INTRODUCTION

*Aedes aegypti* (L.) is an anthropophilic mosquito species that is originally from Africa but found worldwide (Brown et al. 2011, 2014) and in many portions of the USA, where its range appears to be expanding (Kraemer et al. 2015, CDC 2018). This mosquito species has been incriminated as the primary disease vector of several viruses of human importance. For example, as of 2019 approximately a half million individuals were estimated to develop the severe form of dengue and require hospitalization annually, with 2.5% of those cases resulting in death (Shepard et al. 2016, Powell 2018, WHO 2019). Control of disease vectors has been primarily focused on chemical applications of insecticides. However, with the development of insecticide resistance and a limited pool of viable insecticide chemistries with only a few modes of action, chemical controls are failing (Liu 2015, WHO 2018, Kandel et al. 2019). Therefore, it is imperative that novel control chemistries be discovered to assist in the continued fight against disease vectors. One avenue of research that has seen an increase in popularity is with botanical compounds. Several studies have looked at botanical compounds, such as plant essential oils as synergists, to enhance the toxicity of current insecticides, and as insecticides themselves (Isman 2000, 2006; Tong and Bloomquist 2013; Dias and Moraes 2014; Gross et al. 2017; Norris et al. 2019). There are several advantages that botanical insecticides would have in terms of being successful insecticide candidates, and for total cost of development and registration. Reports have indicated that various botanical compounds have several different modes of action and molecular target sites than conventional pesticides do (Enan 2001, 2005; Parnas et al. 2009; Tong and Coats 2010; Tong et al. 2013).

Also, many botanical compounds are nontoxic to mammals, in addition to being volatile, reducing environmental retention times (Isman et al. 2011).

Methyl benzoate is a floral volatile organic compound that has been registered in the USA and European Union as a food-grade flavor additive (Feng and Zhang 2017). It, along with several analogs, has been shown to have insecticidal and repellent properties against several different species of insects. Topically, it has shown to be active against the red imported fire ant *Solenopsis invicta* (Buren), spotted wing drosophila *Drosophila suzukii* (Matsumura), the brown marmorated stink bug *Hyalomorpha halys* (Stål), the diamondback moth *Plutella xylostella* (L.), and the tobacco hornworm *Manduca sexta* (L.) (Feng and Zhang 2017, Zhang and Feng 2017). It has also been shown to have fumigant activity against several stored product pests, the red imported fire ant, and the common bed bug *Cimex lectularius* (L.) (Chen et al. 2019, Larson et al. 2020, Morrison et al. 2019). Since various species of insects are susceptible to methyl benzoate, it was our goal to determine the efficacy of methyl benzoate and several of its analogs against adult *Ae. aegypti* females.

MATERIALS AND METHODS

Insects and rearing

*Aedes aegypti* eggs were obtained from the USDA–ARS Center for Medical, Agricultural, and Veterinary Entomology, in Gainesville, FL. Larvae were reared within a Percival environmental chamber (Percival Scientific, Inc., Perry, IA) at 27°C, 70% RH, with a photoperiod of 12 h light and 12 h dark. Larvae were fed ground Tetramin® fish food (Spectrum Brand Pet, LLC, Blacksburg, VA). Upon emergence, adult mosquitoes were fed a 10% sucrose solution and maintained under the same conditions as were the larvae.

Compounds

Methyl benzoate, acetophenone, butyl benzoate, hexyl benzoate, ethyl benzoate, methyl 2-methyl-
Mortality was recorded 24 h after the treatment. And were given a 10% sugar solution on cotton balls. Transferred to plastic containers covered with netting replicate as a control. After dosing, mosquitoes were times. A solvent-only treatment was included in each mosquito each and replicated a minimum of 3 yields 0–100% mortality) were applied to 10 pounds, 5 different doses of each compound (that were purchased from sigma-aldrich (St. Louis, MO). Methyl 3-methoxybenzene was purchased from TCI America (Portland, OR). Acetone (Honeywell Burdick and Jackson16, Fisher Scientific, Morristown, NJ) was used as the solvent for dilution for all concentrations of test compounds, and as a control.

### Adult bioassay protocols

Adult Ae. aegypti females were anesthetized using ice, and 0.2 µL of compound (dissolved into acetone) was applied to the pronotum, using a handheld PB600-1 Hamilton repeating syringe (Hamilton Company, Reno, NV). For the determination of the median lethal dose (LD50) for each of the compounds, 5 different doses of each compound (that yielded 0–100% mortality) were applied to 10 mosquitoes each and replicated a minimum of 3 times. A solvent-only treatment was included in each replicate as a control. After dosing, mosquitoes were transferred to plastic containers covered with netting and were given a 10% sugar solution on cotton balls. Mortality was recorded 24 h after the treatment.

### Data analyses

For all toxicity bioassays, control mortality (≥10%) was corrected using Abbott’s formula (Abbott 1925). All LD50 values were calculated using probit analysis in PoloPlus software (LeOra Software Co., Petaluma, CA). The LD50 values were compared utilizing the formula for comparing lethal dose ratios found within Robertson et al. (2017). Regression analysis was performed in Microsoft Excel software (Microsoft, Redmond, WA).

### RESULTS

The LD50 values for the compounds tested against adult female Ae. aegypti are given in Table 1. There was an approximate 14-fold change between the most effective compound and least effective compound screened. The most effective compound was butyl benzoate with an LD50 of 5.1 µg per adult female. n-Pentyl benzoate was the next most effective compound with an estimated LD50 of 7.34 µg per adult female. The LD50 of vinyl benzoate of 10.7 µg per adult female was not significantly different from n-pentyl benzoate. This is followed by methyl 3-methoxybenzene, acetophenone, propyl benzoate, methyl 2-chlorobenzene, methyl 2-methylbenzene, hexyl benzoate, and ethyl benzoate with LD50 values of 14.7, 18.4, 19.2, 29.2, 36.4, 45.6, 50.9, and 71 µg per adult female, respectively. Figure 1 shows that there was no correlation (R2 = 0.1283) between alkyl chain length on the alcohol portion of the methyl benzoate analogs.

### DISCUSSION

Aedes aegypti has been incriminated as the major transmission vector of several viruses that are responsible for yellow fever, dengue, chikungunya, and Zika infections in humans (Powell 2018). The estimated economic losses worldwide due to dengue, alone, is more than US$8 billion annually (Shepard et al. 2016). In the USA, common adulticides for mosquito control include organophosphate and pyrethroid insecticides (USEPA 2017), though reports of pyrethroid resistance in Ae. aegypti are emerging (Kandel et al. 2019). Methyl benzoate has been shown in laboratory tests to be toxic to several economically important agricultural pests, and to the common bed bug (Feng and Zhang 2017, Zhang and Feng 2017, Chen et al. 2019, Larson et al. 2020, Morrison et al. 2019). This current study demonstrates that methyl benzoate and its more active analogs may have a role in adult mosquito control. Feng and Zhang (2017) found that contact toxicity on developing and emerging spotted wing drosophila was negatively correlated with alkyl chain length, while Chen et al. (2019) reported that topical toxicity was positively correlated with alkyl chain length for the red imported fire ant. The current study found that
there was no correlation between toxicity and the alkyl chain length on the alcohol. This could be the result of differences between the species of arthropods tested; however, further studies may be warranted to explore further alkyl chain relevance to toxicity.

While formulations would need to be explored and developed for use of these compounds within vector control, the incorporation of environmentally friendly active ingredients into a program directed at mosquito control would reduce our reliance on synthetic pesticides. Volatile organic compounds evidently can be used to modify vector behavior in plant-feeding insects (Aksenov et al. 2014), though the potential for use with medically important arthropods remains speculative (Hurd 2003, Lefèvre and Thomas 2008). It would be prudent to test these compounds further against the larval stages of mosquitoes as well as determine any behavioral effects they may have on the adults.

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REFERENCES CITED

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.

Aksenov AA, Martini X, Zhao W, Stelinski LL, Davis CE. 2014. Synthetic blends of volatile, phytopathogen-induced odorants can be used to manipulate vector behavior. *Front Ecol Evol* 2:1–9. https://doi.org/10.3389/fevo.2014.00078

Brown JE, Evans BR, Zheng W, Obas V, Barrera-Martinez L, Egizi A, Zhao H, Caccone A, Powell JR. 2014. Human impacts have shaped historical and recent evolution in Ae. aegypti, the dengue and yellow fever mosquito. *Evolution* 68:514–525.

Brown JE, McBride CS, Johnson P, Ritchie S, Paupy C, Bossin H, Lutomiah J, Fernandez-Salas I, Ponlawat A, Cornel AJ, Black WC 4th, Gorrochotegui-Escalante N, Urdaneta-Marquez L, Sylla M, Slotman M, Murray KO, Walker C, Powell JR. 2011. Worldwide patterns of genetic differentiation imply multiple ‘domestications’ of Ae. aegypti, a major vector of human diseases. *Proc Biol Sci R Soc* 278:2446–2454.

CDC [Centers for Disease Control and Prevention]. 2018. Potential range in US—estimated potential range of *Aedes aegypti* and *Aedes albopictus* in the United States, 2017 [Internet]. Atlanta, GA: Centers for Disease Control and Prevention [accessed August 8, 2019]. Available from: https://www.cdc.gov/zika/vector/range.html.

Chen J, Rashid T, Feng G, Feng Y, Zhang A, Grodowitz MJ. 2019. Insecticidal activity of methyl benzoate analogs against red imported fire ants, *Solenopsis invicta* (Hymenoptera: Formicidae). *J Econ Entomol* 112:691–698.

Dias CN, Moraes DFC. 2014. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides. *Parasitol Res* 113:565–592.
Enan E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. *Comp Biochem Phys C* 130:325–337.

Enan EE. 2005. Molecular response of *Drosophila melanogaster* tyramine receptor cascade to plant essential oils. *Biochem Molec* 35:309–321.

Feng Y, Zhang A. 2017. A floral fragrance, methyl benzoate, is an efficient green pesticide. *Sci Rep* 7:1–9. https://doi.org/10.1038/srep42168

Gross AD, Norris EJ, Kimber MJ, Bartholomay LC, Coats JR. 2017. Essential oils enhance the toxicity of permethrin against *Aedes aegypti* and *Anopheles gambiae*. *Med Vet Entomol* 33:453–466. https://doi.org/10.1111/mve.12380

Hurd H. 2003. Manipulation of medically important insect vectors by their parasites. *Annu Rev Entomol* 48:141–161.

Isman MB. 2000. Plant essential oils for pest and disease management. *Crop Sci* 19:603–608.

Isman MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* 51:45–66.

Isman MB, Miresmailli S, MacHial C. 2011. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytocem Rev* 10:197–204.

Kandel Y, Vulcan J, Rodriguez SD, Moore E, Chung H-N, Mitra S, Cordova JJ, Martinez KJL, Moon AS, Kulkarni A, Ettestad P, Melman S, Xu J, Buenemann M, Hanley KA, Hansen IA. 2019. Widespread insecticide resistance in *Aedes aegypti* from New Mexico, U.S.A. *PLoS ONE* 14:e0212693.

Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, Moore CG, Vavalho RG, Coelho GE, Van Bortel W, Hendrickx G, Scaffner K, Elyazar IRF, Teng H-Q, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GRW, Golding N, Hay SI. 2015. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *elife* 4:e08347.

Larson NR, Zhang A, Feldlaufer MF. 2020. Fumigation activities of methyl benzoate and its derivatives against the common bed bug (Hemiptera: Cimicidae). *J Med Entomol* 57:187–191. https://doi.org/10.1093/jme/tjz138

Lefèvre T, Thomas F. 2008. Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases. *Insect Genet Evol* 8:504–519.

Liu N. 2015. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu Rev Entomol* 60:537–559.

Morrison WR, Larson NL, Brabec D, Zhang A. 2019. Methyl benzoate as a putative alternative, environmentally friendly fumigant for the control of stored product insects. *J Econ Entomol* 112:2458–2468. https://doi.org/10.1093/jee/toz179

Norris EJ, Gross AD, Bartholomay LC, Coats JR. 2019. Plant essential oils synergize various pyrethroid insecticides and antagonize malathion in *Aedes aegypti*. *Med Vet Entomol* 33:453–466. https://doi.org/10.1111/mve.12380

Parnas M, Peters M, Dadon D, Lev S, Vertkin I, Slutsy I, Minke B. 2009. Carvacrol is a novel inhibitor of Drosophila TRPL and mammalian TRPM7 channels. *Cell Calcium* 45:300–309.

Powell PR. 2018. Mosquito-borne human viral diseases: why *Aedes aegypti*? *Am J Trop Med Hyg* 98:1563–1565.

Robertson JL, Jones MM, Olguin E, Alberts B. 2017. Bioassays with arthropods. Boca Raton, FL: CRC Press.

Robertson Jr. 2017. Essential oils enhance the toxicity of carbaryl and permethrin against *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 50:826–832.

Tong F, Coats JR. 2010. Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl- uptake in American cockroach ventral nerve cord. *Pestic Biochem Physiol* 98:317–324.

Tong F, Gross AD, Dolan MC, Coats JR. 2013. The phenolic monoterpenoid carvacrol inhibits the binding of nicotine to the housefly nicotinic acetylcholine receptor. *Pest Manag Sci* 69:775–780. https://doi.org/10.1002/ps.3443

USEPA [US Environmental Protection Agency]. 2017. *Mosquito control* [Internet]. Washington, DC: US Environmental Protection Agency [accessed August 27, 2019]. Available from: www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes.

WHO [World Health Organization]. 2018. *World malaria report 2018* [Internet]. Geneva, Switzerland: World Health Organization [accessed September 9, 2019]. Available from: https://www.who.int/malaria/publications/world-malaria-report-2018/en/.

WHO [World Health Organization]. 2019. *Dengue and severe dengue* [Internet]. Geneva, Switzerland: World Health Organization [accessed September 9, 2019]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue.

Zhang A, Feng Y, inventors; US Department of Agriculture (USDA). 2017 April 25. Methods for killing insects using methyl benzoate. United States patent US 9629362B1. 13 p.