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FIELD TRIALS OF VECTOLEX CG®, A *BACILLUS SPHAERICUS* LARVICIDE, IN ILLINOIS WASTE TIRES AND CATCH BASINS

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ABSTRACT. The susceptibilities of *Aedes triseriatus* (Say), *Anopheles punctipennis* (Say), *Culex restuans* (Theobald), and *Culex pipiens* (L.) larvae to VectoLex CG® were determined. VectoLex, formulated on corncob granules (10–14 mesh) was applied to 2 tire dumps and numerous catch basins located in east-central Illinois. VectoLex, formulated as effervescent tablets, was also applied to catch basins. In a sunlit dump, there was a 99.6% reduction in *Ae. triseriatus, Cx. restuans*, and *Cx. pipiens* larvae as long as 32 days after treatment, and no pupae were recovered during this interval. There was still a 71.6% larval reduction 74 days after treatment. There was an overall reduction of 22% for *An. punctipennis* larvae. In a shaded dump, no larval *Ae. triseriatus, Cx. restuans*, or *Cx. pipiens* were recovered for 25 days after treatment, and no pupae were recovered 25–67 days after treatment. There was still a 68.7% larval reduction 74 days after treatment. *An. punctipennis* was unaffected. In one catch basin study, VectoLex was comparable to Altosid® (average of 46 days vs. 50 days until larvae were recovered from catch basins). As the summer progressed, the duration of VectoLex control was reduced to 30 days. VectoLex effervescent tablets (evaluated mid-August through September) gave 18-day control. VectoLex was effective against *Ae. triseriatus, Cx. restuans*, and *Cx. pipiens*, but was not effective against *An. punctipennis* in waste tires. VectoLex was effective against *Cx. restuans* and *Cx. pipiens* in catch basins.

INTRODUCTION

Until waste tire dumps are eliminated, control of mosquitoes that utilize tires as larval habitat will be a continuous challenge. The Rubber Manufacturers Association in Washington, DC, estimated that 200 million tires are added yearly to the solid waste stream in the United States (Novak et al. 1990). One concern about control of mosquitoes in waste tires is the dwindling armamentarium of larvicides. In Illinois, *Bacillus thuringiensis* serovar *israelensis* (*B.t.i.*) is the only microbial insecticide registered for waste tires. Recently a new microbial larvicide, VectoLex® (serotype H5a5b, strain 2362, Abbott Laboratories, North Chicago, IL), based on the fermentation of *Bacillus sphaericus*, has become available. Its larvicidal activity is due to the presence of 2 proteins of 41.9 and 52.4 kd that act as a binary toxin (Davidson and Yousten 1990). Previous studies have shown that species in the genera *Aedes, Anopheles, Culex, Mansonia*, and *Psorophora* are susceptible to this toxin (Lacey 1990, Yap 1990, Groves and Meisch 1996). VectoLex is currently registered for control of *Culex* species in high organic habitats such as sewage lagoons, septic ditches, animal waste lagoons, and similar sites.

In east-central Illinois, *Aedes triseriatus* (Say), *Anopheles punctipennis* (Say), *Culex pipiens* (L.), and *Culex restuans* (Theobald) are the species most commonly found in waste tires. *Aedes triseriatus* is the dominant species, comprising 78.7% of all pupae collected (Siegel et al. 1992). In this paper, we present our findings for one field season, May–September 1996. We were interested in determining if VectoLex was effective against *Culex* species, *Ae. triseriatus*, and *An. punctipennis* in tires. Additionally, the efficacy of VectoLex for control of *Culex* species in catch basins was investigated.

MATERIALS AND METHODS

Study site-tire dumps: Tire dumps were located in Trelease Woods, a mixed deciduous woodlot in Champaign County, IL. One tire dump was located in a sunlit meadow approximately 3 m from the tree line. The other tire dump was located in constant shade approximately 5 m inside the woods margin. The surrounding trees (10–15 m tall) were predominantly oak and maple. Within each dump, 10 tires were tied upright to metal fence stakes that were spaced 1 m apart. Alternating tires were designated as treated or control and marked with spray paint. These tires were sampled weekly beginning May 28 and ending August 12.

Tire dosage and sampling: Each treated tire received 376 mg of VectoLex CG corncob granules (approximately 154 granules) with a label potency of 50 *B. sphaericus* international toxic units (ITU)/mg (18,800 *B. sphaericus* ITU/tire). This dose approximated the average number of granules per tire observed in the bottom layer of one treated tire pile in Chicago, IL, and the median number of granules counted in a second tire pile (Siegel et al. 1996). The granules were weighed using a Mettler AE 240 electronic balance with a readability of 0.01 mg and were applied by hand to each treated tire.

In this study, the outcome variables were the numbers of larvae and pupae counted in the treated and control tires. Mosquitoes were sampled by scooping tire contents into white enamel pans with plastic cups and counting the larvae and pupae. The contents of the enamel pans were then returned to the tires. The number of culicine larvae were pooled while the anophelines were recorded separately, because we hypothesized that anophelines may be less susceptible to VectoLex. On 6 occasions, pupae were collected, placed in marked cages, and allowed to emerge in the laboratory in order to confirm our larval identification. The tires were not sampled on days 39 and 53 because they were dry.

Study site-catch basins: Catch basins in the cities of Champaign and Urbana in Champaign County, IL, were used in 3 separate experiments. In the 1st, which began on May 28, VectoLex formulated on corncob granules was compared to Altosid® briquets (Zoecon Corp., Dallas, TX). One hundred ninety-two catch basins holding water were used at the start of this experiment. Whenever possible, adjacent catch basins were used in order to eliminate localized effects. In the 2nd experiment, which began in late June and ended in mid-August, 105 catch basins holding water were treated solely with VectoLex corncob granules. This experiment was replicated twice. In the final experiment, which began in mid-August, 20 catch basins holding water were treated with an effervescent tablet of VectoLex (50 *B. sphaericus* ITU/mg).

Catch basin dosage and sampling: In the 1st and 2nd experiments, each VectoLex-treated catch basin received 680 mg (34,000 *B. sphaericus* ITU) of corncob granules. The dosage was determined by assuming an average area of 1,197.5 cm^2 of water per catch basin and a maximum application rate of 22.45 kg/ha. The calculated area is undoubtedly an underestimate, since the surface area of inlet and outlet pipes was not included in these calculations. Initially, 680 mg of VectoLex granules were weighed as previously described and dispensed by hand in the catch basins. In the first experiment, the Altosid-treated catch basins received one briquet, which is the standard dosage used by the Champaign–Urbana Encephalitis Prevention Program. According to the label, a briquet treats up to 36,576 cm^2 water for 30 days, provided that the depth is ≤60.96 cm. In the final experiment, an effervescent VectoLex tablet (1 g, 50,000 *B. sphaericus* ITU) was placed in each catch basin.

Catch basins were sampled by lowering a metal strainer on 9.09-kg-test braided fishing line into the basin and dragging both the surface and the subsurface. The strainer was then removed and examined. This was done 3 times. The presence of a single larva or pupa resulted in a positive designation. The time between treatment and a positive designation was recorded for each catch basin. When a catch basin dried out within the 1st 2 wk after treatment, it was eliminated from the study. If the catch basin dried after >3 wk, the score for the previous week was used. If catch basins were still negative when the experiment ended, the duration of control was considered to have ended at that point.

Larvicide failure: In the first experiment, Altosid was considered to have failed if larvae collected from a catch basin and reared in water obtained from the same basin pupated successfully and adults emerged. VectoLex was considered to have failed if late-instar larvae or pupae were recovered. When a catch basin was scored positive, it received a new treatment of VectoLex (corncob granules or effervescent tablet, depending on the experiment). The catch basin was then considered a new replicate, and the duration of control was calculated from the new treatment date.

Statistical methods: Overall differences in the number of larvae and pupae counted in treated and untreated waste tires were compared using chi-square analysis with a single degree of freedom. Differences in the duration of control between Altosid and VectoLex were determined using an unpaired Student's *t*-test. Differences between the durations of control for the two VectoLex formulations were compared using 1-way analysis of variance (ANOVA). All calculations employed Statview (Abacus Concepts, Berkeley, CA).

Table 1. Culicine larval recovery from sunlit tire dump, May through August 1996, Champaign Co., IL.

Day post-treatment	No. of control larvae	No. of VectoLex larvae	Larval differential
6	1,033	0	−100%
11	1,383	0	−100%
18	607	0	−100%
25	193	0	−100%
32	520	2	−99.6%
39	Dry	Dry	Dry
46	18	59	+327%
53	Dry	Dry	Dry
60	422	34	−91.9%
67	237	7	−97.0%
74	190	54	−71.6%
82	30	15	−50%

RESULTS

Tire studies: In the sunlit tire dump, a total of 4,804 culicine larvae were counted during 10 sample dates. The control tires contained 4,633 larvae, and the treated tires contained 171 larvae, for an overall difference of 96.3% ($\chi^2 = 4,144$, $P \ll 0.001$). No *Culex* species larvae were collected from the treated tires on days 6, 46, 60, 67, 74, and 81 after treatment, Table 1. A total of 388 anopheline larvae were collected in the tires. The control tires contained 218 and the treated tires contained 170, for an overall difference of 22% ($\chi^2 = 5.94$, $0.025 > P > 0.01$).

A total of 107 pupae were in the meadow tires on 8 sample dates. The control tires contained 68 pupae, and the treated tires contained 39 pupae, for a difference of 42.6% ($\chi^2 = 7.86$, $0.01 > P > 0.005$). On days 46 and 60, there were more pupae in the treated tires than in the control tires. Fifty-eight control pupae were collected from the tires and brought to the laboratory. *Culex* species accounted for 84.5% of these pupae, *Ae. triseriatus*

Table 2. Culicine larval recovery from shaded tire dump, May through August 1996, Champaign Co., IL.

Day post-treatment	No. of control larvae	No. of VectoLex larvae	Larval differential
6	795	7	−99.1%
11	896	9	−99.0%
18	763	1	−99.9%
25	954	0	−100%
32	60	23	−61.7%
39	Dry	Dry	Dry
46	66	1	−98.5%
53	Dry	Dry	Dry
60	128	4	−96.9%
67	267	173[1]	−35.2%
74	278	87	−68.7%
82	122	79[1]	−35.2%

[1] Primarily early 2nd-instar larvae recovered.

Table 3. Pupal recovery from shaded tire dump, May through August 1996, Champaign Co., IL.

Day post-treatment	No. of control pupae	No. of VectoLex pupae	Differential
6	166	43	−74.1%
11	149	0	−100%
18	221	10	−95.5%
25	152	0	−100%
32	105	0	−100%
39	Dry	Dry	Dry
46	1	0	−100%
53	Dry	Dry	Dry
60	0	0	0
67	1	0	−100%
74	3	1	−67%
82	7	4	−42.9%

accounted for 12.1%, and *An. punctipennis* accounted for 3.4%. In contrast, 24 pupae were collected from the treated tires and brought back to the laboratory, of which 16.7% were *Culex* species, 70.8% were *Ae. triseriatus,* and 12.5% were *An. punctipennis.*

In the shaded tire dump, a total of 4,713 culicine larvae were counted during 10 sample dates. The control tires contained 4,329 larvae, and the treated tires contained 384 larvae, for an overall difference of 91.1% (χ^2 = 3,302, $P \ll 0.001$). *Culex* species larvae were collected from the treated tires only on days 67 and 74 after treatment (Table 2). A total of 50 anopheline larvae were collected in the tires, and there was no significant difference in their distribution between treated and control tires.

A total of 863 pupae were counted in the woods (Table 3). Control tires contained 805 pupae, and treated tires contained 58, for an overall difference of 92.8% (χ^2 = 647, $P \ll 0.001$). The difference was greatest for the first 67 days after treatment. Seven hundred sixty-three control pupae were collected from the control tires and brought back to the laboratory. *Culex* species accounted for 3.8% of these pupae, *Ae. triseriatus* accounted for 95.2%, and *An. punctipennis* accounted for 1%. In contrast, 58 pupae were collected from the treated tires, of which 96.6% were *Ae. triseriatus* and 3.4% were *An. punctipennis.*

Catch basin study: Failure in these studies is defined as a catch basin being positive for larvae or pupae 1 wk after treatment. There were no Altosid or VectoLex failures. In the 1st trial, the mean VectoLex catch basin time to positive was 46.4 days (SD = 11.2, CV = 24.0, range 28–77 days, n = 28) and the mean Altosid time to positive was 50.5 days (SD = 14.6, CV = 28.9, range 29–71 days, n = 29). This difference was not significant when evaluated using an unpaired Student's t-test (P = 0.25) or the nonparametric equivalent, the Mann–Whitney U-test (P = 0.34). The catch basin attrition rate in this study was high (135/192, 70.3%), primarily due to desiccation. In the 2nd trial, which began in late June, catch basins treated with VectoLex corncob granules had a mean time to positive of 30.8 days (SD = 18.2, CV = 59.2, range 6–70 days, n = 61). There were 10 failures during this period. No catch basin failed 2 times in a row. The catch basin attrition rate was high (54/105, 51.4%) due to desiccation. In the 3rd trial, catch basins treated with VectoLex corncob granules in August also had a mean time to positive of 30.8 days (SD = 9.3, CV = 30.2, range 15–50 days, n = 45). There were no failures. Overall, the duration of control was significantly greater in the 1st trial than in the subsequent trials (1-way ANOVA, F = 13.1, df = 2, 131, P = 0.001).

When effervescent tablets were evaluated in mid-August, the mean catch basin time to positive was 18.5 days (SD = 3.0, CV = 16.4, range 12–21 days, n = 20). There were no failures. The efficacy of this treatment was underestimated, because 10 catch basins were still negative when the study was terminated on September 18. One catch basin was included late in this study and was only followed for 12 days. Ten catch basins were followed until they turned positive, and their mean time to positive was 17.8 days (SD = 3.5, CV = 19.4, range 12–21 days).

DISCUSSION

Based on larval and pupal counts, VectoLex was effective in controlling *Ae. triseriatus, Cx. pipiens,* and *Cx. restuans* in tires. The duration of control was longer in the meadow than in the woods, which may have been an artifact due to our observing 2nd-instar larvae in the woods on days 67 and 82. If these larvae died later in the week, the effect of VectoLex was underestimated. Early-instar larvae were not found at either site on any other sample date. Initially, numerous dead larvae were observed in all treated tires, but dead larvae were not observed in the later stages of the study. VectoLex was ineffective against *An. punctipennis* in tires. This finding agrees with the study of Groves and Meisch (1996), who found *An. punctipennis* to be approximately 15 times less susceptible to *B. sphaericus* strain 2362 than *Cx. quinquefasciatus* and *Cx. restuans*.

There was a noticeable difference in culicine pupal recovery between the 2 tire dumps; 56 pupae were recovered from the sunlit tires, compared to 841 pupae recovered from the shaded tires, during the first 25 days. Larval counts were comparable. This difference in pupal recovery is likely a result of faster development in the meadow because of warmer water temperature. This effect was compounded by our 1-wk sample interval, which undoubtedly missed pupae. Additionally, the sunlit tires dried sooner than the shaded tires, making these tires less attractive as oviposition sites as the season progressed. Both of these factors could explain the 15-fold difference in pupal recovery.

In general, reductions in the number of pupae observed in the treated tires followed the same trend as larval recovery at both sites but were more variable. In the woods, the large number of pupae recovered from the treated tires on day 6 (Table 3) probably resulted from a cohort of late-instar larvae that were exposed to the toxicant after they stopped feeding or were exposed to a concentration of *B. sphaericus* toxin that did not produce mortality. Once the level of toxin presumably increased in the water, almost no pupae were recovered for the remainder of the summer. The pupal data at both sites were most reliable for the first 32 days after treatment. After that date, the low number of pupae recovered, especially from the meadow, distorts the effect of VectoLex.

Evaluating the duration of efficacy of microbial insecticides in tires is complicated by the impact of study design on the experimental outcome. The activity of VectoLex (and *B.t.i.*) depends on the ingestion of a toxin by susceptible mosquito larvae. Activities such as agitating water, thereby resuspending the toxin in the feeding column, can prolong activity. Conversely, under some circumstances, agitating tire contents may accelerate the rate at which toxin is adsorbed to soil particles, degraded, or otherwise removed from the feeding zone, which, in turn, causes the duration of efficacy in the field to be underestimated. In the case of VectoLex, which may recycle in the field, removal of dead larvae could also affect the duration of control. In any sampling scheme, spillage of water and the loss of toxicant are concerns. In order to minimize some of these problems, we chose to scoop the water out of the tires using plastic cups because we felt this method to be less disruptive than siphoning tire contents. In addition, we did not exhaustively remove tire contents in order to minimize resuspension of VectoLex. Although our methods may have missed larvae, these possible sampling flaws were equally distributed among both treated and control tires.

Establishing a realistic dosage for tires was an additional challenge in this study. The label for VectoLex stated that the maximum dosage is 22.45 kg/ha (0.22 mg/cm^2). This dosage for a uniform area must, in turn, be applied to a volume of water that can be as large as 6.9 liters (Siegel et al. 1992) and can vary based on the size of the tire, the angle of the tire on the ground, the evaporation rate, and the rate of replenishment. If we try to simplify calculations by assuming that an average tire has a radius of 19.12 cm and also ignore the fact that tires have holes, the resulting area is 1,149.1 cm^2. The dosage for this area, 253 mg (0.22 mg × 1,149.1), is approximately 169 10–14-mesh granules (Siegel et al. 1996). This dosage, based on simplified calculations, is reasonably close to the actual numbers of granules in tires observed by Siegel et al. (1996). Average numbers of granules per tire in random and shingle-stacked piles ranged from 6 to 269 (9 mg to 404 mg). Consequently, we feel that our dosage of 376 mg VectoLex (250 granules) per tire in this study agrees with both commercial practice and the maximum label rate.

VectoLex was an inexpensive tire treatment (based on a price of $6.39 per kg) that successfully controlled *Culex* species and *Ae. triseriatus*. Using our dosage, 416 tires could be treated for 1 dollar. Since we applied VectoLex by hand, we were 100% efficient in delivering the toxicant to the target, but we realize that this is unlikely to be achieved in an operational setting. Previously, we found that between 16.4 and 19% of the toxicant (10–14-mesh corncob granules sprayed by backpack applicator) is delivered to stacked tires (Siegel et al. 1996). Consequently, 1 dollar's worth of VectoLex may actually treat between 68 and 79 tires. The duration of control in this study exceeded the 33-day control of *Ae. aegypti* in Puerto Rico obtained by Novak et al. (1985) using 500 mg of *B.t.i.* formulated on 4–8-mesh corncob granules. Based on that study, the mosquito control provided by VectoLex was comparable to that provided by temephos (0.09 mg active ingredient on 3 corncob granules).

In the first catch basin study, there was no discernible difference between VectoLex and Altosid. Both gave excellent control (46 and 50 days, respectively). It is likely that we underestimated the duration of control of Altosid. Altosid may have broken down in our cups in the laboratory, or when we collected larvae we may have collected water from a portion of the catch basin that had a low concentration of Altosid. The only definitive assessment would have been collection of pupae, but none were observed in this study. It is noteworthy that Altosid provided larval control for a length of time almost double the time specified on the label and may in fact have lasted even longer. Our average duration of control of 7 wk is in agreement with the study of McCarry (1994), who obtained season-long control with a single treatment of Altosid pellets (7 g per catch basin).

Head-to-head comparisons between VectoLex and Altosid were complicated by the fact that we used different dosages. The Altosid dosage in a single briquet was 30.5× the VectoLex dosage (a maximum area of 36,576 cm^2 vs. 1,197 cm^2). It is possible that if Altosid granules were used at a dosage comparable to that of VectoLex, the duration of activity between the 2 larvicides would have differed. Altosid cost considerably more per catch basin (90.75 cents compared to 0.44 cents, a 206.3-fold × difference), but if Altosid granules were used at a lower dosage, the cost differential would decrease. The increased cost associated with Altosid briquets must also be balanced against the convenience for the applicator of applying a briquet instead of scooping granules.

In our 2nd trial of VectoLex, there were 10 failures; no failures occurred in the 3rd trial. This failure rate was disturbing, because it meant that 19.6% (10/51) of our initial treatments were ineffective. The most plausible explanation is that failures resulted from heavy rains during the end of June and early July. From that point on, rainfall was sparse. We did not reapply VectoLex after rainfalls, and it is possible that localized flooding combined with human activity (car washing, lawn watering) may have flushed VectoLex granules from some catch basins. There were no heavy rains from mid-July through August, and there were no failures during this period.

During these trials, the duration of control decreased to 30 days. This decrease may have resulted from increased oviposition in catch basins during July and August, as alternative oviposition sites desiccated. Nonetheless, 30-day control is comparable to the duration specified on the label for Altosid. Unfortunately, we did not have Altosid controls during this period, so we do not know whether Altosid would have shown a similar decrease in duration of control.

We received the effervescent VectoLex tablets at the end of our field season and were able to treat only 20 catch basins. The duration of control of the tablet was appreciably shorter than that obtained from the corncob granules, despite the fact that the catch basins received a higher dose of VectoLex when the tablets were used. This decrease may be a result of the effervescent formulation liberating toxin at a different rate than the corncob granules and, consequently, the toxin degrading at a faster rate. It is also possible that a greater proportion of the corncob granules stay in the catch basin, making this treatment more effective. Our results must be interpreted cautiously and, at best, may underscore the need for further studies to come up with an optimal formulation for VectoLex for use in catch basins.

There is anecdotal information that VectoLex recycles in the field, and data presented in 1996 at the 62nd Annual Meeting of the American Mosquito Control Association in Norfolk, VA, indicated that VectoLex was particularly effective when *Culex* larval populations were dense. When the data for the tires are examined, there appears to be diminished larval control on day 46 in the meadow and on day 32 in the woods, followed by increased control for the next 2 sample periods. Further experiments must be conducted in order to determine if recycling is in fact occurring. We were unable to evaluate whether VectoLex was more effective in catch basins with high larval densities, because we treated before larvae were present. We note that during this study, *Cx. restuans* populations remained high and *Cx. pipiens* egg rafts did not constitute >50% of the egg rafts recovered in oviposition buckets until the 1st week of September.

In conclusion, VectoLex was an effective larvicide in waste tires and catch basins for *Ae. triseriatus, Cx. pipiens,* and *Cx. restuans.* In waste tires, the duration of control was superior to published values for *B.t.i.* and appeared closer to values reported for temephos. In catch basins, the duration of VectoLex control was comparable to that of Altosid, while the cost of VectoLex treatment was substantially lower per catch basin. In our study, VectoLex formulated on corncob granules gave control superior to that of the tablet formulation, but the convenience of having a tablet for use in catch basins warrants further study of this formulation.

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