Journal of the American Mosquito Control Association, 10(1):42–44, 1994

FIXED-WING, AERIAL APPLICATION OF LIQUID *BACILLUS THURINGIENSIS* H-14 (ACROBE®) FOR CONTROL OF SPRING *AEDES* MOSQUITOES IN MICHIGAN

RANDALL G. KNEPPER,[1] SALLY A. WAGNER,[1]
ERNEST ABEL[2] AND EDWARD D. WALKER[3]

ABSTRACT. Liquid *Bacillus thuringiensis* H-14 (Acrobe®) was applied from fixed-wing aircraft at a rate of 4.68 liters of water–insecticide mixture (1.17 liter concentrate) per hectare to woodland pools in Michigan. A post-treatment larval survey indicated an 88.5% reduction in *Aedes* species larvae. A volume median diameter of 208 microns was determined.

INTRODUCTION

Spring *Aedes* mosquito larvae, inhabitants of woodland pools formed by melted snow and rainfall, may be successfully controlled in Michigan using rotary-wing, aerial application of granular or liquid formulations of *Bacillus thuringiensis* H-14 (*B.t.i.*) (Knepper and Walker 1989, Knepper et al. 1991). The application must be made in mid-April, before leaf break on trees and before larvae develop to the 4th instar or pupate. In Saginaw County, Michigan, the Saginaw County Mosquito Abatement Commission (SCMAC) annually treats about 14,000 ha of woodland pools during a 7–10-day period. Fixed-wing aircraft as opposed to rotary-wing aircraft may provide significant advantages during this narrow treatment window. Fixed-wing aircraft can carry larger payloads, remain aloft longer, and cover more territory per sortie than helicopters. However, it is not known if these faster aircraft, flying at higher altitudes, could provide efficacious treatments of *B.t.i.* for spring *Aedes* control.

In this study, we examined the efficacy of liquid *B.t.i.*, Acrobe® (American Cyanamid, Princeton, NJ 08540), when applied at a low rate of application from fixed-wing aircraft fitted with Micronair (Micronair, Miami, FL 33166) rotary atomizers on wing booms.

MATERIALS AND METHODS

Thirty-nine pools, each located in separate wooded areas, were chosen for study in April 1992. Each pool contained spring *Aedes* larvae, with a predominance of *Ae. stimulans* (Walker), *Ae. intrudens* Dyar, *Ae. provocans* (Walker), and *Ae. canadensis* (Theobald). Larvae were predominantly 2nd and 3rd instars. During the time of the study, water temperature varied from 1 to 17°C, pool depth ranged from 9 to 51 cm, and pool surface area ranged from 600 to 2,000 m^2. Pools were randomly assigned to treated or nontreated (control) groups, with 33 pools in the former and 6 pools in the latter group. Pools were sampled quantitatively using standard 350-ml mosquito dippers in the fashion described by Knepper et al. (1991). Briefly, each pool was divided into 4 parallel transects across the long axis of the pool. Three sampling stations were established along each transect so that samples could be taken near the edges and in the middle of each pool. At each station, 4 dips were taken for a total of 48 dips per pool per day. Larvae were counted in each dip, recorded, and the larvae poured back into the pool. Sampling was done once in each pool at 5–7 days before treatment and once 5–7 days after treatment with *B.t.i.*, described below.

Sampling data were transformed with $\log_{10}(x + 1)$ and analyzed with repeated-measures, one-way analysis of variance (ANOVA), taking into account that each pool was sampled twice (Steel and Torrie 1980). Efficacy of the *B.t.i.* formulation and application method was expressed as mean percent reduction in numbers of larvae in treated vs. control pools using the formula of Mulla et al. (1971): percent reduction = $100 \times (1 - [T_a \times C_b/T_b \times C_a])$, where T_a is the mean number of larvae from treated pools after treatment; T_b is the mean number of larvae from treated pools before treatment; C_a is the mean number of larvae in nontreated pools after treatment; and C_b is the mean number of larvae in nontreated pools before treatment. We noted considerable variation in sampling data from treated pools before and after treatment. Therefore, we also calculated percent reduction for each treated pool separately, using mean sampling data from the 6 nontreated pools in the calculation as

[1] Saginaw County Mosquito Abatement Commission, 211 Congress Street, Saginaw, MI 48602.

[2] Michigan Department of Agriculture, 350 Ottawa Street, N.W., Suite 2-C, Grand Rapids, MI 49503.

[3] Department of Entomology, Michigan State University, East Lansing, MI 48824.

above but using single sampling values before and after treatment from each treated pool.

Applications were made with an AgCat Turbo airplane having a payload capacity of 1,360 kg. Atomization was accomplished with Micronair AV5000 nozzles fitted with size 4 blades. There were 3 nozzles fixed to each wing boom and rotating at approximately 6,000 rpm. The variable restrictor unit was set at 11. The formulation of *B.t.i.* was liquid Acrobe, mixed 1:3 with water (Acrobe : water) and applied at a dosage of 4.68 liters finished spray (1.17 liter concentrate) per hectare.

Previous aerial applications with rotary aircraft, using a different formulation of liquid *B.t.i.*, indicated that droplets in the range of 200–230 volume median diameter (VMD) were appropriate for pools with spring *Aedes* larvae (Knepper et al. 1991). Here, application equipment was calibrated to determine if droplet size was in this acceptable range, as follows. Water-sensitive dye cards (CIBA-GEIGY Limited, CH-4002 Basle, Switzerland) were placed in wooden holders at 3-m intervals on the ground along a 90-m transect in an open field. The transect was established in a direction perpendicular to the prevailing wind direction. Another transect was established similarly in a nearby woods. Because of the time of year (early spring), the trees had no foliage. The airplane approached the transects at a 90° angle and toward the middle of the transect, at an altitude of 30 m and speed of 177–217 kph, while making an application of the *B.t.i.*/water mix. Weather conditions were light wind and 50% RH with temperature ranging from 10 to 13°C. The dye cards were allowed to dry for 5 min and were retrieved.

Droplet diameter and numbers of droplets per square centimeter were determined using computer-aided image analysis with "Swath Kit" software (U.S. Forest Service 1991). Each dye card was placed by hand and viewed with a high resolution video camera. The image was captured with a video board configured to a 386SX DOS portable computer equipped with a math co-processor. The image analysis software recorded droplet diameters and adjusted them (using a binomial equation incorporating the spread factor for water) and counted numbers of droplets per square centimeter of dye card. Numbers of droplets per square centimeter and VMD on dye cards taken from the transects in the open field and the woods were compared with Mann-Whitney *U*-tests (Steel and Torrie 1980).

RESULTS

Five separate flights were done over the transects in the open field and in the woods for calibration and determination of droplet size and density on dye cards. We considered these flights to be separate calibration replicates suitable for data collection. The number of droplets per square centimeter on dye cards in the open field ranged from 1.9 to 8.2 (median 2.6) and in the woods from 1.6 to 4.9 (median 2.2). There was no significant difference between the number of droplets per square centimeter on dye cards taken from the field or the woods (Mann-Whitney *U*-test, $U = 19$, $n = 5$ in each group, $P > 0.05$). The VMD of droplets on dye cards in the open field ranged from 150 to 218 μm (median 194 μm), whereas the VMD of droplets on dye cards in the woods ranged from 167 to 233 μm (median 200 μm). There was no significant difference between the VMD of droplets on dye cards taken from the field or the woods (Mann-Whitney *U*-test, $U = 19$, $n = 5$ in each group, $P > 0.05$). Four additional aircraft utilized in the larviciding program were also calibrated in the open field with the droplets per square centimeter ranging from 4.0 to 5.0 (median 4.3). The VMD for these aircraft ranged from 174 to 233 μm (median 212 μm). The effective swath width (i.e., distance along the transect over which dye cards collected appropriate numbers of droplets) was about 30 m.

During the 14-day study, the mean number of larvae in samples from treated pools was 249 ± 53.5 (SEM) before treatment, and the mean from these pools after treatment was 17 ± 5.8 larvae. The mean number of larvae in samples from nontreated pools was 244.5 ± 64.2 before treatment, and the mean from nontreated pools after treatment was 143 ± 43.5 larvae. We interpret the decrease in number of larvae in nontreated pools to natural mortality and possibly to rainfall during the sampling interval that increased pool volume and altered sampling results. Repeated-measures ANOVA showed that there was a highly significant effect of treatment with *B.t.i.* on number of larvae in the treated and nontreated pools ($F = 13.2$, $df = 1,37$, $P < 0.001$); that there was significant variation in number of larvae sampled in the pools before and after treatment ($F = 44.3$, $df = 1,37$, $P < 0.001$); and that there was a significant interaction between the effect of *B.t.i.* treatment and time of sampling ($F = 23.8$, $df = 1,37$, $P < 0.001$).

Efficacy of the *B.t.i.* application, as indicated by the percent reduction in mean number of larvae in treated and control pools, was 88.5%. For individual treated pools, the percent reduction in numbers of larvae owing to *B.t.i.* treatment ranged from 58.4 to 100% (median 97%). A histogram plot of percent reduction in treated pools (Fig. 1) indicates that efficacy was high for most treated pools but that in some pools efficacy was variable.

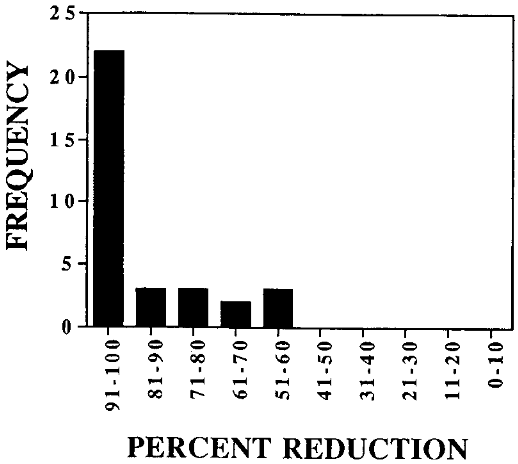

Fig. 1. Histogram showing frequency of different observations of percent reduction of spring *Aedes* larvae in 33 spring pools treated with Acrobe® compared with nontreated pools ($n = 6$).

DISCUSSION

Our sampling data demonstrate that *B.t.i.*, in the commercial liquid formulation Acrobe, controlled spring *Aedes* mosquito larvae when applied by fixed-wing aircraft fitted with application apparatus that was calibrated to apply a low volume insecticide–water mixture in the droplet range of 200 μm VMD. The percent reduction data indicate that this method was as efficacious as application of either granular or liquid formulations of *B.t.i.* applied from rotary aircraft (Knepper and Walker 1989, Knepper et al. 1991). However, considerable variation was observed in percent reduction among individual pools, with ⅓ of the treated pools showing a percent reduction of 90% or less (Fig. 1). We speculate that this variation occurred because of drift of droplets during application, resulting in variable amounts of *B.t.i.* reaching those pools. Indeed, drift may prove to be an important element to consider in the aerial application of liquid *B.t.i.* to scattered small pools containing *Aedes* larvae.

Application of liquid *B.t.i.* from fixed-wing aircraft, compared with rotary aircraft, may allow more efficient and inexpensive control of spring *Aedes* mosquitoes. We have found that the AgCat airplane required fewer sorties, had longer application flights, and treated more area per sortie than did helicopters (R. G. Knepper and S. A. Wagner, unpublished observations). One possible drawback is that, in comparison with helicopters, fixed-wing aircraft may be less precise in applications to woodland pools where larvae are found.

ACKNOWLEDGMENTS

We are grateful for the expert contributions to this study from Al and Mike Schiffer, of Al's Aerial Spraying (Ovid, MI). We also thank William Jany (American Cyanamid, Wayne, NJ) for his assistance and advice and the field crew of SCMAC for assistance with sampling.

REFERENCES CITED

Knepper, R. G. and E. D. Walker. 1989. Effect of *Bacillus thuringiensis israelensis* (H-14) on the isopod *Asellus forbesi* and spring *Aedes* mosquitoes in Michigan. J. Am. Mosq. Control Assoc. 5:596–598.

Knepper, R. G., S. A. Wagner and E. D. Walker. 1991. Aerially-applied, liquid *Bacillus thuringiensis* var. *israelensis* (H-14) for control of spring *Aedes* mosquitoes in Michigan. J. Am. Mosq. Control Assoc. 7:307–309.

Mulla, M. S., R. L. Norland, D. M. Fanara, H. A. Darwazeh and D. W. McKean. 1971. Control of chironomid midges in recreational lakes. J. Econ. Entomol. 64:300–307.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill, New York.

U.S. Forest Service, U.S. Department of Agriculture. 1991. Aerial spraying for gypsy moth: a handbook of technology. U.S. Gov. Printing Office, Washington, DC.