INTRODUCTION

The black salt-marsh mosquito, *Aedes taeniorhynchus* (Wiedemann), is a prolific, pestiferous species of mosquito that has plagued southern Florida mosquito control districts for decades (Provost 1952). It is responsible for expending a large portion of the time and resources of mosquito control districts in the state of Florida (Connelly and Carlson 2009). Although a capable vector of dog heartworm (*Dirofilaria immitis* (Leidy)) (Nayar and Rutledge 2008), *Aedes* primarily bloodfeed on wild mammals and humans when available but will seek reptile and bird hosts to a lesser degree based on habitat proximity to host source, indicating an opportunistic feeding behavior (Edman 1971, Asigau et al. 2019). They emerge in marshes and emerge after flooding from rain events or tidal flows (Ritchie and Johnson 1991). Females primarily bloodfeed on wild mammals and humans when available but will seek reptile and bird hosts to a lesser degree based on habitat proximity to host source, indicating an opportunistic feeding behavior (Edman 1971, Asigau et al. 2019). They emerge in such numbers that massive hatching events have been attributed as the cause of death of cattle from prolonged mosquito feeding (Addison and Ritchie 1993). Their long flight range allows them to travel up to 60 miles (100 mi) assisted by wind, potentially migrating into populated areas. This migration particularly affects the northernmost Florida Keys mosquito numbers during the wet summer months with assisting west winds (Harden and Chubb 1960).
truck-mounted ULV mission is reactionary, and effectiveness may vary depending on wind speed and direction (Schatmeyer and Urone 1973), temperature (Stevens and Stroud 1967), and atmospheric stability and turbulence (Mount and Pierce 1971, 1974). During the summer months when mosquito numbers are highest, FKMCD opts to use a bifenthrin-based barrier spray called Wisdom TC Flowable® (7.9% AI, bifenthrin; AMVAC, Los Angeles, CA) inside the Ocean Reef community. This reduces adult *Ae. taeniorhynchus* landing counts and man-hours spent using truck-mounted ULV single-spray missions.

Adjuvants, used to increase the efficacy of pesticides and herbicides, are available in many formulations meant to cater to the needs of pesticide applicators. Two commercially available adjuvants, Lesco Spreader-Sticker® (LSS) (Lesco, Inc., Cleveland, OH) and Xtended Performance® (XP) (Residex, LLC, Novi, MI), were purchased in 2016 to determine if these products could increase the efficacy of FKMCD’s Wisdom TC Flowable barrier spray applications. Xtended Performance is advertised to “enhance wetting, adhesion and on-target product delivery” and to “extend the performance of all your pest control...applications” (Anonymous 2017). Although the type of adjuvant is not explicitly listed, advertised claims on the label suggest that this product is a wetting agent, sticking agent, and as well as a drift control agent. Lesco Spreader-Sticker is explicitly advertised as a wetting and sticking adjuvant. The object of this trial was to determine if these wetting/sticking adjuvants can increase the effectiveness of barrier spray applications in Key Largo, FL. Leaf bioassays were conducted using the 3 different mixtures to determine their effectiveness against locally captured adult female *Ae. taeniorhynchus*.

**MATERIALS AND METHODS**

**Test site**

A field site along Central Avenue (25.1042881°N, 80.4296970°W) was chosen for its uniformity in sun and rain exposure, lack of residential homes, and relative abundance of the common hammock tree, pigeon plum (*Coccoloba diversifolia* (Jacquin)), for leaf collection. The west side of this road was divided into 4 100-ft (30.5-m) sections. These sections were randomly selected for a control site, Wisdom TC Flowable only (Wisdom), Wisdom + LSS, and Wisdom + XP. Each of the 4 sections was separated by a 150-ft (45.72-m) buffer.

![Image](https://example.com/image.png)

*Fig. 1. The community of Ocean Reef in Key Largo, FL, shown in relation to the 3 large protected areas that produce mosquitoes but are restricted from various pesticide treatments used in mosquito abatement.*
Pesticide application

Treatment solutions were mixed according to maximum label rate (1 fl oz [7.8 ml/liter] Wisdom TC Flowable/gal of water, 0.08 fl oz [0.63 ml/liter] Lesco Spreader-Sticker/gal Wisdom-and-water solution, and 2.56 fl oz [20 ml/liter] XP/gal Wisdom-and-water solution). Mixtures were blended on the morning of each treatment application according to label directions and were agitated again immediately prior to sprayer applications.

Treatment areas were sprayed with the same Stihl model SR-420® (Stihl Corp., Virginia Beach, VA) backpack sprayer. The treatment tank was triple-rinsed between treatments to avoid cross contamination as well as maintaining the same application rate (0.5 gal/min [1.89 liter/min]) by using the same sprayer. All solutions were applied at the Wisdom TC Flowable maximum label rate (1 fl oz [29.57 ml] per 1,000 ft² [92.9 m²]) by using a vertical sweeping motion from 0 to 10 ft (0–3.05 m) above ground level along the 100-ft (30.5-m) designated treatment area, approximately 10 ft (3.05 m) away from the tree line at a slow walking pace of approximately 0.7 mph (1.13 km/h). Wind speed at the time of all applications was less than 5 mph (8 km/h) at the treatment sites. Trials 1–3 were run September 20, 2018, to November 8, 2018; April 11, 2019, to May 30, 2019; and August 16, 2019, to October 4, 2019, respectively. Treatment applications were on the 1st day of trials and leaves were sampled on the same day of the week, 1 wk apart, for 8 wk including the day of application (week 0). This was replicated 3 times.

Locally captured adult female *Ae. taeniorhynchus* were used for the leaf bioassays. A Centers for Disease Control and Prevention light trap (John W. Hock, Gainesville, FL), baited with dry ice (CO₂), was deployed adjacent to known, untreated habitat areas and collected after approximately 24 h. Female *Ae. taeniorhynchus* were transferred to a cage and allowed to rest for 24 h and fed a 10% sucrose solution, ad libitum. Mosquitoes were maintained at 20–25°C and 70–85% RH until bioassays were completed. At least 10 mosquitoes were transferred into each leaf-mounted petri dish using a CO₂ knockdown method for 10–20 sec, depending on robustness of the specimens. A 0.5-inch dental wick (TIDI Products, Neenah, WI) soaked in 10% sucrose solution was provided through a hole drilled in the top of each petri dish. Pretrial tests had shown 10–30% mortality (data not shown) without an available food source. Mortality was assessed at 10 min postintroduction to leaf assays to record mortality caused by handling during transfer to the petri dishes. If dead mosquitoes were seen, they were noted and removed from the sample set. Mosquitoes were classified as dead if they could not fly or did not respond to disturbance of the petri dish. Mortality was recorded at 1, 4, and 24 h. When leaf-mounted petri dishes could not be immediately used for bioassays, they were stored at −38°C in plastic freezer bags until leaf assays could be completed. A previous study has shown no reduction in the efficacy of frozen bifenthrin treatment samples (Doyle et al. 2009).

Leaf bioassays

Leaves from pigeon plum trees were picked weekly from each of the treatment sites and control site starting on the day of application (week 0), 4–6 h after application. Leaves were chosen at random, while avoiding new growth. Leaves were placed into plastic containers with the adaxial side facing up. Care was taken to touch only the stems, and leaves were placed inside the container so the adaxial side did not touch adjacent leaves or the container sides. Leaves were collected on the same day of the week, 1 wk apart, for weeks 1–7 postapplication. If wet on the day of collection, leaves were allowed to dry indoors, then mounted inside 110-mm × 15-mm petri dishes (Weber Scientific, Hamilton, NJ) using double-sided carpet tape. Leaf bioassay methods were partially modeled after Doyle et al. (2009). Leaves were cut from the stem with the aid of disposable straight razors for easier manipulation during mounting and to ensure only the bottom portion of the petri dish was covered. At least 3 different leaves were used in each petri dish to cover the inside base, totaling approximately 15 different leaves per treatment area per week. Glass stirring rods were used to secure the leaves to the tape by applying gentle pressure. Any exposed tape remaining was covered with shredded paper to avoid mosquito tarsal adhesion during bioassays. Five petri dishes were mounted with leaves for each treatment area per week for 8 wk including the day of application (week 0). This was replicated 3 times.

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Statistical analyses

Statistical analyses were completed using Excel (Microsoft, Redmond, WA). Percentage data were arcsine transformed prior to analysis (*p* = arcsin √*p*). One-way analysis of variance (ANOVA) was used to compare mosquito mortality at 1, 4, and 24 h postexposure. A Tukey’s HSD test was used to determine where significant differences between treatments could be distinguished when ANOVA revealed significance between treatments. Results were found to be significant when *P* < 0.05. General linear models for 1, 4, and 24 h (Fig. 2) were used to measure the decline of mortality rates over time. Data
Fig. 2. Regression models of *Aedes taeniorhynchus* mortality to Wisdom TC Flowable® (Wisdom), Wisdom + Lesco Spreader-Sticker® (LSS), and Wisdom + Xtended Performance® (XP) treatments plotted over weeks 0–7 at 1, 4, and 24 h. Regression statistics provided in Table 2.
### RESULTS

Control groups during this study had <5% mortality and are not included in data provided. Adult mortality across all 3 treatments was not significant on the day of the application, and >99% mortality was achieved at 4 h, indicating successful application of the pesticide and adjuvant mixtures. Mortality at 1 h quickly decreased across all treatment types and post–week 3 was not significantly different from control mortality. No significant difference was found between treatment types for 1-h mortality in weeks 0–7 (Fig. 2 and Table 1).

Mean mortality at 4 h over the 3 trials depicts a gradual decrease in mortality for 6 wk until week 7, where all treatments are no different than the control group. Significant differences of mortality rates are noted in Table 1 at 4 h postexposure in week 1 (F = 5.31, P = 0.0088), where mean mortality of Wisdom (99.6%) and LSS (99.62%) mortality are significantly higher than XP (80.64%); and week 4 (F = 4.85, P = 0.0127), where each treatment type is significantly different from the other: XP (19.07%) > LSS (45.7%) > Wisdom (2.12%).

Significance was observed at 24 h postexposure on week 2 (F = 5.45, P = 0.008) and week 3 (F = 4.83, P = 0.013), where LSS mean mortality (100%) and XP (93.77% and 66.85%) in both weeks 2 and 3, respectively.

Linear regression models and regression statistics are reported in Fig. 2 and Table 2. Correlation coefficients in the regression models show a strong relationship (≥0.70) between the weeks since application and mortality at each exposure interval. The LSS means showed the greatest rate of decline in effectiveness at both 1 h (x = −11.11) and 4 h (x = −0.16) postexposure compared to Wisdom (x = −0.92 and x = −0.15) and XP (x = −9.3 and x = −0.15) treatments at 1- and 4-h intervals, respectively. However, LSS mean mortality degraded the slowest (x = −0.11) at the 24-h interval over the 8-wk trial compared to Wisdom (x = −0.13) and XP (x = −0.13).

### DISCUSSION

In our efforts to determine if adjuvants could be utilized to increase the efficiency of FKMC’s barrier treatments against *Ae. taeniorhynchus*, we determined that LSS and XP do have some effects on the efficacy of Wisdom. One-way ANOVA and Tukey’s HSD test reveals significant variances among the leaf assays 5 times during the 8-wk trials. Data show that the LSS mixture maintained higher mean mortalities than either the XP + Wisdom or Wisdom-only treatment where significance was found except one instance on week 4 at 4 h postexposure when the XP mixture was significantly provided in this paper are back-transformed from arcsine transformation used during analysis p = (sin^-1 p)^2.
higher than either the LSS mixture or Wisdom-only treatments. While the difference between the LSS mixture and Wisdom alone was not significant, it can be seen as a trend over time, shown in Fig. 2. Interestingly, mosquitoes exposed to Wisdom (99.58%) and LSS (99.62%) had a significantly higher mean mortality rate than XP (89.88%) on week 1 at the 4-h mortality reading, but no differences were noted at the 1 or 24 h for the same week. Additionally, week 4 showed Wisdom mean mortality (2.12%) at 4 h postexposure to be significantly lower than both LSS (4.57%) and XP (19.07%), but by the 24-h observation of the same week there were no differences in any of the treatments. Linear regression models of the data depict high variability in the data at 1 h postexposure (Wisdom, $R^2 = 55.2%$; LSS, $R^2 = 59.5%$; XP, $R^2 = 49.4%$) and at 24 h postexposure (Wisdom, $R^2 = 52.9%$; LSS, $R^2 = 54.1%$; XP, $R^2 = 50.8%$), but reveals a better fit at 4 h postexposure (Wisdom, $R^2 = 66.3%$; LSS, $R^2 = 73.3%$; XP, $R^2 = 59.0%$). The lower $R^2$ values may be explained by variability in field conditions caused by sunlight, precipitation, or other variables affecting individual leaves at different rates as described by Stoops et al. (2019). It is interesting to note, however, that the regression models at the 4-h exposure interval report higher $R^2$ values than either 1- or 24-h intervals for their respective treatments. The LSS treatment showed less variability at all postexposure intervals than either of the other 2 treatments, meaning that LSS likely has an effect on stabilizing the degradation of bifenthrin from one or more environmental variables.

The increased efficacy at 24-h exposure time suggests that LSS should be considered for addition to FKMC’s barrier treatment applications. However, in reality, a mosquito resting on a treated surface for a full 24-h period is unlikely. Mortality rates at 1 and 4 h postexposure were likely better at predicting mortality rates in an open system, though 24-h exposure rates have been used in past studies to provide a comparison for mortality (Perich et al. 1993, Cilek and Hallmon 2006, Doyle 2007, Trout et al. 2007, Cilek 2008). The difference between LSS and XP at the 24-h exposure interval seen in Table 1 is not seen between the Wisdom and LSS treatments for the same interval. The ANOVA did not reveal any difference between Wisdom and the LSS mixture, but the increase in efficacy can be observed over time with regression models at 24 h postexposure but not at 1 h postexposure. In this study, XP was generally the lowest performing mixture when significance was found, but the highest performing on week 4 at 4 h postexposure. Despite its performance on week 4, it is possible that XP has a negative effect on Wisdom by causing it to become more water soluble and easier for rain events to influence, as other adjuvants do on bifenthrin (Mulrooney and Elmore 2000).

Many studies have been done on the efficacy of bifenthrin products and their ability to reduce mosquito populations over time, with results varying from results presented here. This study found >60% knockdown of Ae. taeniorhynchu at 24 h, 3 wk posttreatment. Trout and Brown (2009) found 50–80% mortality over 8 wk; Cilek (2008) found >70% control 4 wk after treatment; Allan et al. (2009) found 70% knockdown and 40% knockdown after 4 wk on wax myrtles (Myrica cerifera (L.)) and azaleas (Rhododendron simsii (Planch)), respectively; Qualls and Xue (2013) found >90% reduction up to 4 wk posttreatment. Results of a barrier spray treatment may vary depending on the target species, populations, active ingredient, spray equipment, and environmental conditions (Stoops et al. 2019). This illustrates the importance of knowing what field conditions negatively affect barrier treatments and whether specific adjuvants can be utilized to improve a treatment based on the potential negative effects.

Commercially available pesticide adjuvants are either underutilized or understudied in the mosquito abatement industry, considering the lack of studies found studying them. Most pesticides are purchased ready to use, as manufacturers formulate pesticides to operate with specific parameters as opposed to consumers mixing adjuvants for the desired effect. From an operational view, this approach is likely more time and labor efficient as well as safer for individuals mixing the adjuvants into the pesticides. When researching the adjuvants studied here, little information could be found on XP. Unless registered in certain states with strict labeling laws like California, the Environmental Protection Agency (EPA) does not require that adjuvant ingredients be registered or stated on the label, provided the active ingredient is already present on a preapproved list maintained by the EPA. Ingredients can then be undisclosed and listed as a proprietary on the label.

**Table 2.** Regression analysis statistics for each treatment at 1-, 4-, and 24-h postexposure intervals. Treatments include Wisdom TC Flowable® (Wisdom), Wisdom + Lesco Spreader-Sticker® (LSS), and Wisdom + Xtended Performance® (XP).1

| Value | 1 h | 4 h | 24 h |
|-------|-----|-----|------|
|       | Wisdom | LSS | XP  | Wisdom | LSS | XP  | Wisdom | LSS | XP  |
| Slope | -9.8241 | -11.109 | -9.3025 | -0.1513 | -0.1636 | -0.1513 | -0.1284 | -0.1085 | -0.1306 |
| Intercept | 51.376 | 57.75 | 48.255 | 1.0639 | 1.1701 | 1.0639 | 1.2278 | 1.2431 | 1.2088 |
| R²-value | -0.7429 | -0.7717 | -0.7028 | -0.8143 | -0.8564 | -0.7680 | -0.7272 | -0.7356 | -0.7127 |
| R² (%) | 55.2 | 59.5 | 49.4 | 66.3 | 73.3 | 59.0 | 52.9 | 54.1 | 50.8 |

1 Data reported correspond to data seen in Fig. 2.
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