

GROUND-BASED PYRETHROID ADULTICIDES REDUCE MOSQUITOES BUT NOT NONTARGET INSECTS IN CENTRAL FLORIDA

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ABSTRACT. As stewards of public and environmental health, mosquito control agencies are rightfully concerned about impacts on nontarget organisms. This study examined the impact of a modern, pyrethroid based ground adulticide program using ultra-low volume applications in a metropolitan county in central Florida. Nontarget insects and mosquitoes were collected in a before-after control-impact design at 21 sites over 1.5 years. While mosquitoes were reduced, we found no evidence for reduction of nontarget insects, regardless of taxon. Night-flying Lepidoptera may experience greater risk than other nontarget taxa, but overall effects of adulticide missions on this group were low and inconsistent. Instead, meteorology, habitat, and phenology dominate patterns of nontarget abundance. Mosquito reduction was more clearly observed and corrected post-mission reduction was consistent with results expected in complex urban and suburban treatment zones.

KEY WORDS Adulticide, modeling, nontarget, pyrethroid, ultra-low volume

INTRODUCTION

Public and private mosquito control agencies aim to balance their fundamental mission of public health against the potential nontarget impacts inherent to insecticide applications. An important tool for this goal is the use of ultra-low volume (ULV) insecticides, applied aerially (e.g., from helicopters and fixed-wing aircraft) or terrestrially (e.g., from truck-mounted and handheld systems). ULV applications to control adult mosquitoes rely on calibrated droplet sizes, proper timing, and stable weather conditions to achieve effective reduction of nuisance and vector species populations (Bonds 2012). Droplet size is particularly important for mitigating nontarget risk: larger droplets can lead to greater exposure and higher risk for nontarget organisms either through direct contact or increased deposition (Dukes et al. 2004, Zhong et al. 2011). Aerial applications can efficiently treat greater areas than ground-based applications but are not justifiable or available in many locations, where ground-based applications may be used instead. Aerial applications may pose a higher risk to nontarget insects if volume median diam (VMD) approaches or exceeds the targeted maximum of 60 microns, while ground applications are calibrated for VMDs between 8 and 30 microns and may pose lesser risk to nontarget insects.

Given the elevated risk and wider area impacted by aerial treatments, much previous nontarget research has focused on aerial adulticiding. Results of these studies have varied. Honeybee responses to both organophosphates (Zhong et al. 2009) and pyrethroids (Chaskopoulou et al. 2014) indicated low risk from aerial applications with overall colony health

unaffected by incidental individual mortality. A study of aerial pyrethrin applications found that larger bodied sentinel species survived, but ground tarps detected mortality in small-bodied flying arthropods (Boyce et al. 2007). However, studies that emphasize multiple samples under a single aerial application suffer from limitations of pseudoreplication. Additionally, studies without pre-impact sampling provide only an acute measure of mortality. This design may detect which nontarget invertebrates experience mortality but without baseline sampling cannot directly infer if mortality leads to appreciable reduction in nontarget abundance. Studies that did use pre-post sampling designs with UV light traps (Jensen et al. 1999) or malaise and pan traps (Breidenbaugh and de Szalay 2010) detected no significant effects of adulticide treatments on nontarget abundance or diversity.

Truck-mounted equipment is common in mosquito control agencies, and ground-based application missions are more frequent than aerial missions. Repeated ground-based permethrin applications did not clearly affect terrestrial and aquatic nontargets in one study (Davis and Peterson 2008), though populations of some flying insect taxa were reduced. Another study of ground applications of 3 pyrethroid-based products found no significant impact on honeybee colony health following applications but did note sublethal effects in response to resmethrin (Pokhrel et al. 2018). Overall, existing research indicates the safety of ULV applications for nontarget arthropods, but varied methods leave lingering potential of risk.

Here we studied the effects of ground-based pyrethroid applications on mosquitoes and nontarget insects in a real-world context, with a modern ground ULV program in a growing metropolitan county (Seminole County, FL). Pyrethroids are widely used in ground ULV applications and should pose little to no risk to terrestrial and aquatic vertebrates (e.g., mammals, reptiles, fish) when applied at ultra-low volumes (Schleier and Peterson 2010, 2013). However, potential on nontarget invertebrates remain uncertain. By sampling nontarget and mosquito populations

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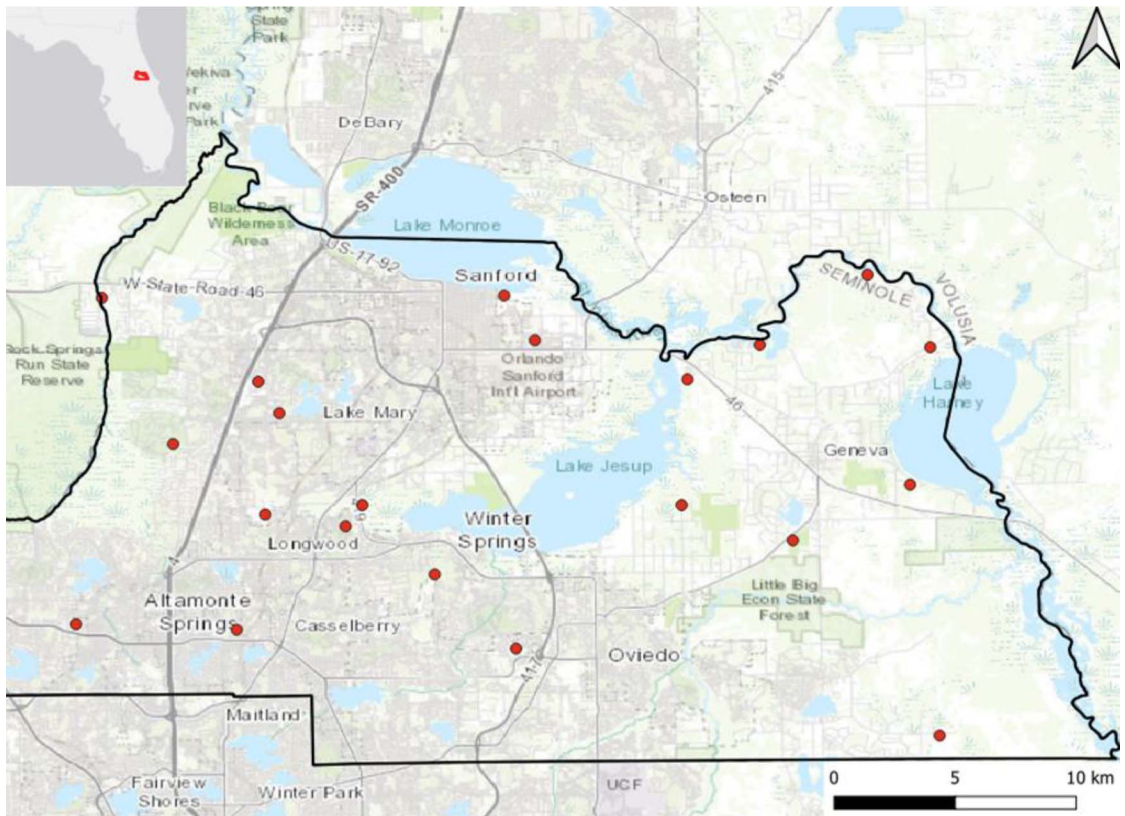


Fig. 1. A map of Seminole County, Florida. Points in red depict 21 sites sampled for this study.

in a before-after control-impact (BACI) design, this study aimed to provide insights valuable for mosquito control operations. The pre-post mission trapping used here mirrors operational data collected by many mosquito control agencies and may prove widely applicable to ground ULV programs. By sampling operational missions and analyzing for the effects of land cover and meteorology, results here aim to provide a comprehensive view of target and nontarget effects of a mosquito control program.

METHODS AND MATERIALS

Study area

Research was conducted at 21 sampling sites in Seminole County, Florida (Fig. 1) from December of 2019 through July of 2021. Seminole County is on the northern side of metropolitan Orlando and like many of its neighbors has a growing population (12% increase between 2010 and 2021[U.S. Census Bureau 2010, U.S. Census Bureau 2021]). Increasing development in the last decade has led to a heterogeneous landscape. Sample sites were selected from a subset of Seminole County Mosquito Control’s weekly surveillance locations to distribute sites

widely across the county and represent a broad range of habitats in the existing urban-wildland gradient (e.g., from high-density urban neighborhoods to suburbia surrounded by mixed forest and forested wetlands amid low-density dwellings). All sites were a minimum of 1.5 km apart. To preserve independence between samples, we avoided sampling sites closer than 2 km in distance on the same night.

Ground ULV applications and sample collection

Ground ULV missions occurred between sunset and 10:00 pm and were made at mid-label rates. Products used for missions in this study were limited to pyrethroid active ingredients, including formulations of deltamethrin (0.00089 lbs/acre), permethrin and piperonyl butoxide (0.0035 lbs/acre), and prallethrin and sumethrin (respective rates of 0.0022 and 0.00044 lbs/acre). Because of cost and availability, multiple formulations were in rotation during the study period. Study design focused on results of ground ULV treatments as a whole and was not intended to assess on a per-product basis. This study aimed to answer questions on acute effects of treatments only and was not designed to detect long-term effects on nontarget diversity or abundance. In order to avoid interference from repeated adulticide missions,

areas which had been treated within 30 days were not considered for sampling.

We selected a BACI sampling design for its sensitivity to biodiversity responses and greater power than simpler designs (Christie et al. 2019). These benefits rely on known timing of the impact for optimal isolation of effects (Smokorowski and Randall 2017), and close coordination with SMC made this possible. When a treatment was scheduled for a designated sampling site, collections were taken the night before and again the night after treatment. Samples considered “Impact” were treated by the Seminole County Mosquito Control’s truck ULV treatment regime. The same timing was applied to “Control” sites, which were not sprayed between sampled nights. All data presented here follow this consecutive three-night cadence of a pre-sample night, a treatment/control night, and a post-sample night.

Mosquitoes were sampled using Biogents BG Sentinel 2 mosquito traps, baited with perforated coolers containing ~2.25 kg of dry ice and lactic acid BG scent lures. Nontarget insects were collected using both attractive UV light traps and passive malaise traps. The UV light traps used 15W bulbs fitted to photovoltaic switches to ensure dusk-to-dawn operation, and specimens were collected in a propylene glycol solution for preservation. The UV light traps were placed to visibility at the site (e.g., forest clearings, open areas). Malaise traps also used propylene glycol preservative and were placed in areas likely to be natural flyways. All traps were set within 100 m of a road to ensure they were within an area affected by ground ULV missions. Traps were placed a minimum of 25 m from one another to reduce interference and organisms were enumerated across traps (e.g., mosquitoes in UV light traps were also counted). In total, this study included 126 trap nights for 63 paired pre-post samples. Of these samples, 21 were untreated controls, and 42 were treated by ground ULV.

Mosquito samples were frozen, then counted and identified to species using a dissecting microscope. Given their huge abundance, nontarget insect samples were counted and identified to order. However, beetles (Coleoptera) were identified to family to provide additional taxonomic resolution because they are typically diverse and numerous (as confirmed in samples) and expected to be active at the time of ULV applications. Flies (Diptera) of various taxa were also of interest because they are typically small in body size (and thus potentially vulnerable to ULV applications). Lepidoptera were considered of interest because many moths are active at night when ULV applications occurred. Non-insect arthropods, primarily arachnids, were collected rarely and incidentally and are excluded from analyses.

Statistical analyses

Before constructing models, we analyzed differences between nontarget trap designs using nonmetric dimensional scaling (NMDS) on a matrix of abundance per

nontarget insect order. The NMDS was grouped by trap type (UV, malaise), where a significant difference between traps would indicate bias toward different taxa of nontarget insects (Fig. 2). Results of this test were used to inform further analyses, indicating whether UV and malaise samples should be combined or analyzed separately. Another grouping was examined, comparing status as pre-sample or post-sample and treated or untreated. This grouping was used to test the hypothesis that post-sample nontarget taxa would vary significantly at treated sites compared to untreated sites.

Data were then analyzed with generalized mixed-effect models (GLMMs), which are well-suited for a BACI design (McDonald et al. 2000). This approach was selected for its ability to resolve non-normality in count data and to incorporate covariates that are known to affect insect abundance. In this way, high variability between samples because of the impact of spatial effects (habitat, spatial autocorrelation) and temporal effects (weather, phenology) could be addressed and quantified. Analyses were conducted using R v4.2.2 (R Core Team 2022). We analyzed three primary response variables: total abundance, taxa richness, and effective diversity for mosquitoes and nontarget insects overall. Diversity was calculated as effective number of species, derived from the Shannon-Wiener index (Jost 2006). Beetles (Coleoptera), flies (Diptera), and moths and butterflies (Lepidoptera) were also analyzed separately for total abundance.

For a BACI design, the primary question of the study (i.e., does ULV application affect mosquito and nontarget insects?) was represented by the statistical interaction between a sample’s status as pre- or post-application and its status as treated or untreated. In principle, pre- and post-application results should be similar without ULV application but reduced after ULV application. Alternatively, an absence of clear interaction effects would indicate no apparent ULV application effects, and the model with interactions may be no more plausible than simpler models. We compared a model with full interactions to simpler additive and null models using a model selection framework based on the corrected Akaike Information Criterion (AICc; Burnham and Anderson 2004), where AICc weights and δ AICc values > 2 were emphasized in inference. Full initial models also included as random effects the repeated measures design and spatial location, plus fixed effect covariates representing environmental conditions (weather, land cover), and day length (seasonality).

The first group of covariates included in the model selection process were meteorological in nature to address the large role that weather can play in insect abundance (Welti et al. 2022, Müller et al. 2024) and to avoid confounding effects that may otherwise skew analyses. Weather data were obtained from NOAA’s Global Historical Climatology Network Daily Database (Menne et al. 2012a, 2012b). Observations were referenced to the nearest reporting station for each collection site, with all stations within 2 km of collection

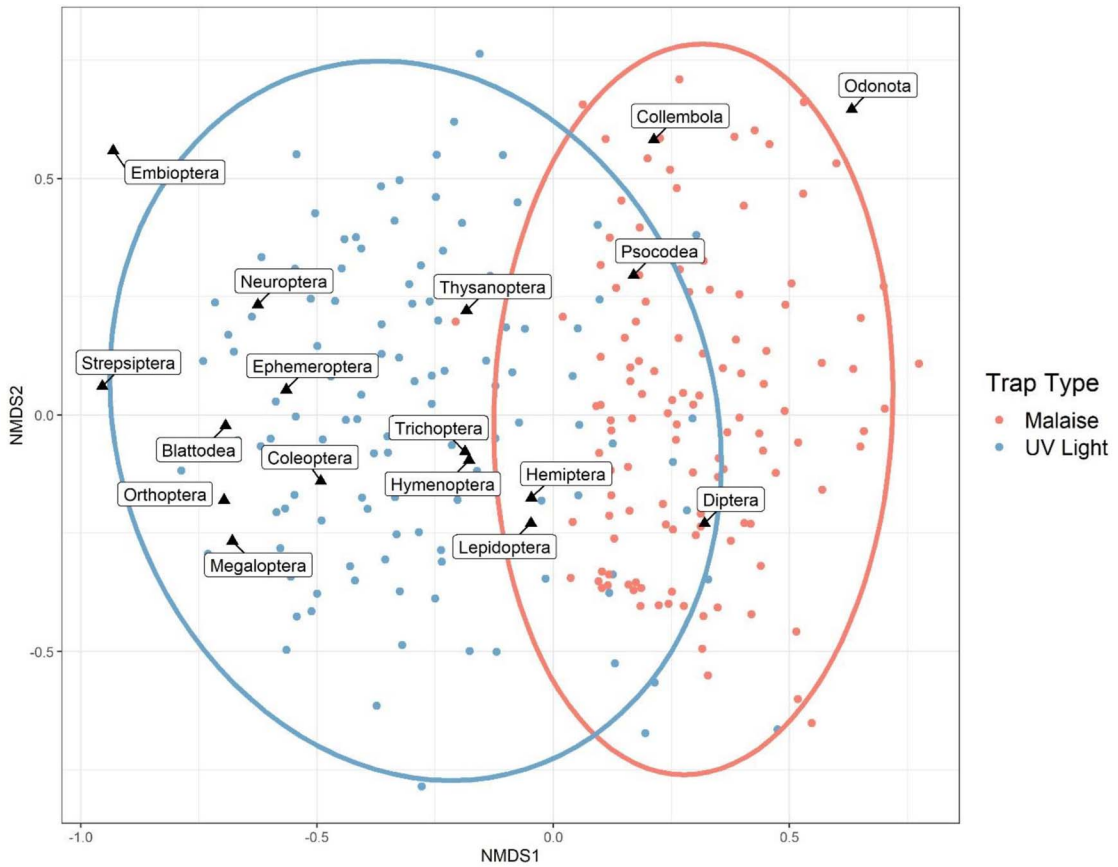


Fig. 2. An ordination plot of the NMDS separating taxa sampled by UV traps (blue) and malaise traps (red). Individual samples are represented by points; solid lines are ellipsoids representing 95% confidence per group. Nontarget insect orders are represented by triangular points and labelled along the ordination scales.

sites. Terms evaluated in the models include minimum, maximum, and average temperature, wind, and gust speed (sustained over 5 sec), daily, cumulative, and 7-day lagged cumulative rainfall. Cumulative rainfalls were summed for 10-day periods.

The second group of covariates used land cover classifications to characterize habitats surrounding sample sites. These covariates were selected for testing based on research that has shown the influence of urbanization on insect abundance and diversity (Fenoglio et al. 2021, Svenningsen et al. 2022). Inclusion of land cover also addressed the potential confounding effect of habitat associations in certain taxa, e.g., those with aquatic life stages (caddisflies, mayflies, mosquitoes) appearing in larger numbers near sources of water. Land cover data were obtained from the Florida Department of Environmental Protection’s Land-Use Land Cover dataset (Florida Department of Environmental Protection, Division of Environmental Assessment and Restoration, Watershed Services Program 2017). Level 2 classifications were extracted in 1 km buffers surrounding sample sites using QGIS software, and the area of each class was calculated

(QGIS Development Team, n.d.). This was then consolidated using a principal component analysis, where the first four principal components (PCs) represented 80% of total variation in 14 land cover variables. PC1 was strongly correlated with impervious surfaces and highly urban environments; PC2 with rural residential areas near non-forested wetlands; PC3 delineated differences between urban and suburban residential areas; and PC4 was strongly correlated with suburban neighborhoods near large bodies of water and their associated drainage basins. We considered PC4 as indicating hydric conditions consistent with potential hotspots for mosquito population control efforts.

Statistical model terms for each response variable were checked for collinearity and autocorrelation and models were iteratively refined until collinearity and autocorrelation were minimized. Coefficients included in the final models (Table 1) were broadly divided into categories of seasonality (measured by day of year), climatic variables, and land cover principal components described above. Random effects of space and time were also tested using the geographically weighted regression model structure provided by R

Table 1. Most plausible models for the nontarget insects' and mosquitoes' responses. Nakagawa Pseudo-R² is reported here, divided into marginal (R²_M; fixed effects) and conditional (R²_C; fixed + random effects). β_i is the coefficient of the pivotal treatment:night interaction, reported with 95% confidence intervals. Model covariates are reported here, including the time effect of day-of-year (*doy*), minimum and maximum temperature as linear and quadratic (*tmin*², *tmin*, *tmax*², *tmax*), nightly precipitation (*prcp*), cumulative precipitation for intervals of 8, 10, and 14 days (*cr8*, *cr10*, *cr14*), cumulative precipitation lagged by 7 days (*cr10_lag7*, *cr14_lag7*), wind speed as an average, 5-sec, and 2-min gusts (*wavg*, *wsf5*, *wsf2*), and land cover principal components as described above (*PC1*, *PC2*, *PC3*, *PC4*).

Response	Trap	R ² _M	R ² _C	β _i (95% CI)	Model covariates
Nontarget Abundance	UV	0.556	0.75	0.372 (-0.284, 1.03)	<i>doy</i> + <i>tmin</i> + <i>tmax</i> + <i>wavg</i> + <i>wsf2</i> + <i>cr10_lag7</i> + <i>PC2</i>
Nontarget Richness	Malaise	0.2	0.662	-0.0493 (-0.447, 0.349)	<i>tmin</i> ² + <i>prcp</i>
	UV	0.449	0.599	1.62 (0.0241, 3.22)	<i>tmin</i> ² + <i>wavg</i> + <i>prcp</i>
Nontarget Diversity	Malaise	0.193	0.421	-0.666 (-1.86, 0.528)	<i>tmin</i> ² + <i>wavg</i> + <i>cr8</i> + <i>PC1</i>
	UV	0.057	0.899	0.00423 (-0.232, 0.231)	<i>doy</i> ² + <i>tmax</i> + <i>wavg</i> + <i>cr10</i> + <i>prcp</i> + <i>cr10_lag7</i> + <i>PC1</i> + <i>PC2</i>
Lepidoptera Abundance	Malaise	0.627	0.696	-0.0409 (-0.0350, 0.117)	<i>Doy</i> ² + <i>tmax</i> ² + <i>wavg</i> + <i>wsf5</i> + <i>cr10_lag7</i> + <i>PC1</i> + <i>PC2</i>
Diptera Abundance	UV	0.412	0.537	-0.372 (-1.02, 0.280)	<i>tmax</i> ² + <i>wavg</i> + <i>prcp</i> + <i>cr10_lag7</i>
	Malaise	0.27	0.592	-0.127 (-0.747, 0.493)	<i>doy</i> ² + <i>tmin</i> ² + <i>cr10_lag7</i>
Coleoptera Abundance	UV	0.353	0.999	-0.0133 (-0.752, 0.725)	<i>doy</i> ² + <i>wavg</i> + <i>prcp</i> + <i>cr14</i> + <i>cr10_lag7</i> + <i>cr14_lag7</i> + <i>PC2</i>
	Malaise	0.267	0.662	-0.152 (-0.561, 0.258)	<i>doy</i> ² + <i>tmin</i> ² + <i>tmax</i> ² + <i>wsf5</i> + <i>cr10</i> + <i>PC1</i> + <i>PC2</i>
Coleoptera Richness	UV	0.839	0.999	1.12 (0.214, 2.03)	<i>doy</i> ² + <i>tmin</i> ² + <i>wavg</i> + <i>prcp</i> + <i>cr10_lag7</i> + <i>PC1</i> + <i>PC2</i>
	UV	0.864	0.864	1.66 (-3.05, 6.37)	<i>doy</i> ² + <i>tmax</i> ² + <i>wsf2</i> + <i>cr10_lag7</i> + <i>PC1</i>
Mosquito Abundance	UV	0.312	0.522	-0.0349 (-0.0944, 0.0246)	<i>tmax</i> + <i>tmin</i> ² + <i>cr10</i> + <i>prcp</i>
	BG	0.407	0.434	-0.45 (-1.21, 0.310)	<i>tmax</i> + <i>wavg</i> + <i>wsf2</i> + <i>cr14_lag7</i> + <i>PC4</i>
Mosquito Richness	BG	0.586	0.586	-0.862 (-2.47, 0.750)	<i>doy</i> ² + <i>tmin</i> ² + <i>tmax</i> ² + <i>wsf2</i> + <i>cr8</i> + <i>prcp</i> + <i>cr8</i> + <i>cr14</i> + <i>PC1</i> + <i>PC3</i> + <i>PC4</i>
	BG	0.209	0.342	-0.0432 (-0.108, 0.0218)	<i>doy</i> ² + <i>tmin</i> + <i>tmax</i> + <i>wsf2</i> + <i>cr14</i> + <i>prcp</i>

package sdmTMB (Anderson et al. 2022), based on an explicit spatial mesh using sample site coordinates and testing for temporal random effects to represent repeated measures at sample sites during the study. This model structure was further tested by backwards stepwise regressions (based on changes in AICc) to test if terms should be retained. Explained variance was calculated using an adapted method of Nakagawa's R^2 , which partitions explained variance based on model fixed effects, random spatial effects, and random temporal effects when present. Model estimates were produced from most plausible models using standardized median coefficients of fixed effects across observations (Fig. 3). Plausible treatment effects reported here are based on these posterior estimates, and corrected using the Henderson-Tilton formula (Henderson and Tilton 1955), also known as Mulla's formula (Mulla et al. 1971). Here, reduction is corrected such that:

$$\%Reduction = (1 - [C_1 \times T_2]/[C_2 \times T_1]) \times 100\%$$

where C_1 = control pre-sample, C_2 = control post-sample, T_1 = treatment pre-sample, and T_2 = treatment post-sample.

We interpreted statistical model results based on the signs of the key interaction effect, where a negative coefficient (β_i) indicates ULV application reduced abundance or diversity, after accounting for covariates and random effects of space and time across a county for 1.5 years. Mosquito control programs do not expect to fully exterminate mosquito populations with a single treatment. Thus, we report confidence intervals of effects and whether those intervals include zero, but we considered treatment effects based on direction and effect size strength rather than simple statistical significance. Strongly negative β_i values indicated potential effects, with caution given confidence intervals.

RESULTS

Nontarget insects

We examined ULV application effects separately for samples collected from UV and malaise traps because they differed significantly when compared via NMDS (Fig. 2; $P < 0.001$). The UV traps caught more individual insects than did malaise traps (median capture of 1000 individuals per night vs 336 per night, respectively). In addition, UV light traps collected representatives from a greater number of insect orders than malaise traps (median of 10 orders per night vs 7 per night, respectively). Overlap in insect orders collected between the two traps was greatest in Hemiptera, Lepidoptera, Diptera, Thysanoptera, and Psocodea. Malaise traps captured Colembola and Odonata, which were largely absent from UV trap samples, while all other orders were numerically biased to UV traps.

Examining the same NMDS analysis for differences between nights and treatments found no

evidence of the crucial interaction effect: communities were not different between first and second trap nights, regardless of treatment. However, treated and untreated sites differed significantly ($P = 0.03$) for overall nontarget communities because treated sites typically had greater abundance and diversity of nontargets.

The most plausible covariates for models of a given response (i.e., total abundance, diversity, lepidopteran abundance; Table 1) differed among analyses of different response variables, along with the effect size and confidence intervals for the primary treatment:night interaction (hereafter β_i). Below we summarize those results.

Total nontarget abundance

The most plausible model for nontarget abundance sampled by UV light traps was dominated by coefficients of temperature and seasonality, which were approximately 7 and 10 times greater than the crucial treatment: night interaction. Pseudo R^2 indicates that this model captured variance well in both the fixed climatic and landscape effects ($R^2_M = 0.556$) and was further improved by the spatial random field ($R^2_C = 0.750$). The model predicted an interaction coefficient not clearly different from zero ($\beta_i = 0.372 \pm 0.656$), indicating no apparent effect on nontarget abundance at sites that received ULV treatments. Model estimates showed that control sites exhibited an average decrease in abundance ($\Delta C_{Est} = -0.619$), but treated sites experienced a lesser decrease ($\Delta T_{Est} = -.104$). This trend contradicts a potential ULV effect on nontarget insects. Also, this effect was not consistent, so that 95% β_i confidence intervals (CIs) were broad and included zero ($-0.206, 1.03$).

Malaise traps exhibited a weaker effect for nontarget abundance, with only moderate variance because of fixed effects ($R^2_M = 0.200$), but most "explained" variance because of spatial random effects ($R^2_C = 0.662$). The most plausible model failed to detect a change in abundance between control and treated sites, with wide confidence intervals ($\beta_i = 0.049$, 95% CI $[-0.447, 0.349]$). Finally, other variables (minimum temperature, day of year) dominated model estimates (Table 1). Overall, models for both UV and malaise traps fail to indicate plausible reduction in nontarget insect abundance as a result of mosquito control missions.

Richness and diversity

Results for nontarget taxa richness were variable between traps, consistent with the differences in sampling mechanism. The most plausible model for UV samples found a consistent positive coefficient for the treatment:night interaction ($\beta_i = 1.62$, CI $[0.0241, 3.22]$), or opposite of that expected for ULV application effects. Model estimates showed that this was a result of a mean decrease in richness at control sites and slight increase at treated sites ($\Delta C_{Est} = -0.160$, $\Delta T_{Est} = 0.0104$). By comparison, malaise samples demonstrated

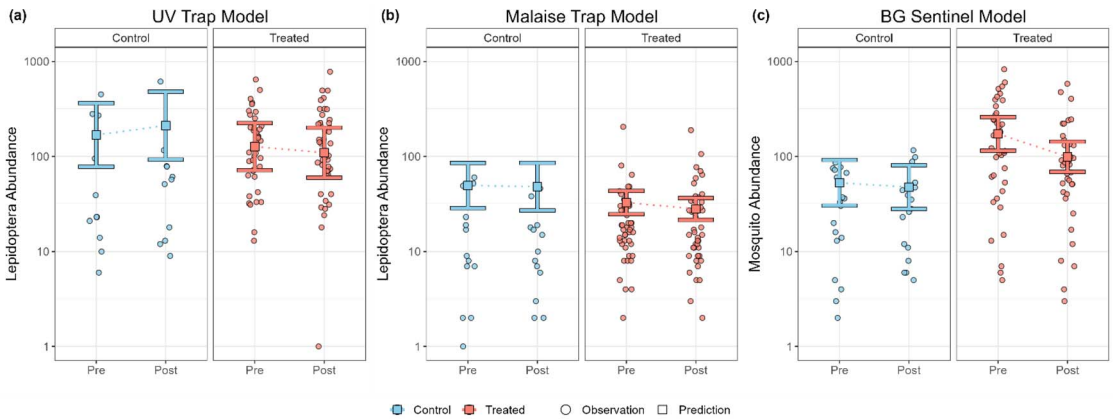


Fig 3. Observations of sampled abundance and estimates from the three models which showed the greatest impact: Lepidoptera in (a) UV and (b) malaise traps, and (c) mosquitoes captured using Biogents Sentinel 2 traps. Observations used to compute estimates are displayed as small circular points. Error bars are centered on mean predicted estimates of abundance as a response to treatment. Predictions are estimated from most plausible models (Table 1) with standardized, median covariates. Error bars represent 95% confidence intervals. Dotted lines visualize slope between pre-samples and post-samples.

a weaker negative effect with broader confidence intervals ($\beta_i = -0.666$, CI $[-1.86, 0.528]$). Fitted estimates confirmed this was because of a mean increase in taxa richness from night to night at control sites, while treated sites experienced a very slight decrease on average ($\Delta C_{Est} = 0.0925$, $\Delta T_{Est} = -0.00705$). Once again, the winning model for UV trap data represented variance better in fixed effects than that of the malaise trap ($R^2_M = 0.449$ and $R^2_M = 0.193$, respectively). In both models, temperature dominated as a quadratic effect predicting a peak richness at high minimum temperatures.

Effective diversity did not share this trend, though again, no clear ULV application effect was observed. Instead, effect sizes of the treatment:night interaction were small for both models, with broad confidence intervals indicating little consistent change (Table 1). In UV traps, effective taxa diversity was strongly predicted by a linear effect of minimum temperature. By contrast, malaise trap diversity varied more plausibly with seasonality (measured by day of year) than temperature. This trend indicated that diversity in malaise traps decreased slightly during the summer, possibly because of abundant taxa overwhelming traps and lowering evenness and as a result effective diversity.

Nontarget taxa of interest

Lepidoptera: Collected Lepidoptera consisted primarily of night-flying moths and small-bodied butterflies. Of the nontarget taxa examined, only Lepidoptera in UV trap samples showed a potential effect of ULV application, but that effect was not clearly different from zero ($\beta_i = -0.372$, 95% CI $[-1.02, 0.280]$; Table 1). Model estimates help understand mechanisms that led to this result: on average, lepidopteran catch at control

sites increased from night to night but decreased at treated sites ($\Delta C_{Est} = 0.203$, $\Delta T_{Est} = -0.136$; Fig. 3), consistent in direction with a treatment effect. Beyond ULV treatments, Lepidoptera abundance in UV traps was most strongly influenced by land cover, temperature, and seasonality.

By contrast, abundance of Lepidoptera in malaise traps responded only to climatic conditions, with temperature leading in effect size over rainfall and wind. The essential interaction effect size for the lepidopteran malaise trap model was negative (i.e., consistent with a ULV application effect) but lower than for UV traps ($\beta_i = -0.127$ and also not clearly different from zero; Table 1, Fig. 3).

Overall, data from both active and passive traps do not clearly support inference of an effect of ULV treatment on Lepidoptera, because of broad confidence intervals. Detected effects were in the correct direction to indicate reduction by ULV treatments, but data remained variable across this real-world scenario and effects were not clear. However, models explained substantial variance, due in part to covariates and random effects: UV traps outperformed malaise in fixed effects (UV $R^2_M = 0.412$, malaise $R^2_M = 0.270$) but overall variance was roughly comparable because of the contribution of spatial random effects (UV $R^2_C = 0.537$, malaise $R^2_C = 0.592$).

Diptera: Collected Diptera were diverse and numerous, including many different families of flies. While larger-bodied groups including blow flies (Calliphoridae), and deer and horseflies (Tabanidae) were present in collections, the vast majority of specimens were comparable or smaller in body size compared to mosquitoes. These included but are not limited to drain flies (Psychodidae), fungus gnats (Sciaroidea), and fruit flies (Drosophilidae). These groups are provided as examples, and analyses here

are performed at the order level only. Diptera results were inconclusive for the impacts of ULV treatments, despite their typical small body size. The most plausible model for UV trap abundance fit data remarkably well R^2_C (0.999), due largely to temporal random effects ($R^2_\varepsilon = 0.445$), followed by fixed effects ($R^2_M = 0.353$) and spatial random effects ($R^2_\theta = 0.201$). Within fixed effects the influence of ULV treatments was low and not clearly different from zero ($\beta_i = -0.0133$, CI $[-0.752, 0.752]$). Moreover, seasonality and minimum temperature had greater influence, indicating that abundance of night-flying dipterans was largely phenological and meteorological in nature.

Malaise trap results for Diptera told a slightly different story but with a similar conclusion. The most plausible model included spatial random effects and explained variance well ($R^2_C = 0.267$; $R^2_C = 0.662$). This model estimated a stronger negative effect for ULV treatments, indicating a slight reduction in Diptera abundance versus control sites ($\beta_i = -0.152$), though it too was quite variable (CI $[-0.561, 0.258]$). Estimates from this model (Table 1) reveal an average night-to-night increase at control sites, with only minor reduction estimated at treatment sites ($\Delta C_{Est} = 0.121$, $\Delta T_{Est} = -0.021$). Seasonality, rainfall, and land cover were important to dipteran abundance. Overall, results indicate that Diptera abundance is highly variable in time and space, and lack a clear signal for ground ULV applications on night-flying dipterans.

Coleoptera: Because of their abundance and diversity, Coleoptera were identified to family for added taxonomic resolution. We identified 64 families of beetles present between UV and malaise traps, with the most abundant being water scavenger beetles (Hydrophilidae), rove beetles (Staphylinidae), and marsh beetles (Scirtidae). Samples were analyzed by NMDS, which found that composition varied significantly by trap type ($P < 0.01$) but did not vary significantly with the treatment: night interaction ($P = 0.660$). Variance by trap type was because of malaise traps' low capture rate (median of 3 individuals per night). As a result, subsequent analyses of beetle abundance, richness and diversity were performed on data from UV traps.

The most plausible model for Coleoptera abundance found a positive effect of the treatment:night interaction ($\beta_i = 1.12$, CI $[0.214, 2.03]$); contrary to a negative ULV application effect (Table 1). Consistent with results for total nontarget abundance sampled by UV traps, there was a greater relative decrease at control sites night-to-night than at treated sites. Fixed effect covariates explained a large proportion of variance and were dominated by temperature, seasonality, and land cover ($R^2_M = 0.839$, $R^2_C = 0.999$). For land cover, beetle abundance was positively correlated with both high and moderate urbanization.

Beetle family richness also increased between sample nights at treated sites ($\beta_i = 1.66$), but broad confidence intervals show inconsistency in that

change (CI $[-3.05, 6.37]$; Table 1). Fixed effects were again important to the model, with no gain added by a spatial random effect (R^2_M and $R^2_C = 0.864$). It is possible that spatial variance was adequately captured by the inclusion of land cover PC1, which showed a positive correction between coleopteran family richness and highly urban areas.

In contrast, the most plausible model for effective diversity of Coleoptera families was improved by spatial random effects ($R^2_M = 0.312$, $R^2_C = 0.522$). This model indicated a very minor reduction in diversity as a result of treatment, and again confidence intervals indicate this effect was inconsistent ($\beta_i = -0.0349$, CI $[-0.0944, 0.0246]$). Fixed covariates here were dominated by the quadratic effect of minimum temperature.

Mosquito reduction

Twenty-six mosquito species were collected and analyzed for changes in overall abundance, species richness, and effective diversity as a result of ULV treatments. Most abundant and frequently captured species included *Culex nigripalpus* Theobald, *Anopheles crucians* Wiedemann, and *Aedes infirmatus* Dyar and Knab. The most plausible model of mosquito abundance indicated the strongest reduction out of any taxa examined and in the direction consistent with ULV application effects ($\beta_i = -0.450$), though it was variable (CI $[-1.21, 0.310]$). Standardized estimates of the model clearly illustrate this overall reduction in mosquito abundances: absolute reduction at treated sites was within expectations for ground ULV missions, and post treatment reductions clearly pushed average estimates below the lower confidence level for pretreatment sites ($\Delta C_{Est} = -0.113$, $\Delta T_{Est} = -0.427$; Fig. 3c). Correcting this plausible reduction effect using Mulla's formula gives a corrected reduction of 33%. This model explained variance well, and a spatial random effect slightly improved model fit ($R^2_M = 0.407$, $R^2_C = 0.434$), related to land cover PC4, as explained above. For weather effects, observed mosquito abundance most plausibly increased linearly with maximum temperature, cumulative lagged rainfall, and average wind speed, but decreased with maximum wind gust speed and nightly precipitation (Table 1, Supplement Table 1).

Results for mosquito species richness were less conclusive, indicating strong reduction but with correspondingly large confidence intervals ($\beta_i = -0.862$, CI $[-2.47, 0.750]$). Fixed model predictors included seasonality, minimum and maximum temperature (consistent with seasonal species richness), and wind and rainfall. Cumulative rainfall had a negative coefficient, potentially related to slightly lower species richness during the peak of the rainy season. This model also included land cover principal components: PC1 indicated a moderate increase in species richness in urban areas; PC3 indicated reduced species richness in highly landscaped suburban areas; and PC4 showed a strong increase in species richness

in the wet habitat hotspots described above. The three land cover variables apparently reduced variation otherwise explained by spatial random effects, but fixed effects of this model still represented substantial variance ($R^2_M = 0.586$).

Mosquito effective diversity results were similar to those for richness: small effect size of the treatment:night interaction and broad confidence intervals ($\beta_i = -0.0432$, CI $[-1.08, 0.0218]$). However, the most plausible model differed in 2 clear ways: land cover variables were not supported, and thus variance explained by spatial random effects improved the model ($R^2_M = 0.209$, $R^2_C = 0.342$). Otherwise, similar conclusions may be drawn: ground ULV treatments did not significantly reduce mosquito diversity.

Overall, we found no clear evidence for effects of ground ULV applications on nontarget insects, measured in multiple ways among taxa or as a whole. Among nontarget taxa, Lepidoptera capture decreased in a manner consistent with treatment effects, but such effects were too variable to be considered meaningful across the county-wide study during the 1.5-year sample period. Mosquito abundance was reduced by ULV applications, but this too was quite variable across the study, and did not strongly indicate effects cascading up to species richness or effective diversity.

DISCUSSION

Pyrethroid adulticides, when used with proper care for label rate, droplet size, and application timing, are expected to minimize impact to nontarget organisms. Data collected and analyzed here support this goal, indicating no clear effects of a ground ULV program on nontarget arthropod abundance or individual taxa of interest. As a result of the above goal, a ground ULV program aims to control mosquito populations, but is not expected to annihilate them. Mosquito abundance was reduced by ULV applications, though modestly and variably. In general, analyses here represented confounding factors well to help tease out the observed signals amid much natural variation.

Methodologically, the use of both attractant UV traps and passive malaise traps provided insight beyond that in previous work. Experimentally, pairs of before- and after-ULV spraying trap events represented distinct mosquito control treatments and were repeated through time at multiple sites. Entomologically, UV light traps captured a numerically greater and more representative sample of night-flying insects than their passive counterparts. On the other hand, malaise traps sampled more Diptera, Psocodea (primarily bark lice), and Odonata (primarily damselflies) than UV traps. Despite a meaningful distinction between trap type in results both trap types showed no clear effect of ULV application on nontarget insects. The study design and sampling also showed that treated and untreated sites clearly differed in abundance and diversity of nontargets, consistent with known features of mosquito control: areas

prioritized for mosquito treatment also support nontarget insects as a result of shared conditions e.g., habitat, resources, or timing. Analyses here showed that areas which crossed SCMC's threshold for treatment by ground ULV based on surveillance trapping and resident complaints a) had a higher abundance of mosquitoes and b) had a higher abundance of nontarget insects than control site counterparts.

Much like growing evidence for global insect decline (Hallmann et al. 2017), total insect abundance is a coarse (i.e., not taxonomically resolved) but valuable measure for a study of nontarget insecticide impacts. The weak, positive treatment effect found for nontarget abundance observed here indicates that, at the acute level, mosquito control contributes little to this described "death by a thousand cuts" (Wagner et al. 2021). However, we must note that this study did not examine potential chronic effects related to repeated ULV applications, which could compound over time. Nontarget abundance for both malaise and UV traps was dominated by phenology and climatological variables, related to seasonal trends in reproduction and weather conditions encouraging flight. Land cover also played a key role in nontarget abundance and diversity, and may exhibit a persistent effect on insect populations and diversity. These patterns are consistent with existing conceptual frameworks in which increasing urbanization and human activity such as agricultural intensification is correlated with lower insect diversity (Seibold et al. 2019, Fenoglio et al. 2021).

Of the nontarget taxa examined in detail, results here for Lepidoptera indicate that follow-up research may be justified. Our results do not show a clear reduction effect of ULV treatments on Lepidoptera, and models indicated that phenology, temperature, and land cover exert greater relative impact on lepidopteran abundance. Our results also show that a night-to-night increase in Lepidoptera at control sites and greater average abundance at control sites compared to treated sites contributed to potential apparent effects of ULV application. This result may indicate habitat effects but is also consistent with long-term consequences of repeated ULV treatments, potentially related to larval sensitivity to adulticide deposition (Zhong et al. 2009). Despite short half-lives, permethrin residues can be detected months post-treatment (Bargar and Hladik 2023) and herbivorous larvae can be exposed to lethal and sublethal pyrethroid doses through deposition on vegetation (Oberhauser et al. 2009, Hoang and Rand 2015). We reiterate that results here do not indicate a consistent negative impact on lepidopteran abundance, but the negative coefficient for UV traps indicates further research on relatively under-studied night-flying species (primarily moths) that comprise an estimated 75–80% of lepidopteran species (Kawahara et al. 2018). Recent research using caged insects observed relatively low risk for butterflies compared to mosquitoes, with resting butterflies receiving dosages of

permethrin below the LC_{50} (Bargar and Jiang 2023). However, night-flying Lepidoptera may intercept ULV droplets in greater doses than caged butterflies.

We selected Diptera as a taxon of interest because their typically small body size may render them vulnerable to ULV droplets targeted for mosquito species of similar size. Small-bodied (<8 mm) Diptera (e.g., Chironomids) are known to be susceptible to aerially applied pyrethrin (Kwan et al. 2009). However, results from UV light trap samples indicated highly stochastic patterns of dipteran abundance, unrelated to ULV treatments and dominated by timing and temperature. The high contribution of random effects to explained variance in this model emphasized the stochastic nature of dipteran abundance in this study. The model based on malaise trap collections also found no conclusive effect of ULV treatments on dipteran abundance, though dipterans increased with more rural land cover (PC2). Diptera families exhibited complex spatial patterns of abundance and diversity across urban-wildland gradients (Rubio et al. 2012) and likely exhibit counteracting patterns at the order level. Further research into this group requires additional taxonomic resolution not provided by this study but may be justified given small body sizes but high abundances.

Analysis of Coleoptera families indicated no effects of ULV treatments on beetle abundance, richness, or diversity. This was consistent with expected body size, flight times, and behaviors. A study of ladybugs caged at ground level, for example, found significantly reduced mortality by permethrin treatments, while ladybugs in covered cages (to simulate the obstruction provided by dense vegetation) exhibited nearly no mortality (Peterson et al. 2016). We placed UV light traps at ground level, with lights and vanes ~27 cm high. Therefore, it is possible that our sampling was preferential to low-flying or ground-dwelling Coleoptera that are less likely to encounter droplets on the wing. However, results here stand abundance, richness, and diversity of sampled Coleoptera were dominated by environmental covariates and were not negatively impacted by adulticide treatments.

Mosquito surveillance results provide a clear picture to contextualize and complement the nontarget data presented here: ground ULV missions plausibly reduced mosquito abundance, while observed reductions of nontarget insects were minimal and inconsistent. However, two major caveats apply. First, predicted mean mortality, after accounting for covariates, was a corrected 35%. These mortality values indicate less effectiveness than preferred in open field conditions, but are comparable to results of caged mosquito trials in vegetation (Barber et al. 2008). Second, confidence intervals for β_i bounded zero indicating inconsistent reduction of mosquito populations. These caveats are related: the estimates presented here are the averages across all missions sampled during 1.5 years, and results vary because of

many uncontrollable factors (e.g., wind, timing, and landscape). Our complex models captured some of the effect of such factors and showed they were important to results. Vegetation and structures are known to be important impediments to ULV insecticide efficacy (Mount 1998). Samples here were taken in the real-world context of operational sites in Seminole County, a majority of which were in partly or densely vegetated residential areas. As a final note, no application made as part of this study was part of a mosquito-borne disease response; all missions were because of surveillance and resident complaints for nuisance mosquitoes. Application rates and frequencies were set accordingly. As such, we expect our study may inform many other similar ground-based ULV application programs to control mosquito populations.

Overall, results here contribute to a growing body of research indicating the safety of modern, data-driven mosquito control programs for nontarget arthropods. Assessment of mosquito abundance indicates a mean 33% reduction by ground ULV, across complex and often challenging habitats and weather conditions. Meanwhile, in the absence of clear reduction effects we conclude that truck mounted ULV treatments with pyrethroid insecticides are not harmful to populations of nontarget insects, but urge further research into potentially vulnerable taxa.

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