LARVICIDAL ACTIVITY AND FIELD EFFICACY OF *BACILLUS SPHAERICUS* STRAINS AGAINST MOSQUITO LARVAE AND THEIR SAFETY TO NONTARGET ORGANISMS[1]

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ABSTRACT. Several strains of the microbial control agent *Bacillus sphaericus* were evaluated in the laboratory against 2nd and 4th-instar larvae of 5 species of mosquitoes. All strains tested exhibited high levels of activity against *Culex* larvae. Strains 2362 (IF-97), 2013–4 and 2013–6 were as effective as the standard preparation strain 1593–4 (RB–80), producing 90% mortality in 4th-instar larvae of *Cx. quinquefasciatus* at a concentration in the range of 0.04–0.05 mg/liter. Strain 1593 (IF–94) was somewhat less active (2 fold) than the other 3 strains with an LC_{90} of 0.11 mg/liter. All strains tested displayed lower activity against *Anopheles* larvae, while *Ae. aegypti* was the least susceptible of all species tested with an LC_{90} higher than 40 mg/liter.

It was demonstrated that 2nd-instar larvae in general were more susceptible than 4th-instars, and that maximum mortality at a given concentration was obtained when an exposure period of 48 hr was used. Unlike *B. thuringiensis* (H-14) bioassays, the extended exposure period is needed rather than 24 hr because *B. sphaericus* strains cause little or no mortality during the 24 hr period.

Under natural field conditions, both strains of *B. sphaericus* 2362 (IF–97) and 1593 (IF–94) yielded excellent initial control of *Cx. tarsalis* and *Cx. peus* at the rates of 0.1 and 0.2 lb/acre of the primary powders. In some of the field tests, larval control persisted up to 14 days posttreatment. At both rates, these 2 strains had no noticeable adverse effects on prevailing macroinvertebrate fauna such as mayfly naiads, diving beetle larvae and adults, ostracods and conchostracans.

INTRODUCTION

Earlier studies on several isolates of *Bacillus sphaericus* Neide demonstrated inconsistent results against larvae of mosquitoes in the laboratory and under natural field conditions (Mulligan et al. 1978, Wickremesinghe and Mendis 1981). Biological activity of these strains was reported to be influenced by many factors, some of which are growth media, production and preparation methods of the pathogen, and microbial flora and nutrients in mosquito breeding sources (Goldberg et al. 1974, 1977; Ramoska and Pacey 1979). Since then, many advances have been made in isolating and producing several strains of *B. sphaericus* with a wide spectrum of activity against larvae of several mosquito species as tested by various workers (Lacey and Singer 1982, Davidson et al. 1981). Wraight et al. (1981a) tested strain 1593 powder (MV–716) and determined the LC_{95} against 4th-instar larvae of *Culex pipiens* Linn. and *Cx. salinarius* Coquillett to be about 0.2 mg/liter, and it was found that older instars were less susceptible and maximum mortality was obtained in 48 hours of exposure. LC_{95} (96 hr) values of this strain against 4th-instar larvae of laboratory reared and field collected larvae of *Aedes stimulans* (Walker) were in the order of 6.3 and 1.4 mg/liter respectively (Wraight et al. 1982). *Bacillus thuringiensis* (H-14) was much more active than *B. sphaericus* against this species.

At the present, efforts are being made to improve the activity of available strains and to isolate and identify others with higher biological activity. The following studies were initiated to screen several strains of *B. sphaericus* in the laboratory against mosquito larvae, and to select the most effective preparations for evaluation under field conditions. In addition, studies were conducted to establish effective rates of application, and to study the impact of larvicidal rates on selected aquatic biota.

METHODS AND MATERIALS

Dry powders produced from several strains of *Bacillus sphaericus* were provided by S. Singer and H. T. Dulmage. These preparations are listed in Table 1 and they were evaluated as follows:

LABORATORY. One percent stock suspension of each material (w/v) was prepared in water, and serial dilutions were made as needed. Aliquots of 0.2–1.0 ml of the proper strength suspensions were added to 100 ml of tap water in 4 oz disposable cups (Sweetheart Cup Div., Baltimore, MD), containing 20 2nd or 4th-instar larvae. Each material was tested 2–3 times on different occasions, utilizing a freshly

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Table 1. Activity of various strains of *Bacillus sphaericus* against 2nd and 4th-instar larvae of 2 mosquito species in the laboratory.

Isolate	Preparation method	Supplier	48 hr LC_{50} LC_{90} (mg-liter)[a]	
			Cx. quinquefasciatus	*An. quadrimaculatus*
			2nd-instar larvae	
2362 (IF-97)	Acetone precipitation	Dulmage	0.004–0.02	0.116– 0.39
1593 (IF-94)	Acetone precipitation	Dulmage	— —	0.054– 0.31
2013–6	Lyophilized	Singer	0.006–0.02	0.187– 1.39
2013–4	Lyophilized	Singer	0.009–0.03	0.090– 4.33
			4th-instar larvae	
2362 (IF-97)	Acetone precipitation	Dulmage	0.006–0.04	0.539– 2.05
1593–4 (RB-80)	Lyophilized	Institut Pasteur	0.011–0.04	— —
1593 (IF-94)	Acetone precipitation	Dulmage	0.040–0.11	0.660– 1.91
2013–4	Lyophilized	Singer	0.010–0.04	1.240–15.32
2013–6	Lyophilized	Singer	0.011–0.05	1.280–25.39

[a] All materials failed to produce (50%) mortality at a concentration of 40 mg/liter against *Aedes aegypti* larvae.

made suspension in each test. In each test, the materials were applied at several concentrations (4–5) in triplicates, and along with each test 3 cups were left untreated as checks. After 24 and 48 hr of exposure of test organisms to various concentrations in a controlled temperature holding room (26 ± 1°C), mortality readings were taken, and the LC_{50} and LC_{90} values in mg/liter were obtained through log probit regression analysis using Compucorp 145E computer.

For determining the range of activity, all strains were initially tested against 2nd and 4th-instar larvae of *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say. A lyophilized preparation of *B. sphaericus* 1593–4 (RB–80) from the Institut Pasteur was also used as a standard to measure the effectiveness of new strains against 4th-instar larvae of *Cx. quinquefasciatus*. The two readily available strains (1593 IF–94 and 2362 IF–97) were further evaluated against 2nd and 4th-instar larvae of *Aedes aegypti* Linn., *An. quadrimaculatus, An. freeborni* Aitken and *Cx. tarsalis* Coquillett. Larvae utilized in the evaluation were obtained from laboratory colonies at the University of California, Riverside.

FIELD. Only two strains (1593 IF–94, and 2362 IF–97) were available from H. T. Dulmage in sufficient quantities for field evaluation. Both materials were used as primary powders and were evaluated in experimental ponds at the Aquatic and Vector Control Research facilities of the University of California, Riverside, in Riverside and in the Coachella Valley of southern California. Detailed description of these facilities were reported elsewhere (Mulla et al. 1982). In brief, the ponds at Riverside were 12 × 24 ft (27 m^2), maintained free of vegetation, filled with water from a reservoir and water pH averaged 8.2. Ponds in the Coachella Valley were 18 × 18 ft (30 m^2), and covered with plant growth. The ponds were filled with water from an artesian well and water pH was 9.4. At both locations, water depth was maintained constant at 12 in (30 cm) by float valves. Water temperature during all tests was monitored with a Mini-Max thermometer (Markson Science Inc., Del Mar, CA) and this information is included in Tables 4 and 5.

Six field trials were conducted with the 2 strains of *B. sphaericus*, 2 in Riverside and 4 in the Coachella Valley facilities. In Riverside, strain 1593 (IF–94) was applied at rates of 0.1 and 0.2 lb/acre (112 and 224 g/ha) of the primary powder, while 2362 (IF–97) was applied at the rate of 0.05 and 0.1 lb/acre (56 and 112 g/ha) of the primary powder. Three replicates were used per application rate along with 3 checks, and mosquito population consisted mostly of immature *Cx. peus* Speiser; but *Cx. tarsalis* was also present in small numbers in the Riverside ponds.

The two strains were evaluated twice each at the Coachella Valley facility, and both materials were applied at the same rate (0.1 and 0.2 lb/acre). In the first 2 tests, 2 replicates per application rate were used along with 2 checks, while 3 replicates and 3 checks were utilized in the other tests. During all 4 tests in the Coachella Valley, larval populations consisted mostly of *Cx. tarsalis*, but *Anopheles franciscanus* McCracken were also present in good numbers. In the first test in the Coachella Valley (April–May 1982) using strains 2362 (IF–97) and 1593 (IF–94), microbiological studies prior to and after application were also made and results are published elsewhere (Davidson et al. 1984).

In applying the preparations, the required

amount for each rate of application was suspended in 120 ml of tap water, and applied with a polyethylene squeeze bottle. To determine the effect of these bacterial larvicides on mosquito larvae and nontarget organisms prevailing in the ponds during these studies, 5 dips per pond were taken prior to treatment and 2, 4, 7 and 14 days after treatment. The 5 dips, at each sampling time, were concentrated into one sample in a concentrator cup (16 oz) provided with 2 windows (2 × 6 cm each) covered with 150 mesh stainless steel strainer cloth. 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.

Since the development of stagnant water mosquitoes is asynchronous, newly hatched larvae were present in large numbers, and in general were not exposed to the microbial agent for sufficient time to effect mortality by the time of samplings. To determine the activity of *B. sphaericus* 1593 (IF–94) and 2362 (IF–97) against those larval cohorts exposed to lethal concentrations, the younger larvae (1st and 2nd-instars) although counted and included in the tables, were excluded from calculations yielding percent reduction. Because of natural fluctuations in larval counts in the check ponds, percent reduction was calculated according to the following formula (Mulla et al. 1971):

$$(\%R) = 100 - \frac{\{C1 \times T2\}}{\{T1 \times C2\}}100,$$ C1: No. of larvae in check pretreatment. C2: No. of larvae in check posttreatment, T1: No. of larvae in treated pretreatment, and T2: No. of larvae in treated posttreatment.

RESULTS AND DISCUSSION

LABORATORY. The 4 strains of *B. sphaericus* tested against 2nd-instar larvae of *Cx. quinquefasciatus*, displayed similar levels of activity; causing 90% mortality (48 hr) at low concentrations in the range of 0.02 and 0.03 mg/liter of the preparations (Table 1). These strains are in general more active than the older preparation of strain 1593 (MV–716) produced by Stauffer Chemical Company where the LC_{95} was in excess of 0.2 mg/liter for *Cx. pipiens* (Wraight et al. 1981a).

After determining the initial activity of various strains, some available powders of *B. sphaericus* 2362 (IF–97) and 1593 (IF–94) were evaluated against 2nd and 4th-instar larvae of several species. The activity as a function of time exposure was also determined. Values for *Cx. quinquefasciatus* and *An. quadrimaculatus* (exposure period 48 hr) thus obtained with strain 2362 (IF–97) are given in Table 2. Strain 2362 (acetone precipitated) powder, showed higher activity against 2nd-instars of all 5 mosquitoes than against 4th-instar larvae. Second-instar larvae were up to 10X more susceptible at LC_{90} level than 4th-instars using the 24 hr exposure period. After 48 hr of exposure, maximum mortality was obtained and 2nd-instars at the LC_{90} level were 2–10X more susceptible than the 4th-instar larvae. Other workers have also found that in general younger instar larvae are more susceptible to *B. sphaericus* than the older larvae (Wraight et al. 1981a, 1981b).

An important feature of *B. sphaericus* strains

Table 2. Activity of *Bacillus sphaericus* 2362 (IF-97 AP) against larvae of various species of mosquitoes in the laboratory.

Mosquito species	LC_{50} and LC_{90} values (mg/liter)[a]					
	2nd-instar larvae			4th-instar larvae		
	LC_{50}-LC_{90}	Correlation coefficient	Slope	LC_{50}-LC_{90}	Correlation coefficient	Slope
			24 hr assessment period			
Cx. quinquefasciatus	0.04–0.18	0.92	1.97	0.06–0.34	0.64	1.63
Cx. tarsalis	0.02–0.05	0.97	2.66	0.13–0.56	0.94	1.99
An. freeborni	0.83–2.58	0.85	0.90	3.04–5.67	0.95	1.38
An quadrimaculatus	0.43–2.98	0.98	1.52	2.39–7.98	0.97	2.45
Ae. aegypti	>20.00	0.72	1.07	>40.00	0.86	1.34
			48 hr assessment period			
Cx. quinquefasciatus	0.005–0.02	0.94	1.92	0.02–0.04	0.89	3.50
Cx. tarsalis	0.003–0.01	0.82	2.67	0.02–0.07	0.95	2.62
An. freeborni	0.092–0.55	0.89	1.66	0.65–4.45	0.93	1.53
An. quadrimaculatus	0.117–0.39	0.95	2.43	0.53–2.05	0.89	2.21
Ae. aegypti	>20.00	0.92	0.85	>40.00	0.92	1.61

[a] Values (mg/liter) obtained from log probit regression analysis.

is their slow action against larvae. As evident from data in Table 2, by increasing the assessment period from 24 to 48 hr, there was a marked increase in mortality of 2nd as well as 4th-instar larvae. In 2nd-instar larvae at the LC_{90} level there was approximately 5–8X increase in mortality of the various species when assessment was made 48 hr after treatment rather than at 24 hr. In 4th-instar larvae the increase in mortality was up to 8X for the longer period (48 hr) of assessment.

Strain 1593 (IF–94) when tested against 4th-instar larvae of 4 species showed higher activity when the assessment period was increased to 48 hrs (Table 3). The increase in mortality was 1.5–7X at the longer assessment period (48 hr) in 3 species of mosquitoes. There was no increase in mortality of *Ae. aegypti* larvae with increased assessment period. This species is so refractory that accurate dosage-mortality relationships cannot be clearly established.

However, these materials showed various degrees of activity against 2nd-instar larvae of *An. quadrimaculatus* (Tables 2 and 3). Strain 2362 and 1593 showed essentially similar activity causing 90% mortality at 0.39 and 0.31 mg/liter respectively. Strains 2013–6 and 2013–4 were less active (5–10X) against the young anopheline larvae than 2362 (IF–97) and 1593 (IF–94). Strain 2013–6 produced better results than 2013–4, causing 90% mortality at 1.39 mg/liter as compared to 4.33 mg/liter for the latter.

Against 4th-instar larvae of *Cx. quinquefasciatus*, strains 2362, the standard preparation (1593–4 RB–80), 2013–4 and 2013–6 produced similar results, causing 90% mortality at low concentrations in the range of 0.04–0.05 mg/liter. The 1593 (IF–94) preparation was somewhat less active against 4th-instars of *Cx. quinquefasciatus* with an LC_{90} of 0.11 mg/liter. All strains were less active against 4th-instar larvae of *An. quadrimaculatus* than *Cx. quinquefasciatus*. Against 4th-instar larvae of *An. quadrimaculatus*, strains 1593 and 2362 were equally active and displayed higher activity than strains 2013–4 and 2013–6. At concentrations of 1.91 and 2.0 mg/liter, 1593 (IF–94) and 2362 (IF–97) respectively produced 90% mortality, while a concentration of 15 and 25 mg/liter, respectively, was required to produce similar results with strain 2013–4 and 2013–6 (Table 1). From the data presented it seems that all strains of *B. sphaericus* tested were almost equally and highly active against *Cx. quinquefasciatus* and *Cx. tarsalis*, having intermediate levels of activity against *An. quadrimaculatus* and *An. freeborni*, but with a very low level of activity against *Ae. aegypti*. The same trend was reported for strains 2013–4 and 1023–6 by Lacey and Singer (1982). However, their $LC_{50\text{-}95}$ values against 2nd-instar larvae of *Cx. quinquefasciatus* and *Cx. tarsalis*, having intermediate lower than the results shown in Table 1. Variation in activity levels could be attributed to several factors such as larval strain, age and vigor at the time of testing, and procedures used in bioassays. In the present studies it was found that 2nd-instar larvae were more susceptible than 4th-instar larvae and that maximum mortality was obtained with the 48 hr exposure.

A comparison of the activity of *B. sphaericus* strains 1593 and 2362 shows these strains to be more potent than *B. thuringiensis* (H–14) against *Cx. quinquefasciatus*. The LC_{90} for the two *B. sphaericus* strains against 4th-instar larvae ranged from 0.04 to 0.11 mg/liter as compared with LC_{90} of 0.45 mg/liter of the *B. thuringiensis* H–14 standard (Mulla et al. 1982). For *An. quadrimaculatus* 4th-instar larvae, the LC_{90} for the two *B. sphaericus* strains was about 1–2 mg/liter as compared to 1.4 mg/liter for the standard *B. thuringiensis* (H–14) (IPS–78).

FIELD. *Bacillus sphaericus* strain 2362 produced complete initial control of 3rd-4th-instar larvae of *Cx. tarsalis* and *Cx. peus* at the rates of 0.05, 0.1, and 0.2 lb/acre of the primary powder (56, 112 and 224 g/ha) (Table 4). In one test (April–May) in the Coachella Valley facility, excellent control was obtained for about 4 days, while in the other test (May–June), high level of control (92%) prevailed for one week, and moderate level of control (59–82%) prevailed for 2 weeks. Essentially similar results were obtained at Riverside at the rates of 0.05 and 0.1 lb/acre. At both locations, some level of control persisted for 14 days after treatment in some but not all of the tests.

Bacillus sphaericus strain 1593 although

Table 3. Activity of *Bacillus sphaericus* 1953 (IF-94) against 4th-instar larvae of various mosquito species in the laboratory.

Mosquito species	(mg/liter)[a] LC_{50}-LC_{90}	Correlation coefficient	Slope
	24 hr assessment period		
Cx. quinquefasciatus	10.15– 0.67	0.910	2.000
Cx. tarsalis	0.09– 0.24	0.767	3.015
An. quadrimaculatus	1.04– 3.02	0.909	2.860
Ae. aegypti	14.24–26.37	0.896	4.784
	48 hr assessment period		
Cx. quinquefasciatus	0.04– 0.09	0.914	3.938
Cx. tarsalis	0.06– 0.14	0.780	3.612
An. quadrimaculatus	0.65– 1.91	0.881	2.758
Ae. aegypti	7.95–22.73	0.853	2.806

[a] Values (mg/liter) obtained from log prohibit regression analysis.

Table 4. Evaluation of *Bacillus sphaericus* 2362 (IF-97) against *Culex tarsalis* and *Cx. peus* larvae in experimental field ponds.

Rate		Avg. no. larvae/ 5 dips Pre-treatment		% reduction after treatment (days)			
lb/acre	g/ha	1–2	3–4	2	4	7	14
		July–Aug.[a]					
0.05	56	61	100	100	—	77	39
0.10	112	144	105	100	—	63	48
Check	—	93	116	—	0	—	—
		April–May[b]					
0.10	112	64	17	100	100	47	0
0.20	224	14	20	100	97	0	0
Check	—	2	3	—	—	—	—
		May–June[c]					
0.10	112	39	35	100	—	92	82
0.20	224	19	17	100	—	92	59
Check	—	20	14	—	—	—	—

[a] UCR facility, population *Cx. peus* (75%), *Cx. tarsalis* (25%). Water temp. range 25°–33°C, mean min 27°C-mean max 33°C.

[b] Coachella facility, population *Cx. tarsalis*, water temp. range 14°–31°C, mean min 16°C-mean max 27°C. Calculation of percent reduction is based in part on larval counts in the check. Due to light breeding in check, treatments show no control 7 and 14 days posttreatment.

[c] Coachella facility, population *Cx. tarsalis*, water temp. range 17°–33°C, mean min 24°C-mean max 32°C.

Table 5. Evaluation of *Bacillus sphaericus* 1593 (IF-94) against *Culex tarsalis* and *Cx. peus* larvae in experimental ponds.

Rate		Larvae/ 5 dips Pre-treatment		% reduction after treatment (days)			
lb/acre	g/ha	1–2	3–4	2	4	7	14
		July–Aug.[a]					
0.10	112	31	7	83	100	0	0
0.20	224	59	28	98	94	4	0
Check	—	2	3	—	—	—	—
		April–May[b]					
0.10	112	52	68	97	—	88	95
0.20	224	47	93	100	—	92	96
Check	—	14	19	—	—	—	—
		May–June[c]					
0.10	112	19	18	100	—	77	78
0.20	224	27	45	100	—	88	79
Check	—	20	14	—	—	—	—

[a] Coachella facility, *Cx. tarsalis*, water temp. range 14–31°C, mean min 16°C-mean max 27°C. Steep decline in reduction in treatments is due to lack of breeding in check ponds. The formula used yielded these results when calculating % reduction.

[b] Coachella facility, population *Cx. tarsalis*, water temp. range 17°–33°C, mean min 24°C-mean max 32°C.

[c] UCR facility, population *Cx. peus*, water temp. range 14°–23°C, mean min 16°C-mean max 22°C.

somewhat less active than *B. sphaericus* 2362 in laboratory, produced excellent control of larvae at the rates of 0.1 and 0.2 lb/acre. In one test, 83 and 98% reduction was obtained, respectively, 2 days after treatment, and 100 and 94% reduction respectively 4 days posttreatment but the population recovered completely 7 days later (Table 5). In the succeeding 2 tests and at the same rates, almost complete control was obtained 2 days after treatment and the population was markedly suppressed for more than 2 weeks posttreatment (Table 5). In all the above field trials undertaken in the Coachella Valley (Tables 4 and 5), spores of *B. sphaericus* settled rapidly to the bottom as evidenced by microbiological determination (Davidson et al. 1984). Initially, spore counts declined at the water surface and increased on the bottom mud surface, then declined in numbers at the mud surface also.

Both strains of *B. sphaericus* (1593 and 2362) at the rates applied showed no apparent adverse effects on nontarget organisms present in the ponds at the time of treatment. In tests in the Coachella Valley, numbers of naiads of the mayfly *Callibaetis pacificus* in treated ponds increased or declined in line with check ponds over the 2-week study period (Table 6). Similarly, no marked effects due to treatments with both strains were noted on ostracods, conchostracans, diving beetle (Dytiscidae and Hydrophilidae) larvae and adults (*Berosus metalliceps* and *Tropisternus lateralis*) and tadpoles were not noticeably affected. Data on these groups are not included in the table.

In another series of tests, similar lack of adverse effects was noted. Mayfly naiads either increased or decreased in numbers as they did in the check ponds (Table 7). Ostracod populations again increased or decreased in line with those in the check ponds over the 21-day study period. Conchostracans followed similar natural fluctuations of populations, with no apparent effects of *B. sphaericus* treatments.

Findings of this research indicate that both strains of *B. sphaericus* (1593 and 2362) could be effectively utilized for the control of culicine mosquito larvae with no adverse impact on associated aquatic biota. At the rates of 56 and 112 g/ha (0.05 and 0.1 lb/acre) 2362 can provide

Table 6. Impact of *Bacillus sphaericus* on nontarget organisms in experimental ponds (Coachella Valley, May 1982).

		Mean no. nontarget organisms/5 dips pre- and posttreatment (days)[a]							
		Mayflies				Ostracods			
Strain	Rate lb/acre	Pre-treatment	2	7	14	Pre-treatment	2	7	14
1593	0.10	5	16	12	2	40	13	9	12
	0.20	8	15	18	9	54	47	46	21
2362	0.10	5	12	5	5	58	80	89	124
	0.20	4	5	14	4	23	26	29	44
Check	—	30	32	38	5	67	30	28	61

[a] Tadpoles were not affected during the duration of these studies. Dragonfly naiads appeared 2 weeks posttreatment in some of the treated and check ponds.

Table 7. Effect of *Bacillus sphaericus* strains on nontarget organisms in experimental ponds (Coachella Valley, April–May 1982)

	Mean no. of nontarget organisms/5 dips				
	Strain 1593		Strain 2362		
Posttreatment (days)	0.1	0.2	0.1	0.2	Check
			Mayfly naiads[a]		
Pretreatment	0	0	1	2	7
2	2	2	2	2	2
4	3	2	6	3	4
7	1	11	13	3	1
14	2	8	24	1	2
21	2	8	24	1	2
			Ostracods		
Pretreatment	129	339	263	75	21
2	119	499	273	308	33
4	124	341	232	125	55
7	56	153	111	606	84
14	52	273	135	773	63
21	10	15	60	495	32
			Conchostracans		
Pretreatment	3	66	7	30	7
2	3	14	3	9	5
4	1	11	4	15	4
7	3	8	0	8	4
14	0	1	0	4	1
21	0	0	0	0	0

[a] *Callibaetis pacificus* Seeman.

satisfactory control of *Cx. tarsalis* and *Cx. peus* in clear standing water, while higher rates of 112 and 224 g/ha (0.1 and 0.2 lb/acre) are required to control the same species with 1593. Rate of application of both strains, however, is largely dependent on larval density and other factors such as water quality, pH and water temperature. Studies are underway to determine the activity and longevity of these two strains in fresh and polluted water under field conditions, and to determine their activity against floodwater mosquitoes [*Psorophora columbiae* (Dyar and Knab) and *Ae. nigromaculis* (Ludlow)] in the laboratory and under field conditions.

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