the fermentation medium, growth and sporulation are maximized (Yousten et al. 1984). Optimal synthetic media for maximal sporulation and

[1] Mention of a pesticide, commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

toxin production are presented by Singer (1981b), Kalfon et al. (1983) and Yousten et al. (1984).

Provided the minimum nutritional requirements are present, a multitude of raw materials can be utilized as fermentation media. Lane et al. (1984) obtained good primary powder production (2–3 g/liter of whole fermentation culture) when 2% skim milk and 0.5% yeast extract were used in a vitamin-salts basal medium. Hertlein et al. (1981) and Obeta and Okafor (1983) produced larvicidal *B. sphaericus* on a variety of agricultural wastes including extracts of dung as well as raw filtered sewage. Singer (1980) observed growth in hay infusion medium, a situation that mimics conditions found in natural detritus.

Maximum toxin production will be dependent on the most propitious combination of media and other fermentation parameters and the strain of *B. sphaericus* selected. A marked difference in growth, sporulation, time at which toxin is produced during fermentation and level of toxin production is observed for the various strains with mosquito larvicidal activity (Singer 1979, 1980; Myers and Yousten 1981, Bourgouin and de Barjac 1980). For example, one of the most promising isolates, 1593, is 3000 times more active than the SSII-1 isolate (Myers and Yousten 1981). Several other isolates appear to be at least as active as 1593 (Lacey and Singer 1982, Mulla et al. 1984a, 1984b; Yousten et al. 1984, Wickremesinghe and Mendis 1980). Yousten et al. (1984) found that strain 2362 sporulated better and produced toxin at higher fermentation temperatures and in a medium with a higher protein content than did strain 1593. They suggest that this might provide an advantage for production under field conditions in the tropics.

Bacillus sphaericus may be grown in a broad spectrum of vessels ranging from petri plates on solid media to shake flasks and small to large fermentors in liquid media. Even local production with a relatively small fermentor can produce substantial amounts of larvicide. Using a continuous flow fermentation procedure, B.C. Hertlein (personal communication) produces up to 1893 liters of whole fermentation culture over several days. Small scale cottage production using available raw materials may also enable use of *B. sphaericus* by developing countries that cannot purchase large quantities of commercially produced material. As Hertlein et al. (1981) point out, locally produced *B. sphaericus* offers the additional advantage of eliminating packaging, shipping, long-term storage and mixing.

Larger scale production has been somewhat limited. Dulmage and Correa (personal communication) have grown up to 200 liters at a time using submerged fermentation resulting in 4 kg of primary powder per batch. The largest batches to date were grown experimentally by Stauffer Chemical Company and Abbott Laboratories. The Stauffer material (isolate 1593; MV 716) has been tested extensively (Table 1). Both materials displayed good stability and efficacy (Davidson et al. 1981, Singer 1980, World Health Organization 1980). Avenues of future research for the improved fermentation of *B. sphaericus* are presented by Singer (1982).

A wide variety of bioassay protocols have been utilized for determination of toxicity of *B. sphaericus* preparations making it difficult to compare results of independent researchers. A standardized bioassay procedure would enable repeatable and rapid determination of toxicity of different fermentation runs or comparison of different isolates grown under identical conditions.

Table 1. Activity of the *Bacillus sphaericus* 1593 powder MV 716 (Stauffer Chemical Co.) toward 2nd or 3rd instar mosquito larvae.

Species	Source[a]	Temperature	LC_{50}, ppb	Reference
Culex quinquefasciatus	L	NR[b]	58	Singer 1980
Cx. quinquefasciatus	L	25	80–90	Sinegre et al. 1980
Cx. pipiens	F	26	49–83	Wraight et al. 1981b
Cx. pipiens (Hampton strain)	L	26	59–65	Wraight et al. 1981b
Cx. pipiens (Whitehall strain)	L	26	82–163	Wraight et al. 1981b
Cx. salinarius	L	26	50–61	Wraight et al. 1981b
Aedes stimulans	F	21	333–338	Wraight et al. 1981a, 1982
Ae. stimulans	F	13	1117–1455	Wraight et al. 1981a
Ae. aegypti	L	NR	47,860	Singer 1980
Anopheles albimanus	L	NR	275	Singer 1980

[a] L = Laboratory reared; F = Field collected.
[b] NR = Not reported.
Modified from Davidson (1984).

A standardized bioassay protocol proposed by E. W. Davidson and L. A. Lacey (unpublished) utilizes 48 hr old 2nd instar *Culex quinquefasciatus* Say exposed to 5–7 concentrations of each bacterial preparation for 48 hr at 27–32°C. Twenty larvae are exposed in covered wax paper cups in 100 ml of non-chlorinated water after the addition of 50 mg of debittered brewer's yeast. Alternatively, one-half the amount of larvae, water and yeast may be used. Three test cups/concentration and control are used/replication. Three replications are run over a period of time to ensure sufficient genetic variability in the colony larvae that are used. The concentrations of *B. sphaericus* should produce at least 2 mortality points above and 2 below the 50% level. After correction for control mortality with Abbott's formula, the concentration that produces 50% mortality (LC_{50}) is determined by plotting the mortality points on logarithm-probability paper. Alternatively and more accurately, the data can be subjected to computer programmed probit analysis if available. Control mortality over 10% warrants rejection of a replicate. Simultaneous exposure of additional *Cx. quinquefasciatus* larvae to the international standard of *B. sphaericus* (RB-80; isolate 1593 produced by the Pasteur Institute) will enable determination of International Units using the following formula:

$$\frac{LC_{50}\text{ RB-80 (in mg/liter or ppm; ng/ml or ppb; } \mu l/ml)}{LC_{50}\text{ test material}} \times 1000 = \text{IU/mg or IU/ml}$$

FORMULATION

To paraphrase Couch and Ross (1980): The blame for failure of a microbial agent is frequently assigned to the microbe when often the underlying cause is improper formulation or misuse of an otherwise effective formulation. The formulation requirements for *B. sphaericus* will depend on the habitats and behavior of the target mosquito as well as the level of control and duration of residual activity that are desired. Table 2 outlines the objectives that are desirable for future formulations of *B. sphaericus*. Although various characteristics of formulations of microbial control agents used in aquatic habitats differ considerably from those used in terrestrial-agricultural habitats, the principal goals of formulation are the same: increased ease of handling, and enhanced activity via greater stability and maximized contact with the target insect. Many of the adjuvants currently employed in the formulation of microbials for agricultural use may be useful in the formulation of *B. sphaericus*. The various classes of diluents, suspending agents, emulsifiers and other adjuvants, and their characteristics are reviewed by Angus and Lüthy (1971), Bull (1978), Couch (1978) and Couch and Ignoffo (1981).

Very little formulation research has been conducted as a consequence of lack of industrial interest in *B. sphaericus*. The Stauffer MV 716 powder is actually unformulated primary powder with a dispersant (N. Goodman, personal communication) and although it provided good control it did not disperse well in water (Mulligan et al. 1980). The Abbott powder was formulated as a wettable powder (primary powder, wetting agent, ataclay and a flowing agent) and was considerably easier to mix and apply (T. Couch, personal communication). The remainder of formulation work has been conducted by universities and the U.S. Department of Agriculture.

Burges (1983) recommends that formulation requirements should be considered at all stages of the fermentation process. Particle size of the nutrient ingredients employed could strongly influence the characteristics of the end product.

Table 2. Formulation objectives for microbial control agents of mosquito larvae.

I. *Increase Ease of Handling*
   1. Storage—space considerations
   2. Mixing—flowability, little or no mixing or dilution required
   3. Application—equipment compatibility (avoid clogging of nozzles, etc.); permit variety of application techniques

II. *Ensure Stability*
   1. Long shelf life—compatibility of adjuvants with toxin; prevent secondary fermentation and contamination
   2. Field—UV protection where needed; protection from microbial denaturing of toxin; minimize settling of toxin

III. *Maximize Contact with Target Larvae*
   1. Penetrate foliage—granular formulations
   2. Optimize dispersal—maximal distribution with minimal drift
   3. Maintain toxin in larval feeding zone—sustained release floating formulation
   4. Attract or arrest target larvae—inclusion of feeding stimulants/arrestants

IV. *Increase Residual Activity*
   1. Maximize prolonged contact with larvae (see III)
   2. Optimize field stability (see II–2)

V. *Considerations*
   1. Cost
   2. Adjuvant compatibility with toxin and environment
   3. Ensure normal larval feeding

Likewise, the drying process that is used can alter the nature of the primary powder considerably. Singer (1982) found that lyophilizing produced a more readily suspendable primary powder than that produced with the lactose-acetone precipitation method (Dulmage et al. 1970). Lyophilization of large quantities of fermentation residue, however, may be impractical. Good retention of larvicidal activity followed spray drying of the Stauffer and Abbott powders. The spray dried powders produced by Dulmage and Correa suspended more readily than their acetone-lactose precipitated powder, produced higher mortality and provided longer shelf life (H. T. Dulmage and J. A. Correa, personal communication; Davidson unpublished data; Mulla et al. 1984a, 1984b).

Particle size of wettable powders and flowable concentrates may be a function of the interaction of several factors, e.g., media constituents, drying procedure and formulation adjuvants. The particle size of the suspended formulations can exert considerable influence on application and ultimately, efficacy. Rapid settling of formulated *B. thuringiensis* (H-14) is a major factor influencing its lack of residual larvicidal activity against mosquitoes. Molloy et al. (1983) reported that the settling rate of a formulation corresponded directly with its mean particle size. Hinkle (1983) observed that the flowable concentrate Teknar™ (Sandoz, Inc.), the formulation with the smallest mean particle size, settled more slowly than 2 wettable powders and consequently provided more prolonged control.

The choice of isolate may also influence shelf life and field stability. Mulligan et al. (1978) observed greater residual activity of the 1593 isolate than with SSII-1 and Singer (1980) noted better stability of 1593 than SSII-1 when the 2 isolates were stored as broths. Good shelf life has been reported for the Stauffer MV 716 powder (Singer 1980) even after suspension in deionized water for protracted periods of time (Hertlein et al. 1980).

Currently available formulations of *B. thuringiensis* (H-14) and their uses against mosquitoes are presented in Table 3. A similar diversification of formulations of *B. sphaericus* is also needed to overcome several obstacles to effective application and to enhance residual larvicidal activity. The lack of residual activity of *B. sphaericus* that is reported in the literature (Davidson et al. 1981, Mulla et al. 1984a) is undoubtedly due in large part to the rapid settling of *B. sphaericus* spores (Davidson et al. 1984, Lacey et al. 1984). Although the spores may retain larvicidal activity long after settling into the substratum (Hertlein et al. 1979, Mulligan et al. 1980), they may be inaccessible to mosquito larvae, especially those that feed at or near the surface (e.g., *Anopheles* spp.). A formulation that enables flotation of spores and other toxic constituents or that releases toxic ingredients near the surface over a sustained period of time would enhance residual larvicidal activity considerably. A slow release, floating formulation of *B. sphaericus* was produced by pelleting a mixture of 30% primary powder (1593), 30% powdered sugar (releasing agent), and 40% polypropylene foam for buoyancy (Lacey et al. 1984). The pellets provided sustained suppression of container-breeding *Culex quinquefasciatus* under natural conditions for over 8 weeks (Fig. 1).

In addition to surmounting settling problems, penetration barriers must also be overcome in several habitats. Granular formulations of *B. sphaericus* would not only provide the necessary penetration but may also minimize drift. A formulation that enables penetration of foliage and remains floating on the surface and provides adequate coverage of the breeding site is currently under study in our laboratory. *Bacillus sphaericus* granules (5% 2362 primary powder) formulated using the corncob granule, binder and release mechanism employed in the Bactimos™ (Biochem Products) *B. thuringiensis* (H-14) granule, produced 100% mortality against 2nd instar *Cx. quinquefasciatus* in clear water at a rate of 2.5 kg of the granules/ha. Against older instars (3rd-4th instar) in a polluted habitat, 97% reduction was obtained at a rate of 10 kg/ha (Lacey, unpublished data). Granule properties such as density, flotation characteristics and binding mechanisms will strongly influence coverage and degree of larvicidal activity.

The active ingredient for each of the commercial granular (H-14) formulations of *B.*

Table 3. Currently available commercially produced formulations of *Bacillus thuringiensis* (H-14) and their uses.

Wettable Powders
Conventional aerial and ground application to unobstructed breeding sites
Liquids
Oil and aqueous base, conventional aerial and ground application, point source and low volume aerial application
Granules
Botanical and clay carriers, aerial application, especially to breeding sites with dense foliage (e.g., rice fields and salt marsh)
Briquettes, pellets
Provide sustained larvicidal activity in containers and small impoundments

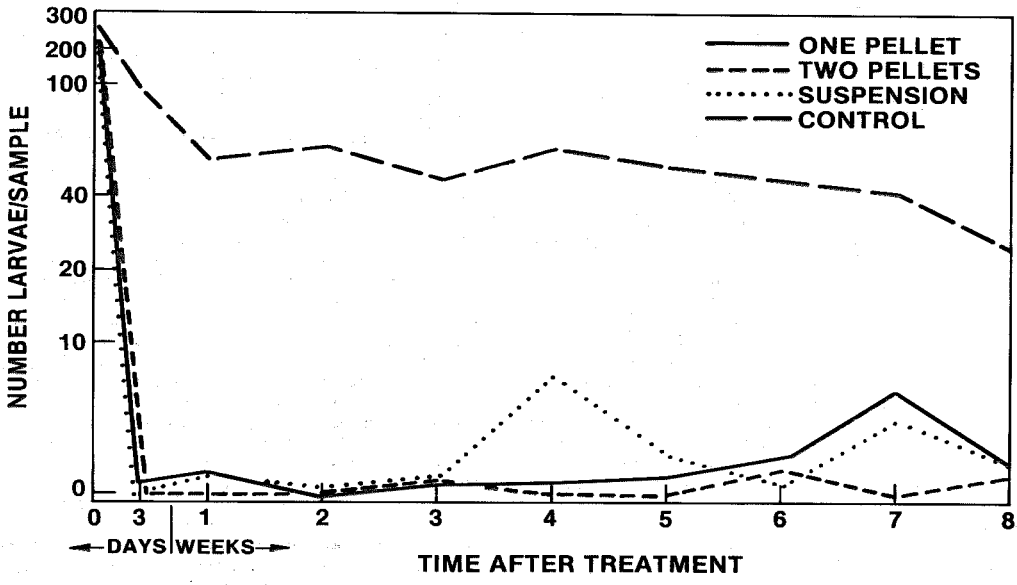

Fig. 1. Efficacy of an aqueous suspension and slow release forumlation of *Bacillus sphaericus* (1593) against *Culex quinquefasciatus* larvae breeding in 15 liter peridomestic buckets (from Lacey et al. 1984).

thuringiensis is bound loosely to the surface of their botanical carriers (corncob grits) and consequently provides little, if any, residual activity. By using a floating granule that incorporates *B. sphaericus* primary powder into a matrix such as that utilized by Lacey et al. (1984), sustained control could be obtained in habitats that support multivoltine species. Less expensive carriers than polypropylene, their characteristics, uses, benefits and formulations are reviewed by Ross (1983) and Sawyer (1983).

The liquid formulations of *B. thuringiensis* (H-14) have enabled the use of both conventional and novel application methods for mosquito control. McLaughlin and Vidrine (1984) applied diluted Teknar at the source of irrigation water for second crop rice fields and obtained good control of *Psorophora columbiae* (Dyar and Knab) and eliminated the need for aircraft application. Against the same species, M. Yates (personal communication) aerially applied undiluted Teknar with a Beecomist® spray system (80–100μ sleeve) and observed excellent control using as little as 0.58 liter/ha. In studies conducted in experimental ponds and rice fields, *Ps. columbiae* was significantly more susceptible to *B. sphaericus* than to *B. thuringiensis* (H-14) (M. S. Mulla and H. A. Darwazeh, personal communication; Lacey and Dame, unpublished data). Flowable concentrate formulations of *B. sphaericus* could find widespread use against this ubiquitous species and several others and provide a variety of options for the method of application.

A considerable amount of research on additional formulations is warranted. Areas to be considered could include: floating briquettes, microencapsulation of attractants/arrestants to enhance larval feeding on inoculum, adjuvants for protection of spores and toxins from UV radiation, and adjuvant compatibility with toxin and the environment. Genetic engineering may play a complementary role in the development of more efficacious formulations not only by facilitating greater toxin production but also by broadening the host range, and enhancing germination, sporulation and amplification of *B. sphaericus* in mosquito cadavers (Davidson 1984, Des Rochers and Garcia 1984).

References Cited

Angus, T. A. and P. Lüthy. 1971. Formulation of microbial insecticides, pp. 623–638. *In:* H. D. Burges and N. W. Hussey (eds.) Microbial control of insects and mites. Academic Press, London. 861 pp.

Bourgouin, C. and H. de Barjac. 1980. Evaluation du potential de *Bacillus sphaericus* comme larvicide anti-moustiques. WHO mimeo. doc. WHO/VBC/80.792. 24 pp.

Bull, D. L. 1978. Formulation of microbial insecticides: microencapsulation and adjuvants, pp.

11–20. *In:* C. M. Ignoffo and L. A. Falcon (eds.), Formulation and application of microbial insecticides. Misc. Pub. Entomol. Soc. Amer. 10(5):1–80.

Burges, H. D. 1983. Production, formulation, standardization, and safety of microbial larvicides, pp. 81–90. *In:* F. Michal (ed.), Basic biology of microbial larvicides of vectors of human diseases. UNDP/World Bank/WHO Publ. 188 pp.

Couch, T. L. 1978. Formulation of microbial insecticides: conventional formulations, pp. 3–10. *In:* C. M. Ignoffo and L. A. Falcon (eds.), Formulation and application of microbial insecticides. Misc. Pub. Entomol. Soc. Am. 10(5):1–80.

Couch, T. L. and C. M. Ignoffo. 1981. Formulation of insect pathogens, pp. 621–634. *In:* H. D. Burges (ed.), Microbial control of pests and plant diseases 1970–1980. Academic Press, London. 949 pp.

Couch, T. L. and D. A. Ross. 1980. Production and utilization of *Bacillus thuringiensis.* Biotechnol. Bioeng. 22:1297–1304.

Davidson, E. W. 1984. *Bacillus sphaericus* as a microbial control agent for mosquito larvae. *In:* M. Laird and J. W. Mills (eds.) Integrated mosquito control methodologies, Vol. 2. Academic Press, London (in press).

Davidson, E. W., A. W. Sweeney and R. Cooper. 1981. Comparative field trials of *Bacillus sphaericus* Strain 1593 and *B. thuringiensis* var. *israelensis* commercial powder formulations. J. Econ. Entomol. 74:350–354.

Davidson, E. W., M. Urbina, J. Payne, M. S. Mulla, H. Darwazeh, H. T. Dulmage and J. A. Correa. 1984. Fate of *Bacillus sphaericus* 1593 and 2362 spores used as larvicides in the aquatic environment. Appl. Environ. Microbiol. 47:125–129.

Des Rochers, B. and R. Garcia. 1984. Evidence for persistence and recycling of *Bacillus sphaericus.* Mosq. News 44:160–165.

Dulmage, H. T., J. Correa and A. J. Martinez. 1970. Coprecipitation with lactose as a means of recovering the spore-crystal complex of *Bacillus thuringiensis.* J. Invertebr. Pathol. 15:15–20.

Gibb, G. D. 1983. Fermentation studies of *Bacillus sphaericus* at the 10 liter stir jar stage. Master's Thesis, Western Illinois University. 41 pp.

Hertlein, B. C., R. Levy and T. W. Miller. 1979. Recycling potential and selective retrieval of *Bacillus sphaericus* from soil in a mosquito habitat. J. Invertebr. Pathol. 33:217–221.

Hertlein, B. C., J. Hornby, R. Levy and T. W. Miller, Jr. 1980. Shelf-life of larvicidal preparations based on the strain 1593 of *Bacillus sphaericus.* WHO mimeo. doc. WHO/VBC/80.790. 3 pp.

Hertlein, B. C., J. Hornby, R. Levy and T. W. Miller, Jr. 1981. Prospects of spore-forming bacteria for vector control with special emphasis on their local production potential. Dev. Ind. Microbiol. 22:53–60.

Hinkle, N. C. 1983. Factors affecting the efficacy of *Bacillus thuringiensis* var. *israelensis* as a mosquito larvicide. Master's Thesis, Auburn University. 75 pp.

Kalfon, A., I. Larget-Thiery, J.-F. Charles and H. de Barjac. 1983. Growth, sporulation, and larvicidal activity of *Bacillus sphaericus.* Eur. J. Appl. Microbiol. Biotechnol. 18:168–173.

Lacey, L. A. 1983. The larvicidal activity of *Bacillus* pathogens against *Toxorhynchites* mosquitoes (Diptera: Culicidae). J. Med. Entomol. 20:620–624.

Lacey, L. A. 1984. *Bacillus thuringiensis* serotype H-14 for the microbial control of mosquitoes. *In:* H. C. Chapman, A. R. Barr, D. E. Weidhaas and M. Laird (eds.), Biological control of mosquitoes. Am. Mosq. Control Assoc. Bul. 6 (in press).

Lacey, L. A. and S. Singer. 1982. Larvicidal activity of new isolates of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) against anopheline and culicine mosquitoes. Mosq. News 42:537–543.

Lacey, L. A., M. J. Urbina and C. M. Heitzman. 1984. Sustained release formulations of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) for the control of container-breeding *Culex quinquefasciatus.* Mosq. News 44:26–32.

Lane, D. A., G. Gibb and S. Singer. 1984. Effect of medium components on the preparation of primary powders of *Bacillus sphaericus.* Abstract, Annu. Meet., Am. Soc. Microbiol.

McLaughlin, R. E. and M. F. Vidrine. 1984. A point-source introduction system for control of *Psorophora columbiae* by *Bacillus thuringiensis* var. *israelensis:* Results of a large area operational test. Environ. Entomol. (in press).

Molloy, D., S. P. Wraight, B. Kaplan, J. Gerardi and P. Peterson. 1984. Laboratory evaluation of commercial formulations of *Bacillus thuringiensis* var. *israelensis* against mosquito and black fly larvae. J. Agric. Entomol. (in press).

Mulla, M. S., H. A. Darwazeh, E. W. Davidson, H. T. Dulmage and S. Singer. 1984a. Larvicidal activity and field efficacy of *Bacillus sphaericus* strains against mosquito larvae and their safety to nontarget organisms. Mosq. News 44:(in press).

Mulla, M. S., H. A. Darwazeh, E. W. Davidson and H. T. Dulmage. 1984b. Efficacy and persistence of the microbial agent *Bacillus sphaericus* against mosquito larvae in organically enriched habitats. Mosq. News 44:166–173.

Mulligan, F. S., III, C. H. Schaefer and T. Miura. 1978. Laboratory and field evaluation of *Bacillus sphaericus* as a mosquito control agent. J. Econ. Entomol. 71:774–777.

Mulligan, F. S., III, C. H. Schaefer and W. H. Wilder. 1980. Efficacy and persistence of *Bacillus sphaericus* and *B. thuringiensis* (H-14) against mosquitoes under laboratory and field conditions. J. Econ. Entomol. 73:684–688.

Myers, P. and A. A. Yousten. 1978. Toxic activity of *Bacillus sphaericus* SSII-1 for mosquito larvae. Infect. Immun. 19:1047–1053.

Myers, P. and A. A. Yousten. 1981. Toxic activity of *Bacillus sphaericus* for mosquito larvae. Dev. Ind. Microbiol. 22:41–52.

Obeta, J. A. N. and N. Okafor. 1983. Production of *Bacillus sphaericus* strain 1593 primary powder on media made from locally obtainable Nigerian agricultural products. Can. J. Microbiol. 29:704–709.

Ross, H. W. 1983. Available granular carriers: Properties and general processing methods, pp. 32–44. *In:* K. G. Seymour (ed.), Pesticide formulations and application systems: Second conference. ASTM Special Tech. Publ. 795. 107 pp.

Sawyer, E. W. 1983. Introduction to granular carriers, granular pesticide formulations, and processing, pp. 26–31. *In:* K. G. Seymour (ed.), Pesticide formulations and application systems: Second conference. ASTM Special Tech. Publ. 795. 107 pp.

Sinègre, G., B. Gaven and G. Vigo. 1980. Evaluation preliminaire de l'activité larvicide de la souche 1593 de *Bacillus sphaericus* vis-a-vis de quatre èspeces de moustiques du littoral Mediterraneen Français. WHO mimeo. doc. WHO/VBC/80.762. 8 pp.

Singer, S. 1979. Use of entomogenous bacteria against insects of public health importance. Dev. Ind. Microbiol. 20:117–122.

Singer, S. 1980. *Bacillus sphaericus* for the control of mosquitoes. Biotechnol. Bioeng. 22:1335–1355.

Singer, S. 1981a. Mosquitoes: Time to look again at *B. sphaericus*. Prac. Biotechnol. April 16–20.

Singer, S. 1981b. Potential of *Bacillus sphaericus* and related spore-forming bacteria for pest control, pp. 283–298. *In:* H. D. Burges (ed.), Microbial control of pests and plant diseases 1970–1980. Academic Press, London. 949 pp.

Singer, S. 1982. The biotechnology for strains of *Bacillus sphaericus* with vector control potential. Proc. III Int. Coll. Invetebr. Pathol., Sussex University, England. pp. 485–489.

Singer, S., N. S. Goodman and M. H. Rogoff. 1966. Defined media for the study of bacilli pathogenic to insects. Ann. N.Y. Acad. Sci. 139:16–23.

Wickremesinghe, R. S. B. and C. L. Mendis. 1980. *Bacillus sphaericus* spore from Sri Lanka demonstrating rapid larvicidal activity on *Culex quinquefasciatus*. Mosq. News 40:387–389.

World Health Organization. 1980. Data sheet on the biological control agent *Bacillus sphaericus* strain 1593. WHO mimeo. doc. WHO/VBC/80.777. 16 pp.

Wraight, S. P., D. Molloy and H. Jamnback. 1981a. Efficacy of *Bacillus sphaericus* strain 1593 against the four instars of laboratory reared and field collected *Culex pipiens* and laboratory reared *Culex salinarius*. Can. Entomol. 113:379–386.

Wraight, S. P., D. Molloy, H. Jamnback and P. McCoy. 1981b. 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.

Wraight, S. P., D. Molloy and P. McCoy. 1982. A comparison of laboratory and field tests of *Bacillus sphaericus* strain 1593 and *Bacillus thuringiensis* var. *israelensis* against *Aedes stimulans* larvae (Diptera: Culicidae). Can. Entomol. 114:55–61.

Yousten, A. A., N. Madhekar and D. A. Wallis. 1984. Fermentation conditions affecting growth, sporulation and mosquito larval toxin formation by *Bacillus sphaericus*. Dev. Ind. Microbiol. (in press).