IMPACTS OF BARRIER INSECTICIDE MIXTURES ON MOSQUITO, *AEDES AEGYPTI* AND NON-TARGET HONEY BEE, *APIS MELLIFERA*

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Guest Editor: Lawrence E. Reeves

ABSTRACT

Four novel commercial insecticide mixtures, composed of pyrethroid and nicotinoid active ingredients, were evaluated in a series of experiments in the laboratory, semi-field and field to determine acute toxicity (LC50) against pyrethroid-susceptible (ORL1952) and resistant (Puerto Rico) strains of *Aedes aegypti* L., and non-target adult European honey bees, *Apis mellifera* L. The four products were Tandem, Temprid FX, Transport Mikron, and Crossfire. The acute toxicity data showed that pyrethroid-resistant *Ae. aegypti* PR exhibited decreased sensitivity to all 4 insecticide mixtures, compared to pyrethroid-susceptible *Ae. aegypti* ORL1952. Tandem, Temprid FX, and Transport Mikron were more toxic to *Ae. aegypti* ORL1952 than to *A. mellifera*, but Crossfire was the least toxic. Transport Mikron was also more toxic to *Ae. aegypti* PR than to *A. mellifera*. The Honey bee Tolerance Indexes, determined with LC_{50} data of pyrethroid-susceptible mosquitoes, demonstrated that while Transport Mikron, Tandem, and Temprid FX were more toxic to *Ae. aegypti* ORL1952 than to *A. mellifera*, Crossfire was less toxic. The honey bee Tolerance Indexes decreased substantially when calculated with LC_{50} data from pyrethroid- resistant mosquitoes, but honey bees remained tolerant of Transport Mikron. Notably, while the insecticide mixtures did not control the PR resistant *Ae. aegypti* strain when applied as residual sprays to perimeter vegetation at label rates, susceptible *Ae. aegypti* ORL1951 were controlled, but applications affected honeybees (*A. mellifera*) for up to 28 days after treatment. Temprid FX resulted in 74% and 99% mortality, in adult *Ae. aegypti* ORL1952 and *A. mellifera*, respectively, for 28 days post-treatment. Transport Mikron and Tandem residues killed *Ae. aegypti* ORL1952 for up to 21 days post-treatment, while the effect of Crossfire lasted only 14 days. All three insecticides killed *A. mellifera* for up to 28 days post-treatment but at decreased mortality rates. For operational mosquito control, these data indicate that Transport Mikron has a reasonable safety margin (~25%) when targeting susceptible mosquitoes, compared to Tandem, Temprid FX, and Crossfire. The tested insecticide formulations need to be applied in higher doses to control resistant strains of mosquitoes that may be detrimental to honey bees. The ULV data indicated that pyrethroid resistance can be overcome with the insecticide mixtures.

Key Words: *Aedes aegypti*, *Apis mellifera*, insecticide mixture, non-target, barrier spraying

INTRODUCTION

Mosquito control programs aim to reduce mosquito-borne illness and nuisance mosquitoes through Integrated Mosquito Management (IMM) while limiting environmental impacts and preserving the integrity of non-target communities, which include economically and ecologically important populations such as honey bees and other pollinators (Sanchez-Arroyo et al. 2019 & 2021). Insecticide applications targeting adult mosquitoes are one of the major tools used in IMM. However, these types of broad-scale applications place mosquito control programs under public scrutiny with the public perception that adulticides contaminate the environment and have unintended impacts on beneficial insect populations. When mosquito control products are evaluated in laboratory and field settings, non-target impacts are often not evaluated and therefore, data on the effects of mosquito adulticides on non-target organisms is severely lacking, especially for honeybees (Qualls et al. 2010, Sanchez-Arroyo et al. 2019 & 2021, Giordano et al. 2020, McGregor et al. 2021). Although mosquito adulticide label restrictions and timing of applications aim to minimize impacts on non-target organisms, chemical exposure may occur through wind drift, plant contamination, and other unintended actions and uncontrollable factors.

In addition to the potential impacts of mosquito control insecticides on non-targets, the development of resistance in both nuisance and vector mosquito species to these insecticides is a global problem (Hemingway & Ranson 2000, Nauen R. 2001, Cui et al. 2006, Liu 2015). Thus, new commercial insecticides are needed for mosquito control programs. Recent studies evaluating insecticide formulations with multiple modes of action, mainly with the combination of active ingredients for adult and larval control, have been demonstrated to be efficacious against resistant mosquito populations (Chung et al. 2001, Dantur et al. 2013, Jiang et al. 2017, Lei et al. 2019). Darriet & Chandre 2013 demonstrated that the combination of deltamethrin, piperonyl butoxide (PBO) and Group 1 neonicotinoids enhance control of resistant *Aedes aegypti* and *Anopheles gambiae*. By combining multiple modes of action, resistance mechanisms have been demonstrated to be overcome but little work has been done to evaluate the combination of multiple insecticide formulations and the impact this might have on non-target populations

Honey bees, in particular, are keystone pollinators in human agriculture and green spaces in urban and rural communities. Recently studies evaluating mixtures of biological and chemical insecticides (Chung et al. 2001, Luo et al. 2019) and/or larvicides and adulticides (Dantur et al. 2013, Darriet & Chandre 2013 ,Lucia & Harburguer 2009) with different modes of action have been reported against mosquitoes that demonstrate improved efficacy and a reduction in resistance. This study aimed to assess the impact of applications of mixtures of insecticides on mosquitoes and using the Western honeybee (*Apis mellifera*) as a model non-target organism, therefore providing mosquito control programs information on the selectivity of novel and registered insecticide mixtures. This information can guide mosquito control programs on operational control methods to minimize impacts on non-targets. Because the active ingredients proposed in this project have been assayed against *A. mellifera* as part of the registration process, it is expected that registered products will have a minimal effect on the bees.

MATERIALS AND METHODS

Mosquitoes. Two strains of *Ae. aegypti* were used in this study, the Orlando 1952 (ORL 1952) strain and the Puerto Rico (PR) strain which were obtained from the United State Agricultural Research Service Center for Medical and Veterinary Entomology in Gainesville, Florida and were maintained in colony at the Urban Entomology Laboratory at the University of Florida. *Aedes aegypti* eggs were added to trays containing 2.5 L of well water and maintained in an incubator at 28±2°C, a 14 h light:10 h dark cycle and ~15 % RH until pupation. The developing larvae were fed with a food slurry consisting of 1: 1 brewer's yeast/ liver powder. Pupae were collected and maintained at 26±2°C and 30 - 70% RH until adult mosquitoes emerged.

Honey Bees. Newly emerged *A. mellifera* adults and honey bee combs with capped brood (Figure 1a) were obtained from the honey bee Research and Extension Laboratory of the Entomology and Nematology Department of the University of Florida. The combs were kept at 33±2°C, 25 - 30% RH and red light until adult bees emerged. One to three days post-emergence, adult bees were collected, and either used directly in the experiments or transferred to 'Bee Cups', and kept at 33±2°C, 25 - 30% RH, and red light until assayed. The bee cups had ventilation holes and syringes filled with a 50% sucrose solution as food source for the bees (Figs. 1b,c).

Laboratory Evaluation. The insecticide mixtures used were Crossfire (MGK Insect Control Solutions), Tandem (Syngenta), Temprid FX (Bayer), and Transport Mikron (FMC) (Table 1). Tandem, Temprid FX, and Transport Mikron are registered for mosquito control while Crossfire is only registered for the control of bedbugs. All formulations are designed to be used as surface treatments and kill on contact and through residual activity.

Aqueous insecticide dilutions were applied uniformly to Whatman filter paper # 1 strips which were air-dried. Mosquito and honey bee bioassay strips had an area of 5 cm^2 and 14 cm^2, respectively. The mosquito and honey bee bioassay strips were treated with the same concentration (9 µL insecticide solution/cm^2).

Laboratory experiment. For the laboratory experiments, >3-day old adult susceptible and resistant female mosquitoes, *Ae. aegypti* were knocked down with CO_2, and the mosquitoes were transferred to 20-mL scintillation vials. Insecticide-treated paper strips were introduced to the scintillation vials after the mosquitoes had recovered completely from the knock-down. Ten females were used in three replicates of an insecticide concentration. Mosquitoes were fed with a 10% sucrose solution on a cotton ball for the duration of the experiments. *Aedes aegypti* mortality was assessed at 24±2 h.

Honeybees were knocked down with CO_2 and transferred to 4-ounce jelly jars. Insecticide-treated paper strips were introduced to the jars after the bees had recovered completely from the knock-down. Ten worker honey bees (3-10 d old) were exposed to each concentration of insecticides. *Apis mellifera* were fed with a 50% sucrose solution on a cotton ball for the duration of the experiment. *Apis mellifera* mortality was assessed at 48±2 h.

Experiment in greenhouses. The insecticide mixtures were diluted. based on the LC_{90}s generated in the acute toxicity studies and within range of typical Ultra Low Volume (ULV) applications. Tandem and Transport Mikron were diluted at a 1:8 ratio, while Temprid FX was diluted 1:56 and Crossfire was left undiluted.

For the ULV aerosol applications, aqueous insecticide mixture dilutions and water (negative control) were

Figure 1: a) Honey bee combs with capped brood and emerging bees. b) Bee cups with ventilation holes and syringes filled with 50% sucrose solution. c) Bee cups in 'Honey bee Hive Observation Room'.

Figure 2: Portable ULV Sprayer and Field Cages (Blue board was not present during application and was used only for better contrast in the picture).

applied to caged *Ae. aegypti*, ORL1952 and PR, and *A. mellifera* (from 3 beehives) with a Curtis Dyna-Fog Hurricane Ultra II electric portable aerosol applicator (Westfield, IN, ULV / mister) designed for spraying industrial and residential areas (Figure 2).

Droplet sizes of the different insecticide formulations were determined in triplicate with the Curtis Dyna-Fog Hurricane Ultra II electric portable aerosol applicator at the Anastasia Mosquito Control District, St. Augustine, Florida (AMCD) using an Artium Phase Doppler Interferometer (PDI), model TK1 (Artium Technologies, Sunnyvale, CA) which is capable of precisely measuring droplets from 0.7 - 150 µm (Table 2). The volume mean diameter (DV) $DV_{0.1}$, $_{0.5}$, and $_{0.9}$ represent the droplet size below which 10, 50 and 90% of the spray volume consists of droplets smaller than the listed size.

The insecticide trials were set up in a greenhouse located at the Entomology and Nematology Department of the University of Florida. The application rate was 1 oz (~30 ml) per 1000 cu ft which is typical for ULV aerosols and indoor use. Three replicates were conducted for each treatment and insect. Negative control treatments were set up before, between, and after insecticide treatments to check for ambient contamination with pesticides. The temperature inside the greenhouse ranged from 26-33°C during the experiments.

To cage the insects, mosquitoes and bees from three beehives, were first knocked down with CO_2. After mosquitoes had been immobilized, 10, 3-6 d old females were transferred to each treatment cage. After bees were immobilized, 10 newly emerged females from each bee hive were transferred to each treatment cage.

For each treatment with insecticide, the aerosol applicator was positioned 30 cm from the insects confined to field cages, which were attached to a wooden stake. The greenhouse ventilation was turned off during insecticide application. Fifteen minutes post-application, the greenhouse was evacuated of any residual insecticide mist for 15 minutes by turning on the ventilation remotely to avoid exposure of the operator to the pesticide application, after which the cages with treated insects were retrieved. Each treatment was set up in triplicates. A similar procedure was observed for negative controls where caged insects were treated with water rather than insecticide.

Treated cages with mosquitoes were kept at room temperature and ambient RH. Mosquitoes were fed with a 10% sucrose solution on a cotton ball for the duration of the experiments. Mortality was recorded immediately after treatment and at 24±2 h. Treated cages with bees were kept in the dark in a honey bee hive observation room maintained at 31±2°C and 15-30% RH. Honey bees were fed with a 50% sucrose solution on a cotton ball for the duration of the experiments. Mortality was recorded immediately after treatment and at 48±2 h.

Barrier Treatment Evaluation. For the barrier applications, aqueous insecticide dilutions were applied with Stihl *SR 450* backpack sprayers (Virginia Beach, VA) mounted on all-terrain vehicles. Applications were directed to perimeter vegetation and three potted azaleas (*Rhododendron* sp.) at the St. John's County Golf Course, St. Augustine, FL for each treatment (Tandem, Temprid FX, Transport Mikron, Crossfire, water = negative control). The insecticide mixtures were diluted to the high label rate concentrations for barrier applications (Table 3).

Potted azaleas were placed 30 m apart from each other within each treatment group, and the treatment groups were separated from each other by buffer zones of at least 304 m. The potted azaleas were not blooming at the time of treatment, but flowers developed 1-2 weeks after treatment. After treatment, potted azaleas were taken to Gainesville and placed outside at the Urban Entomology Building.

The residual effects of the insecticide mixtures were assessed on day 1, 7, 14, 21, and 28 post treatment using leaf bioassays on susceptible and resistant adult *Ae. aegypti* for the potted azaleas in Gainesville and on susceptible *Ae. aegypti* (ORL 1952) and *Culex quinquefasciatus* (Gainesville 1995 + Ocala 2003) for perimeter vegetation in St. Augustine.

For the experiments conducted at AMCD, two leaves adjacent to each other were collected from each plant and each time after treatment. The leaves were selected from the woody portion of the stems to ensure they were present when the plants were treated with insecticides. Two plastic Petri dishes were prepared for each plant and time after treatment: one for susceptible, and one for resistant mosquitoes. One leaf was placed into each dish with the treated side up. *Culex quinquefasciatus* and ORL 1952 strain were knocked down with CO_2, and 10, 3-6 d old females of each species were transferred into their own Petri dish. The Petri dishes were kept at room temperature and ambient RH. Mosquitos were fed with a 10% sucrose solution on a cotton ball for the duration of the experiments. Mortality was recorded at 24±2 h.

The residual effects of the insecticide mixtures on adult *A. mellifera* were assessed with azalea leaf bioassays on day 1, 7, 14, 21, and 28 post treatment. For the experiments, three souffle cups (one for each of three beehives used in the experiment) were prepared for each azalea and time point. Triplicates of five leaves were collected from each azalea and time point. The leaves were selected from the woody portion of the azalea stems to ensure they were present when the plants were treated with insecticides.

Table 1: Tested insecticide active ingredients and classes

Commercial Insecticide Name	Active Ingredient (A.I.)	A.I. - Class	A.I. (%)
Tandem	Thiamethoxam	Neonicotinoid	11.60
	λ-Cyhalothrin	Pyrethroid	3.50
Temprid FX	Imidacloprid [%],	Neonicotinoid	21.00
	β - Cyfluthrin	Pyrethroid	10.50
Transport Mikron	Acetamiprid	Neonicotinoid	5.00
	Bifenthrin	Pyrethroid	6.00
Crossfire	Clothianidin	Neonicotinoid	4.00
	Metofluthrin	Pyrethroid	0.10
	(Piperonyl Butoxide - synergist)	NA	10.00

Table 2: The volume mean diameter (DV) for diluted insecticides applied using a ULV sprayer is presented.

Product	DV 0.1 (x ± std. dev.) µm	DV 0.5 (x ± std. dev.) µm	DV 0.9 (x ± std. dev.) µm
CrossFire	34.2 ± 1.74 a	113.9 ± 1.08 a	140.9 ± 3.76 a
Temprid FX	18.7 ± 0.35b	39.3 ± 0.60 c	116.6 ± 10.11 bc
Tandem	18.2 ± 1.29 b	50.9 ± 4.20 b	131.5 ± 08.30 ab
Mikron	15.3 ± 0.78 c	34.0 ± 1.40 d	105.5 ± 9.06 c

*Means followed by the same letter within a column are not significantly different

Table 3: Amount of active ingredients applied in barrier trials

Insecticide	Dilution[a]	Percent A.I.[b] in Diluted liquid	Product (oz)/ 1000 sqft [c]	A.I.[d](oz)/ sqft	A.I. - Class
Tandem	1:115	Thiamethoxam (0.10) λ-Cyhalothrin (0.03)	2.2	0.347	Neonicotinoid Pyrethroid
Temprid FX	1:236	Imidacloprid (0.09) β – Cyfluthrin (0.04)	1.08	0.405	Neonicotinoid Pyrethroid
Transport Mikron	1:106	Acetamiprid (0.05) Bifenthrin (0.06)	2.4	0.291	Neonicotinoid Pyrethroid
Crossfire	1:9	Clothianidin (0.44) Metofluthrin (0.11) PBO[e] (1.11)	26	3.905	Neonicotinoid Pyrethroid Synergist

[a] Dilutions based on product labels
[b] A.I. = active ingredient
[c] All products applied at the rate of 2 gallons /100 sqft
[d] Combined a.i.s
[e] PB = Piperonyl Butoxide

Table 4. Acute toxicity of four insecticide mixtures for *Aedes aegypti* ORL1952 (pyrethroid-susceptible), *Aedes aegypti* PR (pyrethroid-resistant), and *Apis mellifera.*

Insecticide	*Aedes aegypti*		*Apis mellifera*
	ORL1952 - Susceptible	**PR - Resistant**	
	LC_{50} ± 95% Confidence Limits (µg/cm²) [a]		
Tandem	0.219 (0.131, 0.382) b	8.211 (0.168, 15.631) ab	1.723 (0.865, 2.655) b
Temprid	0.046 (0.023, 0.102) a	3.903 (1.548, 7.396) ab	0.300 (0.058, 0.653) a
Transport	0.128 (0.079, 0.206) ab	1.022 (0.466, 1.646) a	3.171 (1.481, 11.553) b
Crossfire	2.096 (1.731, 2.483) c	10.180 (5.379, 22.419) b	1.869 (1.055, 3.048) b
	LC_{90} ± 95% Confidence Limits (µg/cm²) [a]		
Tandem	0.663 (0.381, 4.753) a	43.522 (21.585, 16,467.304) a	4.240 (2.739, 12.965) a
Temprid	0.193 (0.092, 5.647) a	22.243 (10.461, 335.423) a	1.082 (0.549, 2273.330) a
Transport	0.341 (0.211, 1.391) a	3.270 (1.934, 23.425) a	12.506 (5.636, 17,421.779) a
Crossfire	2.934 (2.479, 4.781) a	29.416 (15.956, 718.725) a	3.820 (2.548, 34.958) a

[a]Of highest active ingredient (a.i.).
*Means followed by the same letter within a CL group for each species/strain are not significantly different

Table 5: *Aedes aegypti* insecticide resistance ratios and *Apis mellifera* tolerance ratios in relation to doses needed to kill *Aedes aegypti*.

Insecticide	Mosquito Index	Honey Bee Tolerance Index	
	Resistant/Susceptible LC_{50} Ratio[b]	**LC_{50} Ratio**[d] **to resistant *A. aegypti***	**LC_{50} Ratio**[c] **to susceptible *A. aegypti***
Tandem[e]	38.0	0.21	7.98
Temprid FX[e]	86.7	0.08	6.67
Transport Mikron[e]	8.1	3.10	25.17
Crossfire[e]	4.4	0.18	0.81

[a]Of highest active ingredient
[b]*Ae. aegypti* PR LC_{50} / *Ae. aegypti* ORL1952 LC_{50}
[c]*A. mellifera* LC_{50} / *Ae. aegypti* ORL1952 LC_{50}
[d]*A. mellifera* LC_{50} / *Ae. aegypti* PR LC_{50}
[e]Insecticide mixture (pyrethroid/nicotinoid)

Each set of leaves was placed into a souffle cup with the treated sides up. Ten newly emerged female honey bees from each of three hives were transferred to the souffle cup. Honey bees were fed with a 50% sucrose solution on a cotton ball for the duration of the experiments. The cups were kept in the dark in the honey bee hive observation room at 31±2°C and 15-30% RH. Mortality was recorded at 48±2 h.

Data Analysis. Data of the laboratory study were analyzed using generalized linear model procedures as implemented in SAS® PROC NLMIXED (SAS/STAT 15.1; SAS Institute, Cary NC) using a binomial distribution function and associated canonical logit link function. Because studies were repeated over time, time was considered a random effect. The fixed continuous effect was log10(rate). The LC50 was calculated as $-b_0/b_1$, where b_0 and b_1 are the intercept and rate parameter from the logistic regression model, respectively. LC50 was back-transformed to rate ± lower and upper 95% confidence limits and is reported as $\mu g/cm^2$.

Data from the field study were analyzed using generalized linear model procedures as implemented in SAS® PROC GLIMMIX (SAS/STAT 15.1; SAS Institute, Cary NC) using a binomial distribution function and associated canonical logit link function. For the mosquito study, fixed effects were insecticide, strain, day after treatment application (DAT), and all two- and three-way interactions. Replicate plot within each insecticide treatment was the sole random effects. For the honey bee portion of this study, fixed effects consisted of Insecticide, DAT and the Insecticide x DAT interaction. Because the honey bee response was based on replicated evaluations with bees collected from three hives, beehive was treated as a random effect in addition to replicate plots within insecticide.

RESULTS

Laboratory Evaluation. We determined the acute toxicity (LC_{50}) of Tandem, Temprid FX, Transport Mikron, and Crossfire for pyrethroid-susceptible and pyrethroid-resistant *Ae. aegypti* mosquitoes and *A. mellifera* honey bees. Based on these results, Honey bee Tolerance Indexes were calculated as the ratio of honey bee LC_{50} to mosquito LC_{50} (Table 4,5).

Higher concentrations of all four insecticide mixture formulations are needed to kill *Ae. aegypti* PR than *Ae. aegypti* ORL1952 (~38-fold more Tandem, ~87-fold more Temprid FX, ~8-fold more Transport Mikron and ~ 4-fold more Crossfire). The honey bee Tolerance Index decreased when calculated with *Ae. aegypti* PR LC_{50} rather than *Ae. aegypti* ORL1952 LC_{50} data, due to the high level of pesticide-resistance that has been observed in field populations of *Ae. aegypti*. Tandem, Temprid FX, and Transport Mikron were about 8, 7, and 25-fold more toxic to *Ae. aegypti* ORL1952 than to *A. mellifera* while Crossfire was less toxic (0.8-fold) to mosquitoes. The insecticide mixtures were all less toxic to *Ae. aegypti* PR than to *A. mellifera* at rates of ~ 0.2, 0.1, 3, and 0.2-fold (Table 5).

Greenhouse Evaluation. All insects died when treated with ULV sprays of Tandem, Temprid FX, Transport Mikron, and Crossfire at the rate of 1 oz (~30 ml) / 1000 cu ft. Difficulties encountered during these studies prevent conclusions to be drawn from this experiment.

Barrier Treatment Evaluation. Mortality at different time-points post treatment of susceptible and resistant mosquitoes and honey bees was determined after exposure to leaves of vegetation treated with Tandem, Temprid FX, Transport Mikron, and Crossfire at label rates for residual surface treatments (Table 6A, B). There was no *Ae. aegypti* PR mortality through exposure to the treated leaves with the sole exception of Temprid / day 1 (7% mortality). *Apis mellifera*, *Ae. aegypti* ORL1952, and *Cx. quinquefasciatus* were both affected by the insecticide residues left on the treated foliage, with high mortality (>75%) for 2-4 weeks with most products. Temprid had effective residual activity with 74% mortality for *Ae. aegypti* ORL1952 on day 28. The residual activity of Temprid also resulted in 99% mortality of *A. mellifera* up to day 28. Crossfire had the least effective residual activity and was the least toxic to both *A. mellifera* and *Ae. aegypti* ORL1952.

DISCUSSION

The research on target and non-target impacts of two-AI barrier insecticide mixtures for use in operational mosquito control was conducted to further understand the utility of novel combination insecticides for control of pyrethroid-resistant *Ae. aegypti* and the potential impacts on non-targets. The data shows that the barrier treatments with combination insecticides did not provide control against *Ae. aegypti* PR but they are effective for *Ae. aegypti* ORL1952. In addition, the dual-AI product Transport Mikron would be the best choice for controlling susceptible *Ae. aegypti* while minimizing non-target impacts.

Compared to acute toxicity data of commercial pyrethroid and organophosphate insecticide formulations by Sanchez-Arroyo et al. (2019), none of the insecticide mixtures tested in the present study had a lower LC_{50} for *Ae. aegypti* ORL1952 than Talstar or Mosquito Mist. The insecticide formulations tested by Sanchez-Arroyo et al. (2019) were Aqualuer (permethrin 20.6%, PBO 20.6%), Deltagard (deltamethrin 2.0%), Duet (prallethrin 1.0%

Table 6A: Mortality of *Ae. aegypti*, ORL1952 and PR, and *A. mellifera* exposed to treated leaves at different days after treatment (DAT) (Gainesville)

	Percent Mortality (95% Confidence Limits)				
DAT	NEG. CONTROL*	TANDEM*	TEMPRID FX*	TRANSPORT MIKRON*	CROSSFIRE*
***Aedes aegypti* ORL1952 (n = 30 per DAT)**					
1	0 b	88 (71 - 95) b	99 (92,100) a	99 (95 - 100) a	07 (02 - 20) b
7	0 b	99 (92-100) a	99 (92 - 100) a	91 (78 - 97) b	0 b
14	16 (7-36) a	22 (9 - 44) c	96 (86 - 99) a	45 (23 - 69) c	23 (10 - 45) a
21	0 b	7 (02 - 19) d	73 (51 - 88) b	32 (15 - 57) c	0 b
28	0 b	0 d	74 (51 - 88) b	0 d	0 b
***Aedes aegypti* PR (n = 30 per DAT)**					
1	0 a	0 a	9 (3 - 24) a	0 a	0 a
7	0 a	0 a	0 b	0 a	0 a
14	0 a	0 a	0 b	0 a	0 a
21	0 a	0 a	0 b	0 a	0 a
28	0 a	0 a	0 b	0 a	0 a
***Apis mellifera* (n = 90 per DAT)**					
1	8 (3 - 20) a	98 (91 - 99) a	98 (91 - 99) a	87 (74 - 94) a	99 (94 - 100) a
7	7 (2 - 16) a	69 (50 - 83) b	99 (93 - 100) a	89 (76 - 95) a	26 (13 - 44) b
14	14 (6 - 29) a	72 (53 - 85) b	99 (93 - 100) a	60 (41 - 77) b	29 (15 - 47) b
21	13 (5 - 27) a	15 (7 - 30) c	99 (94 - 100) a	13 (6 - 26) c	12 (5 - 25) c
28	0 b	11 (5 - 24) c	99 (93 - 100) a	4 (1 - 12) d	3 (1 - 10) d

*Means followed by the same letter within a treatment group for each species/strain are not significantly different

Table 6B: Mortality of *Aedes aegypti* Orl1952 and *Culex quinquefasciatus* exposed to treated leaves at different days after treatment (DAT) (St. Augustine).

	Percent Mortality (95% Confidence Limits)				
DAT	NEG. CONTROL*	TANDEM*	TEMPRID FX*	TRANSPORT MIKRON*	CROSSFIRE*
***Culex quinquefasciatus* (n = 30 per DAT)**					
0	0 b	67 (28 - 100) a	100 a	100 a	80 (41-100) a
7	0 b	80 (62-97) a	100 a	100 a	94 (76-100) a
14	0 a	63 (13 - 100) a	50 (0-100) a	61 (11-100) a	0 a
21	0 b	37 (0 - 78) ab	90 (48 - 100) a	74 (32 - 100) a	35 (6-77) ab
28	0 a	0 a	13 (0 - 35) a	17 (0 – 38) a	20 (0-41) a
***Aedes aegypti* (n = 30 per DAT)**					
1	0 b	100 a	100 a	100 a	100 a
7	0 b	100 a	100 a	100 a	3 (0-6) b
14	0 (0 – 26) c	80 (54 – 100) ab	54 (28 -80) b	92 (65 – 100) a	0 c
21	0 (0-20) c	84 (64 -100) a	100 b	92 (72 - 100) a	57 (37 -77) c
28	0 a	46 (5 – 86) a	41 (0 – 82) a	41 (0 – 82) a	29 (11 - 70 a

*Means followed by the same letter within a treatment group for each species are not significantly different

+ Phenothrin 5.0%), Talstar (bifenthrin 7.9%), and Mosquito Mist (chlorpyrifos 24.6%). These were all single-AI products tested. Comparing the pesticides tested in the present study and those tested by Sanchez-Arroyo et al. (2019) demonstrates that Talstar and Mosquito Mist were the most toxic to *A. mellifera*. However, the dual- AI products tested in the present study, represent lower risk to honey bees than the single-active ingredient products tested previously (Sanchez-Arroyo et al. 2019), with exception of Temrpid FX. Transport Mikron, the insecticide mixture that contained the pyrethroid bifenthrin and the neonicotinoid acetamiprid, did not have a lower LC_{50} than Talstar, a bifenthrin insecticide, but its safety margin for *A. mellifera* was approximately 3 times higher than the safety margin of single-AI product, based on the honey bee Tolerance Index.

Overall, all of the insecticide formulations evaluated resulted in mortality to honey bees. For operational mosquito control in Florida, these data indicate that only Transport Mikron has a reasonable safety margin (~25%) when targeting susceptible mosquitoes, but Tandem, Temprid FX, and Crossfire should not be used. Thus, it is important to adhere to the restrictions stated on the pesticide labels to preserve honey bees and other non-targets. Most barrier application labels recommend applying the treatment to non-flowering vegetation to avoid non-target impacts. For the majority of mosquito control programs, the active ingredient (AIs) bifenthrin is the barrier treatment of choice and is one of the AIs in Transport Mikron. Since bifenthrin alone was demonstrated to be highly toxic to *A. mellifera* (Sanchez-Arroyo et al. (2019) but less toxic when combined with the neonicotinoid, acetamiprid, this combination may be more suitable for best management practices when using barrier applications for control of mosquito populations.

Another thing for mosquito control professionals to consider is when targeting insecticide-resistant mosquito populations, higher doses of the barrier products would be necessary. Given the non-target impacts described in the current study and Sanchez-Arroyo et al. 2019 and the fact that none of the products tested were sufficient at controlling the resistant PR strain, barrier applications in areas where resistant mosquito populations are documented would not be recommended and other methods such as ULV applications (Sanchez-Arroyo et al. 2019 and 2021) would be preferred.

ACKNOWLEDGMENTS

The research reported in this publication was supported by the Florida Department of Agriculture and Consumer Services under the grant fund number 026366. The content is solely the responsibility of the authors and does not necessarily represent the official views of the FDACS, University of Florida and Anastasia Mosquito Control District. We thank J. Ellis to provide the honeybees, M. Farooq for droplet testing, H. Ward, O. Sypes, and L. Bangonan provided the technical help in the field experiment.

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Received on 19 October 2021, accepted on January 25, 2022, and published on 30 March 2022.