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FIELD TRIALS OF THREE CONCENTRATIONS OF LAGINEX™ AS BIOLOGICAL LARVICIDE COMPARED TO VECTOBAC™-12AS AS A BIOCONTROL AGENT FOR *CULEX QUINQUEFASCIATUS*

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ABSTRACT. Laginex™ AS Biological Larvicide (*Lagenidium giganteum*) and Vectobac™-12AS (*Bacillus thuringiensis* var. *israelensis*) were compared in plastic pools containing laboratory-reared *Culex quinquefasciatus* larvae located in a wooded area in Panama City, FL, in August and September 1997. The pools were dipped at 2-day intervals to assess larval control. Sentinel larvae in screen-sided cups were added to all treatment and control pools at 4-day intervals and evaluated for infection at 48 h. Laginex produced larval control up to 20 days as compared to the Vectobac-12AS compound, which required retreatment by the 10th day. The most effective control was attained by the Laginex 25 treatment, which reduced larvae in the pools by 100% for 22 days. Control in the Laginex 15 and Laginex 35 treatments dropped to 90% and 74% at day 13 and day 9, respectively. The numbers of infected larvae remained above 50% as larval control was assessed, but dropped and fluctuated when water temperatures were lowered at the onset of cold weather. Organic pollution did not prove to be a significant factor in the evaluations.

KEY WORDS Laginex™, *Lagenidium giganteum,* biological control, *Bacillus thuringiensis* var. *israelensis, Culex quinquefasciatus,* mosquito control

INTRODUCTION

Laginex™ AS Biological Larvicide (AgraQuest, Davis, CA; U.S. Environmental Protection Agency registration number 69592-2) is an aqueous suspension consisting of the California strain of *Lagenidium giganteum* Couch (40%), a microbial parasite of mosquito larvae, related to Oomycetes, and inert ingredients (60%). The formulation was shipped to our laboratory in refrigerated containers and required application within 2 wk of receipt. Mosquito infection is initiated by zoospores, which attach to the epicuticle of mosquito larvae, differentiating them from other aquatic species (Kerwin 1991). After death of the larva, within 2–3 days after spore attachment, asexual spores are released and they amplify the initial infection. Two cells can merge to form oospores, the sexual stage, which in some cases can remain viable for several years, even in extreme conditions (Fetter-Lasko and Washino 1983, Kerwin and Washino 1983, Kerwin et al. 1986, Hornby 1992).

Water temperature and organic pollution affect zoosporogenesis, which is necessary for mosquito infection. Lord and Roberts (1985) reported the virtual elimination of zoospores surviving to germination with a 0.6-g/liter concentration of NaCl. Jaronski and Axtell (1983) reported the highest level of infection rates in the range of 21–29°C and a decrease in infection rates above 29°C and below 21°C. Merriam and Axtell (1982) reported decreased mycelial growth in 2 different strains due to an increase in salinity level. Jaronski and Axtell (1982) developed a quantitative equation to predict larval infection dependent upon a number of water quality variables.

Our field studies assessed infection rates and control of *Culex quinquefasciatus* Say exposed to *Lagenidium* or Laginex in simulated woodland pools. The efficacy of Laginex was compared with that of Vectobac™-12AS (*Bacillus thuringiensis* var. *israelensis* [*Bti*] de Barjac, Abbott Laboratories, North Chicago, IL), a commonly used mosquito control in the state of Florida. The effects of water quality on the continuation of the life cycle of *L. giganteum* were also investigated.

MATERIALS AND METHODS

Field experiments were conducted during August and September 1997 in Panama City, FL, using plastic wading pools (area = 0.00049 ha, diameter = 100.3 cm) that were filled to a level of 11.4 cm with aged well water (63 liters). One centimeter of washed sand was added to each pool and covered with 1 cm of dried oak leaf litter. Water samples were collected from each pool at the beginning and midpoint of the study. The pH, calcium carbonate (mg/liter), total alkalinity (mg/liter), conductivity (μmhos/cm), nitrogen ammonia (mg/liter), and chemical oxygen demand (COD) (mg/liter) were determined. Water temperatures were monitored by maximum–minimum thermometers placed in 1 pool of each dosage. Treatments were applied within 2 days of receipt of the Laginex formulation. The treatment dosages were Laginex 15 (1,090.2 ml/ha), Laginex 25 (1,816.9 ml/ha), Laginex 35 (2,543.7 ml/ha), and Vectobac-12AS (32,457.6 ml/ha). Actual treatments applied to each pool, adjusted for surface area, were 85.2 μl, 142 μl, 198.9 μl, and 91 μl, respectively. The dose for each pool was added to a glass beaker filled with 50 ml of tap water and taken to the field for dispersal into the

Table 1. Mean ± SE of percent control of immature mosquitos during a 4-wk posttreatment interval in simulated woodland pools. Unshared letters among the treatments denote a significant difference ($P < 0.05$) based on Student–Newman–Keuls multiple range tests.

Treatment	*n*	Mean[1]
Laginex 15	44	0.87 ± 0.28 a
Laginex 25	44	0.89 ± 0.26 a
Bacillus thuringiensis var. *israelensis*	44	0.75 ± 0.36 b
Laginex 35	44	0.74 ± 0.35 b
Control	44	0.03 ± 0.02 c

[1] Percent control means depicted are unadjusted for the arcsine transformation.

individual pools. Four pools per treatment were placed in isolated locations of a wooded area to minimize contamination by resident birds and animals. Approximately 4,000 1st- and 2nd-stage laboratory-reared *Cx. quinquefasciatus* larvae were added to each pool at weekly intervals. Five 350-ml dipper samples were taken before treatment and at 2-day intervals after treatment. The dip counts from the treatment pools were compared to dip counts in the controls to assess larval reduction. At 4-day intervals, 30 2nd- and 3rd-stage larvae were added to 2 screen-sided plastic sentinel disposable 600-ml cups located in each pool. After 48 h, sentinel larvae were removed, rinsed, and taken to the laboratory for microscopic examination to detect infection.

Statistical analysis: The data were evaluated by an analysis of variance to determine differences between treatments. Percentages were then subjected to an arcsine transformation to normalize the data before statistical analysis (Steele and Torrie 1980). Mean separation of the treatments was done using Student–Newman–Keuls procedure (SAS Institute 1990).

RESULTS

Significant differences were noted among the different treatments and a significant treatment by day interaction was found ($F = 23.00$, df 54,165, $P = 0.0001$). The overall model explained 88% of the variation associated with percent mortality of mosquito larvae within our simulated woodland pools. Differences between the treatments are illustrated in Table 1. No difference between the Laginex 15 and the Laginex 25 treatments was noted; however, these 2 treatments controlled significantly more mosquito larvae than the Laginex 35, the *Bti,* and the control. The Laginex 35 and *Bti* treatments differed significantly from the control. In addition to laboratory-reared *Cx. quinquefasciatus* larvae, larval dips produced small numbers of *Anopheles crucians* Weidermann and *Culex restuans* Theobald. The 3 Laginex treatments reduced larvae 98–100% for the 1st 5 days in each study (Fig. 1). Similar studies have shown larval reduction to be 86% and 87% for 3 and 5 days, respectively (Guzman and Axtell 1987a) and 73% over a 138-day period (Guzman and Axtell 1987b). Larval reduction for the Laginex 15 treatment decreased to 90% at day 15 but returned to 100% by day 20. Larval reduction in pools treated with Laginex 25 remained at 100% through day 22 then dropped sharply to 49% on day 25. In experimental pools treated with Laginex 35, larval reduction ranged from 90 to 100% through day 18. Vectobac-12AS was put in on days 3 and 12. After the 1st treatment, larval reduction was high, from 90 to 100%, until day 12 when it dropped to 80%. After retreatment on day 12, larval reduction returned to 98% by day 17. By day 20,

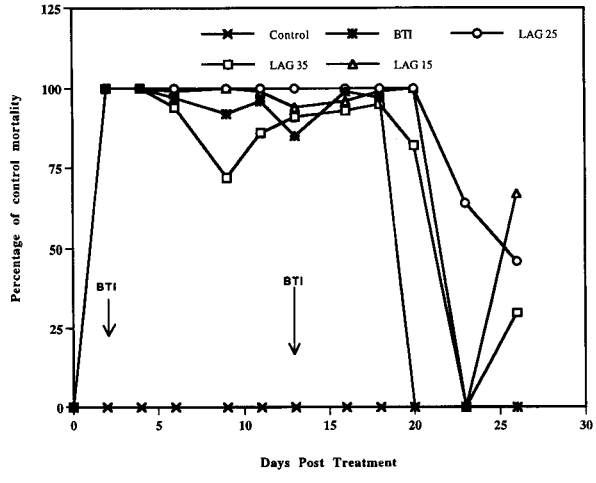

Fig. 1. Percent of control mortality of *Culex quinquefasciatus* larvae for Laginex™ AS bioassays in plastic pools during August and September 1997 in Panama City, FL.

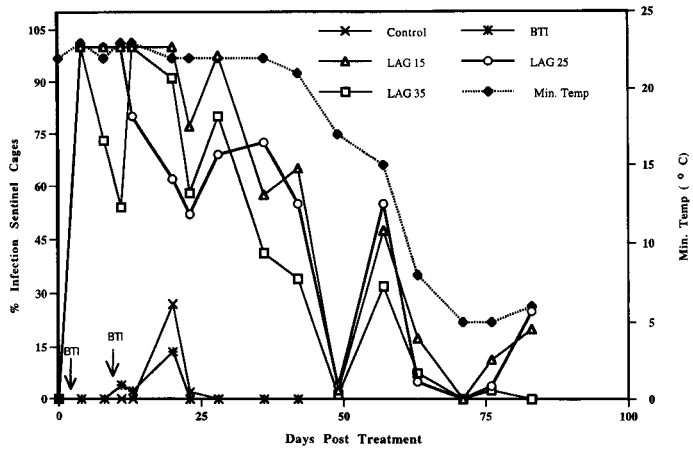

Fig. 2. Percent infection of *Culex quinquefasciatus* larvae in sentinel cages for Laginex™ AS bioassays in plastic pools during August through November 1997 in Panama City, FL.

larval reduction dropped to 0% and stayed at this level throughout the rest of the study.

The infection rate of sentinel larvae for the Laginex 15 treatment was constantly maintained at 100% until day 24 when it dropped to 77% and subsequent larval infections declined (Fig. 2). The infection rate returned to 90% by day 27. The infection rate of Laginex 25 sentinel larvae ranged from 80 to 100% through day 15, then dropped to 65% by day 20. Infection rates of sentinel larvae equaled or exceeded 90% during this period utilizing the Laginex 35 treatment. By day 25, the infection rate decreased to 59%. The infection rate then increased to 80% by day 27. *Lagenidium giganteum* contamination was noted from days 12 through 20 in the *Bti* formulation pools. The infection rate ranged from 5 to 20% during this period. Similarly, a control pool had an infection rate of 30%. We attribute these infections to the intrusion of birds and wild animals, particularly raccoons, even though all attempts were made to isolate treatments.

Temperature did not affect larval production or infection rates during the test period. Water temperatures ranged from 18 to 33°C with a mean maximum of 30.3 ± 1.38°C and a mean minimum of 21.6 ± 1.93°C. Conditions were favorable for increased infection rates in treated pools because of low levels of organic pollution (Table 2). Mean COD (29.35 mg/liter) and mean alkalinity level (137 mg/liter) were considered too low to reduce infection rates. Nitrogen ammonia and $CaCO_3$ alkalinity were below detectable levels.

DISCUSSION

Control was best attained by the Laginex 25 treatment (Fig. 1). The drop in larval control for the Laginex 35 treatment from day 7 to day 9 was possibly caused by a rapid reduction in larval numbers, resulting in a longer period of time required to reestablish zoospores. The Laginex 25 treatment remained relatively stable for a period exceeding 25 days. After a sharp decline around day 21, pathogen activity of the Laginex 15 and Laginex 35 treatments began to increase at day 24.

Vectobac-12AS maintained excellent control for 2–3 days but required retreatment at ca. 10 days. Beyond day 20, the Vectobac-12AS treatment maintained 0% control, whereas all 3 dosages of the Laginex treatments continued control past day 25, after regeneration of the Laginex 15 and Lagi-

Table 2. Water chemistry parameters measured for the Laginex™ AS bioassays conducted in plastic pools during August and September 1997 in Panama City, FL.

Parameter	*n*	Mean ± SD	Range
Water temperature (maximum–minimum) (°C)	44		18–33
Maximum (°C)		30.3 ± 1.38	28–33
Minimum (°C)		21.6 ± 1.93	18–24
pH	20	8.07 ± 0.07	7.97–8.16
$CaCO_3$ (mg/liter)	20	0.0 ± 0.0	Tested but undetected
Total alkalinity (mg/liter)	20	137 ± 7.1	130–154
Conductivity (μmhos/cm)	20	368 ± 26.97	341–410
Nitrogen ammonia (mg/liter)	20	0.00 ± 0.0	Tested but undetected
Chemical oxygen demand (mg/liter)	20	29.35 ± 20.84	2–80

nex 35. Further control in the Vectobac-12AS pools would have required retreatment, whereas the Laginex 15 and Laginex 35 treatments produced increases in larval control (Fig. 1). The Laginex 25 treatment would be expected to have enhanced larval control if water temperatures had remained stable and the experiment had not been terminated because of the colder temperatures. At the onset of cold weather, nearing mid-November, we noted a sharp decline in the numbers of sentinel larvae infected. Domnas (1981) indicated temperatures ranging from 25 to 28°C to be optimal for zoospore procuction. Jaronski and Axtell (1982) reported results indicating that *Lagenidium* had potential for mosquito control in unpolluted waters. Because of low COD (mean = 29.35 mg/liter) and low NH_3-N (mean = 0) levels, our tests experienced little of the effects that can be caused by organic pollution. Lower temperatures resulted in a decreased and slower development of *L. giganteum,* and thus an increase in larval development. When applied to field situations, Laginex AS Biological Larvicide significantly reduced larval populations in the absence of the commonly used chemical controls.

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