Evaluation and Adaptation of Attractive Toxic Sugar Baits For *Culex tarsalis* and *Culex quinquefasciatus* Control In The Coachella Valley, Southern California

Author(s): Whitney A. Qualls, Jodi Scott-Fiorenzano, Gunter C. Müller, Kristopher L. Arheart, John C. Beier, and Rui-De Xue

Source: Journal of the American Mosquito Control Association, 32(4):292-299.
Published By: The American Mosquito Control Association
DOI: http://dx.doi.org/10.2987/16-6589.1
URL: http://www.bioone.org/doi/full/10.2987/16-6589.1

Journal of the American Mosquito Control Association, 32(4):292–299, 2016

EVALUATION AND ADAPTATION OF ATTRACTIVE TOXIC SUGAR BAITS FOR *CULEX TARSALIS* AND *CULEX QUINQUEFASCIATUS* CONTROL IN THE COACHELLA VALLEY, SOUTHERN CALIFORNIA

WHITNEY A. QUALLS,[1] JODI SCOTT-FIORENZANO,[2] GUNTER C. MÜLLER,[3] KRISTOPHER L. ARHEART,[1] JOHN C. BEIER[1] AND RUI-DE XUE[2]

ABSTRACT. The project goal was to determine how a new vector control strategy that targets the sugar-feeding behavior of mosquitoes, attractive toxic sugar baits (ATSBs), can be used to more effectively control West Nile virus (WNV) vectors in the Coachella Valley, California. Three laboratory studies were conducted to determine the utility of this method for control against *Culex quinquefasciatus* and *Culex tarsalis*: 1) efficacy evaluations of 2 formulations of ATSB, microencapsulated garlic oil, and a combination of microencapsulated garlic oil and 1% boric acid; 2) choice assays to determine the attractiveness of ATSB with the microencapsulated garlic oil against attractive sugar baits (ASB; the attractant alone; without toxin) and a 10% sucrose solution; and 3) vegetation efficacy tests on 3 common plant species in the Coachella Valley, *Atriplex lentiformis*, *Tamarix ramosissima*, and *Pluchea sericea*. At 48 h the average mortality for *Cx. quinquefasciatus* was 91% after exposure to ATSB with microencapsulated garlic oil and 99% on ATSB garlic + 1% boric acid solution. *Culex tarsalis* averaged 86% and 91% mortality following the ATSB microencapsulated garlic oil solution and the ATSB garlic + 1% boric acid solution, respectively. Choice assays indicated that the there were differences in preferences between the solutions and between species. Both *Cx. quinquefasciatus* and *Cx. tarsalis* were found to prefer the ASB and ATSB solutions to the 10% sucrose solution. However, when comparing the ASB to ATSB, *Cx. quinquefasciatus* significantly preferred the ASB solution ($t = 3.6$, df = 25, $P = 0.0008$). There were no significant differences in the preference of *Cx. tarsalis* to feed on the ASB or ATSB solutions as indicated in the choice assays ($t = 1.9$, df = 25, $P = 0.07$). Assays indicated that applications of ATSB to the 3 common plants in the Coachella Valley resulted in high mortality in both *Cx. quinquefasciatus* and *Cx. tarsalis*. There were significant differences in the treatments compared to the control ($F = 40.15$, $df_{1,2} = 4{,}72$, $P < 0.001$) but no significant differences among the different plants and ATSB treatments ($F = 1.06$, $df_{1,2} = 4{,}72$, $P = 0.38$). Laboratory findings suggest that ATSB is effective for use against WNV vectors in California. Further evaluations are needed in the field to determine how the environment may impact ATSB applications to influence mosquito mortality and nontarget organisms in arid environments in the United States.

KEY WORDS *Culex tarsalis, Culex quinquefasciatus,* mortality, sugar-feeding

INTRODUCTION

West Nile virus (WNV) was first identified in North America in 1999 and subsequently identified in California in 2003 (Reisen et al. 2004). West Nile virus continues to present a problem to mosquito abatement districts in California with a noticeable increase in 2015 with 782 cases and 53 deaths (California.Gov 2014). Specifically, in the Coachella Valley, the dry desert biome combined with overirrigation provides the ideal habitat for the production of the main WNV vectors, *Culex quinquefasciatus* Say and *Culex tarsalis* Coq.

The stochastic nature of WNV amplification has resulted in a wide range of outcomes as demonstrated by the sporadic epidemics throughout the USA over the last 17 years. Complex spatial and temporal relationships among temperature, rainfall, and underlying geographically variable ecologic factors which promote WNV transmission have historically been difficult to sort out (Reisen 2010). This has resulted in the lack of preparedness for timely WNV control measures. Current control measures for WNV generally require ground- or aerial-based broadcast applications at peak vector activity. This can be problematic as the peak activity time of many of the *Culex* vectors is during dusk and dawn, which limits these types of applications due to human behavior. Even though these types of applications have controlled and reduced WNV transmission (Carney et al. 2008, Lothrop et al. 2008, Macedo et al. 2010), control failure due to insecticide resistance has been a reoccurring theme for abatement districts. In addition, larval control targeting WNV vector development in storm drains and sewage ponds is effective but very manpower intensive. These challenges highlight that new methods of *Culex* control are needed.

One new method of control is through the use of attractive toxic sugar baits (ATSB). This method works by using the drive of both male and female

[1] University of Miami Miller School of Medicine, Department of Public Health Sciences, 1120 NW 14th Street, Miami, FL 33136.

[2] Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092.

[3] Hebrew University, Department of Parasitology, The Kuvin Center for Study of Infectious and Tropical Diseases, Hadassah, Medical School, P.O.B. 12272, Jerusalem 91120, Israel.

mosquitoes for daily sugar meals (Yuval 1992, Foster 1995). As mosquitoes search for a sugar source, the highly attractive combination of juices competes with natural sugar and lures the mosquitoes to feed on the ATSB with mortality occurring after ingesting a low dose of insecticide that is contained in the bait (Xue et al. 2013). Because ATSB targets the sugar-seeking behavior of mosquitoes and uses an oral, instead of a contact toxin, it circumvents problems traditionally associated with the indiscriminate use of contact insecticides (Enayati and Hemingway 2010) and allows for applications to be made to substrate instead of requiring direct contact with the mosquito at the time of application.

Previously successful field trials in arid environments of Africa and Asia (the Middle East) controlling populations of *Anopheles* and *Culex* spp. with ATSB demonstrate that this approach may be useful to successfully control WNV vectors in the arid environments in North America (Müller and Schlein 2008, Müller et al. 2010 a,c). Preliminary studies in storm drain systems and cisterns and wells in Florida using crude ATSB formulations demonstrated that *Cx. quinquefasciatus* would feed on the ATSB and die following the ingestion of the toxin (Müller et al. 2010b, Qualls et al. 2012). To determine the utility of ATSB for WNV vector control we collaborated with the Coachella Valley Mosquito and Vector Control District (CVMVCD), Coachella Valley, California, to conduct laboratory studies that would 1) determine the efficacy of 2 formulations of ATSB against WNV vectors; 2) compare the attractiveness of ATSB, attractive sugar baits (ASB; no toxin), and a 10% sucrose solution to the *Culex* vectors; and 3) determine the efficacy of the ATSB application to common vegetation in the Coachella Valley.

MATERIALS AND METHODS

Mosquitoes

Culex tarsalis and *Cx. quinquefasciatus* were reared and maintained in the laboratory under controlled temperature (27°C) and relative humidity (50%), with a photoperiod of 14:10 (L:D) h. Larvae were fed a 3:1:1:1 ration of ground alfalfa, brewer's yeast, ground fish food flakes, and dried defatted liver powder, respectively, per day. Pupae were removed from larval pans and transferred to cages and given ad libitum acces to a 10% sugar water solution. Five- to 7-day-old male and female mosquitoes were used in the tests. Sugar water was removed from 12 h prior to commencement of tests. Water was not removed.

Material

The commercially available All Clear Naturals ATSB® (active ingredient 0.8% microencapsulated garlic oil; ADAPCO, Sandford, FL) was evaluated. Formulations of the product were made following manufacturer recommendations. Additionally, the same formulation was prepared but with the addition of 1% boric acid (Fisher Scientific, Asheville, NC) for evaluation. The ASB without the active ingredient was provided by the producer Westham, Ltd. (Tel-Aviv, Israel) as the control solution for choice and vegetation assays.

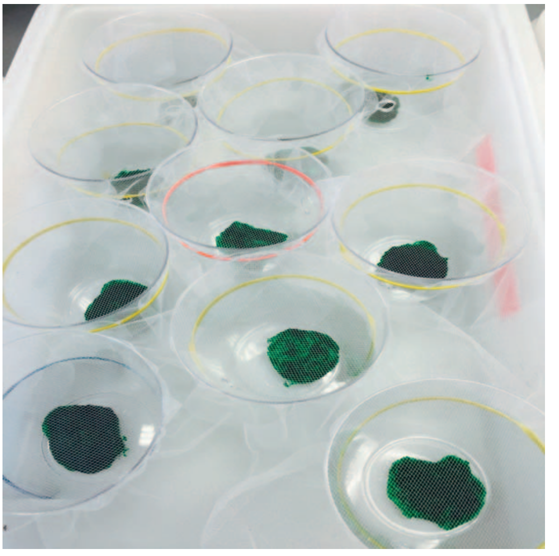

Fig. 1. Bioassay cups containing a color-dyed ATSB solution.

Efficacy evaluation of ATSB

The formulations described above were evaluated against laboratory-reared *Cx. quinquefasciatus* and *Cx. tarsalis* with 10% sucrose solution serving as the control. The ATSB solutions were prepared in a 1:1 (v/v) mixture with water. Each solution evaluated had a food color added (0.5% v/v) to visualize mosquito feeding on the solutions (food-coloring dye; McCormick and Company, Inc., Sparks, MD).

Following solution preparation, 5–10 mosquitoes, 5–7 days old, male and females, were aspirated into 100-ml Solo® cups with a screen mesh on the top that contained 1 cotton ball soaked in the desired solution (Fig. 1). The cups containing the mosquitoes and treatment solutions were placed in the insectary and mortality was monitored for 48 h. A total of 10 replications per treatment were conducted on 3 separate occasions.

ATSB choice assay

Choice tests were conducted with ATSB, ASB, and 10% sucrose solution on both species of *Culex*. To determine a choice between test solutions, choice test chambers were fabricated from clear plastic tubes that were enclosed with lids in each end (diam 4 cm × length 30 cm) (Paper Mart, Orange, CA; Fig. 2b). For evaluation purposes, a wood-burning tool was used to burn a 1-cm hole into the center of the

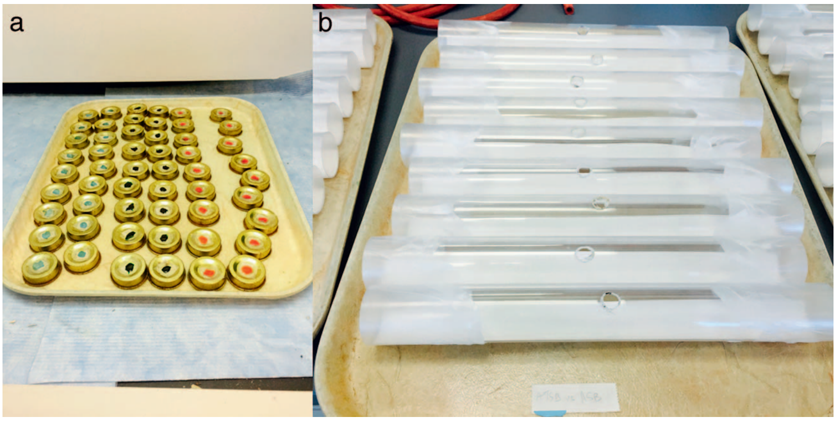

Fig. 2. (a) Choice chamber lids containing the 50-µl aliquots of each formulation and (b) choice chamber design.

tube so that a mosquito could be aspirated into the tube. The tubes were lined on the bottom, adjacent to the hole, with lengthwise bent card stock (3 cm × 30 cm) (Pacon Corporation, Appleton, WI). The card stock was adhered to the outside of the arena with masking tape. The tube ends prior to each experiment and placement of the lids were covered with Parafilm (2 cm × 2 cm). Following this procedure, the tubes were considered experimental arenas. Test solutions were prepared as previously mentioned and included a separate food coloring per each solution to determine solution choice. Food coloring was added to the solutions to observe feeding on the different solutions. Following the material preparation, 50-µl aliquots of each formulation were pipetted onto abraded acetate paper cut into 2-cm × 2-cm squares, which were fastened with double-sided masking tape onto the inner side of the lids that enclosed the choice chamber (Fig. 2a). Formulations were allowed to dry for a minimum of 3 h before being placed onto the tube arenas.

One 5–7-day-old female mosquito was individually aspirated into the 1-cm hole in the tube, which was then stoppered with a piece of cotton. The cotton stopper prevented the mosquito from escaping. Mosquitoes were allowed to acclimate to the arenas for a minimum of 1 h.

Choice assays were carried out for 48 h. After the assay both dead and surviving mosquitoes in the choice chambers were placed into a –20°C freezer for up to 3 h. After removal from the freezer, the card-stock liner at the bottom of the arena was carefully removed and investigated under a dissecting scope for the presence of dye. Dye was counted as green, red, blue, or a combination of the colors, indicating that the mosquito ingested both choices given in the choice assay. The combination colors were divided in half and distributed evenly to both bait choices. Individual mosquito fecal droppings were identified by density of color on the card stock. The dyed fecal droplet displayed heavy dying with limited radiation/seepage of color from the initial fecal droplet. Given the length of the choice test chamber, there were very few instances where the fecal droplets combined into a massive color blot. In the instances that this occurred, the fecal droplet density was determined by the density of color spots within the color blot. A total of 9 replications per choice assay were conducted on 3 separate occasions.

ATSB efficacy on common species of native plants

Three species of vegetation common to the Coachella Valley, *Atriplex lentiformis* (S. Wats) (common name, saltbrush; Family Amaranthaceae), *Tamarix ramosissima* Ledeb. (common name, saltcedar; Family Tamaricaceae), and *Pluchea sericea* Nutt. (common name, arrowweed; Family Asteraceae), were selected for ATSB evaluations against both species of *Culex* (Fig. 3). Plant clippings (~7.6 cm) were removed from an area where no pesticide applications had been made. The clippings were brought back to the laboratory and treated with the different ATSB formulations (ATSB and ATSB + 1% boric acid) and ASB. The solutions were prepared at a 1:1 label rate and a 700-ml squirt bottle was calibrated for each solution prior to application. Plant clippings were allowed to dry for 3 h prior to being placed in a glass bottle for evaluation. One treated plant clipping was placed individually into the glass containers. Five to 7 5–7-day-old mosquitoes were aspirated into each glass container housing plant clippings. Mortality was recorded at 72 h. For each treatment, vegetation type, and species there were 3 clippings evaluated on 3 separate occasions.

Statistical analysis

The data for this study were counts of dead mosquitos. There were two mosquito species and three types of plants that received three different treatments. We used a generalized linear model to fit a Poisson regression for a 3x3 factorial design for each species. The model included main effects for plant type and treatment plus the two-way interaction. A natural log link was used, and an offset of the

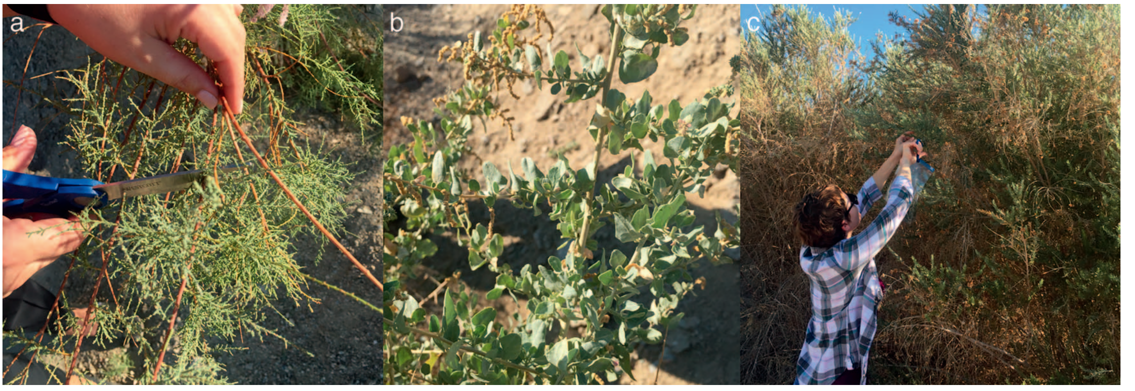

Fig. 3. The 3 plants commonly found in the Coachella Valley, California, utilized in the laboratory bioassays: (a) *Tamarix ramosissima*, (b) *Atriplex lentiformis*, and (c) *Pluchea sericea*.

natural log of the number of mosquitos observed for each case was included to produce mean percents and standard errors. Back-transformed model means and standard errors were reported with the results of planned comparisons of treatments within each plant type. The 0.05 alpha level was used to determine statsitical significance. SAS 9.3 (SAS Institute, Inc.; Cary, NC) was used for all analyses.

RESULTS

Efficacy evaluation of ATSB

Assays indicated that the microencapsulated garlic and a 1% boric acid solutions were ingested and resulted in high mortality at 48 h postexposure in both *Cx. quinquefasciatus* (Fig. 4a) and *Cx. tarsalis* (Fig. 4b). Mortality was a result of feeding on the respective solutions since visual inspection of the mosquitoes during the assays revealed stained abdomens, an indicator of feeding on the solutions.

The mean mortality for *Cx. quinquefasciatus* following ATSB with microencapsulated garlic oil was 91%, and 99% after exposure to the ATSB + 1% boric acid solution. Control mortality for these experiments averaged 9%. *Culex quinquefasciatus* female mortality averaged 91.5% (SE 9.8) after exposure to the ATSB with microencapsulated garlic oil solution and 99% (SE 10.2) after exposure to the ATSB + 1% boric acid solution. There were no significant differences between the 2 ATSB treatments ($P = 0.60$) but a significant difference between

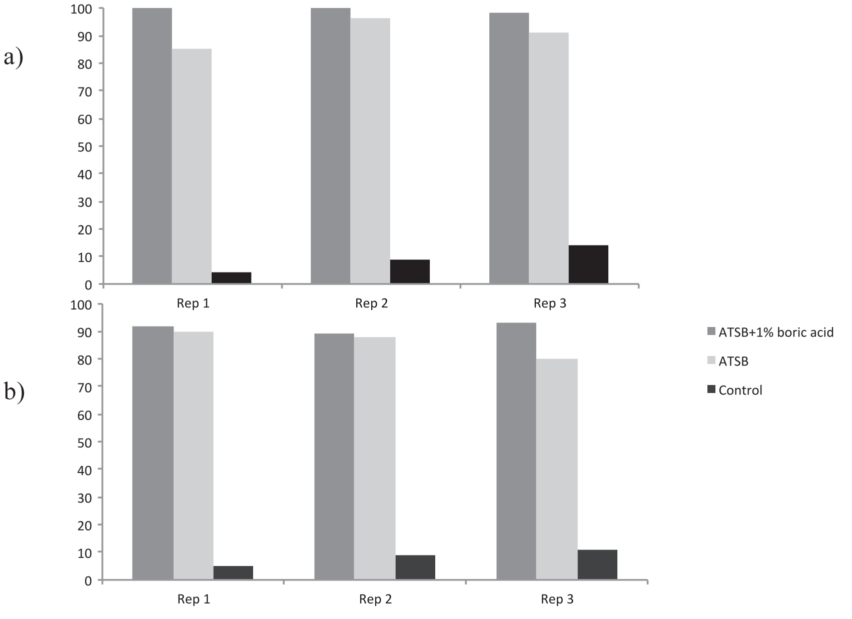

Fig. 4. Average percentage of mortality for each replication of the evaluation of the different ATSB treatments and control against (a) *Culex quinquefasciatus* and (b) *Culex tarsalis*.

Table 1. Mean percentage of mortality (%) and standard error (SE) for *Culex quinquefasciatus* following application of control, ATSB[1] microencapsulated garlic oil, and ATSB + 1% boric acid solution to the 3 common vegetation types in the Coachella Valley, California.

Treatment	*Atriplex lentiformis*		*Tamarix ramosissima*		*Pluchea sericea*	
	% mortality	SE	% mortality	SE	% mortality	SE
Control	18.2 a[2]	4.9	14.7 a	4.4	20.0 a	5.0
ATSB microencapsulated garlic solution	81.9 bA[3]	10.7	83.8 bA	10.6	54.8 bB	8.7
ATSB garlic + 1% boric acid solution	83.1 b	10.8	81.9 b	10.7	81.8 b	11.1

[1] ATSB, attractive toxic sugar bait.
[2] Means within each column followed by a different lowercase letter are significantly different at $P < 0.05$ (ANOVA).
[3] Uppercase letters represent significant differences between rows $P < 0.05$ (Poisson Regression).

the 2 treatments and control ($P < 0.001$). Male mortality averaged 52.1% (SE 8.4) after exposure to the ATSB with microencapsulated garlic oil solution and 100% (SE 13.5) after exposure to the ATSB + 1% boric acid solution. There were significant differences observed between treatments. The ATSB + 1% boric acid solution performed significantly better for males compared to the microencapsulated garlic oil ($P = 0.003$) and control ($P < 0.001$). The ATSB with microencapsulated garlic oil resulted in significantly more mortality compared to the control solution ($P = 0.014$).

Culex tarsalis averaged 86% and 91% mortality following exposure to the ATSB microencapsulated garlic oil solution and the ATSB + 1% boric acid solution, respectively. Control mortality averaged 8% for these experiments. Culex *tarsalis* female mortality averaged 89.3% (SE 9.3) after exposure to the ATSB with microencapsulated garlic oil solution and 92.5% (SE 10.8) after exposure to the ATSB + 1% boric acid solution. There were no significant differences between treatments ($P = 0.82$) but a significant difference between the 2 treatments and control ($P < 0.001$). Male mortality averaged 91.3% (SE 10.8) after exposure to the ATSB with microencapsulated garlic oil solution and 94.2% (SE 10.5) after exposure to the ATSB 1% boric acid solution. There were no significant differences between treatments ($P = 0.83$) but a significant difference between the 2 treatments and control ($P < 0.001$).

ATSB choice assay

Choice assays indicated that the there were differences in preferences between the solutions and species. Both *Cx. quinquefasciatus* and *Cx. tarsalis* were found to prefer the ASB and ATSB solutions to the 10% sucrose solution. However, when comparing ASB to ATSB, *Cx. quinquefasciatus* significantly preferred the ASB solution ($t = 3.6$, df = 25, $P = 0.0008$). *Culex quinquefasciatus* was 3 times more likely to feed on the ASB solution compared to the ATSB solution. However, *Cx. quinquefasciatus* was 1 time more likely to feed on the ASB ($P = 0.02$) and 2 times more likely to feed on the ATSB when compared to the 10% sucrose solution ($P = 0.003$). For *Cx. tarsalis* there were no significant differences in the preference to feed on the ASB or ATSB solutions as indicated in the choice assays ($t = 1.9$, df = 25, $P = 0.07$). *Culex tarsalis* was 2.5 times more likely to feed on the ASB ($P = 0.004$) and 1.5 times more likely to feed on the ATSB when compared to the 10% sucrose solution ($P = 0.05$).

ATSB efficacy on common species of native plants

Assays indicated that applications of a microencapsulated garlic solution and a 1% boric acid solution to 3 common plants in the Coachella Valley, *A. lentiformis, T. ramosissima,* and *P. sericea*, resulted in high mortality in both *Cx. quinquefasciatus* and *Cx. tarsalis*. There were significant differences in the treatments compared to the control ($F = 40.15$, $df_{1,2} = 4,72$, $P < 0.001$) but no significant differences among the different plants and ATSB treatments ($F = 1.06$, $df_{1,2} = 4,72$, $P = 0.38$). The average mortality for *Cx. quinquefasciatus* and *Cx. tarsalis* following ATSB application to the 3 different plants with microencapsulated garlic oil, ATSB + 1% boric acid solution, and control is shown in Tables 1 and 2.

DISCUSSION

Laboratory results demonstrate that both *Cx. tarsalis* and *Cx. quinquefasciatus* are attracted to the ASB and ATSB formulated with garlic oil and will readily feed on the ATSB resulting in high mortality of both species. Mosquito mortality data suggest that the addition of 1% boric acid solution to the ATSB with microencapsulated garlic oil will increase mortality for both genders and species of mosquitoes evaluated. In addition, plant type did not affect ATSB mortality on the mosquitoes evaluated.

Importantly, the choice assay results suggest that the dose of the microencapsulated garlic oil in the ATSB did not repel the mosquitoes, as both the ASB and the ATSB with the garlic oil were more attractive to both species than a 10% sucrose solution. Shin et al. (2011) showed that certain active ingredients could repel *Culex* spp. when incorporated into ASBs. This current study, as well as field studies conducted by Revay et al. (2015) and Junnila et al. (2015), indicate that ATSBs with microencapsulated garlic

Table 2. Mean percentage of mortality (%) and standard error (SE) for *Culex tarsalis* following application of control, ATSB[1] microencapsulated garlic oil, and ATSB + 1% boric acid solution to the 3 common vegetation types in the Coachella Valley, California.

Treatment	*Atriplex lentiformis*		*Tamarix ramosissima*		*Pluchea sericea*	
	% mortality	SE	% mortality	SE	% mortality	SE
Control	22.1 a[2]	5.4	18.8 a	4.8	18.4 a	4.9
ATSB microencapsulated garlic solution	96.0 b	11.3	90.0 b	10.6	75.1 b	10.3
ATSB garlic + 1% boric acid solution	84.4 b	10.5	79.2 b	10.1	83.1 b	10.8

[1] ATSB, attractive toxic sugar bait.
[2] Means within each column followed by a different letter are significantly different at $P < 0.05$ (ANOVA).

oil are effective at attracting and killing vector mosquito species.

Extensive mosquito control is performed in the rural southeastern section of the Coachella Valley, which is predominantly composed of saline soils and shrubby desert plants. Barrier treatments using pyrethroids applied to the 3 common species of local vegetation have proven to be an effective method of WNV vector control in this region. The plants evaluated in this study are often found growing in monoculture clusters and the dominant species varies with location. In line with previous studies conducted at CVMVCD our findings verified that ATSB applied to any of the 3 common plants are effective in killing WNV vectors, suggesting that this method could supplement traditional pyrethroid applications as a barrier treatment. Rotation with ATSB and pyrethroid barrier applications has the potential to reduce the development of resistance in *Culex* populations, an issue in agricultural areas of the Coachella Valley.

The efficacy of spraying toxic sugar baits, both attractive and unattractive, on plants to control several species of adult mosquitoes has been extensively studied over the past decade (Xue and Barnard 2003; Xue et al. 2006, 2008, 2011, 2013; Müller et al. 2010a, 2010c). Other field studies have also demonstrated the efficacy of using 1% boric acid as the active ingredient against *Culex, Anopheles*, and *Aedes* spp. (Müller et al. 2010a, 2010c; Naranjo et al. 2013; Qualls 2015); however, these trials were not with the ATSB garlic oil formulation.

Attractive toxic sugar bait has been evaluated as a method for many years, utilizing homemade blends of ASBs with the addition of toxins. The move to a commercialized production line of ATSBs increases the stability and residual action of the attractive properties of the ATSBs. Two recent studies have demonstrated residual efficacy over 25 days after application of ATSB with microencapsulated garlic oil against *Ae. albopictus* (Skuse) (27 days control) and *An. sergentii* (Theobald) (34 days control) (Revey et al. 2015, Junnila et al. 2105). Further field studies are needed to determine the stability and residual activity of the commercialized ATSB product.

Very few laboratory trials have been reported for ATSB evaluations with boric acid (Xue et al. 2011, Stewart et al. 2013, Hossain et al. 2014) and none with garlic oil. Xue and Barnard (2003) were the first to report that presenting boric acid in nonattractive sugar meals would result in high mosquito mortality in the field with a significant reduction in landing-rate counts of *Ae. albopictus*, *An. quadrimaculatus* Say, and *Cx. nigripalpus* Theobald. Following this demonstration of boric acid working as a gut toxin, laboratory trials of a 1% boric acid solution in a 5% sucrose solution resulted in 98% mortality in *Ae. albopictus* (Xue et al. 2011). Hossain et al. (2014) found 52% mortality in *Ae. taeniorhynchus* (Wiedemann) in laboratory studies with a 1% boric acid solution mixed in 5% sucrose. Stewart et al. (2013) found in laboratory trials that mixing an ATSB with 1% boric acid resulted in less mortality than in field trials utilizing the same formulation. In the laboratory trials the ATSB with 1% boric acid resulted in >80% control with *An. gambiae* (Giles) but ~40% control for *An. arabiensis* Patton and *Cx. quinquefasciatus*. Our study results are similar to the findings of Xue et al (2011) with 95% mortality following feeding of the ATSB garlic formulation with the addition of 1% boric acid for both *Culex* species evaluated.

Differences in species, different physiological stages, the addition of an attractive sugar bait, and access to water may all impact the differences observed in the described laboratory trials. Field trials of ATSBs have demonstrated significant reductions in mosquito populations (Müller and Schlein 2008; Müller et al. 2010a, 2010c; Khallaayoune et al. 2013; Qualls et al. 2014). Under field conditions mosquitoes may have a greater need for energy (Healy and Jepson 1988, Yuval et al. 1994, Foster 1995, Fernandes and Briegel 2005) resulting in larger sugar meals increasing the likelihood of ingesting the lethal dose presented in the ATSBs. Additionally, the increased stress on field populations may reduce the lethal dose required in the field compared to laboratory mosquito populations further identifying differences observed in field versus laboratory trials.

The demand for new techniques and methods for mosquito and vector control is constantly increasing, as mosquito-borne pathogens continue to impact global public health (Xue and Barnard 2003, Xue et al. 2006). Laboratory evaluations of active ingredients that can be used in ingestible formulations of ATSBs are of paramount importance to determine those that will be effective in the field. We present the first laboratory trials that demonstrate the efficacy of garlic oil and the combination of garlic oil and boric acid mixed in an ASB resulting in significant mortality of the important WNV vectors in California.

ACKNOWLEDGMENTS

Special thanks are due to the Coachella Valley Mosquito and Vector Control District in Indio, California. Greg White, Christopher Cavanaugh, and Melissa Snelling were vital partners in this project and their support is also highly regarded. The research reported in this publication was supported by a grant from the Coachella Valley Mosquito and Vector Control District.

REFERENCES CITED

California. Gov. 2015. *2015 West Nile virus activity by County*. [Internet] [accessed November 27, 2016]. Available from: http://westnile.ca.gov/.

Carney RM, Husted S, Jean C, Glaser C, Kramer V. 2008. Efficacy of aerial spraying of mosquito adulticide in reducing incidence of West Nile virus, California, 2005. *Emerg Infect Dis* 14:747–54.

Enayati A, Hemingway J. 2010. Malaria management: past, present, and future. *Annu Rev Entomol* 55:569–591.

Fernandes L, Briegel H. 2005. Reproductive physiology of *Anopheles gambiae* and *Anopheles atroparvus*. *J Vector Ecol* 30:11–26.

Foster WA. 1995. Mosquito sugar-feeding and reproductive energetics. *Annual Rev Entomol* 40:443–474.

Healy TP, Jepson PC. 1988. The location of floral nectar sources by mosquitoes: the long-range response of *Anopheles arabiensis* Patton (Diptera: Culicidae) to *Achillea millefolium* flowers and isolated floral odour. *Bull Entomol Res* 78:651–657.

Hossain TT, Fulcher A, Davidson C, Beier JC, Xue RD. 2014. Evaluation of boric acid sprayed on plants against salt marsh mosquitoes, *Aedes taeniorhynchus* (Diptera: Culicidae). *Fla Entomol* 97:1865–1868.

Junnila A, Revay EE, Müller GC, Kravchenko V, Qualls WA, Xue RD, Allan SA, Beier JC, Schlein Y. 2015. Efficacy of attractive toxic sugar bait (ATSB) against *Aedes albopictus* with garlic oil encapsulated in beta-cyclodextrin as the active ingredient. *Acta Trop* 152:195–200.

Khallaayoune K, Qualls WA, Revay EE, Allan SA, Arheart KL, Kravchenko VD, Xue RD, Schlein Y, Beier JC, Müller GC. 2013. Attractive toxic sugar bait: control of mosquitoes with the low-risk active ingredient dinotefuran and potential impacts on non-target organisms in Morocco. *Environ Entomol* 42:1040–1045.

Lothrop HD, Lothrop BB, Gomsi DE, Reisen WK. 2008. Intensive early season adulticide applications decrease arbovirus transmission throughout the Coachella Valley, Riverside County, California. *Vector Borne Zoonotic Dis* 8:475–489.

Macedo PA, Schleier JJ III, Reed M, Kelley K, Goodman GW, Brown DA, Peterson RK. 2010. Evaluation of efficacy and human health risk of aerial ultra-low volume applications of pyrethrins and piperonyl butoxide for adult mosquito management in response toWest Nile virus activity in Sacramento County, California. *J Am Mosq Control Assoc* 26:57–66.

Müller GC, Beier JC, Traore SF, Toure MB, Traore MM, Bah S, Doumbia S, Schlein Y. 2010a. Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa. *Malaria J* 9:210.

Müller GC, Junnila A, Qualls WA, Revay EE, Kline DL, Allan S, Schlein Y, Xue RD. 2010b. Control of *Culex quinquefasciatus* in storm drain system in Florida using attractive toxic sugar baits. *Med Vet Entomol* 24:346–351.

Müller GC, Junnila A, Schlein Y. 2010c. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval habitats. *J Med Entomol* 47:63–66.

Müller GC, Schlein Y. 2008. Efficacy of toxic sugar baits against cistern-dwelling *Anopheles claviger*. *Trans R Soc Trop Med* 102:147–149.

Naranjo DP, Qualls WA, Müller GC, Samson DM, Roque D, Alimi T, Arheart KL, Beier JC, Xue RD. 2013. Evaluation of boric acid baits against *Aedes albopictus* (Diptera: Culicidae) in tropical environments. *Parasitol Res* 112:1583–1587.

Qualls WA, Müller GC, Revay EE, Allan SA, Arheart KL, Beier JC, Smith ML, Scott JM, Kravchenko VD, Hausmann A, Yefremova ZA, Xue RD. 2014. Evaluation of attractive toxic sugar bait (ATSB)-barrier for control of vector and nuisance mosquitoes and its effects on non-target organisms in sub-tropical environment in Florida. *Acta Trop* 131:104–110.

Qualls WA, Müller GC, Traore SF, Traore MM, Arheart KL, Doumbia S, Schlein Y, Kravchenko VD, Xue RD, Beier JC. 2015. Indoor use of attractive toxic sugar bait (ATSB) to effectively control malaria vectors in Mali, West Africa. *Malar J* 40:46–58.

Qualls WA, Xue RD, Revay EE, Allan SA, Müller GC. 2012. Implications for operational control of adult mosquito production in cisterns and wells in St. Augustine, FL using attractive sugar baits. *Acta Trop* 124:158–161.

Reisen W, Lothrop H, Chiles R, Madon M, Cossen C, Woods L, Husted S, Kramer V, Edman J. 2004. West Nile virus in California. *Emerg Infect Dis* 10:1369–1378.

Reisen WK. Landscape epidemiology of vector-borne diseases. 2010. *Annu Rev Entomol* 55:461–483.

Revay EE, Schlein Y, Tsabari O, Kravchenko V, Qualls W, Xue RD, Beier JC, Traore SF, Doumbia S, Hausmann A, Müller GC. 2015. Formulation of attractive toxic sugar bait (ATSB) with safe EPA-exempt substances significantly diminishes the *Anopheles sergentii* population in desert oasis. *Acta Trop* 150:29–34.

Shin E, Park C, Ahn YJ, Lee DK, Chang KS. 2011. Insecticidal and repellent activities of insecticide-sucrose solutions to *Culex pipiens molestus* (Diptera: Culicidae) under laboratory and field conditions. *Pest Manag Sci* 67:665–671.

Stewart ZP, Oxborough RM, Tungu PK, Kirby MJ, Rowland MW, Irish SR. 2013. Indoor applications of attractive toxic sugar bait (ATSB) in combination with mosquito nets for control of pyrethroid-resistant mosquitoes. *PLos ONE* 8:e84168. doi:10.1371/journal.pone.0084168.

Xue RD, Ali A, Kline DL, Barnard DR. 2008. Field evaluation of boric acid-and fipronil-based bait stations against adult mosquitoes. *J Am Mosq Control Assoc* 25:415–418.

Xue RD, Barnard DR. 2003. Boric acid kills adult mosquitoes (Diptera: Culicidae). *J Econ Entomol* 96:1559–1562.

Xue RD, Kline DL, Ali A, Barnard DR. 2006. Application of boric acid baits to plant foliage for adult mosquito control. *J Am Mosq Control Assoc* 22:497–500.

Xue RD, Müller GC, Kline DL, Barnard DR. 2011. Effect of application rate and persistence of boric acid sugar baits applied to plants for control of *Aedes albopictus*. *J Am Mosq Control Assoc* 27:56–60.

Xue RD, Müller GC, Qualls WA, Smith ML, Scott JM, Lear J, Cope SE. 2013. Attractive targeted sugar baits: field evaluations and potential for use in mosquito control. *Wing Beats* Spring:13–18.

Yuval B. 1992 The other habit: sugar-feeding by mosquitoes. *Bull Soc Vector Ecol* 17:150–156.

Yuval B, Holliday-Hanson M, Washino RK. 1994. Energy budget of swarming male mosquitoes. *Ecol Entomol* 19:74–78.