EFFICACY AND PERSISTENCE OF THE MICROBIAL AGENT *BACILLUS SPHAERICUS* AGAINST MOSQUITO LARVAE IN ORGANICALLY ENRICHED HABITATS[1]

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ABSTRACT. Two preparations of the microbial control agent *Bacillus sphaericus* (Strain 2362, IF-117) were evaluated in organically enriched replicated field ponds for assessment of their efficacy and short-term persistence against mosquito larvae. Spores of the cream and powder preparations settled quickly and accumulated in the mud zone out of reach of feeding mosquito larvae.

Both cream and powder formulations yielded 90% + initial control of larvae at the rate of 0.2 lb/acre (0.22 kg/ha) in ponds without or with low level of enrichment. In clear water and low level of pollution, this rate of application yielded good control of larvae for about 3 weeks. This extended level of control with this agent is an improvement over currently available larvicides. Extent of larval control was not high in ponds with high level of enrichment. In all ponds, predaceous insects peaked or started to increase in about 2–3 weeks posttreatment exerting regulatory force on mosquito larvae with the exception of the highly enriched ponds. In the highly enriched ponds, the predators built up to high levels, but they could not cope with the extremely heavy populations of mosquito larvae.

Bacillus sphaericus 2362 had no adverse effects on dominant macroinvertebrate fauna, especially predaceous insects. The use of 2362 and other active strains therefore offers a good potential for use in integrated mosquito control programs.

Recently, several strains and formulations of the microbial control agents *Bacillus thuringiensis* Berliner var. *israelensis* serotype H-14 and *B. sphaericus* Neide were reported to possess high level of biological activity against larvae of a wide spectrum of mosquito species in the laboratory and under field conditions (Davidson and Sweeney 1983, Davidson et al. 1981, Lacey and Singer 1982, Mulla et al. 1982a, 1982b, Mulligan et al. 1978, 1980).

As a result of extensive research on the efficacy and field evaluation of microbial control agents, *B. thuringiensis* (H-14) was found to be effective in the field and registered for mosquito control in 1980, and since then this material has found widespread use in mosquito control programs. *Bacillus sphaericus*, although known for its larvicidal activity for many years, has only attracted greater attention in recent years when potent strains were isolated and tested against mosquito larvae. This microbial agent has been reported by some to have a potential to recycle (Hertlein et al. 1979) in some mosquito breeding sources, while other workers have reported this material to lack recycling potential, with little or no persistence in some situations (Mulla et al. 1984, Mulligan et al. 1978, 1980). In recent studies it was clearly established by entomological evaluation (Mulla et al. 1984) and microbiological assessment (Davidson et al. 1984) that *B. sphaericus* strains 1593 and 2362 do not show significant persistence or recycling potential in clear water situations in California. It has been hypothesized that persistence and recycling potential of *B. sphaericus* are more achievable in polluted than in clear waters. These two are distinct phenomena, recycling being an important feature of some microbial control agents. Recycling connotes a mode where the organism multiplies and produces sufficient amounts of the toxin in the habitat following treatment and where such toxin is available to mosquito larvae in quantities that would produce high level of mortality. In recycling, it is also important that toxin production continues during a period when several generations of the target species are produced. Persistence, on the other hand, involves production or no production of toxin in sufficient titer that produces larval mortality in the exposed generation or maybe one or two additional generations in multivoltine species.

The present studies were carried out to determine the initial activity and persistence of *B. sphaericus* 2362, a potent strain, against mosquito larvae in outdoor ponds provided with various levels of organic pollution. In addition, data and information were gathered on the impact of this microbial larvicide on prevailing nontarget organisms. The intensity of larval breeding in ponds provided with different levels of enrichment, was also determined.

METHODS AND MATERIALS

In recent studies, Mulla et al. (1984) reported that *B. sphaericus* 2362 (IF-97) powder was

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highly active against larvae of *Culex tarsalis* Coquillett and *Cx. peus* Speiser at the low rates of 0.05 and 0.1 lb/acre (56 and 112 g/ha). To further enhance the activity of this pathogen against mosquito larvae, several new preparations of *B. sphaericus* 2362 were produced by various methods by one of the co-authors (Dulmage) in sufficient amounts for laboratory evaluation. Code number, strain, and preparation method of each material are included in Table 1. Bioassay methods utilized in the laboratory are described elsewhere (Mulla et al. 1982a). For testing, 1% stock suspension of each preparation (w/v) was prepared in tap water, and serial dilutions in water were prepared as needed. The required amounts of the proper strength dilution (0.2–1.0 ml) were added to 4-oz disposable cups (Sweetheart Cup Div. of Maryland, Baltimore), containing 20 4th-instar larvae of *Cx. quinquefasciatus* Say in 100 ml of tap water. After 48 hr of exposure in a holding room at 25° ± 1°C, mortality readings were taken, and values were subjected to log probit regression analysis by using a Compucorp 145 E desk model computer. The LC_{50} and LC_{90} in mg/liter were obtained from log probit analysis made by this computer.

Each strain, along with the International Standard strain *B. sphaericus* 1593-RB80 (Institut Pasteur, Paris) were tested 3–4 times on different days, at 4–5 concentrations, utilizing 3 replicates per concentration; 3 replicates were left untreated as checks. Larvae utilized in these studies were obtained from a laboratory reared colony at the University of California, Riverside.

Field trials were carried out at the Aquatic and Vector Control Research Facility in the Coachella Valley of southern California. Thirty-two identical ponds with natural vegetation supporting wild populations of mosquito larvae were employed in these studies. Detailed description of the experimental facility was published by Mulla et al. (1982a). In brief, the facility consists of 64 ponds in 8 rows, each pond measuring 18×18 ft (30 m^2), with plant cover and water (pH 9.4). The latter was provided to these ponds from an artesian well through an underground pipeline. Water level in the ponds was maintained constant (12 in) by float valves, and water temperature was monitored with a Mini-Max thermometer (Markson Science Inc., Del Mar, CA) during the duration of the experiment. To prepare the ponds for the intended research, the following procedures were instituted.

Prior to flooding, 0.025, 0.05 and 0.1% (w/v) (on the basis of water volume in the filled ponds) of chicken mash (5, 10 and 20 lb/pond) were added, utilizing 6 ponds per pollution rate, and 5 ponds were left unpolluted. Cage layer chicken mash 5 (16% protein minimum) was used and was obtained from Poultrymen Cooperative Association. Riverside, CA. The ponds were then filled with water in a period of 24 hr. Eight days after flooding, when mosquito larval populations reached the second or third instar, two of each of the enriched and unenriched ponds were treated with either a powder (spray dried) or a cream formulation of *B. sphaericus* 2362 (IF-117) at the rate of 0.2 lb/acre (220 g/ha), and two of each of the enriched and unenriched ponds were left untreated as checks.

The required amount of the microbial agent was mixed with 120 ml of water and applied with a polyethelene squeeze bottle from the sides and the middle of the pond, covering the entire water surface area. To assess the microbiological aspects of *B. sphaericus* in treated and check ponds, surface water and mud samples were taken prior to and 2, 4, 6, 14 and 21 days after treatment. Samples were pasteurized, diluted and plated on a selective medium for *B. sphaericus* (Davidson et al. 1984).

To assess the impact of the two formulations on mosquito larvae and non-target organisms in fresh and polluted water, 5 dips per pond were taken before treatment and 2, 4, 7, 14, 21, 28 and 35 days after treatment. The five dips were combined into one sample in a concentrator provided with 150 mesh stainless steel strainer

Table 1. Evaluation of various strains and preparations of *Bacillus sphaericus* against 4th-instar larvae of *Culex quinquefasciatus* in the laboratory.

Strain	Code	Preparation method	48-hr LC_{50}-LC_{90} mg/liter	Correlation coefficient	Slope
2362	IF-117	Spray dried powder	0.008–0.017	0.94	3.78
2362	IF-109	Spray dried powder	0.008–0.027	0.94	2.48
2362	IF-117	Cream	0.014–0.029	0.95	4.10
2362	IF-97	Acetone precipitation	0.006–0.038	0.86	1.64
1593[a]	RB-80	Lyophilized	0.011–0.040	0.87	2.55
1593	IF-94	Acetone precipitation	0.041–0.111	0.75	2.97

[a] Used as a standard.

cloth, and the sample in a small amount of water was transferred into 50 ml plastic vials, and preserved with 95% ethyl alcohol. All organisms in the sample were examined, identified, and counted under a stereoscopic microscope in the laboratory. The mosquito population consisted mostly of *Culex tarsalis* Coquillett, but *Anopheles franciscanus* McCracken larvae were also present in small numbers amounting to 1% in some of the treated ponds.

Since the growth of stagnant water mosquitoes is asynchronous, newly hatching larvae were present in large numbers at all times, and newly hatched larvae, in general, were not exposed to the pathogen for sufficient time to suffer mortality. To determine the activity of *B. sphaericus* 2362 formulations against exposed larval cohorts prevailing at time of treatment, the younger larvae (1–2 instars), although counted and identified, were not included in the calculations of percent reduction. Percent reduction was based only on the number of 3–4 instars present in the samples in posttreatment versus pretreatment counts.

RESULTS AND DISCUSSION

All preparations of *B. sphaericus* 2362 available for screening displayed excellent activity against 4th-instar larvae of *Cx. quinquefasciatus* in the laboratory, and were 2-fold more active than the International Standard preparation of *B. sphaericus* 1593-RB80 (Table 1). *Bacillus sphaericus* 2362 (IF-117) spray-dried powder was the most active material tested, causing 90% mortality at 0.017 mg/liter. The preparation IF-109 spray dried powder and IF-117 cream were slightly less active than IF-117 spray dried powder, causing 90% mortality at 0.027 and 0.029 mg/liter respectively. Cream preparation contained water, therefore fewer spores/mg were actually applied in this preparation than the powder preparations. The preparation IF-97, was dried by the acetone precipitation method, and this material essentially produced similar results as the lyophilized standard of *B. sphaericus* 1593-RB80, yielding 90% mortality at 0.038 and 0.04 mg/liter respectively. The acetone precipitated, *B. sphaericus* 1593 (IF-94), was the least active of all preparations tested, with an LC_{90} of 0.111 mg/liter (Table 1).

Since *B. sphaericus* 2362 (IF-117) showed the highest activity in the laboratory, this material was then employed in field tests assessing persistence and recycling potential of this agent in polluted and unpolluted waters. Both powder and cream preparations were tested in the field at the rate of 0.2 lb/acre (224 g/ha).

Microbiological assessment of *B. sphaericus* spores in water and mud of treated ponds revealed that the spores dropped rapidly from the surface water to accumulate in the bottom mud where they were not available to larvae, as observed in an earlier study (Davidson et al. 1984). On ponds treated with the IF-117 cream preparation, spores in the upper water layer (the larval feeding zone) fell below 100 spores/ml after the second day. In ponds treated with the IF-117 spray dried powder, higher initial spore numbers were found in the surface water, and spore numbers remained above 100/ml through the fourth day after treatment. These differences in spore numbers were reflected in control of larvae (see below). In confirmation of earlier data, a minimum of ca. 100 spores/ml in the upper water layer appeared to be required to control *Culex* spp. larvae in the field.

The cream preparation could not be easily suspended in water by shaking and the suspension had large aggregation of the material. Despite this problem it proved to be highly effective. The cream preparation produced high initial control of larvae in unpolluted ponds, and control persisted for 3 wk (Table 2). The magnitude of larval control was quite high even 4 and 5 wk after treatment, but such high level of control cannot be solely attributed to the treatment, because relatively high levels of reduction in larval populations also occurred in the check ponds. In the slightly enriched ponds (0.025%) initial control was somewhat lower 2–7 days posttreatment, which increased to high levels thereafter. It seems that this delayed high level of reduction was partly due to natural decline in the populations. At the two higher levels of enrichment (0.05 and 0.10%) initial control was nil to poor up to 3 wk posttreatment. In the ponds with 0.05% pollution, level of control reached 94% 28 and 35 days after treatment. However, due to marked reduction in larvae in check ponds with 0.05% enrichment, the extent of reduction in the treated ponds for these dates cannot be attributed to *B. sphaericus* treatment. At the highest rate of pollution the extent of control was low to nil throughout the duration of the experiment.

The powder preparation yielded somewhat better results than the cream preparation (Table 2). In the unenriched ponds, *B. sphaericus* 2362 powder produced 100% initial control, and a high level of control continued into the 28th and 35th days posttreatment, but it is difficult to attribute this solely to the treatment, because marked reduction of larvae also occurred in the check ponds. In the ponds with 0.025% enrichment initial control was 100% for

Table 2. Field evaluation of *Bacillus sphaericus* 2362 (0.2 lb/acre) against larvae of *Culex tarsalis* in polluted and unpolluted water ponds. (Coachella Valley, April–May 1982)[a].

Pollution %	Pre-treatment	Mean no. 3–4 instar larvae/5 dips and % (reduction) after treatment (days) 2	4	7	14	21	28	35
		B. sphaericus 2362 (IF-117) Cream						
0.0	26	2 (92)	0 (100)	4 (85)	0 (100)	2 (92)	2 (92)	3 (88)
0.025	22	8 (64)	7 (68)	9 (45)	3 (73)	0 (100)	0 (100)	2 (91)
0.05	16	15 (6)	9 (44)	95 (0)	23 (0)	11 (19)	1 (94)	1 (94)
0.10	33	35 (0)	54 (0)	112 (0)	73 (0)	47 (0)	24 (27)	21 (36)
		B. sphaericus 2362 (IF-117) Powder						
0.0	24	0 (100)	0 (100)	0 (100)	1 (96)	1 (96)	2 (92)	3 (88)
0.025	12	0 (100)	0 (100)	2 (83)	8 (33)	9 (25)	10 (17)	18 (0)
0.05	17	2 (88)	1 (94)	4 (76)	16 (60)	19 (0)	7 (59)	20 (0)
0.10	12	3 (75)	3 (75)	229 (0)	116 (0)	113 (0)	74 (0)	42 (0)
		Check						
0.0	13	19 (0)	47 (0)	28 (0)	19 (0)	11 (15)	5 (62)	3 (77)
0.025	26	29 (0)	51 (0)	63 (0)	17 (35)	7 (73)	5 (81)	4 (85)
0.05	10	50 (0)	157 (0)	84 (0)	51 (0)	4 (60)	2 (80)	5 (50)
0.01	31	116 (0)	242 (0)	195 (0)	150 (0)	99 (0)	53 (0)	65 (0)

[a] Water temperature range 10–29°C, mean min. 12°C, mean max. 28°C.

4 days, but declined to 83% and 33% on days 7 and 14 posttreatment, and level of control remained low thereafter. At the 0.05% level of enrichment, initial control was excellent (88–94%) for days 2 and 4 posttreatment. Thereafter, control of larvae in this treatment declined, reaching nil. At the 0.10% pollution level, initial control was mediocre (75%) which lasted for 4 days only, declining to 0% thereafter.

From this experiment it is evident that the efficacy of *B. sphaericus* against mosquito larvae is greatly affected by the nature and level of organic pollutants and that efficacy is inversely proportional to the level of pollution in the water or high density of larvae as a result of the enrichment. Initial control in the two higher rates of pollution was mediocre, and the efficacy of this microbial agent did not last beyond 4 days with the cream preparation and beyond 7 days with the powder preparation in the 0.05% pollution. However, in the cream preparation a low level of reduction of larvae was noted on day 21 and high level of reduction then occurred on days 28 and 35 posttreatment in the 0.05% level of pollution. At the 0.1% pollution, initial reduction was mediocre and there was no reduction in larvae on day 7 and beyond. As mentioned above, these increases in reduction cannot solely be attributed to the treatment with the microbial agent. From the data of this experiment, it can be concluded that both preparations of *B. sphaericus* 2362 yielded good control of larvae up to 21 days posttreatment in unpolluted and slightly polluted waters. Extent of control beyond that date cannot be accurately attributed to treatments as larval populations in all the ponds including checks was markedly suppressed due to the appearance of predaceous insects. At the highest enrichment level, heavy breeding of mosquitoes occurred through most of the experimental period and the predators in general were not able to suppress heavy mosquito larval populations significantly. This lack of efficacy of *B. sphaericus* in highly polluted water could be attributed to the high larval density and the large amount of available nutrients. These were found to be very important factors in the efficacy of *B. thuringiensis* (H-14) (Mulla et al. 1982a, 1982b; Ramoska and Pacey 1979).

Both formulations of *B. sphaericus* 2362 (IF-117) had no harmful effects on nontarget organisms prevailing during the test period. These organisms remained constant or increased in numbers (Tables 3, 4), and played an important role in the gradual decline of mosquito larvae in treated as well as check ponds, 2–3 wk after treatment. Organisms which were present in fairly large numbers in the samples during this study were: diving beetle adults (*Berosus metalliceps* Sharp and *Tropisternus lateralis* Fabricious), diving beetle larvae (dytiscids and hydrophilids), mayfly naiads (*Callibaetis pacificus* Seemann) and ostracods (Tables 3, 4). The predaceous insects, dragonfly and damselfly naiads, began to appear in all ponds 2–3 wk after treatment, therefore, they are omitted from the data in the tables. However, these predaceous organisms along with the other predators which were abundant two weeks posttreatment, played an important role in supplementing the action of *B. sphaericus* 2362 (IF-117) against mosquito larvae.

JOINT ACTION OF *B. SPHAERICUS* AND PREDATORS. During the course of studies on the efficacy of *B. sphaericus* 2362 in experimental ponds, an interesting relationship between mosquito larvae and predator populations developed. In ponds without organic enrichment, treatment with 2362 cream resulted in complete elimination of mosquito larvae two days after treatment (Fig. 1A). This level of reduction prevailed for about 21 days posttreatment, at which time the density of predators both in the treated and check ponds reached a peak. Although mosquito larval population remained very high in the checks for about 14 days after initiation of test, they nevertheless started to decline sharply as the predator population increased. It should be noted that the initial suppression of mosquito larvae was affected by 2362, and this sup-

Table 3. Effects of *Bacillus sphaericus* 2362 (0.2 lb/acre) on nontarget organisms in polluted and nonpolluted water ponds. (Coachella Valley, April–May 1983).

Pollution (%)	Mean no. of organisms/5 dips pre- and posttreatment (days)											
	Diving beetle adults[a]						Diving beetle larvae[a]					
	Pre-	2	7	14	28	35	Pre-	2	7	14	28	35
	B. sphaericus 2362 (IF-117) Cream											
0	4	3	3	1	0	2	0	0	3	8	5	5
0.025	3	6	2	2	1	2	0	0	2	12	13	9
0.050	8	6	2	5	3	5	0	0	4	18	3	2
0.100	7	6	1	3	6	6	0	0	3	58	15	6
	B. sphaericus 2362 (IF-117) Powder											
0	4	8	2	3	2	3	0	0	2	7	12	8
0.025	4	10	0	2	2	1	0	0	1	12	5	9
0.050	8	10	2	3	2	5	0	0	2	13	9	6
0.100	6	2	2	2	3	10	0	0	2	13	11	6
	Check											
0	3	7	2	2	2	4	0	0	6	9	13	5
0.025	6	8	2	3	1	3	0	0	6	21	7	2
0.050	5	7	3	3	2	5	0	0	3	24	5	4
0.010	7	4	2	2	2	8	0	0	3	24	10	4

[a] Dytiscidae and Hydrophilidae.

Table 4. Effects of *Bacillus sphaericus* 2362 (0.2 lb/acre) on nontarget organisms in polluted and nonpolluted water ponds. (Coachella Valley, April–May 1983).

Pollution (%)	Mean no. of organisms/5 dips pre- and posttreatment (days)											
	Mayfly naiads						Ostracods					
	Pre-	2	7	14	21	35	Pre-	2	7	14	21	35
	2362 (IF-117) Cream											
0	12	8	4	1	2	7	319	68	25	60	147	84
0.025	9	14	3	0	1	12	1505	816	56	140	206	23
0.050	12	22	7	8	17	2	116	98	18	46	81	14
0.100	47	61	6	25	50	11	207	550	60	329	308	196
	2362 (IF-117) Powder											
0	9	8	3	1	0	7	213	95	56	126	210	217
0.025	11	28	5	5	4	20	732	599	49	78	242	64
0.050	16	55	14	3	2	38	735	231	42	112	186	32
0.100	71	67	7	15	35	15	452	886	79	1465	4456	98
	Check											
0	17	6	2	2	1	9	266	284	53	137	249	21
0.025	13	23	6	4	15	4	235	234	133	168	158	63
0.050	23	46	8	22	32	8	39	53	7	54	56	249
0.100	53	16	3	23	62	12	880	795	95	450	350	539

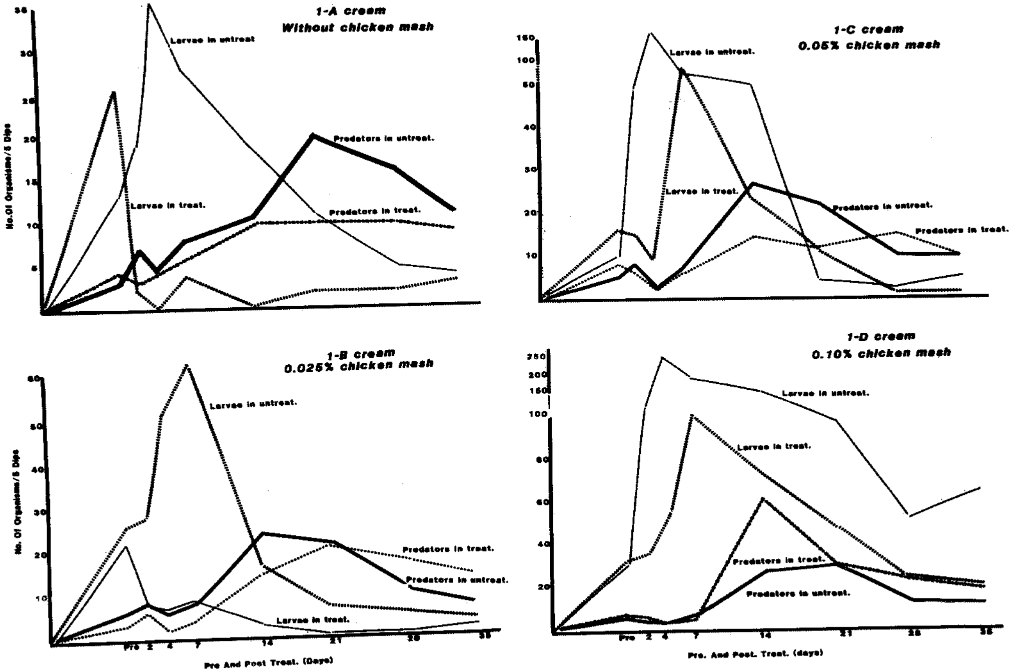

Fig. 1. Impact of the microbial control agent *B. sphaericus* strain 2362 applied as a cream preparation at the rate of 0.2 lb/acre against *Culex tarsalis* larvae (all instars) in freshwater ponds provided with various levels of organic enrichment. A: no enrichment, B: 0.025%, C: 0.05% and D: 0.1% chicken mash.

pression endured for up to 21 days posttreatment, after which time it was maintained by the predators in both treated and check ponds.

An essentially similar picture was noted in ponds enriched at 0.025% of chicken mash (Fig. 1B). Here, as in Fig. 1A, mosquito larvae were initially suppressed with 2362 cream treatment, and level of reduction was maintained up to 14–21 days posttreatment. Predator population peaked 14 days posttreatment, affecting larval population decline in both check and treated ponds, although predator density was somewhat lower in the treated than in the untreated ponds. Note that the values for population density on the vertical scale vary for the various enrichment levels. The action of predators in ponds with 0.025% enrichment was similar to that in the unenriched ponds.

Mosquito larval density produced in ponds enriched with 0.05% chicken mash but untreated with 2362 cream reached a very high level during 2–14 days posttreatment. The 2362 cream treatment maintained larval populations at a low level for 4 days (Fig. 1C), although larval density in untreated ponds reached over 100 larvae/5 dips. Predator population reached a peak level 14 days posttreatment, and exerted suppressive pressure on mosquito larvae. Larval population in both treated and untreated ponds were maintained at a relatively low level beyond day 21 posttreatment.

As the level of organic enrichment increased to 0.1%, mosquito breeding occurred at extremely high density in both treated and untreated ponds (Fig. 1D). Treatment with 2362 cream yielded some suppression of mosquito larvae, the larval population in the treated ponds surging but remaining at about one half that observed in the untreated ponds 4 days and beyond after treatment. The predator population as in the previous situations remained low initially, but peaked at 14 days posttreatment. From this period on, the predators exerted some suppressive force on mosquito larvae, but could not adequately cope with the very heavy and persistently high intensity of recruitment and breeding of mosquito larvae.

From the data presented in Fig. (1A-D), it is evident that 2363 cream treatments are quite effective and necessary during the first brood of mosquito production following flooding. This microbial agent which is completely innocuous to the prevailing predators, permits rapid build up of predators which in turn exert suppressive forces on mosquito larvae. In the high rate of enrichment, however, predators could not reduce the high density of larvae and the rate of 0.22 kg/ha of 2362 was not adequate to control mosquito larvae.

Essentially similar results were obtained in treatments made with the powder preparation of 2362 (IF-117) as with the cream preparation. Treatment with the powder in unenriched ponds produced almost complete initial control of larvae, and the high level of control persisted up to 21 days posttreatment (Fig. 2A). Beyond this time, predators peaked in both treated and untreated ponds and exerted suppressive forces on mosquito larvae.

Treatment with 2362 powder in ponds enriched with 0.025% produced good initial control and this high level of control continued for only 7 days and the population increasing slightly at 14 days (Fig. 2B). After this period, natural enemies kept mosquito larvae in check at essentially the same level in both treated and untreated ponds.

At the 0.05% pollution, high level of control was achieved for only 7 days (Fig. 2C). At the higher level of pollution (0.10%) mosquito larval populations were controlled by 2362 powder treatments for only 4 days (Fig. 2D). The larval populations showed similar trends in build up and decline in both the treated and untreated ponds. In the 0.05% enriched ponds, larval population reached almost a similar low level in both treated and untreated ponds, 21–35 days posttreatment. In the 0.1% enriched ponds, larval density in both the treated and untreated was high, and larval density never declined to a low level as in the other pollution levels.

From the data presented in figures 1 and 2, it is clear that *B. sphaericus* can provide excellent initial control of mosquito larvae in unpolluted waters, and such adequate control is sustained for longer periods than with *B. thuringiensis*

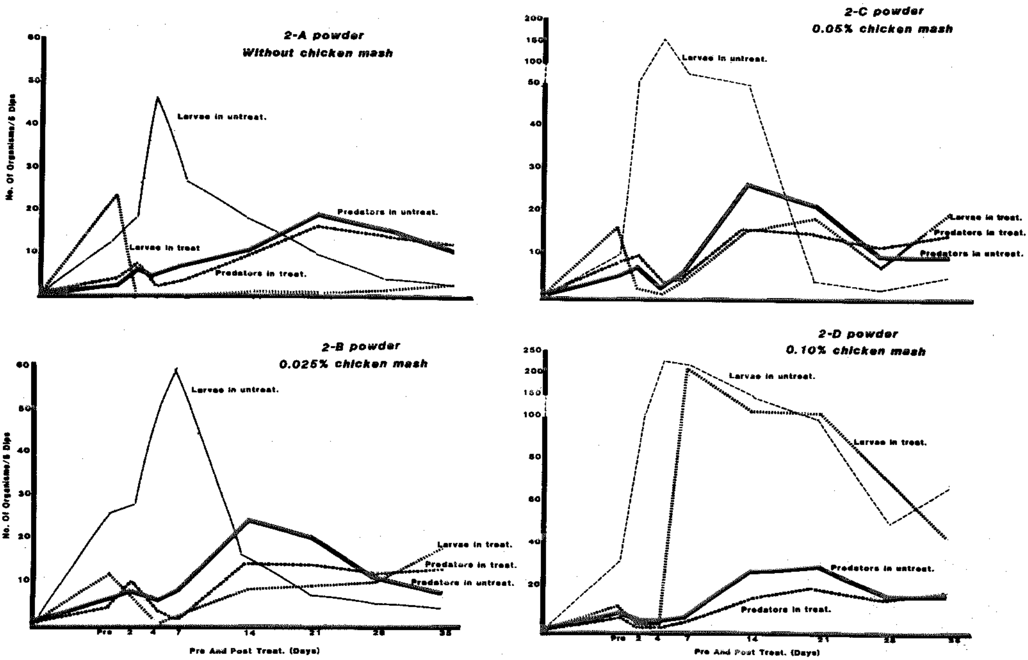

Fig. 2. Impact of the microbial control agent *B. sphaericus* strain 2362 applied as a powder preparation at the rate of 0.2 lb/acre against *Culex tarsalis* larvae (all instars) in freshwater ponds provided with various levels of organic enrichment. A: no enrichment, B: 0.025%, C: 0.05% and D: 0.1% chicken mash.

(H-14) (Mulla et al. 1982a, 1982b). However, there is no evidence that longevity of efficacy of *B. sphaericus* goes beyond the 2–3 wk posttreatment period. Since this material exerts no adverse effects on non-target biota, buildup of natural predators facilitates suppression of subsequent cohorts of mosquitoes. These studies indicate that *B. sphaericus* has the potential to control mosquito larvae for 2–3 weeks or possibly longer. Mosquito larval control over a period of 2–3 wk with one treatment is not achievable with practical rates of most of the conventional larvicides. It is quite significant that 2362 preparations can yield such long-lasting control. The initial effectiveness as well as longevity of *B. sphaericus* strains may be further enhanced through formulation techniques. From the data presented, it is evident that some *B. sphaericus* strains show good larvicidal activity and that they offer good potential for use in mosquito control programs.

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