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# BVA 2 MOSQUITO LARVICIDE—A NEW SURFACE OIL LARVICIDE FOR MOSQUITO CONTROL

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ABSTRACT. BVA 2 mosquito larvicide was evaluated in laboratory pan tests against 3rd-instar *Aedes taeniorhynchus* (Wied.), *Culex quinquefasciatus* Say, and *Culex nigripalpus* Theobald larvae. BVA 2 was as effective as the standard, GB-1111, at 14 liters/ha (>99.1% vs. 99.8%). In small field plot tests BVA 2 mosquito larvicide applied at 28 liters/ha was as effective as GB-1111 (99.0% vs. 99.8%) 24 h posttreatment. Operationally, applied by helicopter at 46.8 liters/ha, BVA 2 mosquito larvicide was more effective (>90%) in the slightly less vegetated site than in the heavily vegetated site. As a pupicide applied at 14 liters/ha in laboratory pan tests, no significant differences were noted between BVA 2 mosquito larvicide and GB-1111 against *Ae. taeniorhynchus, Cx. quinquefasciatus,* and *Cx. nigripalpus* pupae.

**KEY WORDS** Operational field laboratory, Diptera, Culicidae, *Aedes taeniorhynchus,* Culex, pupicide

## INTRODUCTION

The use of petroleum oils as mosquito larvicides in the United States dates to 1793 in Philadelphia (Howard 1928). Early petroleum-based larvicides such as diesel oil, kerosene, or tar oils were odoriferous and often discolored the water's surface for several days (Headlee 1921, Lowry 1929, Howard 1932, Mail 1933, King et al. 1944). Current products, although derivatives of petroleum distillates, are nearly odorless and clear (Anonymous 1990, Floore et al. 1992). Phytotoxicity and other detrimental effects some petroleum oils have on certain nontarget aquatic organisms have lead to the elimination of all of these products except Golden Bear®-1111 (Golden Bear Oil Specialties, Oildale, CA) (GB-1111) and Bonide® (Bonide Products, Inc., Yorkville, NY) (Gjullin 1968, Mulla et al. 1969, Schmidt et al. 1973, Levy et al. 1980, Mulla and Darwazeh 1981). Currently, these 2 surface oils are labeled as larvicides/pupicides. Pupicidal effects of larvicide oils had been documented by Howard (1917), Lowry (1929), and Micks et al. (1968).

For several years studies evaluating candidate surface oils as mosquito larvicides have been conducted by the John A. Mulrennan, Sr. Arthropod Research Laboratory, Panama City, FL (JAMSARL). This paper reports the results of a study comparing BVA 2 mosquito larvicide with GB-1111 against 3rd-instar *Aedes taeniorhynchus* (Wied.), *Culex quinquefasciatus* Say, and *Culex nigripalpus* Theobald larvae and pupae in laboratory and small plot field tests. Also, results of an operational study conducted by the Hillsborough County Mosquito and Aquatic Weed Control Department (HCMCD), Tampa, FL, is presented. In initial laboratory pan bioassay tests 3 candidate BVA oil formulations were evaluated (unpublished data). Less than 2% differences in mortality separated the 3 formulations from one another and less than 5% separated them from the standard, GB-1111. B-V Associates (Wixom, MI) chose the BVA 2 formulation reported on here for further study based on the initial study.

## MATERIALS AND METHODS

*Laboratory studies:* Laboratory bioassay tests were conducted in 61 × 61 × 10-cm pans filled with 16 liters of well water. The procedures for the laboratory and small plot studies were described by Floore et al. (1991). One hundred 3rd-instar mosquito larvae were placed in each pan before treatment. In the *Ae. taeniorhynchus* tests, 60 g of reagent grade NaCl (Fisher Scientific, Inc., Atlanta, GA) was added to the water prior to adding the larvae (5‰ salinity). The 14 liters/ha application rate (1.5 gallons per acre, GPA) was based on 0.37 $m^2$ of water surface area. Five tests were conducted against each species in the laboratory with each test consisting of 2 pans of BVA 2 mosquito larvicide formulation, 1 pan of GB-1111, and 1 untreated control. Larvae were considered dead if they did not move when touched by a glass probe. Larvae treated with GB-1111 sank to the bottom of the pan when dead. Larvae treated with BVA 2 mosquito larvicide floated on the surface when dead. Water temperature during the laboratory tests was 26 ± 1°C.

In the pupae tests, 4 tests were conducted against 100 *Ae. taeniorhynchus, Cx. quinquefasciatus,* and *Cx. nigripalpus* pupae in laboratory pans. Mortality was assessed by determining the emergence inhibition (EI) (Floore et al. 1991):

$$\%EI = 100 - \left(\frac{CS - DA}{CS + PE + DP} \times 100\right),$$

where CS is cast skin, DA is dead adults, PE is partially emerged adults, and DP is dead pupae. Water temperature during the pupae laboratory tests was 26 ± 1°C.

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*Laboratory small field plot studies:* Small plot field study application rates (28 liters/ha, 3 GPA) were based on 0.93 $m^2$ of water surface area. These tests were conducted in a screened enclosure at the JAMSARL in concrete cattle watering tanks (J. B. Hill Contractor, Inc., Leesburg, FL) that measured 0.6-m wide × 1.7-m long × 0.6-m deep. The *Cx. quinquefasciatus* and *Cx. nigripalpus* bioassays were conducted in freshwater and the *Ae. taeniorhynchus* bioassays were conducted in tanks filled with salt water pumped from a saltwater canal described by Rathburn and Boike (1975). Pretreatment assessments and posttreatment mortality were determined by dipping larvae using a standard mosquito larval dipper at 8 stations located at each corner of the tank (4), the middle of each long wall axis (2), and 2 areas along the center axis of each tank approximately 30.5 cm from each end. Water temperature during the tests was 29 ± 3°C and the salinity in the saltwater plots was ±18‰ at treatment.

*Analysis:* Larval mortality was assessed by counting the number of larvae in each pan or plot 24 h posttreatment. All the larvae were counted in the pans and in the plots only those actually dipped were counted. Treatment means were separated by analysis of variance (ANOVA) PROC GLM and least significant differences (LSD) multiple comparison tests (SAS Institute 1990). Differences were considered significant at $P = 0.05$. Pupicidal data were assessed and analyzed in a manner similar to the larval data described above.

*Operational study:* In the operational study, conducted at the Seaboard sewage effluent sprinkler field in Hillsborough County, Florida, BVA 2 mosquito larvicide was applied at 46.8 liter/ha (5 GPA) by a Bell 47 helicopter equipped with 20 disc core nozzles (TeeJet D10/45, Spraying Systems Co., Wheaton, IL) attached to a 9.8-m boom. Application was from a height of 3.3 m at an airspeed of 74 kilometers/hr and a swath width of 21.3 m. The treatments were initiated at 0800 h.

Ditching characteristics of the 28.3-ha study sites allowed for a portion of each cell (A, B, and C cells) to be utilized as either a treated or untreated control site. The initial test was conducted in April 1993 when the vegetation (various grasses and woody shrubs) in the study site was 18–61 cm in height. In October when the last test was conducted, the majority of the vegetation exceeded 1 m.

The mosquito species complement at the sprinkler field was *Aedes sollicitans* (Walker), *Anopheles crucians* Wied, *Anopheles quadrimaculatus* Say, *Cx. nigripalpus, Cx. quinquefasciatus, Psorophora ciliata* (Fabricus), and *Psorophora columbiae* (Dyar & Knab) throughout the season.

*Operational data analysis:* Twenty larval samples at HCMCD predetermined dipping stations were taken at each site (treated and untreated) prior to application and again 24 and 48 h posttreatment.

Table 1. Laboratory tests of BVA mosquito larvicide compared to GB-1111 against 3rd-instar *Aedes taeniorhynchus, Culex quinquefasciatus,* and *Culex nigripalpus* larvae 24 h posttreatment.[1-3]

| BVA mosquito larvicide oil | | | GB-1111 | |
|---|---|---|---|---|
| Formulation | % control | ± SE | % control | ± SE |
| | *Aedes taeniorhynchus*[A] | | | |
| 2 | 95.91[a] | 1.43 | 100.00[a] | 0.00 |
| | *Culex quinquefasciatus*[A] | | | |
| 2 | 97.92[a] | 1.12 | 99.88[a] | 0.11 |
| | *Culex nigripalpus*[A] | | | |
| 2 | 99.33[a] | 0.67 | 100.00[a] | 0.00 |

[1] Application rates: BVA oil and GB-1111 at 14 liter/ha (1.5 gallons per acre).

[2] Species followed by different uppercase letter represent significant differences ($P = 0.05$) between species using least significant differences (LSD) multiple comparison test.

[3] Means followed by different lowercase letter represent significant differences ($P = 0.05$) between treatments using LSD multiple comparison test.

Percent reduction was assessed as percent reduction using the following formula (Mulla et al. 1971):

$$\%\text{reduction} = 100 - [(\text{no. larvae control pretreatment} \times \text{no. larvae treatment posttreatment}) \div (\text{no. larvae treatment pretreatment} \times \text{no. larvae control posttreatment})] \times 100.$$

This formula assumed that changes in the mosquito larval population were occurring at the same level and rate in both treated and untreated control sites. Comparisons within and among sample sites, and among sampling times to assess larval abundance were made by ANOVA PROC GLM procedures and LSD multiple comparison tests after arcsin transformation of the data (SAS Institute 1990).

## RESULTS AND DISCUSSION

*Laboratory:* In the laboratory pan test no significant mortality differences existed between species ($P = 0.2018$) (Table 1). The treatments were significantly different from the control ($P = 0.0001$). No differences were noted in the treatment by species interaction ($P = 0.1054$), or between larvicide oils ($P = 0.0272$).

In the laboratory bioassay test the BVA 2 mosquito larvicide was as effective as the GB-1111 formulation in controlling *Ae. taeniorhynchus* (95.9% vs. 100.0%), *Cx. quinquefasciatus,* (97.9% vs. 99.9%) and *Cx. nigripalpus* (99.3% vs. 100.0%) larvae (Table 1). BVA 2 was more effective against the *Culex* species than against *Ae. taeniorhynchus.*

Table 2. Laboratory tests of BVA 2 mosquito larvicide compared to GB-1111 against *Aedes taeniorhynchus, Culex quinquefasciatus,* and *Culex nigripalpus* pupae 24 h posttreatment.[1-3]

| BVA 2 mosquito larvicide[a] | | GB-1111 | |
|---|---|---|---|
| % control | ± SE | % control | ± SE |
| *Aedes taeniorhynchus*[A] | | | |
| 99.50 | 0.71 | 100.00 | 0.00 |
| *Culex quinquefasciatus*[A] | | | |
| 96.50 | 0.71 | 100.00 | 0.00 |
| *Culex nigripalpus*[A] | | | |
| 94.50 | 4.50 | 100.00 | 0.00 |

[1] Application rates: BVA oil and GB-1111 at 14 liter/ha (1.5 gallons per acre).

[2] Larvicides followed by different lowercase letter represent significant differences ($P = 0.05$) between treatments using least significant differences (LSD) multiple comparison test.

[3] Species followed by different uppercase letter represent significant differences ($P = 0.05$) between species using LSD multiple comparison test.

Table 3. Small plot field tests of BVA 2 mosquito larvicide compared with GB-1111 against 3rd-instar *Aedes taeniorhynchus, Culex quinquefasciatus,* and *Culex nigripalpus* larvae 24 h posttreatment.[1-3]

| BVA 2 mosquito larvicide[a] | | GB-1111 | |
|---|---|---|---|
| % control | ± SE | % control | ± SE |
| *Aedes taeniorhynchus*[A] | | | |
| 100.00[a] | 0.00 | 100.00[a] | 0.00 |
| *Culex quinquefasciatus*[A] | | | |
| 97.00[a] | 1.00 | 99.50[a] | 0.50 |
| *Culex nigripalpus*[A] | | | |
| 100.00[a] | 0.00 | 100.00[a] | 0.00 |

[1] Application rates: BVA 2 oil and GB-1111 applied at 28 liter/h (3.0 gallons per acre).

[2] Species followed by different uppercase letter represent significant differences ($P = 0.05$) between species using least significant differences (LSD) multiple comparison test.

[3] Means followed by different lowercase letter represent significant differences ($P = 0.05$) between treatments using LSD multiple comparison test.

No significant differences ($P = 0.3149$) were noted between BVA 2 mosquito larvicide and GB-1111 in the laboratory pan tests against pupae; however, significant differences were noted between treatments and control ($P = 0.0001$) (Table 2). No significant differences were observed between *Ae. taeniorhynchus* and the *Culex* species ($P = 0.7993$) or between the *Culex* species ($P = 0.6634$). BVA 2 was most effective against *Ae. taeniorhynchus* (99.5% vs. 100.0%) and least effective against *Cx. nigripalpus* (94.5% vs. 100.0%).

*Small plot field tests:* In the small plot field study no significant statistical differences were noted between the oils ($P = 0.0255$) (Table 3). Significant differences were noted between the treatments and controls ($P = 0.0001$). No significant differences were noted between the 2 larvicides and the tests with *Cx. quinquefasciatus* and those with *Ae. taeniorhynchus* and *Cx. nigripalpus* larvae ($P = 0.0971$). Actual *Cx. quinquefasciatus* larval mortality was 97.0% with BVA 2 mosquito larvicide and 99.5% with GB-1111, whereas *Ae. taeniorhynchus* and *Cx. nigripalpus* mortality was 100% with both surface oils (Table 3).

Small plot field bioassay tests under identical outdoor conditions showed BVA 2 mosquito larvicide to be as effective as GB-1111 in controlling *Ae. taeniorhynchus, Cx. quinquefasciatus,* and *Cx. nigripalpus* larvae. Emergent vegetation, rainfall events, mixed larval instars, and fluctuating water temperatures were no more detrimental to BVA 2 mosquito larvicide than to GB-1111.

*Operational study:* Because significant treatment by site interaction ($P = 0.0023$) occurred in the study, sample sites were considered separately (Table 4). Based on the larval reduction formula (Mulla et al. 1971) no differences were noted between treatment and control areas in site A, which was heavily vegetated (95% surface obstruction). In sites B and C, which were 80% vegetated, larval reduction was approximately 94% and 60%, respectively, at 24 h posttreatment and 95% and 63% at 48 h posttreatment. Site A differed significantly ($P = 0.0010$) from the less vegetated sites B and C (80%) in percent larval mortality (Table 4). No significant treatment by site interaction ($P = 0.5609$) was noted between the sites with similar vegetation properties (B and C). Significant differences existed between pretreatment and 24 and 48 h posttreatment assessment times ($P = 0.0002$ and $P = 0.0017$, respectively), but not between 24 and 48 h posttreatment ($P = 0.2897$).

In the operational study, BVA 2 mosquito larvicide applied aerially controlled the larval mosquito population in a sewage effluent sprinkler field site where mosquito breeding was a problem. Larval control ranged from less than 60% to greater than 93%. This variation was presumed to be directly related to the amount of surface or emergent vegetation in the target habitat. No reduction was noted in the more densely vegetated site (A) where the mosquito rearing areas were covered with grass canopies, making aerial larvicide application difficult. The sites with good (C) to excellent (B) control were more accessible to aerial larvicide application because the larval rearing sites were more exposed.

## SUMMARY

BVA 2 mosquito larvicide oil successfully controlled *Ae. taeniorhynchus, Cx. quinquefasciatus,* and *Cx. nigripalpus* larvae and pupae in laboratory tests and small plot field studies. BVA 2 was most effective against *Ae. taeniorhynchus* and *Cx. nigripalpus* and least effective against *Cx. quinquefas-*

Table 4. BVA 2 mosquito larvicide operational study at Seaboard Sprinkler Field, Tampa, FL, March 30–October 21, 1993.[1-3]

| Plot | Total no. larvae/20 dips | | | Percent reduction[4] | |
|---|---|---|---|---|---|
| | Pretreatment | 24 h posttreatment | 48 h posttreatment | 24 h | 48 h |
| A—control | 131 | 41 | 46 | | |
| A—treatment[B] | 94[a] | 38[b] | 55[b] | NR[5] | NR[5] |
| B—control | 1,389 | 1,648 | 1,881 | | |
| B—treatment[A] | 2,221[a] | 168[b] | 142[b] | 93.62 | 95.28 |
| C—control | 465 | 166 | 180 | | |
| C—treatment[B] | 35[a] | 5[b] | 5[b] | 59.98 | 63.1 |

[1] Application rates: BVA 2 mosquito larvicide oil and GB-1111 applied at 18.9 liter/hectare (5.0 gallons per acre).

[2] Sites followed by different uppercase letter represent significant differences ($P = 0.05$) between treatments using Duncan's multiple comparison test.

[3] Means followed by different lowercase letter represent significant differences ($P = 0.05$) between time of assessment using Duncan's multiple comparison test.

[4] % reduction = 100 − no. larvae control pretreatment × no. larvae treatment posttreatment/no. larvae treatment pretreatment × no. larvae control posttreatment × 100.

[5] NR, no larvae reduction based on formula.

*ciatus* larvae. However, the actual percentage mortality differences were small and probably not important in an operational program. The BVA 2 mosquito larvicide was as effective as GB-1111 in controlling emergence of pupae.

BVA 2 was effective in controlling natural mosquito larval populations in an operational study conducted by the HCMCD in an area accessible to aerial larvicide application. BVA 2 mosquito larvicide should be another reliable tool in the mosquito control arsenal of larvicides.

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