Journal of the American Mosquito Control Association, 12(3):499–502, 1996

EFFECTS OF THREE LARVICIDES ON THE PRODUCTION OF *AEDES ALBOPICTUS* BASED ON REMOVAL OF PUPAL EXUVIAE

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ABSTRACT. The production of adult *Aedes albopictus* from tires in northcentral Florida was monitored for 169 days by the daily removal of pupal exuviae. More than twice as many adults emerged from tires located in the shade (1.74 adults/tire/day) compared to tires in the sun (0.64 adults/tire/day). The effect of 3 larvicides on the production of adult *Ae. albopictus* was evaluated. The fungal pathogen *Lagenidium giganteum* was ineffective. A liquid formulation of *Bacillus thuringiensis israelensis* (Acrobe®) provided significant control for 47 days, whereas a slow-release pellet formulation of the insect growth regulator methoprene (Altosid®) provided almost complete control for 116 days.

Control of container-inhabiting mosquitoes such as *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) has been attempted with various chemical and biological agents (Hawley 1988). The effectiveness of larvicide-based control programs is often measured by monitoring larval and pupal mortality, monitoring biting populations of adult mosquitoes (Nasci et al. 1994), or using emergence traps (Service 1976). A less frequently used method is to determine daily adult production from containers by the removal of pupae (Focks et al. 1981) or pupal exuviae (Focks et al. 1982). The objectives of this study were to determine the production of adults for a field population of *Ae. albopictus* from tire habitats by counts of pupal exuviae and to examine the utility and sensitivity of this method to measure the effectiveness of various control agents.

A study site was established at the USDA, Medical and Veterinary Entomology Research Laboratory, Gainesville, FL, in May 1992. Golf cart tires (40 cm diam × 20 cm wide) were placed in a grassy area southwest of the facility on May 11, 1992. Forty tires were located in an area shaded by trees and shade cloth with the tires oriented north and south. Forty tires were placed in the same area but exposed to full sun and oriented east and west. Half of these tires were used for sampling the density of immature mosquitoes; the other half were used to evaluate adult production. Pine, oak, and sweetgum leaves were collected from a nearby forest floor, autoclaved, and soaked in well water in 38-liter containers for 24 h. Approximately 38 g of this leaf matter was placed into each tire. Tires were initially filled to capacity with well water (3.5 liters) and water was added as required to keep containers from drying out.

Water temperature in the tires was monitored and recorded from June to December every 5 min and averaged each hour using a Campbell Scientific, Inc., CR10 control module and 105T thermocouple probe.

Absolute numbers of immature mosquitoes were determined weekly for 25 wk. Twenty control tires each from the sun and shade groups were assigned numbers. One tire from each group was randomly selected for weekly sampling. These tires were taken to the laboratory and the contents were removed and larvae and pupae counted. Fourth-instar larvae were identified to species, and tires and contents were then returned to the field.

Daily adult production per tire was determined for the period June–November 1992. *Aedes albopictus* exuviae were collected from randomly selected tires (4 in the sun and 4 in the shade) daily for 169 consecutive days. A loop with handle was fabricated from plexiglass; a white, fine mesh cloth was attached to the loop. Exuviae were located and removed by silhouetting the cast skins with a flashlight against the white mesh. *Aedes albopictus* exuviae were distinguished from *Culex quinquefasciatus* Say exuviae by microscopically examining the shape of the posterior segments.

Three agents with different modes of action were evaluated: the fungal pathogen *Lagenidium giganteum,* the liquid formulation of *Bacillus thuringiensis israelensis* (*B.t.i.*) (Acrobe®), and the slow-release pellet formulation of the insect growth regulator methoprene (Altosid®). A group of 8 tires was randomly selected for each treatment group. Pretreatment emergence data were recorded for these tires until treatment,

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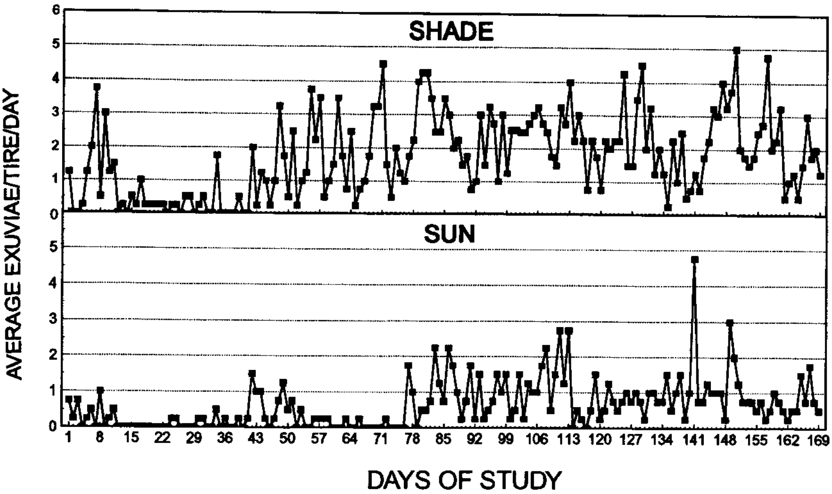

Fig. 1. Daily average number of *Aedes albopictus* exuviae produced per tire per day from tires located in either the shade or sun for 169 days (4 tires/group, June–November, 1992).

when 4 tires were randomly selected as control tires and 4 tires randomly selected for treatment.

Cultures of *L. giganteum* (California strain) were obtained from James Kerwin, University of Washington, Seattle, WA. Moribund *Ae. albopictus* larvae (8) infected with *L. giganteum* were added to each of 4 tires in the shade on July 24, 1992. Laboratory exposures of the same dose and volume were made using 50 2nd- and 50 4th-instar *Ae. albopictus* larvae in both filtered tire water (pH = 7.78; conductivity = 1,843 μmho/cm) and distilled water. Acrobe (1,200 ITUs per mg) was added to each of 4 other tires to give a final concentration of approximately 100 ppm. Altosid (4% methoprene) was applied to another 4 randomly chosen tires at the minimum recommended rate of one pellet (approx. 79 mg) per tire.

The average minimum temperatures for tires in the sun and shade did not differ significantly (17.8 ± 1.3°C and 18.1 ± 1.2°C, respectively, one-way ANOVA, $P > 0.05$); the average maximum temperature for tires in the sun was significantly higher than that of the shade tires (31.4 ± 0.7°C and 28.3 ± 0.8°C, respectively, one-way ANOVA, $P < 0.01$).

Larval and pupal densities were highly variable for both tires in the sun and the shade over the 4-month sampling period. The average number of larvae (all instars) from tires in the sun was 203 ± 45.5 and was not significantly different (one-way ANOVA, F = 0.1, $P > 0.05$) from that of tires in the shade (220 ± 43). Species composition, based on 4th-instar larval samples, revealed that *Ae. albopictus* and *Cx. quinquefasciatus* accounted for >95% of the mosquitoes present. *Culex quinquefasciatus* was the first species to invade tires in both the sun and the shade but after approximately 2 months, *Ae. albopictus* became the dominant species. This was especially true in the shade where on average, 83% of the 4th-instars were *Ae. albopictus* and 17% were *Cx. quinquefasciatus,* whereas in the sun 68% were *Ae. albopictus* and 32% were *Cx. quinquefasciatus.*

There was a significant difference (one-way ANOVA, F = 10.2, $P < 0.001$) between the average number of pupae from tires in the sun (5.8 ± 1.3) and the average number of pupae from tires in the shade (12.8 ± 1.8). Because larval numbers were not significantly different for these 2 groups (203 larvae/tire in the sun vs. 220 larvae/tire in the shade), fewer larvae successfully pupated in tires from the sun as compared to those in the shade.

Few *Cx. quinquefasciatus* adults emerged during the study (0.01 ± 0.006 adults/tire/day) and will not be considered further. A daily average of *Ae. albopictus* exuviae produced per tire per day is given in Fig. 1. Significantly more adult *Ae. albopictus* emerged (one-way ANOVA, F = 99.5, $P < 0.001$) from tires in the shade (1.74 ± 0.10 adults/tire/day) than from tires in the sun (0.64 ± 0.05 adults/tire/day). There was clearly a preference for the shaded sites by *Ae. albopictus,* particularly during the early stages of establishment in this area.

Due to the low adult production from tires in the sun, evaluation of the control agents was conducted only for tires located in the shade. The pretreatment average daily production of adults from the control tires and the tires selected for *L. giganteum* treatment were not significantly different from treated tires (control =

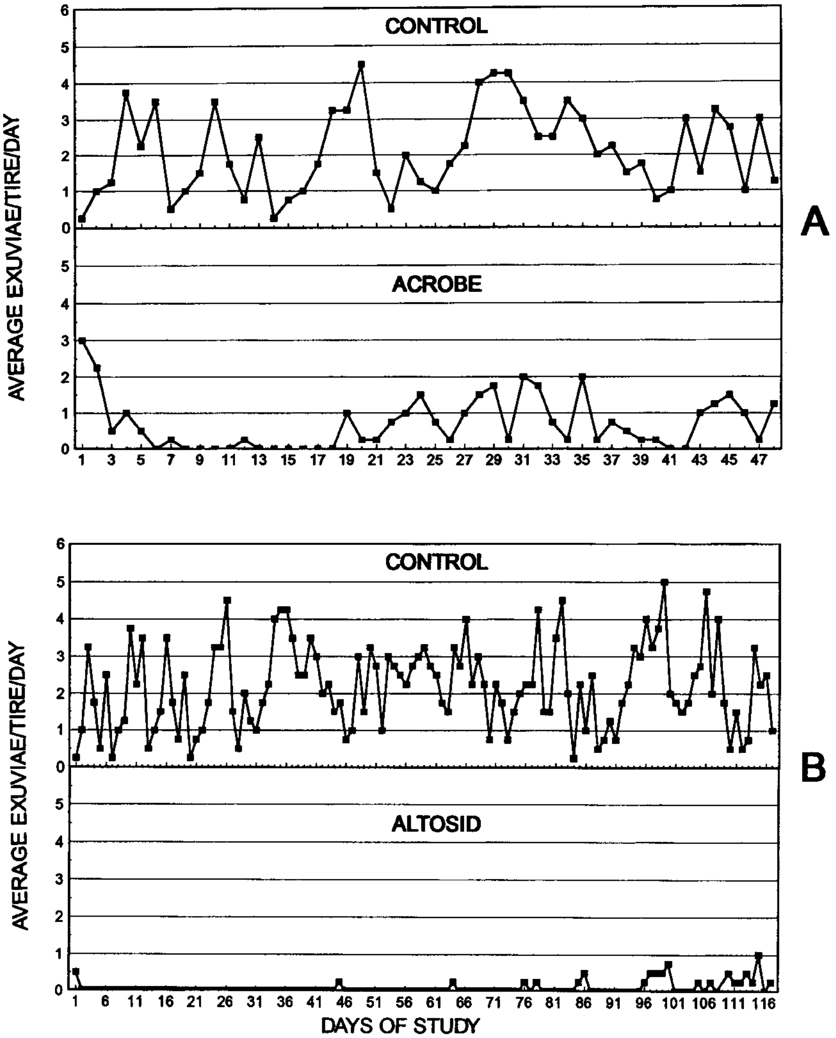

Fig. 2. Daily average number of *Aedes albopictus* exuviae produced per tire per day for 2 treatment groups and their controls (4 tires/group). A. Tires treated with *Bacillus thuringiensis israelensis,* Acrobe®. B. Tires treated with methoprene, Altosid®.

0.60 ± 0.12; *L. giganteum* = 0.42 ± 0.07; one-way ANOVA, F = 0.22, $P > 0.05$). *Lagenidium giganteum* was ineffective as a control agent for *Ae. albopictus.* Posttreatment production was evaluated for 45 days and was not significantly different than controls, with an average daily production of adults from the control tires of 2.04 ± 0.16 and 2.13 ± 0.20 for the *L. giganteum*-treated tires (one-way ANOVA, F = 0.12, $P > 0.05$). In the laboratory distilled water control, 100% mortality was obtained at 72-h postexposure but in the filtered field water there was no mortality due to *L. giganteum.* This is consistent with the previous finding that *L. giganteum* is ineffective in highly organic water (Jaronski and Axtell 1982, Kramer 1990). Efforts to select for a strain of *L. giganteum* tolerant of the highly organic water conditions tolerated by container-inhabiting mosquitoes is in progress.

The Acrobe formulation of *B.t.i.* was effective in reducing the production of adult *Ae. albopictus* (Fig. 2A) for 48 days (one-way ANOVA, F = 50.1, $P < 0.001$). The 51-day pretreatment average daily production of adults from the control tires and from the tires selected for Acrobe treatment were not significantly different (control = 0.71 ± 0.13; *B.t.i.* = 0.75 ± 0.14; one-way ANOVA, F = 0.24, $P > 0.05$). Posttreatment production was significantly different, with an average daily production of adults from the control tires of 2.10 ± 0.17 and only 0.67 ± 0.11 for the Acrobe-treated tires (one-way ANOVA, F = 50.07, $P < 0.001$). A small number of adults emerged during the first week postexposure, presumably due to the presence of prepupae and pupae within the containers when the test began. No adults emerged during the 2nd week of the study, with some adults produced beginning the 3rd week and for the remainder of the test but at much lower levels than for the controls (Fig. 2A). These results reflect the initial kill expected with *B.t.i.* but also demonstrated some residual mortality, perhaps due to the formulation of the *B.t.i.*

The pretreatment average daily production of adults from the control tires and the tires selected for Altosid treatment were not significantly different (control = 0.60 ± 0.12; methoprene = 0.53 ± 0.08; one-way ANOVA, F = 0.24, $P > 0.05$). Posttreatment production was significantly different (Fig. 2B), with an average daily production of adults from the control tires of 2.18 ± 0.11 and only 0.07 ± 0.02 for the Altosid-treated tires (one-way ANOVA, F = 382.7, $P < 0.001$). Nearly complete control was achieved in these tires for 117 days even though larvae were present throughout the test. Nasci et al. (1994) found that Altosid pellets (at more than twice the recommended rates) provided essentially 100% control of *Ae. albopictus* in Louisiana for 150 days based on average pupal mortality. This and the previous studies have demonstrated that Altosid provides excellent long-term control of *Ae. albopictus* in the field.

Measuring production of adult *Ae. albopictus* by the daily removal of exuviae from breeding sites was a good relative indicator of the effectiveness of the 3 control agents evaluated. This method causes little disruption of the habitat but is tedious and can only accurately provide a minimum adult production rate. This is due to exuviae not recovered because of human error or the loss of exuviae because of sinking or removal by foraging insects (Focks et al. 1982). Based on the standing pupal crop for tires in the shade (12.8) and assuming a pupal period of approximately 2.5 days (del Rosario 1963), a survival rate of 83% (Hien 1975, Tsuda et al. 1992), and an 85% proportion of *Ae. albopictus,* the daily emergence rate would be estimated at 3.6 adults/tire/day. The adult production rate determined by removal of pupal exuviae was 1.74 adults/tire/day (48% of the estimated rate). In a 76-day field study in Louisiana, the production rate of adult *Ae. aegypti* was likewise found to be only 71% of the estimated rate (Focks et al. 1982). The reasons for the low recovery rate in this study are unclear but demonstrate the limitations of this method as tool for evaluating and comparing the actual production of adult *Ae. albopictus.*

We thank James Kerwin for providing the *L. giganteum* cultures, American Cyanamid for providing the Acrobe, and the Zoecon Corporation for providing the Altosid pellets. We also appreciate the helpful comments and suggestions of James Kerwin, Jack Fournie, and Tokuo Fukuda.

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