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CONTROL OF *AEDES ALBOPICTUS* LARVAE USING TIME-RELEASE LARVICIDE FORMULATIONS IN LOUISIANA

ROGER S. NASCI,[1,2] GERALDINE B. WRIGHT[2] AND F. SCOTT WILLIS[3]

ABSTRACT. The ability of time-release formulations of larvicides and insect growth regulators (IGRs) to provide long-term control of *Aedes albopictus* was investigated in the field. Larvicides used in the study were Bactimos® pellets (*Bacillus thuringiensis* var. *israelensis,* active ingredient) and Abate® pellets (temephos, active ingredient). The IGR Altosid® (methoprene, active ingredient) was used in pellet and sand formulations. Application rates were higher than label recommendations. In a preliminary test, clay flower bowls were treated with 2 g of material. Bactimos pellets failed to provide control after 60 days. Abate pellets and the Altosid formulations provided essentially 100% control for 150 days. After 360 days in the field, the Abate pellets produced 100% larval mortality, and significant levels of control were provided by the Altosid formulations and the Bactimos pellets. In a small-scale operational trial of this technique, 1 g of Altosid pellets was applied to every container that could be located in 2 urban residential neighborhoods in Lake Charles, LA. *Aedes albopictus* biting populations were monitored weekly in the treated areas and in an untreated control area. Biting population densities declined significantly in treated areas compared with the control area. Results suggested that long-term control of *Ae. albopictus* populations can be achieved economically with one application of Altosid pellets or Abate pellets in containers.

INTRODUCTION

Following its introduction into the continental United States (Sprenger and Wuithiranyagool 1986), *Aedes albopictus* (Skuse) quickly spread throughout the southeastern portion of the country (Moore et al. 1988, Francy et al. 1990). *Aedes albopictus* was first discovered in Lake Charles (Calcasieu Parish), LA, in the fall of 1987 (R. S. Nasci, unpublished data). During that year, only one service request made to the Calcasieu Parish mosquito control program was attributed to this species. In subsequent years, *Ae. albopictus* became established in urban and suburban areas throughout the parish and became a major pest species. The number of service requests attributed to *Ae. albopictus* increased to 98 in 1991, and represented approximately 70% of the service requests from parish urban areas.

Efforts to control *Ae. albopictus* have been largely unsuccessful. In a Florida tire dump, combinations of larviciding and adulticiding had to be repeated every 3 weeks to achieve adequate control (Peacock et al. 1988). Source reduction, either by eliminating tire dumps or by storing tires in a manner insuring that they do not collect water, is recommended as the best solution for controlling this species in tire dumps (Moore et al. 1988, Peacock et al. 1988).

Though tire dumps are capable of producing large populations of this species, the primary sources of *Ae. albopictus* in urban residential areas of Lake Charles are not tires. During a 1988 survey of 86 residences in the city, 57% of the residences had containers producing *Ae. albopictus* and the average residence had 14 containers. Only 13% of the containers were classified as disposable trash (i.e., tires, cans, etc.). The remainder were a variety of nontrash items such as flower pots, buckets, and other domestic containers (F. S. Willis, unpublished data).

Source reduction through community education was attempted in Lake Charles but was unsuccessful. Residents were willing to dispose of trash in their yards, but neglected or quickly forgot to empty permanent containers such as buckets and flower pots (L. G. Terracina, personal communication). As a result, numerous sources of *Ae. albopictus* remained in the urban residential areas. The mosquito control program did not have the personnel to empty the water from each container in the most affected neighborhoods every week. An alternative was to develop a larvicide-based control program for controlling *Ae. albopictus* in heavily infested neighborhoods. Time-release formulations of temephos have been used for successful long-term control of *Aedes aegypti* (Linn.) and *Aedes triseriatus* (Say) in tires (Novak et al. 1985, Beehler et al. 1991). The goals of this study were to determine if a single application of time-release insecticide formulations could provide season-long control of *Ae. albopictus* larvae in domestic containers and to determine if this technique could be applied on an operational scale.

[1] Present address: Medical Entomology–Ecology Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Disease, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, P. O. Box 2087, Fort Collins, CO 80522.

[2] Department of Biological and Environmental Sciences, McNeese State University, Lake Charles, LA 70609.

[3] Calcasieu Parish Mosquito Control, 1925 Merganser, Bldg. 6 Chennault, Lake Charles, LA 70601.

METHODS

Four time-release formulations were examined for their ability to provide long-term control of *Ae. albopictus* in containers. These included 2 larvicides, Bactimos®[4] pellets (5% *Bacillus thuringiensis* var. *israelensis,* 7000 ITU *B.t.i.* serotype H-14/mg) and Abate®[4] pellets (5% temephos), and the insect growth regulator (IGR) Altosid®[4] in pellet (4% methoprene) and sand formulations (1.3% methoprene). At application rates of 11.2 kg/ha (10 pounds per acre), package labels recommend reapplication every 7–14 days for Bactimos pellets and every 30 days for Abate and Altosid pellets. The Altosid sand formulation was an experimental product and did not have recommendations for application rate and reapplication timing at the time of these experiments.

Field assay: The ability of the 4 formulations to provide long-term control in the field was initially determined in 2 field sites. One site was located adjacent to the McNeese State University Department of Biological and Environmental Sciences greenhouse, the other was in the back yard of a suburban residence. *Aedes albopictus* had been collected in both areas before the experiment. Fifteen 22 × 5-cm clay bowls were placed in each field site. The bowls were filled with water and 0.2 g liver powder, and left undisturbed for 2 weeks before the studies began. On day 0, June 11, each bowl was examined for the presence of larvae and pupae. Pupae were removed from the bowls, placed in emergence cages, and taken to the laboratory until adult emergence to determine pretreatment mortality. Two grams of either Bactimos pellets Abate pellets, Altosid pellets, or Altosid sand were added to each of the test bowls. Each treatment was applied to 3 bowls. No insecticide was added to the 3 control bowls. The 2 g dosage per 22-cm-diam bowl was approximately 48 times the dosage that would be applied at the 11.2 kg/ha label rate. Preliminary laboratory experiments indicated that label rates provided control for 30 days and that the higher rate was necessary to provide season-long control. Water was added to the bowls daily throughout the duration of the study to keep the bowls flooded and to insure that eggs that were oviposited in the bowls received a regular hatching stimulus.

Twice a week for 150 days each bowl was checked for the presence of larvae and pupae. In the bowls containing larvicides, data were recorded as 0 if none of the bowls in a treatment contained larvae, 1 if one bowl contained larvae, 2 if 2 bowls contained larvae, and 3 if all 3 bowls in the treatment group contained larvae. In the IGR-treated bowls, all pupae in each bowl were taken to the laboratory and held until adult emergence or death. Average pupal mortality for the 3 bowls was calculated for each IGR treatment at each site during each sampling period.

After the 150-day experiment, the bowls were left in the field throughout the winter (210 additional days). The bowls were then brought into the laboratory and 500 ml of deionized water and 0.2 g liver powder were added to each. The larvicide and IGR activity in each was tested by placing 10 6-day-old larvae in each of the bowls. Pupae were removed daily and held until adults emerged. Larval and pupal mortalities were calculated.

Operational field trial: Because the field assay indicated that long-term control of immature *Ae. albopictus* could be obtained with Abate pellets and with both of the Altosid formulations, an operational field trial was conducted to determine if enough containers in a residential area could be located and treated to have an effect on *Ae. albopictus* biting populations in the area. Altosid pellets were chosen for the trial over Altosid sand, Abate pellets, and Bactimos pellets. Altosid is essentially nontoxic to mammals. Though Abate pellets provided excellent control in the preliminary tests, and temephos is safe to use in drinking water (Laws et al. 1968), we felt it was unwise to dispense large dosages of Abate pellets into small containers in domestic habitats. Bactimos pellets did not produce the desired duration of control in the preliminary tests.

Three urban residential areas were selected in north-central Lake Charles. Each area was 3 city blocks square, covered *ca.* 26 ha, and contained approximately 80 residences. The 3 areas were adjacent to each other. From June 6 to 10, all of the residences in the 2 treatment areas were inspected and treated. Every container that could be found at each residence was treated with approximately 1 g of Altosid pellets (applied with a ½ teaspoon measuring spoon). The application rate was reduced to 1 g per container because laboratory experiments indicated that the duration of control did not change at doses above 1 g (G. Wright, unpublished data). The application rate was not adjusted for containers of different sizes. Containers in the 3rd area were left untreated which served as a control.

We measured *Ae. albopictus* population densities in the 3 areas by collecting mosquitoes attracted to human bait. Four sampling stations were established in each area. Starting on June

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[4] Use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

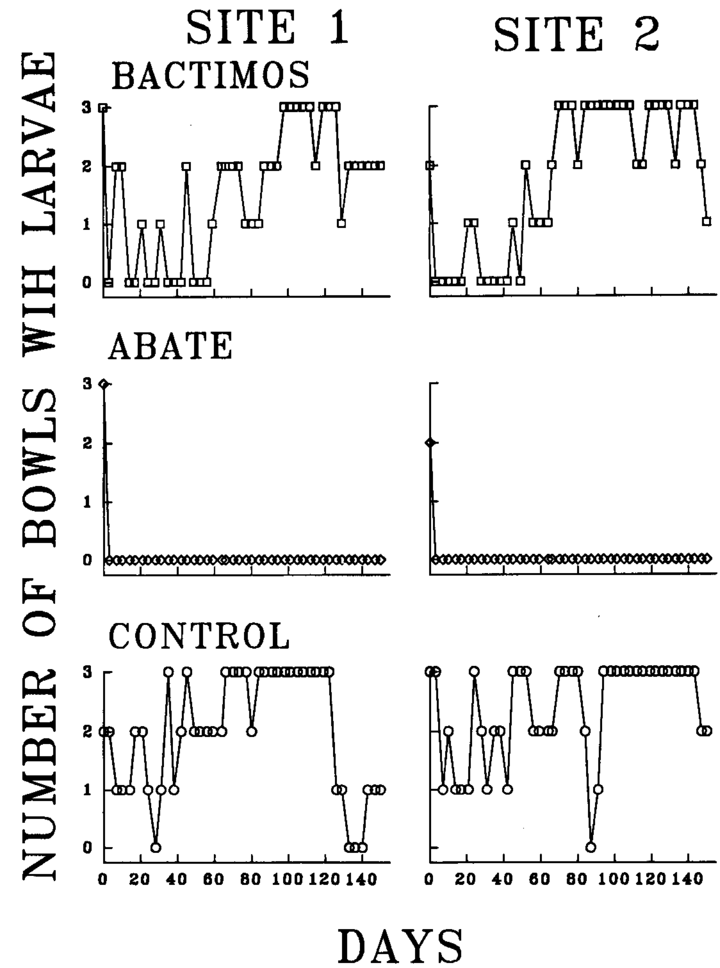

Fig. 1. Number of bowls containing *Aedes albopictus* larvae, out of a possible 3 bowls sampled twice a week for 150 days in 2 field sites. Bowls were treated with either Bactimos pellets, Abate pellets, or were untreated controls.

10, once each week at each of the sampling stations, a battery-powered aspirator was used to collect mosquitoes attempting to land on the host during a 15-min period. Collection effort was kept constant at each site throughout the 15-wk study. Specimens were identified and counted, and the total number of *Ae. albopictus* females collected in each area each week was recorded.

RESULTS AND DISCUSSION

Field assay: Bactimos pellets provided control for only about 60 days (Fig. 1). During the first 60 days, the proportion of Bactimos-treated bowls that contained larvae was significantly less than the proportion of control bowls that contained larvae during the same period in both sites (Site 1: Bactimos = 12/54, control = 30/54, $\chi^2 = 12.6$, $P < 0.001$; Site 2: Bactimos 9/54, control = 36/54, $\chi^2 = 27.7$, $P < 0.001$). During the last 90 days of the experiment, there was no difference in the proportion of bowls that contained larvae in either site (Site 1: Bactimos = 56/78, control = 57/78, $\chi^2 = 0.03$, $P > 0.05$; Site 2: Bactimos 68/78, control = 68/78, $\chi^2 = 0$, $P > 0.05$).

Abate pellets provided complete control for the entire 180-day period (Fig. 1). Larvae were never found in the Abate-treated bowls. Either larvae that hatched were quickly killed, or females never oviposited in the Abate-treated bowls.

Average pupal mortality in bowls containing either Altosid formulation was almost always

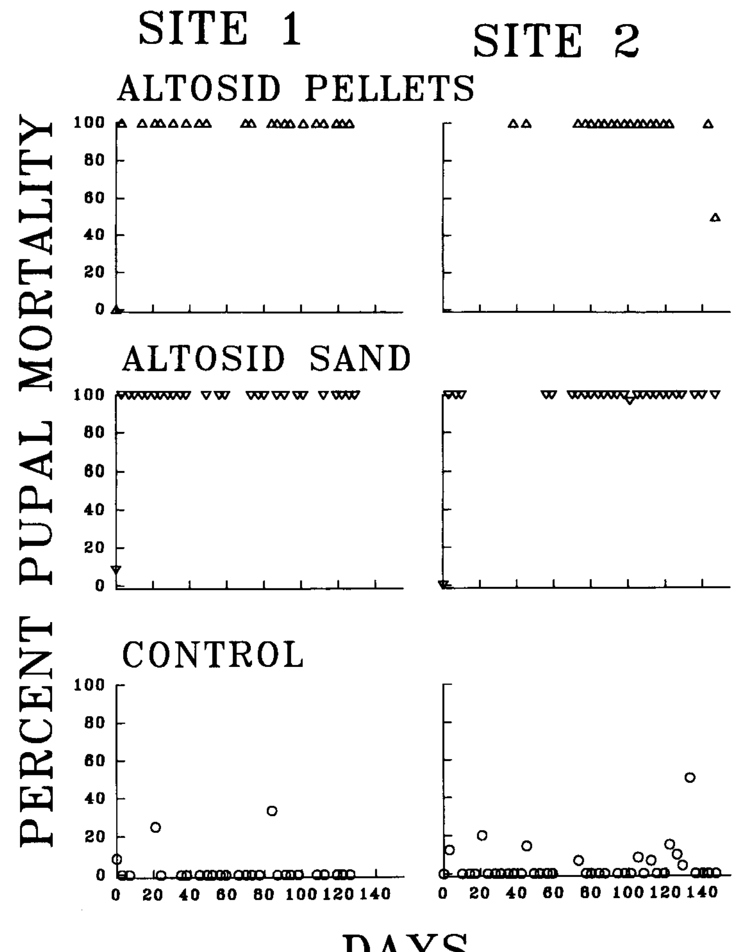

Fig. 2. Average percent *Aedes albopictus* pupal mortality in bowls treated with Altosid pellets or Altosid sand, or in untreated control bowls placed in the field and sampled twice a week for 150 days. Sample size for each sampling date = 3 bowls.

Table 1. Average percentage mortality in *Aedes albopictus* larvae placed in 6 insecticide-treated bowls left in the field for 360 days.

Treatment	Average % mortality[1]	
	Larvicides	IGRs
Bactimos pellets	43	—
Abate pellets	100	—
Altosid pellets	—	73
Altosid sand	—	77
Control	12	2

[1] Average percent mortality in the control bowls was significantly lower than in the larvicide of IGR-treated bowls (ANOVA, $P < 0.05$). Proportions were transformed (arcsine $\sqrt{x}$) prior to testing.

100% throughout the entire 150-day test period (Fig. 2). At site 2 on the last sampling day, one of 2 pupae collected from one bowl survived, accounting for the 50% mortality on that date. Pupal mortality in the control bowls rarely exceeded 20% at either site and was usually 0%.

In laboratory bioassays of the treatments after 360 days in the field, all of the formulations displayed significantly greater activity than the controls (Table 1). Bacterial spores in the Bactimos-treated bowls were probably resuspended when the water, liver powder, and larvae were added, accounting for the 43% average larval mortality. Abate pellets produced 100% larval mortality, and the Altosid formulations still produced greater than 70% pupal mortality. These results

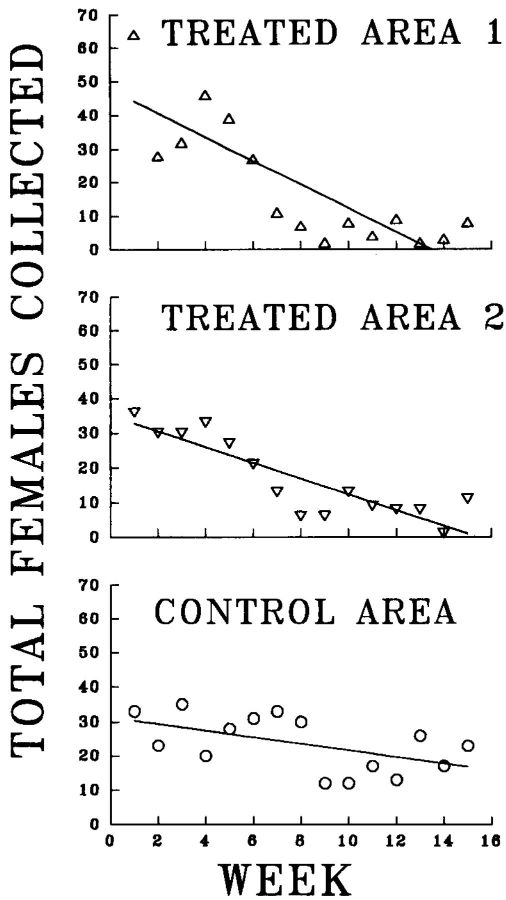

Fig. 3. Total female *Aedes albopictus* collected at human bait during 4 15-min collections each week in the 2 areas treated with Altosid pellets and in the untreated control area. Lines indicate the calculated regression of total number collected over week. Regression formulae: Treated area 1, 47.7 − 3.54 * week, r^2 = 0.69, P = 0.001; Treated area 2, 34.9 − 2.27 * week, r^2 = 0.76, P = 0.0001; Control area, 31.3 − 0.97 * week, r^2 = 0.28, P = 0.04.

indicated that the Abate pellet, Altosid pellet, and Altosid sand formulations were able to provide excellent control of *Ae. albopictus* for long periods when applied at 2 g per container to small containers in the field.

Operational field trial: The total numbers of female *Ae. albopictus* collected during each weekly 1-h sampling period in the 2 treated areas and in the control area are shown in Fig. 3. Because application of Altosid pellets to the containers did not occur until the first week in June, adult *Ae. albopictus* were already present in the areas. Therefore, successful control of the population would be indicated by a decrease in the biting population as the existing adults died and production of new adults from containers in the area was reduced. A gradual decline in biting density occurred in the 2 treated areas; the number collected in the control area was relatively unchanged. After week 8, the total number collected in the treated areas generally stayed below 10 per hour, while the number collected in the control area never went below 10 per hour.

A linear regression equation representing the relationship of the total number collected per week and the time (week) after treatment was calculated for each of the areas (Sokal and Rohlf 1969). The 2 treatment area slopes were statistically the same (t-test for equality of slopes, $P > 0.05$), and were statistically greater than the control area slope (t-test for equality of slopes, $P < 0.01$), indicating a greater rate of population reduction in the treated areas (Fig. 3). The P values indicated that the slopes in the treated areas were highly different from 0; the slope in the control area was only moderately different from 0. In addition, the treatment area regression equations explained 69% or more of the variation in population densities (r^2 values 0.69 and 0.76 in treatment areas 1 and 2, respectively); only 28% of the variation (r^2 = 0.28) was explained in the control area.

The difference in regression equations demonstrated that the biting population in the treated areas decreased at a faster rate than in the control area. Apparently, the treatment had a measurable effect on the *Ae. albopictus* populations in these areas. However, the presence of biting female *Ae. albopictus* in the treated areas at the end of the experimental period indicated that control was not as effective as we had initially anticipated from the preliminary field experiments. The presence of biting females late in the experiment was probably due to a combination of several factors. Though care was taken to treat every container in the treated areas, some containers may have been missed, such as tree holes that were treated only if at ground level. The treatment may have failed in certain types of containers. Containers may have been emptied, resulting in removal of the pellets, and containers may have been added through the course of the season. Some of the females may have dispersed into the area from outside the treated neighborhood, as the treatment areas were relatively small. Adult *Ae. albopictus* have been reported to disperse up to 200 m (Mori 1979).

Results of the operational field trial show that enough containers in a neighborhood can be located and treated with Altosid pellets to significantly reduce the biting *Ae. albopictus* population. Similar results should be produced with Abate pellets, as the field assay indicated that this material gave season-long control at the increased application rates. Treatment of a larger area earlier in the season, before adult emergence, may result in greater population reductions than were observed in this experiment.

Though this control technique is labor intensive, the ability to obtain season-long control with one application makes it a cost-effective technique. Using locally determined values of 14 containers per house, 15 min to treat all the containers at a residence, labor costs of $10.00 per hour, and material costs of $20.00 per pound ($0.63 per 1-g dose), we estimate it costs approximately $3.13 to treat the average house in urban residential areas of Lake Charles, LA. Despite its economy, this control method is not likely to be used in a city-wide *Ae. albopictus* control program. Rather, it may be successfully used in moderately sized, heavily infested residential neighborhoods.

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