References Cited

Butts, J. A. 1979. Survey of *Dirofilaria immitis* in Mecklenburg County, North Carolina. J. Am. Vet. Med. Assoc. 174:1088–1089

Buxton, B. A. and G. R. Mullen. 1980. Field isolations of *Dirofilaria* from mosquitoes in Alabama. J. Parasitol. 66:140–144.

Christensen, B. M. and W. N. Andrews. 1976. Natural infections of *Aedes trivittatus* (Coq.) with *Dirofilaria immitis* in central Iowa. J. Parasitol. 62:276–280.

Crans, W. J. and M. F. Feldlaufer. 1974. The likely mosquito vectors of dog heartworm in a coastal area of southern New Jersey. Proc. Calif. Mosq. Control Assoc. 42:168.

Falls, R. K. and T. R. Platt. 1982. Survey for heartworm. *Dirofilaria immitis* and *Dipetalonema reconditum* (Nematoda: Filorioidea) in dogs from Virginia and North Carolina. Am. J. Vet. Res. 43:738–739.

Hayes, R. O. 1953. Determination of physiological saline solutions for *Aedes aegypti* (L.). J. Econ. Entomol. 46:624–627.

Iyengar, M. O. T. 1957. Developmental stages of filariae in mosquitoes. South Pac. Comm. Tech. Pap. 104, 11 pp.

Magnarelli, L. A. 1978. Presumed *Dirofilaria immitis* infections in natural mosquito populations of Connecticut. J. Med. Entomol. 15:84–85.

Sauerman, D. M., Jr. and J. K. Nayar. 1983. A survey for natural potential vectors of *Dirofilaria immitis* in Vero Beach, Florida. Mosq. News 43:222–225.

Taylor, A. E. R. 1960. The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. J. Helminthol. 34:27–39.

Tempelis, C. H. and W. C. Reeves. 1964. Feeding habits of one anopheline and three culicine mosquitoes by the precipitin test. J. Med. Entomol. 1:148–151.

Villavaso, E. J. and C. D. Steelman. 1970. Laboratory and field studies of the southern house mosquito, *Culex pipiens quinquefasciatus* Say, infected with the dog heartworm, *Dirofilaria immitis* (Leidy), in Louisiana. J. Med. Entomol. 7:471–476.

LABORATORY EVALUATIONS OF FORMULATIONS OF AROSURF® MSF AND *BACILLUS SPHAERICUS* AGAINST LARVAE AND PUPAE OF *CULEX QUINQUEFASCIATUS*[1]

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Formulations of the mosquito larvicide and pupicide Arosurf® MSF (Sherex Chemical Company, Inc., P. O. Box 646, Dublin, OH 43017) and commercial preparations of the mosquito larvicide *Bacillus thuringiensis* var. *israelensis (B.t.i.)* were shown by Levy et al. (1984) to generally enhance the mosquito-controlling efficacy over each of the formulation components. This dual component self-spreading formulation was shown to improve field coverage of *B.t.i.* as well as broaden the range of developmental stages that could be rapidly controlled from a single application. The rationale for these formulations as well as the mixing procedures and techniques for field application are further discussed by Levy et al. (1984), Burgess et al. (1985) and Hertlein et al. (1985).

This report summarizes the results of similar efficacy testing with joint formulations of Arosurf MSF and an experimental commercial formulation of the mosquito larvicide *Bacillus sphaericus* (Biochem Products, P. O. Box 264, Monchanin, DE 19710). The efficacy of *B. sphaericus* as a biological control agent for a variety of mosquito species has been reviewed by the World Health Organization (Anonymous 1985).

The *B. sphaericus* formulation used in these tests was coded as BSP-1 (Strain 2362) and contained 12.3% fermentation solids and 87.7% inert ingredients. This product is currently registered under an EPA Experimental Use Permit (EPA Est. No. 43382-BL-1). Recommended application rates of 0.5–3.0 pt/acre were based on water quality (organic content), concentration of larvae, larval instar and the density of vegetative cover.

Formulations of Arosurf MSF and *B. sphaericus* were prepared for bioassay against laboratory-reared larvae and pupae of *Culex quinquefasciatus* Say for testing as water-base and

[1] Mention of a brand name or proprietary product does not constitute a guarantee or warranty by Lee County Mosquito Control District, and does not imply its approval to the exclusion of other products that may also be suitable.

technical formulations. To prepare water-base formulations, the desired concentrations of *B. sphaericus* and water purified by reverse osmosis filtration (RO) were blended together in 400 ml tripour plastic beakers with a Bamix (M100) biomixer (Biospec Products, Bartlesville, OK 74003) equipped with an impeller stirring tip. While the biomixer was blending the *B. sphaericus* and water (ca. 10 sec), the desired concentration of Arosurf MSF was pipetted into the water-*B. sphaericus* mixture. Blending was continued for an additional 30 sec. Technical formulations of Arosurf MSF and *B. sphaericus* were blended with the biomixer for 30 sec. The application rate of Arosurf MSF in all formulations was standardized at 0.26 gal/acre (0.25 ml/m² water surface) while the application rate of *B. sphaericus* ranged from 0.03 to 1.02 pt/acre. Bioassays were generally designed to test the comparative efficacy of the formulations against the individual formulation components.

All formulations were applied with a pipette to 400 ml glass beakers containing 250 ml of water collected from a sewage treatment system and 10 second instar larvae or mixed populations of 5 fourth instar larvae and 5 pupae (3 replications/formulation). The water surface area of each beaker was 0.000001 acre. Larvae were fed a 4% ground rabbit chow suspension (i.e., ca. 10 drops/beaker) prior to the addition of the test materials. Water temperature throughout the tests ranged from 26 to 27°C.

Cumulative mean percentage mortality of larvae, pupae, and emerging adults was recorded at 24 hr posttreatment intervals. Cumulative percentage adult emergence was also recorded for each test formulation. These criteria were the basis for evaluating the comparative mosquito-controlling efficacy of the *B. sphaericus*-Arosurf MSF formulations and their individual components. Results were analyzed using z and t tests.

Bioassays were also conducted to determine whether Arosurf MSF in an Arosurf MSF-*B. sphaericus* formulation would effectively disperse water-base formulations of *B. sphaericus* over the surface of the water prior to sedimentation, and effectively kill larvae at a site that was distant from the point of application, when compared to water-base formulations containing no Arosurf MSF. These bioassays were performed according to procedures by Levy et al. (1984).

Results of bioassays (Table 1) indicated that extremely low application rates of commercial grade *B. sphaericus* (i.e., 0.03–0.26 pt/acre) could be blended with Arosurf MSF (0.26 gal/acre) to provide homogeneous water-base or technical formulations that could be used to control mixed populations of *Culex* larvae, pupae and/or emerging adults breeding in sewage treatment systems. Similar results were observed in preliminary bioassays with formulations of Arosurf MSF and an "in-house" fermentation culture containing cells and spores of *B. sphaericus* strain 1593 (Levy et al. 1982). Recommended rates of 0.51 and 1.02 pt/acre of *B. sphaericus* could not be mixed satisfactorily with Arosurf MSF and water under high sheer blending to form a homogeneous formulation that would assure the dispensing of accurate surface dosages of the 2 active components. Observations showed that a viscous mixture was formed that was difficult to pipette and that would readily separate upon cessation of blending. The poor formulations that resulted were attributed to too high concentrations of inert ingredients that were present in *B. sphaericus* at the 0.51 and 1.02 pt/acre rates. Similar difficulties in mixing were also recorded when technical Arosurf MSF and *B. sphaericus* were formulated at these rates.

All *B. sphaericus* and *B. sphaericus*-Arosurf MSF water-base and technical formulations killed 100% of the 2nd instar *Culex* larvae within 24 hr posttreatment, and therefore indicated the high sensitivity of this instar to extremely low concentrations of *B. sphaericus*. Fourth instar larvae that had not ceased to feed prior to pupation were susceptible to *B. sphaericus* at all *B. sphaericus* formulations within 24–72 hr posttreatment. However, observations indicated that adverse interactions to Arosurf MSF with water-base formulations of *B. sphaericus* at the higher application rates (i.e., 0.51 and 1.02 pt/acre) inhibited the spreading potential of Arosurf MSF as well as its ability to effectively depress the surface tension of the water, and therefore resulted in poor control of pupae and emerging adults. Spreading and surface tension reduction tests confirmed these observations. No adult escapes were observed in tests with only Arosurf MSF. Mixing compatibility and 100% control of larvae, pupae and emerging adults resulted in 24–72 hr posttreatment with the additional water-base or technical formulations of *B. sphaericus* (0.03–0.26 pt/acre) and Arosurf MSF. The lower percentage mortality that was observed at 24 hr posttreatment in the technical formulations was related to the age of late 4th instar larvae at the time of treatment, and not directly to formulation differences.

The utilization of primary fermentation powders of *B. sphaericus* containing little or no inert ingredients may allow *B. sphaericus* to be more compatible with Arosurf MSF, particularly at the higher *B. sphaericus* application rates. The mixing compatibility of technical and water-base formulations of *B.t.i.*–Arosurf MSF

Table 1. Efficacy of water-base and technical formulations of Arosurf® MSF and *Bacillus sphaericus* against larvae and pupae of *Culex quinquefasciatus*.[1]

Water-base or technical formulation (dosage-active ingredients)[2]	Larval instar/ pupae (P)	Cumulative mean percentage mortality of larvae,[3] pupae, and/ or emerging adults at indicated posttreatment time periods			Cumulative percentage adult emergence from surviving pupae
		24 hr	48 hr	72 hr	
Arosurf MSF and *B. sphaericus*	2nd	100	—	—	0
(0.26 gal/acre + 1.02 pt/acre)	4th/P	40	46.7	—	53.3
Arosurf MSF and *B. sphaericus*	2nd	100	—	—	0
(0.26 gal/acre + 0.51 pt/acre)	4th/P	46.7	63.3	—	36.7
Arosurf MSF and *B. sphaericus*	2nd	100(100)[4]	—	—	0(0)
(0.26 gal/acre + 0.26 pt/acre)	4th/P	50 (26.7)	100(100)	—	0(0)
Arosurf MSF and *B. sphaericus*	2nd	100(100)	—	—	0(0)
(0.26 gal/acre + 0.13 pt/acre)	4th/P	60 (46.7)	100(100)	—	0(0)
Arosurf MSF and *B. sphaericus*	2nd	100(100)	—	—	0(0)
(0.26 gal/acre + 0.06 pt/acre)	4th/P	50 (36.7)	100(100)	—	0(0)
Arosurf MSF and *B. sphaericus*	2nd	100	—	—	0
(0.26 gal/acre + 0.03 pt/acre)	4th/P	36.7	83.3	100	0
B. sphaericus	2nd	100	—	—	0
(1.02 pt/acre)	4th/P	36.7	40	—	60
B. sphaericus	2nd	100	—	—	0
(0.51 pt/acre)	4th/P	43.3	46.7	—	53.3
B. sphaericus	2nd	100	—	—	0
(0.26 pt/acre)	4th/P	33.3	33.3	—	66.7
B. sphaericus	2nd	100	—	—	0
(0.13 pt/acre)	4th/P	36.7	40	—	60
B. sphaericus	2nd	100	—	—	0
(0.06 pt/acre)	4th/P	26.7	40	—	60
B. sphaericus	2nd	100	—	—	0
(0.03 pt/acre)	4th/P	36.7	36.7	43.3	56.7
Arosurf MSF	2nd	76.7	83.3	100	0
(0.26 gal/acre)	4th/P	30	73.3	100	0
Control	2nd	10	16.7	16.7	—
	4th/P	3.3	6.7	10	90

[1] Flowable concentrate of *B. sphaericus* (strain 2362) used in all tests is an EUP product of Biochem Products; all tests conducted in water collected from a sewage treatment system.

[2] All water-base formulations applied at a total application rate of 5.0 gal/acre while technical formulations were applied at a total application rate of 0.26 gal/acre.

[3] 50% control of mixed larvae and pupae with *B. sphaericus* alone = 100% control of larvae and 0% control of pupae.

[4] Percentages of mortality in parenthesis are for technical *B. sphaericus* and Arosurf MSF formulations. All other data are indicative of water-base formulations.

and resulting mosquito-controlling efficacy against mixed populations of larvae, pupae and emerging adults were markedly improved when experimental commercial primary powders of *B.t.i.* were substituted for the standard commercial *B.t.i.* preparations which also contained high concentrations of inert materials.

Results of comparative bioassays relating larvicidal action to the spreading potential of water-base *B. sphaericus* formulated with and without Arosurf MSF showed that 95% of the 3rd–4th stage larvae of *Cx. quinquefasciatus* could be killed with the Arosurf MSF (0.26 gal/acre)-*B. sphaericus* (0.5 pt/acre) water-base formulation (5.0 gal/acre) within 24 hr posttreatment; however, *B. sphaericus* (0.5 pt/acre) water-base formulation applied at 5.0 gal/acre without Arosurf MSF only killed 35% of the larvae at this time period. The data indicate that Arosurf MSF can act as an efficient self-spreading carrier of *B. sphaericus* over the water surface to provide control of larvae and pupae in the areas of a habitat that are distant from the point(s) of application. Therefore, better mosquito control can be expected in habitats that cannot be uniformly sprayed because of dense overlying vegetation or water surface obstructions.

In summary, we found that vigorous mixing of water-base or technical formulations of Arosurf MSF and *B. sphaericus* would be required for proper field application. Results of bioassays in sewage water against *Cx. quinquefasciatus* indicated that *B. sphaericus* has excellent larvicidal activity and Arosurf MSF has excellent pupicidal activity. Larvicidal efficacy of *B. sphaericus* was based on the instar and age of the larvae, i.e., 2nd instar and early to middle-aged 4th instar larvae that fed on *B. sphaericus* were susceptible, while late 4th instar larvae that ceased to feed and pupated prior to ingestion of *B. sphaericus* survived the bacillus but succumbed to the effects of Arosurf MSF. Bioassays also indicated that effective dispersal of *B. sphaericus* over the surface of the water will result when Arosurf MSF is combined with water-base formulations of *B. sphaericus*. Blends of *B. sphaericus* and Arosurf MSF produced no synergistic effects; however, we suggest that joint action self-spreading formulations of *B. sphaericus* and Arosurf MSF can be used in dynamic *Cx. quinquefasciatus* and *Cx. nigripalpus* Theobald breeding situations such as sewage treatment systems where high concentrations of mixed larvae, pupae and emerging adult populations are usually found throughout the year. In addition, the recycling potential of *B. sphaericus* particularly in water containing high organic content (i.e., sewage treatment systems) makes this bacillus an excellent candidate for further formulation studies (Hertlein et al. 1979, Hornby et al. 1984).

References Cited

Anonymous. 1985. Informal consultation on the development of *Bacillus sphaericus* as a microbial larvicide. UNDP/World Bank/WHO Special Program for Research and Training on Tropical Diseases, 7–11 October 1985, Geneva, Switzerland, TDR/BCV/SPHAERICUS/85.3.

Burgess, J. H., R. Levy and T. W. Miller, Jr. 1985. A new method for applying Arosurf MSF (monomolecular surface film formulations). J. Am. Mosq. Control Assoc. 1:245–247.

Hertlein, B. C., R. Levy and T. W. Miller, Jr. 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. 1985. An injection method for spraying biological control agents and a monomolecular surface film for control of immature mosquitoes. J. Am. Mosq. Control Assoc. 1:255–257.

Hornby, J. A., B. C. Hertlein and T. W. Miller, Jr. 1984. Persistent spores and mosquito larvicidal activity of *Bacillus sphaericus* 1593 in well water and sewage. J. Georgia Entomol. Soc. 19:165–167.

Levy, R., C. M. Powell, B. C. Hertlein and T. W. Miller, 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 44:537–543.

Levy, R., C. M. Powell, B. C. Hertlein, W. D. Garrett and T. W. Miller, Jr. 1982. Additional studies on the use of the monomolecular surface film Arosurf® 66-E2 for operational control of mosquito larvae and pupae. J. Florida Anti-Mosq. Assoc. 53:100–106.

AN AUTOMATIC CARBON DIOXIDE DELIVERY SYSTEM FOR MOSQUITO LIGHT TRAP SURVEYS

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The advantages of using carbon dioxide (CO_2) with mosquito traps were first described by Reeves (1951). In addition to increasing the total number of mosquitoes captured (Newhouse et al. 1966, Magnarelli 1975), the species composition of CO_2-baited traps more accurately reflects the true mosquito nuisance populations in an area (Parsons et al. 1974, Slaff et al. 1983). Generally, either dry ice or bottled CO_2 are used as the attractant in mosquito traps. A disadvantage of either source is that CO_2 is emitted for a far greater period of time than is desired, since the traps are usually placed long before sunset and retrieved well after sunrise. The system we describe offers regulated CO_2 delivery while providing timed switching for the CO_2 and light trap devices.

We connected an ASCO[3] solenoid, part no. 8210-093, powered by a 6-volt battery, to a standard single-stage CO_2 regulator using 3/8 in (9.5 mm) pipe bushing and 1/4 in (6.4 mm) pipe nipple (Fig. 1). A standard CDC photocell was wired to the solenoid.[4]

One benefit of the solenoid and photocell delivery system is that the unit may be set out and picked up during normal working hours. In addition, no CO_2 is wasted before or after the trapping period if the trap photocell and the CO_2 tank photocell are synchronized to initiate operation under the same lighting conditions. The photocell has a potentiometer adjustment that makes such a procedure straightforward. As a result, greater trapping time per CO_2 tank is possible, saving money and allowing an increase in the number of CO_2-

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[2] North Carolina Div. of Health Services, Vector Control Branch, c/o Marine Resources Center/Bogue Banks, Atlantic Beach NC 28512

[3] Automatic Switch Co., Florham Park, NJ 07932. Price $65.00.

[4] John W. Hock Co., P. O. Box 12852, Gainesville, FL 32604. Price $21.00.