REPELLENCY AND INITIAL TOXICITY OF ABATE® AND DURSBAN® FORMULATIONS TO *AEDES TRISERIATUS* IN OVIPOSITION SITES[1]

THOMAS N. MATHER[2] AND GENE R. DEFOLIART
Department of Entomology, University of Wisconsin, Madison, WI 53706

ABSTRACT. Repellency of chemically-treated ovitraps to ovipositing *Aedes triseriatus* was tested using Abate (temephos) 5G, Dursban (chlorpyrifos) 1G, and Dursban 2E. Using ovitrap grids in three southwestern Wisconsin woodlots, it was found that ovitraps treated with Abate 5G and Dursban 1G at a rate of 1125 ppm did not appear to repel ovipositing mosquitoes. Ovitraps treated with Dursban 2E were repellent to ovipositing *Ae. triseriatus* throughout a 5 week test. Although quantitative data were not obtained in the field tests, a substantial number of adult female mosquitoes were killed, presumably by toxic residue, in the Dursban 1g-treated ovitraps, with fewer being killed in the Abate 5G and Dursban 2E-treated traps. Exposure of caged mosquitoes in the laboratory to Abate 5G or Dursban 1G ovitraps resulted in an average adult mortality of 30.5 and 71%, respectively. Larvicidal activity in all treated ovitraps persisted through the 5 weeks of testing; when tested using mosquito larvae bioassay, 100% mortality was recorded in all treated water samples. Larvicidal activity of the three insecticides in oak tree holes still persisted at the conclusion of the experiment 11 months after application. Potential population reductions of *Ae. triseriatus* obtainable by larvicides as compared to the use of "filler" materials in tree holes are discussed.

Aedes triseriatus (Say), the major vector of La Crosse encephalitis virus, breeds in tree holes, discarded automobile tires and other small water containers. Elimination of such sites is recommended in order to reduce the chances of contact with the vector. Some breeding sites, such as tires and other small man-made containers can simply be removed. Relatively immovable sites such as tree holes can be filled with various materials to prevent the accumulation of water (Scholl and DeFoliart 1979) or treated with an insecticide.

Oldham et al. (1972) and Lewis and Christenson (1975) reported long-lasting residual toxicity with emulsifiable concentrates of temephos and chlorpyrifos to larvae of the western tree hole mosquito, *Aedes sierrensis* (Ludlow). The latter authors reported up to 3¼ and 4¾ years of activity, respectively, from the two insecticides when tested in fabricated tree holes. Except for data on *Bacillus thuringiensis* serotype H-14, which has demonstrated short residual toxicity (De Maio et al. 1981), there is little recent research on the treatment of *Aedes triseriatus* container habitats with insecticides. In the work described here, we tested the repellency and toxicity of oviposition sites treated with formulations of Abate and Dursban to ovipositing *Ae. triseriatus*. Persistence of chemical activity in naturally occurring breeding sites was also monitored through the year. Theoretically, as will be discussed, if there is no repellency to ovipositing mosquitoes, a higher degree of control can be achieved by the use of insecticides in tree holes than can be achieved by plugging or filling the tree holes.

MATERIALS AND METHODS

OVITRAP TREATMENT AND FIELD PLACEMENT. Ovitrap grids consisting of 40 ovitraps (Loor and DeFoliart 1969) were placed well within the confines of each of three similar woodlots in Iowa County, Wisconsin. The woodlots were each larger than 5 ha and were composed mainly of oak trees. Natural tree holes were plentiful, and each site was shown to provide high levels of *Ae. triseriatus* oviposition activity (Mather and DeFoliart 1984). The ovitraps, each containing 200 ml of rain water and attached to 1 m long wooden stakes approximately 0.3 m above the ground, were placed in four equally spaced lines, each line and trap 12 m from the next. Presoaked balsa wood strips (Novak and Peloquin 1981) were secured in each trap by a spring clip to prevent loss of the strip to animal vandalism. The ovitraps, excluding the balsa strips, were allowed to 'age' for 4 wk prior to the beginning of the experiment. One wk prior to initiating monitoring of oviposition activity, 20 traps in each site were treated with Abate®5G, Dursban®1G or Dursban®2E to yield a chemical concentration of 1125 ppm in the ovitrap. Treated ovitraps were arranged in an alternate design, so that every other trap was treated. Traps were visited at weekly intervals for 4 wk posttreatment; each balsa strip was removed, labeled and returned to the laboratory for egg counting. New, presoaked balsa strips were added, the 200 ml

[1] Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI 53706, and by National Institutes of Health Grant AI-07453.

[2] Present Address: Department of Tropical Public Health, Harvard University, 665 Huntington Avenue, Boston, MA 02115.

water level restored, and the position of treated ovitraps changed. Each ovitrap was rotated one position in the grid, so that treated traps occupied the position of untreated traps in weeks 2 and 4. Weekly oviposition activity data were treated as individual replicates. Results were subjected to Chi-square tests for equal proportions to determine if treated ovitraps were repellent. Unpaired Student's *t*-tests using actual egg counts were also employed to determine repellency of the three test chemicals.

LONGEVITY OF CHEMICALS IN OVITRAPS AND TREE HOLES. Following completion of the 4 wk testing period, 100 ml samples of water from each treated ovitrap, and five randomly selected untreated ovitraps were returned to the laboratory in 120 ml capacity plastic specimen cups and tested for chemical activity using an *Ae. triseriatus* bioassay. Fifteen second-third instar *Ae. triseriatus* (Burkholder strain—F_1 generation) larvae were added to each cup. Larvae were observed for mortality 2 and 4 hr after introduction to the test water.

The longevity of chemical activity in tree holes was also tested by mosquito bioassay. Eight basal tree holes were located that appeared to maintain some water level throughout the season. The volume of water in each tree hole was measured at the time of treatment by siphoning the contents into plastic buckets, and then measuring it with a graduated cylinder. Water was immediately returned to the tree hole, and the calculated amount of insecticide needed to achieve a concentration of 1125 ppm was added the next day. The granular insecticides (Abate G and Dursban G) were added to the tree holes by sprinkling the pre-weighed granules into the tree hole. The emulsifiable concentrate (Dursban EC) was added by pipetting the liquid into the tree hole, and then mixing by pipetting tree hole water several times with a turkey baster. All tree holes were treated on 1 July 1982. Water samples were collected from each treated tree hole and one untreated tree hole at monthly intervals through October, and again at the end of May, 1983. These samples were returned to the laboratory for bioassay of chemical activity. Each sample was separated into two subsamples and placed in 120 ml capacity plastic specimen cups. The first subsample of 50 ml was diluted with 50 ml of fresh, double glass-distilled water (1:2 dilution). The second subsample, 100 ml, was tested undiluted. A distilled water control was tested as well. Fifteen second-third instar *Ae. triseriatus* larvae were added to each cup as described above, and mortality observations were made 4 and 24 hr after addition of the bioassay larvae.

LABORATORY EVALUATION OF TREATED OVITRAPS. In the laboratory, 30 bloodfed *Ae. triseriatus* were placed in 0.6 m^2 screened cages with an equal number of males. On the seventh evening following the bloodmeal, an ovitrap was placed in the center of each cage. Ovitraps had been prepared similar to those used in the field, but treated ovitraps contained either 200 ppm of Abate 5G or 200 ppm of Dursban 1G. Mosquitoes were permitted free access to the ovitrap for 12 hr, after which the ovitrap was removed and examined for dead mosquitoes. Dead mosquitoes were also collected from the cage. Eggs laid on the balsa ovistrips were counted in both control and treated ovitraps. Both dead and surviving female *Ae. triseriatus* were dissected to determine if eggs had been laid. The number of mature eggs still held in the ovaries was recorded in each case.

RESULTS

REPELLENCY OF TREATED OVITRAPS. Comparison of *Ae. triseriatus* oviposition in treated and untreated ovitraps was used to detect repellency of treated ovitraps. The number of ovitraps positive for eggs out of the total possible number of traps, and the mean number of eggs (± S.D.) per ovitrap is shown in Table 1. Results from 4 wk of ovitrap observations showed that nearly equal numbers of treated and untreated ovitraps were positive for eggs in both the Abate 5G and Dursban 1G-treated sites. The percent of positive ovitraps (traps with eggs present on the ovistrip) ranged from 95–100% in untreated ovitraps, and 85–100% in treated ovitraps at the Abate 5G site. At the Dursban 1G site the range was 90–100% for both treated and untreated traps. Because nearly equal proportions of positives occurred among treated and untreated ovitraps at the Abate 5G and Dursban 1G sites, Chi-square analysis was not appropriate to test for differences. Therefore, unpaired *t*-tests using actual egg counts from treated and untreated ovitraps were used to determine relative differences between the number of eggs laid. Table 1 shows that p-values from these tests remained above the critical value of $\alpha = 0.05$ for all comparisons between Abate 5G-treated ovitraps and the untreated controls. Although having similar numbers of positive ovitraps and egg counts in treated and untreated ovitraps does not preclude that a chemically-treated ovitrap is not repellent to some degree to ovipositing *Ae. triseriatus*, it provides strong evidence for a lack of repellent effect.

Similar results were observed in the comparison of egg counts from Dursban 1G-treated and untreated ovitraps, except in the August 6 result where the p-value was determined to be

Table 1. Repellency of Abate and Dursban to oviposition by *Aedes triseriatus*.

Date	Type of data	Treatment					
		Abate 5G		Dursban 1G		Dursban 2E	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
July 30	No. pos./total	19/20 (95%)	17/20 (85%)	17/19 (90%)	18/19 (95%)	19/20 (95%)	0/20 (0%)
	Mean no. eggs ± SD	215 ± 177	124 ± 117	175 ± 171	113 ± 64	265 ± 162	0.0 ± 0
	p-value	0.062		0.418		*<0.001*	
Aug. 6	No. pos./total	20/20 (100%)	18/20 (90%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	4/20 (20%)
	Mean no. eggs ± SD	123 ± 111	203 ± 164	131 ± 90	74 ± 103	173 ± 154	0.7 ± 2
	p-value	0.184		*0.014*		*<0.001*	
Aug. 13	No. pos./total	20/20 (100%)	17/20 (85%)	18/19 (95%)	18/20 (90%)	20/20 (100%)	13/20 (65%)
	Mean no. eggs ± SD	130 ± 120	104 ± 89	117 ± 79	87 ± 53	148 ± 115	11 ± 37
	p-value	0.317		0.266		<0.001*	
Aug. 20	No. pos./total	20/20 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	15/20 (75%)
	Mean no. eggs ± SD	178 ± 140	244 ± 126	166 ± 121	101 ± 72	216 ± 133	6.0 ± 9
	p-value	0.052		0.069		*<0.001*	

* p-values indicating significant difference between treated and untreated ovitraps at $\alpha = 0.05$.

0.014. However, there were 100% positive Dursban 1G-treated and untreated ovitraps recorded for that week (Table 1). Also, variability in egg counts from individual treated traps was quite large that week, ranging from only 9 eggs in one treated ovitrap to 485 eggs in another. It is unlikely that the observed statistical difference between egg counts is indicative of a repellent effect during this 1 wk period.

A repellent effect was observed, however, in ovitraps treated with Dursban 2E. The water in the treated traps became milky white upon addition of the concentrated liquid chemicals and smelled oily. Results from the Chi-square analysis confirmed the observed repellent effect of Dursban 2E to ovipositing *Ae. triseriatus*. The Chi-square result was 36.190 for traps checked July 30, 26.667 for August 6, 8.485 for August 13, and 5.714 for August 20. Chi-square values over the critical value of 3.84 ($\alpha = 0.05$) would indicate repellency. Furthermore, comparison of egg counts from treated and untreated ovitraps resulted in p-values of < 0.001 for all 4 weeks (Table 1). However, the repellent effect of the Dursban 2E-treated ovitraps did appear to be diminishing somewhat toward the end of the observation period, as indicated by the increase in number of positive ovitraps on August 13 (13/20; $\chi^2 = 8.485$) and August 20 (15/20; $\chi^2 = 5.714$). Egg counts from the Dursban 2E-treated traps remained low, however, throughout the study. There was only a single *Ae. triseriatus* egg laid in 5 traps checked on August 13, and in 4 traps checked on August 20. On August 20, when the highest number of positive Dursban 2E-treated ovitraps was recorded (15/20), the number of eggs per trap ranged from 0–37, with a mean of only 6.0 eggs. This suggests that although gravid *Ae. triseriatus* females were able to land on the treated oviposition substrate, they were still being repelled from ovitraps 4 weeks following treatment with Dursban 2E.

TOXICITY OF TREATED OVITRAPS TO ADULT MOSQUITOES. All three insecticide treatments produced at least some toxicity to ovipositing females, as evidenced by dead females found floating on the water surface during the experiment and at the bottom of treated ovitraps at the end of the experiment. Dead females were found on the water surface in 26 of 79 ovitraps treated with Dursban 1G, 10 of 80 ovitraps treated with Dursban 2E, and 8 ovitraps treated with Abate 5G. Total numbers found floating were 45, 11 and 9, respectively. None were observed in untreated ovitraps. Most of the dead mosquitoes in the Dursban 1G and Abate 5G ovitraps had deposited most or all of their eggs, while 8 of the 11 females taken from Dursban 2E traps either had retained many

eggs (> 25 eggs), or had deposited them on the water surface as they died. This suggests more rapid mortality for mosquitoes landing in Dursban 2E ovitraps than in those treated with either Abate 5G or Dursban 1G. These direct mortality observations were not quantitative, as many more adult mosquitoes were observed in the bottom of treated ovitraps when water samples were taken at the end of the experiment. However, some exposed mosquitoes probably were able to fly away before death ensued.

Laboratory studies using caged gravid mosquitoes exposed to Dursban and Abate-treated ovitraps showed that a high percentage of female *Ae. triseriatus* were killed, presumably through contact of the treated oviposition surface (Table 2). In two separate trials, 30 and 31% of exposed *Ae. triseriatus* females were killed following 12 hr exposure to ovitraps treated with Abate 5G at a rate of 200 ppm. The mortality rate of gravid mosquitoes exposed to ovitraps treated with 200 ppm of Dursban 1G was 57 and 85% in two trials. Only one male mosquito was killed in one trial with Dursban 1G. Upon dissection, all killed female mosquitoes were shown to have deposited some or all of their eggs. Table 2 shows that although all mosquitoes had obtained a complete bloodmeal 7–8 days prior to exposure to the treated ovitraps, a few in each trial did not contain fully mature eggs. In each case those mosquitoes that had developed mature eggs did lay a majority of their eggs during the 12 hr exposure period. Because of the lack of mortality among males, and females with developing eggs, only mosquitoes that had oviposited some or all of their eggs were considered exposed to treated ovitraps in calculating the direct mortality rate. Statistical comparison of the proportion of mosquitoes killed by the Abate-treated ovitrap vs. the Dursban-treated ovitrap resulted in a p-value of 0.054. The number of mature eggs either laid or retained was similar for mosquitoes exposed to treated and control ovitraps. The number of mosquitoes considered not exposed was also similar in both treatment and control trials.

LONGEVITY OF CHEMICALS. Five weeks following treatment, bioassays for chemical activity in the treated ovitraps showed that the water still retained its ability to kill 100% of bioassay *Ae. triseriatus* larvae. Of the 60 water samples from treated ovitraps, all caused 100% mortality of test larvae, while in the randomly selected untreated water samples, mortality was zero. This indicated that when applied at a rate of 1125 ppm, Abate 5G, Dursban 1G and Dursban 2E all retain lethality for *Ae. triseriatus* larvae for at least 5 wk under field conditions.

Tests designed to assess the longevity of chemical activity in tree holes also showed that Abate 5G, Dursban 1G and Dursban 2E had persistent larval killing activity when applied at an initial rate of 1125 ppm. Water samples taken from three Abate 5G and three Dursban 1G-treated tree holes and two Dursban 2E-treated tree holes were tested, both undiluted and in a 1:2 dilution by larval bioassay. Bioassays conducted at monthly intervals for 3 mo. following treatment, and again following winter, 11 mo. after treatment, indicated persistence of chemical activity in the tree hole water. Water from one untreated control tree hole was also tested at these intervals. None of the 240 larvae used at each of the first three monthly intervals to assay the water from treated tree holes survived. Results were similar in bioassay tests conducted using tree hole water 11 mo. after treatment, with the exception of water from one Abate 5G treated tree hole. All of the bioassay larvae in the undiluted sample from this one tree hole were killed, however, only 2

Table 2. Mortality of gravid *Aedes triseriatus* following 12 hr. exposure to ovitraps treated with Abate 5G and Dursban 1G at a rate of 200 ppm.

		Abate 5G		Dursban 1G	
	Replicate	Control	Treatment	Control	Treatment
No. ♀♀ tested	1	30	30	30	30
	2	30	30	30	30
No. eggs laid	1	759	635	664	783
	2	851	721	844	612
No. eggs held	1	154	214	292	315
	2	176	333	304	175
No. ♀♀ exposed[a]	1	25	26	26	28
	2	26	26	27	28
No. ♀♀ killed (%)[b]	1	4	11 (31%)	0	16 (57%)
	2	3	10 (30%)	2	24 (85%)

[a] Mosquitoes were considered not exposed if eggs were not fully mature at the time of collection.

[b] Percent of mosquitoes killed calculated from the number exposed, and corrected for mortality in the controls.

of 15 bioassay larvae were killed in the diluted sample. Mortality was nearly complete within 4 hr in all tests. No mortality was observed among the 30 larvae used each month to assay the untreated tree hole water.

DISCUSSION

Theoretically, if 80% of the tree holes in a wooded area can be found and plugged between mosquito seasons, thereby eliminating those tree holes at least temporarily as a suitable breeding site for *Ae. triseriatus,* the expected result is an 80% decrease in numbers and of biting and egg laying by the vector population. Oviposition by the remaining 20% of the population is restricted to 20% of the suitable breeding area previously available.

If 80% of the tree holes can be found and treated with a residually acting insecticide that is not repellent to ovipositing females, the treated tree holes will attract 80% of oviposition by the remaining 20% of the vector population thus acting as "trap sites" and reducing oviposition by an additional 80%, or to 96% of that of the original population. Biting is not further reduced, remaining at 80% of the original level. If treated tree holes are not only not repellent to ovipositing females but lethal to them, (as appears to be the case), oviposition, compared to that of the original population is reduced by 97%. There is also a further reduction in biting, amounting to 85% of the original level. These relationships are depicted in Table 3 for a population that numbers 200,000 females emerging over a season from overwintering eggs. Other parameter values upon which Table 2 is based are as described by DeFoliart (1983).

The implications of this additional 16–17% reduction in oviposition and up to 5% in biting, relative to encephalitis prevention, are not known due to uncertainties regarding various aspects of *Ae. triseriatus* biology and La Crosse virus epidemiology. For example, density dependent mortality of larvae is not affected by plugging tree holes as population and suitable breeding habitat are both reduced similarly and simultaneously (by 80% in this example). With the use of insecticide as described, however, the 80% reduction in oviposition in the 20% of sites remaining "safe" might be largely negated by a significant reduction in density-dependent larval mortality. Research is needed on this point.

Our results show that, among the formulations tested, only Dursban 2E was initially repellent and that repellency persisted through at least 5 wk. Both Dursban and Abate treated tree hole water produced 100% mortality of *Ae. triseriatus* larvae for at least 11 months following application and indicate that these insecticides might produce high larval mortality for a period of several years against *Ae. triseriatus,* as shown by Oldham et al. (1972) and Lewis and Christenson (1975) against *Ae. sierrensis.*

The selective use of insecticides warrants further study relative to possible use in conjunction with elimination of removable breeding sites in La Crosse encephalitis prevention programs. Biting by the vector cannot be reduced much further than that shown in Table 3 unless a greater percentage of the original breeding sites in an area can be found and treated. Oviposition can be reduced further,

Table 3. Theoretical results of different strategies used to reduce an *Ae. triseriatus* population.

Life events[a]	Number of females surviving[b]			
	A	B	C	D
No. females emerging	200,000	40,000	40,000	40,000
No. surviving BLM #1	114,580	22,916	22,916	22,916
No. surviving to OVP #1	37,600	7,520	7,520	7,520
No. OVPs in "safe" sites	37,600	7,520	1,504	1,504
No. surviving BLM #2	37,600	7,520	7,520	1,504
No. surviving to OVP #2	10,740	2,148	2,148	429
No. OVPs in safe sites	10,740	2,148	430	86
No. surviving BLM #3	10,740	2,148	2,148	86
No. surviving to OVP #3	3,060	612	612	25
No. OVPs in safe sites	3,060	612	122	5
Total BLMs[c]	162,920	32,584 (80%)	32,584 (80%)	24,506 (85%)
Total safe OVPs[c]	51,400	10,280 (80%)	2,056 (96%)	1,595 (97%)

[a] BLM equals bloodmeal; OVP equals oviposition.

[b] At 0.87 daily life expectancy (Sinsko and Craig 1979); A, in uncontrolled population; B, when 80% of breeding sites are filled with non-toxic "filler" materials; C, when 80% of breeding sites are treated with larvicide, but females attracted for oviposition are not killed; D, same as C except that attracted females are killed.

[c] Percentages in parentheses represent percent reduction compared to the original uncontrolled population.

however, by the addition of a quantity of treated ovitraps, thus increasing the ratio of treated to safe oviposition sites.

Little is known about the utilization of tree hole water by wildlife. Abate has extremely low mammalian toxicity, but whether the presence of insecticides such as Dursban might have detrimental effects in a given situation should be considered. As *Ae. triseriatus* has essentially only one generation per year in the northern states, genetic resistance would probably not become a problem unless insecticide treatments were extended over many years.

ACKNOWLEDGMENTS

The authors appreciated the cooperation of S. Jones, O. Richardson, J. Rowe, and T. Haig for permitting use of their woodlots in this study. We also thank T. Hartberg of American Cyanamid Co., S. Ditmarsen of Dow Chemical Co., and K. Magrow of Clarke Outdoor Spraying Co. for providing insecticides.

References Cited

DeFoliart, G. R. 1983. *Aedes triseriatus:* Vector biology in relationship to the persistence of LaCrosse virus in endemic foci. *In* C. H. Calisher and W. H. Thompson, eds. California serogroup viruses. A. R. Liss, Inc., New York, NY.

DeMaio, J. D., J. C. Beier and S. L. Durso. 1981. Larvicidal activity of *Bacillus thuringiensis* var. *israelensis* against *Aedes triseriatus* in tree hole and tire habitats. Mosq. News 41:765–769.

Lewis, L. F. and D. M. Christenson. 1975. Residual activity of temephos, chlorpyrifos, DDT, Fenthion, and malathion against *Aedes sierrensis* (Ludlow) in fabricated tree holes. Mosq. News 35:381–384.

Loor, K. A. and G. R. DeFoliart. 1969. An oviposition trap for detecting the presence of *Aedes triseriatus* (Say). Mosq. News 29:487–488.

Mather, T. N. and G. R. DeFoliart. 1984. Dispersal of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. J. Med. Entomol. (In press).

Novak, R. J. and J. J. Peloquin. 1981. A substrate modification for the oviposition trap used for detecting the presence of *Aedes triseriatus*. Mosq. News 41:180–181.

Oldham, M., E. E. Lusk and D. J. Womeldorf. 1972. Residual activity of various insecticides in tree holes. Proc. Calif. Mosq. Control Assoc. 40:69–71.

Scholl, P. J. and G. R. DeFoliart. 1979. Pipe insulating cement for closing tree hole breeding sites of *Aedes triseriatus*. Mosq. News 39:149.

Sinsko, M. J. and G. B. Craig, Jr. 1979. Dynamics of an isolated population of *Aedes triseriatus* (Diptera: Culicidae). I. Population size. J. Med. Entomol. 15:89–98.