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THE MOSSIE-BUSTER: A HOSE-DRIVEN INSECTICIDE DELIVERY TOOL FOR THE CONTROL OF CONTAINER-BREEDING MOSQUITOES

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ABSTRACT. A novel insecticide delivery tool, the Mossie-Buster, was recently developed to control larval populations from urban breeding sites in Townsville, Australia. This functional and user-friendly control device directly targets the main breeding sites of *Aedes aegypti* (L.) with a focused delivery of insecticides. The Mossie-Buster comprises a commercially available device and attachments that mix an insecticide solution into the flow of water emitted from a hose. Attached to the device is a trigger for controlled insecticide release. Preliminary laboratory and field trials demonstrated the tool to be effective in eliminating all *Ae. aegypti* present in various typical breeding containers in different environmental conditions for a minimum of 2 wk in exposed areas to 3 months in an unexposed area.

INTRODUCTION

Vector-borne diseases are increasing in their range and health impact on urban populations. This is largely due to the process of urbanization, which is occurring primarily in less developed countries at a staggering rate (Slooff 1987). It has been projected by the World Health Organization (W.H.O.) (1983) that 50% of the world's population will live in an urban environment by the year 2000. Environmental sanitation in developing countries through the installation of piped water supplies has had a significant impact on the reduction of many diseases, but it is not often that we see it as a potential cause of disease. Frequently watered premises in urban areas are rarely completely free of water collection sites such as plant pot bases, toys, discarded household items, buckets and blocked gutters, which, in some areas, are the preferred breeding sites of *Ae. aegypti,* the major vector of dengue viruses in North Queensland (Barker-Hudson et al. 1988). It could be argued that a well-watered and slightly unkempt garden provides the perfect urban niche for *Ae. aegypti* in the developed world (Tun-Lin et al. 1995).

Blanket misting or fogging with insecticides is expensive and often criticized by environmentally conscious members of the public and by the applicators themselves because of solvent odors and the nonspecific targeting nature of the delivery system. Public acceptance of vast amounts of residual pesticides being sprayed over residential and wetland habitats is fast deteriorating, and this practice is only tolerated because no alternative exists in many cases. Another deficiency inherent in misting and fogging methods is the variability in results. Nelson (1991) reported that numerous trials have shown "widely different results, varying from no detectable control to virtually complete elimination of the adult vector population."

In a vector control analysis, Coluzzi (1992) concluded that "vector control . . . has little or no place without new tools" and stated that the best hope is in the development of "totally different, highly specific anti-vector measures." The Mossie-Buster is a new target-specific and nonintrusive insecticide delivery tool that may promote community-based control in developed countries.

METHOD

Mossie-Buster: The Mossie-Buster application system consists of a garden hose, a trigger attachment for controlling dispersal and an on-the-shelf product that includes a 2-liter plastic bottle and a device (registered design, Yates, Australia) that mixes the hose water with the insecticide during dispersal (Fig. 1). The total cost of construction (excluding hose) should not exceed U.S.$14. The trigger can be connected either directly to the mixing device or with a short length of hosing, depending on the area being treated. Water and insecticide displacement of this model is approximately 13.7 liters/min under available water pressure with the tap fully open; however, this will vary with the type of hose fixtures used and tap water pressure. Insecticide is drawn up into the water stream at an average rate of 395 ml/min, which means that a 1-liter insecticide suspension is delivered in 150 sec. The average discharge rate is 65–70 psi (Department of Water and Waste Water, Townsville City Council) and is variable because peak periods in water use can affect water pressure. Also, water supply is not often regulated by the same water authorities in different areas.

Insecticides: Many researchers are investigating biological control methods, and one such agent, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), has been well tested and is used extensively to control mosquito larvae (Margalit et al. 1994).

Calculations to determine a suitable dosage of *B.t.i.* were based on a concentration of 500 mg/liter of 6AS VectoBac®, 78 times the LC_{99} of 6.37 mg/liter as determined for a Townsville strain of *Ae. ae-*

Fig. 1. The Mossie-Buster in action.

gypti (Canyon, unpublished data). Dosage selection was based on a study by Margalit and Bobroglo (1984), who demonstrated that residual effectiveness doubles with a 100 × LC_{100} dose. The following formula was used to calculate the volume of concentrate necessary to produce the required dose when mixed with the water sprayed from a Mossie-Buster-fitted hose:

$$X = (A \times B)/C,$$

where X is the amount of technical material required, A is the concentration desired in mg/liter, B is the quantity of *B.t.i.* suspension concentrate required in liters, and C is the conversion factor (1,000 if in ml/liter).

The formula below, which takes into account the quantity of water already in the target vessel and insecticide overspray, was used to calculate the target concentration after spraying.

$$C_t = (C_s\ (V_s \times P/100))/((V_s \times P/100) + V_t),$$

where C_t is the concentration in target (mg/liter), C_s is the concentration in spray (mg/liter), V_t is the volume in liters in target, V_s is the volume in liters in spray, and P is the percentage of spray landing in target.

The extent of overspray was assessed to determine the quantity of delivered insecticide. Ten pot bases and ten 9-liter buckets were sprayed from a distance of 1.5 m with the Mossie-Buster held 1 m above the ground for periods of 1 and 3 sec, respectively. The volumes of treated water reaching the pot bases and buckets were 91 ± 3.8 ml and 491 ± 13.5 ml, and the volumes oversprayed were 122 ± 3.8 ml and 117 ± 13.5 ml, respectively. An increase of this distance to 3 m resulted in a 61–65% reduction in delivered volume. At 1.5 m, spray losses due to overspray were 57 and 19% in pot bases and buckets, respectively.

The time required to spray each untreated liter of target water to attain a *B.t.i.* dose of 500 mg/liter was calculated to be 0.48 sec based on percentage overspray. When time was rounded off, 1 and 4 sec were designated for target vessels containing 1 and 5 liters of untreated water, which resulted in an estimated concentration of 778 mg/liter (122 × LC_{99}) and 643 mg/liter (101 × LC_{99}), respectively. A bottle containing 790 ml *B.t.i.* suspension (137 ml *B.t.i.* and 653 ml H_2O) that was sprayed for a cumulative period of 120 sec could therefore treat 120 liters of container water for around U.S.$1.70.

Field dose confirmation: Since turbidity might be related to *B.t.i.* concentration, 4 absorbance readings were made on each of 4 Mossie-Buster-treated containers with a Pye Unicam spectrophotometer at 550-nm wavelength. Dilutions of VectoBac 6AS *B.t.i.* ranging from 10 to 5,000 mg/liter were prepared and scanned to produce a baseline from which field samples could be compared. A standard calibration curve was produced using the SPSS (Windows version 6.1, 1994) linear regression program.

Laboratory evaluation: Bioassays on late 3rd- to early 4th-instar *Ae. aegypti* larvae conforming to W.H.O. instructions (WHO/VBC/81.807) were used to assess the longevity of *B.t.i.* effectiveness. Since wild recruitment into treated containers can only occur through oviposition, and since 1st-instar hatchings are more susceptible to *B.t.i.* than 4th-instar larvae (Mulla 1985), 4th-instar bioassays will indicate a decrease in effectiveness before the treatment loses effectiveness against natural recruitment. Hatch rates and survival of 1st-instar larvae were thus used to supplement bioassay data.

Egg bioassays: Larval counts in the field can often be very difficult using containers heavily laden with organic material. Therefore laboratory trials were set up to evaluate the accuracy of an egg-counting method to determine the number of 1st-instar larvae that potentially hatched from a known number of eggs. Eggs used in these experiments were obtained from an *Ae. aegypti* colony (COUNCIL strain) that was maintained under standard conditions (Foster 1980). Adults were maintained in a climate controlled room (24 ± 1°C, 65 ± 5% relative humidity, 10:14 [L:D] h). Eggs were laid on filter paper and stored in sealed plastic bags in the same room conditions.

In this experiment, 12 pieces of filter paper laden with 66 ± 8.2 viable eggs (aged 46–52 days) were placed into each of 6 intervention and 6 control glass beakers containing 300 ml of treated and untreated tap water, respectively. The treated water was obtained by spraying *B.t.i.* suspension for 4 min through the Mossie-Buster (966 mg/liter) into a bucket from a distance of 1.5 m. Eggs were classified as viable under a microscope if they were not entirely collapsed (Christophers 1960). First-instar larvae were observed and removed daily over 6 days to determine egg hatch and mortality rates. The high rate of hatching in treated beakers prompted the placing of the 6-day-old control filter paper pieces, on which hatching had ceased, into the treated beakers to observe any change in hatch rate on the 7th, 8th, and 9th consecutive days. The numbers of unhatched eggs were counted before and after the experiments to assess the use of unhatched egg counting as a method of estimating the successful hatched number of 1st-instar larvae.

Longitudinal effectiveness: Identical sets of 6 control and 6 *B.t.i.*-treated beakers were bioassayed weekly by exposing early 3rd- to late 4th-instar larvae and using the egg-hatching method (68 ± 3.1 eggs/filter paper/beaker; age 11–87 days) to determine long-term effectiveness of the treatment. Prior to each assay, water was added to the beakers to replace the equivalent amount of water lost to evaporation.

Preliminary field trials: The Mossie-Buster was tested during the wet late summer months from March through May 1996 in 3 suburban backyard sites in Mundingburra, Townsville, that sustained a native *Ae. aegypti* population. Site 1 was under a low-set Queenslander house where it was protected from rain, yard watering and organic material (leaf and flower drop). Site 2 was exposed in a semi-sheltered and partially shaded position where it received rain but little organic material. Site 3 was well sheltered but received copious amounts of rain and organic matter from surrounding vegetation.

Six 9-liter buckets (top diameter 27 cm) were placed in each of these 3 sites; 1 untreated control bucket containing 5 liters of tap water and 5 *B.t.i.*-treated buckets that contained a total of 5 liters of water prior to treatment with the Mossie-Buster. A piece of filter paper bearing 76 ± 3.1 eggs (aged 11–87 days) was placed just below the water line in each bucket for 24 h, after which the numbers of unhatched eggs were counted. Egg hatching, 1st-instar survival, and container water levels were recorded weekly. First-instar larvae in site 1 buckets were pipetted out daily as they became visually detectable, until the following weekly egg hatch bioassay. Buckets in site 1 were also bioassayed weekly with late 3rd- to early 4th-instar *Ae. aegypti* larvae.

Table 1. Estimated milligrams per liter in target vessels (C_t) after Mossie-Buster B.t.i. treatment according to application duration and volume.

Seconds to spray	V_s[1] (liters)	V_t[2] (liters)	C_t (mg/liters)
40	9.3	50	643
13	3.0	20	535
7	1.6	10	571
4	0.9	5	643[3]
2	0.5	2	778
1	0.2	1	778[3]

[1] Spray volume.
[2] Water in target.
[3] Based on this information, 1- and 5-liter containers were treated for 1 and 4 sec, respectively, in this study.

Due to contamination and presence of organic material, attempts to count live 1st-instar larvae in sites 2 and 3 were unsuccessful. Upon observation in week 2 of large quantities of live larvae in the treated containers of sites 2 and 3, the contents of the buckets were emptied into larval rearing trays where the larvae were identified and estimated.

Five 300-ml plant pot bases (200 mm diam), each holding earth-filled pots, and 5 800-ml disposable plastic containers were concurrently treated with buckets in site 3 and were used to assess treatment effectiveness over a 1-month period against eggs oviposited by the wild population.

RESULTS

Insecticides: The time required to spray target containers, each containing 6 predetermined quantities of water, to attain the desired *B.t.i.* concentration is shown in Table 1.

Field dose calculation: Bacillus thuringiensis israelensis concentration was highly correlated with percent absorbance ($y = 0.0001X + 0.0102$, $r^2 = 0.99$) (Fig. 2). Using this formula, the Mossie-Buster-delivered *B.t.i.* dose in the field was calculated to be 965.6 ± 32.1 mg/liter, which was substantially higher than the prefield estimated concentration of 643 mg/liter.

Egg bioassays: The egg-hatching experiment revealed a low mean percentage hatch rate in the controls over a 6-day period. Percentage hatch rates in *B.t.i.*-treated beakers were significantly higher than those in untreated beakers on the first day (t =

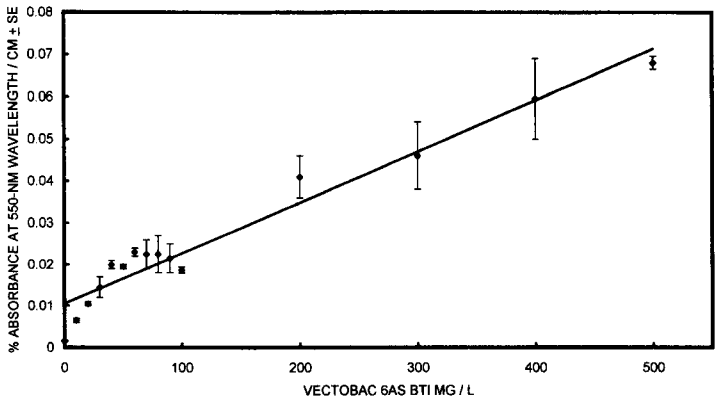

Fig. 2. Calibration curve showing the relationship between % absorbance and *B.t.i.* concentration.

-5.9, $P < 0.01$, df = 5), but the mean percentage hatch rate for treated beakers closely followed the control rate thereafter ($t = 1.09$, $P > 0.05$, df = 4) (Fig. 3). All larvae that hatched in *B.t.i.*-treated beakers died after 24 h exposure, but no larval mortalities were recorded in control beakers. Prior to each assay, any larvae missed in the initial observation, if present in the beakers, were counted and added to the initial observation to correct the initial viable egg count. No significant difference was shown between the initial viable egg count (mean ± SE: 61.3 ± 12.4; range: 40–118) and the corrected viable egg count (mean ± SE: 66.5 ± 12.9; range: 41–123) ($t = -2.17$, $P > 0.05$, df = 5). Placement of the 6-day-old control egg papers, on which eggs had ceased to hatch by the 6th day, into the *B.t.i.*-treated beakers stimulated an additional 5% mean hatch rate on the 7th day, after which no further hatching was observed on days 8 and 9.

Longitudinal effectiveness: First-instar mortalities in the residual effectiveness experiment remained at 100% until the 10th wk, when the experiment was discontinued. Mean hatch rate for treated beakers was high in week 1 (78.6 ± 4.1%) and week 2 (76.2 ± 9.3%) but dropped during weeks 3–8 (16.7 ± 3.2) while remaining higher than the rate for control beakers (7.6 ± 2.6%). A peak in hatch rate occurred in the 9th wk for both treated (80.7 ± 5.7%) and untreated (42.6 ± 9.0%) beakers (Fig. 4). In treated beakers, 4th-instar larval mortalities decreased by 5% from week 10 to week 12, when the experiment ended.

Preliminary field trials: Buckets in site 1 received no plant material and no rainfall due to their sheltered position. Evaporation, estimated from site 1 to be 0.39 ± 0.02 liters/bucket/wk, increased *B.t.i.* concentrations in treated buckets and theoretically extended treatment effectiveness. No significant difference was evident in hatch rates between control and treated containers ($t = 0.10$, $P > 0.05$, df = 9). First-instar larval bioassays were conducted in site 1 until week 10, when mortalities had decreased to 57% (Fig. 5). *Bacillus thuringiensis israelensis* treatment lost 100% efficacy in weeks 8 and 5 for 1st- and 4th-instar larvae, respectively. In week 12, 4th-instar mortalities had decreased to 21%, and all buckets dried out, which ended the experiment. Control mortalities were 0.29 ± 0.86% and 0% for 1st- and 4th-instar larvae, respectively.

Site 2 experienced 20.6 mm of rain on 8 of 14 days. The mean increase in water levels corresponding to week 1 and week 2 bioassays were 0.04 and 1.56 liters, respectively. Evaporation in

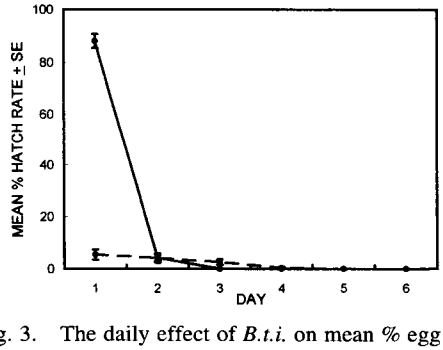

Fig. 3. The daily effect of *B.t.i.* on mean % egg hatch rates in control (– – –) and treated (———) beakers.

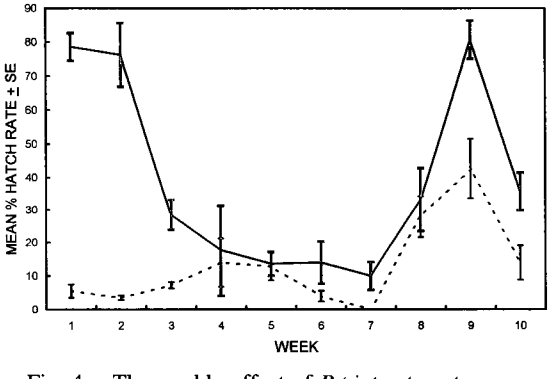

Fig. 4. The weekly effect of *B.t.i.* treatment on mean % hatch rates in control (– – –) and treated (———) beakers.

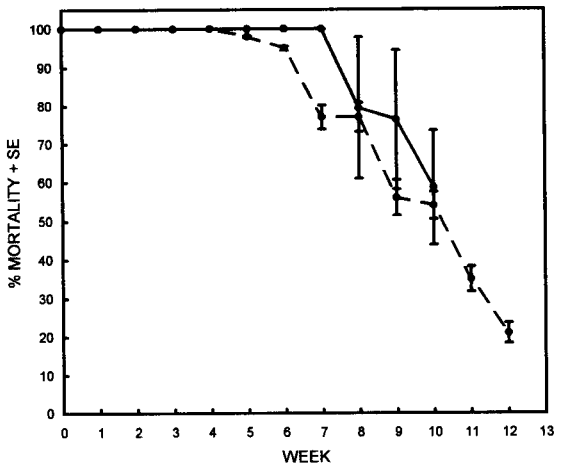

Fig. 5. Weekly bioassays in site 1 to determine the residual efficacy of *B.t.i.* on 1st- (———) and (– – –) 4th-instar *Ae. aegypti* larvae.

this partially unshaded site would have been higher than that in sites 1 and 3, which were fully shaded. Rainfall was estimated to have diluted the initial dose from 966 mg/liter to 962 ± 30 and 758 ± 20 mg/liter in weeks 1 and 2, respectively. Cumulative debris that fell into the exposed buckets was slight; however, 3 buckets were observed to become contaminated by washing and drinking Indian myna birds (*Acridotheres tristis* L.) and 2 sacred ibis (*Threskiornis molucca* L.). Treated buckets continued to be effective until the week 2 bioassay, when *Ae. aegypti* mortalities decreased to 95.1 ± 0.88%.

Site 3 experienced 9.8 mm of rain on 5 of 14 days. The mean increase in water levels corresponding to week 1 and week 2 bioassays were 1.19 and 1.23 liters, respectively. This was estimated to have diluted the initial dose from 996 mg/liter to 806 ± 18 and 683 ± 11 mg/liter in weeks 1 and 2, respectively. Stormy weather caused a large quantity (3–6 cm depth) of leaf and flower litter to fall into each of the buckets in this site. In addition, cane toads (*Bufo marinus* L.) were rescued from two buckets after an undetermined overnight period. Treated buckets were effective until the week 2 bioassay, when *Ae. aegypti* mortalities decreased to 95.9 ± 1.89%.

Experiments in sites 2 and 3 were terminated after week 2 due to the inadvertent colonization of 500–1,000 (exact numbers were not counted) *Culex quinquefasciatus* (Say) larvae. Each of these buckets also contained 0–5 3rd- to 4th-instar *Ae. aegypti* larvae of undetermined origin.

After 1 month of observation, no larval recruitment from the natural *Cx. quinquefasciatus* or *Ae. aegypti* mosquito populations was detected in the 5 *B.t.i.*-treated disposable plastic containers adjacent to the buckets. These containers, which were positioned in site 3, were diluted and refilled to overflowing by rain several times until they dried out after 1 month. The pot bases filled up, overflowed with rain, and dried out after 3–5 days between rainy days. After the pot bases had initially dried out, an undetermined number of naturally recruited larvae were observed in all 6 pot bases every time they were flooded with rain; however, these larvae never reached full development, because the pot bases dried out on a regular basis.

DISCUSSION

The results of the laboratory hatching study concurred with those of Rozeboom (1934), who observed that bacteria and yeasts greatly enhanced the hatching of mosquito eggs. The reason for this is that eggs older than 20 days require a reduction in dissolved oxygen, which is effected by bacteria and yeasts, as a stimulus for hatching (Gjullin 1941). This is a propitious occurrence since Mossie-Buster application of *B.t.i.* at the dosage used in this study raised target container water levels by 19 ± 1.3%, thus stimulating the hatching of affected dormant eggs. During treatment, the target water was greatly agitated by the spray, and heavy frothing caused by undetermined VectoBac ingredients occurred. These localized disturbances also wet and dislodged eggs oviposited above the water level that might have increased hatch rates. All larvae hatching during this process were exposed to lethal *B.t.i.* doses and were killed. Treated containers cannot be implicated in sustaining local *Ae. aegypti* populations with subsequent rainfall within the period of treatment effectiveness, because many of the quiescent eggs will have already hatched and died under the stimulation provided by *B.t.i.* treatment. Retreatment is necessary where containers are exposed to frequent rainfall and regular yard-watering regimes that render the treatment ineffective.

Residual efficacy of laboratory-treated beakers was similar to results obtained in the field buckets in site 1. The drop in hatch rates in treated beakers in week 3 was expected, but the peak in week 9 cannot be explained with available data. Dissolved oxygen levels (Gjullin et al. 1941) were presumably lower at week 9 than at week 1, which may indicate the presence of another, undetected, stimulant to egg hatching.

Bacillus thuringiensis israelensis treatment in site 1 was far more effective than that in the two exposed sites against *Ae. aegypti* 1st- and 4th-instar larvae. They were also not diluted by rainfall or yard-watering, polluted by plant material, or exposed to sunlight. The treated containers in this site theoretically became more effective over time, since constant water evaporation invariably increased the *B.t.i.* dose. The substantial residual efficacy of the treatment indicates that *B.t.i.* would most probably be very effective in water storage sites that were well protected from environmental factors.

Mortality in treated containers in sites 2 and 3

decreased to 95% 2 wk posttreatment. This reduction in *B.t.i.* efficacy can be attributed to environmental contaminants such as soil particles (Ignoffo et al. 1981, Ramoska et al. 1982, Van Essen and Hembree 1982, Margalit and Bobroglo 1984). Ramoska et al. (1982) suggested that this effect was "mediated by the substrate rather than by the host species" since substrate is ingested by larvae of both *Ae. aegypti* and *Cx. quinquefasciatus.* They concluded that the *B.t.i.* toxin crystals adsorbed to soil particles are deactivated so that they are unable to dissolve in the larval gut. The presence of organic matter also detrimentally affected the efficacy of *B.t.i.,* although to a lesser degree than fine clay particles (Margalit and Bobroglo 1984). Another environmental parameter that greatly affects *B.t.i.* efficacy is sunlight (Ignoffo et al. 1981). Exposure of *B.t.i.*-treated cups to UV radiation was shown to reduce larval mortalities in a 168-h bioassay by 58 ± 19.4%. Another possible contributing factor for the mortality decrease in site 3 was plant debris, which accumulated to a depth of 3–6 cm. This mass of organic material may have created a physical barrier between the larvae and nonsuspended *B.t.i.* crystals.

The presence of large numbers of *Cx. quinquefasciatus* larvae from a wild population may be the result of the introduction of bacterial or organic contaminates by birds that were observed washing their bills and drinking from 3 buckets in site 2, and by cane toads that fell into 2 buckets in site 3.

Garden enthusiasts need not discard their plant pot bases, which permit a less frequent watering regime, if they treat their gardens with the Mossie-Buster regularly. Thoughtful placement of any water-holding containers away from sources of plant debris and sunlight will increase the longevity of Mossie-Buster treatment. Blocked gutters, junkyards, and yards strewn with disposable or other containers that are liable to collect water can also be treated effectively, thereby eliminating the breeding sites that are the most difficult to control. The cost of treatment at the dosage specified is far from prohibitive.

Widespread use of the Mossie-Buster in urban areas with container-breeding mosquitoes would achieve two goals: local authorities would incur less expenditure on *Ae. aegypti* control, and local residents would become more liable and responsible for controlling breeding sites. Empowerment of householders or communities with an appropriate technology to treat mosquito breeding sites can only lead to an improvement in community participation in vector control.

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