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Baseline Susceptibility Status of Florida Populations of *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus*

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Abstract

Resistance to insecticides used to control mosquito vectors threatens the ability of mosquito-control organizations to protect public health. *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) are invasive species widely distributed throughout Florida and have been implicated in recent epidemics of Zika, dengue, and chikungunya viruses. Knowledge of the susceptibility status of these mosquito species to pyrethroid and organophosphate active ingredients (AIs) is needed to inform product selection and treatment decisions. The susceptibility of 37 *Ae. aegypti* and 42 *Ae. albopictus* populations from Florida was assessed in response to six pyrethroid and three organophosphate AIs using the CDC bottle bioassay method. Of all bioassays completed with a pyrethroid AI, 95% for *Ae. aegypti* and 30% for *Ae. albopictus* resulted in a resistant outcome. For organophosphate AIs, ~31% of assays conducted for both species were classified as resistant. The highest frequency of susceptibility for both species was observed in response to the organophosphate AI, naled. Lambda-cyhalothrin was the only pyrethroid to result in a susceptible status for *Ae. aegypti* and also had the highest frequency of susceptibility for *Ae. albopictus.* Resistance was detected to every AI tested for both *Ae. aegypti* and *Ae. albopictus*, but there was a pronounced trend of pyrethroid resistance in Florida populations of *Ae. aegypti.* The results of this work provide evidence for the need to decrease reliance on pyrethroids and to implement different methods of control of *Ae. aegypti* in Florida.

Keywords

Aedes aegypti; *Aedes albopictus*; insecticide resistance; pyrethroid; organophosphate

The founding of the Florida Anti-Mosquito Association (now Florida Mosquito Control Association) in 1922 was spurred by the need to control mosquitoes that presented a major threat to public health and prevented people from being able to comfortably inhabit Florida (Patterson 2004). Disruption of larval development sites by ditching or stocking these sites with mosquitofish had dramatic positive impacts on the rates of malaria transmission in

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Florida. Since the early 1900s, mosquito control in Florida has evolved from being focused on disrupting larval habitats (Patterson 2016) to a modern integrated approach known as integrated mosquito management (IMM). As the name implies, IMM is based on an integrated approach that includes surveillance and aims to control mosquitoes through a combination of larviciding, adulticiding, biological control, habitat management, education, and communication with the public. It also emphasizes the importance of evidence-based decision making when it comes to operational control decisions and policy (World Health Organization 2013).

Present-day mosquito control continues to play a vital role in protecting public health and actively works with the health sector to monitor mosquito-borne viruses, both endemic and exotic. In Florida, endemic arboviruses, such as West Nile virus, Eastern equine encephalitis virus, and St. Louis encephalitis virus, are closely monitored through a sentinel chicken program administered by the Florida Department of Health (FDOH), mosquito pool testing for viruses, and disease reporting so appropriate control actions can be taken to prevent outbreaks in humans and animals (FDOH 2014). In recent decades, Florida has faced challenges from exotic arboviruses as well, including dengue (Radke et al. 2012, Rey 2014, Arévalo et al. 2015), chikungunya (Kendrick et al. 2014), and Zika (Likos et al. 2016, FDOH 2019) viruses, which collectively resulted in over 400 locally acquired human disease cases since 2009. As there are currently no vaccines for these three exotic viruses, prevention of human disease relies heavily on personal protection and vector control, including the use of adulticides.

Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse) are competent vectors of a variety of arboviruses, including dengue, chikungunya, and Zika viruses. Both species are widely distributed throughout Florida (Parker et al. 2019) and are considered a nuisance and public health concern because of their close association with human habitation and affinity for taking bloodmeals from humans (Hawley 1988, McBride et al. 2014, Takken and Verhulst 2013). However, controlling these mosquitoes can be difficult because, as larvae, they occupy artificial and natural containers that can be both cryptic and abundant, making habitat elimination or larviciding efforts challenging to implement on a large scale (Simard et al. 2005, Unlu et al. 2014, Garcia-Sánchez et al. 2017). Both *Ae. aegypti* and *Ae. albopictus* are crepuscular mosquitoes with host-seeking peaks occurring just before dusk and around dawn. However, biting can continue into hours outside of these peak activity times, a behavior that is likely driven by host availability and is why they are also considered a day-biting mosquito (Chadee and Martinez 2000, Smith et al. 2018).

Public health vector control adulticide missions are usually conducted in the evening due to label restrictions, the activity patterns of humans, and the host-seeking patterns of pestiferous mosquito species. Despite this asynchrony in treatment and activity periods of *Ae. aegypti* and *Ae. albopictus*, these species are still exposed to adulticides from spray missions, residual barrier treatments, and other sources, like the household use of insecticides (Gray et al. 2018). Currently, only two chemical classes of mosquito adulticides are available to public health vector control professionals in the United States: organophosphates and pyrethroids. Both classes act on the nervous system of insects with organophosphates acting as acetylcholinesterase inhibitors (O'Brien 1966) and pyrethroids as voltage-gated sodium channel agonists (Narahashi 1971). With this limited chemical toolbox for adulticiding, chemical rotation is difficult and insecticide resistance can develop.

Insecticide resistance is a threat to the ability of vector control to manage mosquito populations. During periods of local transmission of arboviruses, decreased adulticiding efficacy can ultimately allow for further spread of the pathogen. Since the first description of insecticide resistance in mosquitoes in 1947, only 1 yr after the introduction of DDT (Brown 1986), insecticide resistance has been reported in 68 countries to at least one class of insecticide (World Health Organization 2019). In *Ae. aegypti* and *Ae. albopictus* specifically, resistance has been documented to numerous active ingredients (AIs) hundreds of times globally (https://www.pesticideresistance.org/). In Florida, a recent study described phenotypic resistance to pyrethroids (6- to 61-fold resistance ratio) in 21 Florida *Ae. aegypti* strains and low pyrethroid resistance (less than 1.6-fold resistance ratio) in five *Ae. albopictus* strains (Estep et al. 2018). Other studies have also detected varying levels of resistance in Florida populations of *Ae. aegypti* and *Ae. albopictus* to pyrethroids and organophosphates (Liu et al. 2004, Marcombe et al. 2012). However, the need for systematic statewide resistance surveillance persists as vector control professionals strive to maintain the efficacy of the limited mosquito control toolbox while preventing mosquito-borne disease.

We conducted a statewide insecticide susceptibility monitoring program for *Ae. aegypti* and *Ae. albopictus* in Florida. The objective of the program was to determine the statewide insecticide susceptibility status of these two species and to aid in informing mosquito control efforts to protect public health.

Materials and Methods

Mosquito Collection and Rearing

A statewide container mosquito survey was initiated by a team at the Florida Medical Entomology Laboratory (FMEL), University of Florida, Institute of Food and Agricultural Sciences and is described in Parker et al. (2019). Briefly, from 2016 to 2018, various organizations were provided a surveillance kit to collect *Ae. aegypti* and *Ae. albopictus* eggs throughout Florida. Egg papers were sent to FMEL where the samples were processed. Upon receipt, the egg papers were observed under a microscope and viable eggs were quantified. Any eggs that were not collapsed/desiccated and had not already hatched were considered viable. Eggs were held in a plastic container with a moist cotton ball to prevent desiccation for at least 1 wk prior to hatching.

Insectary conditions were maintained at 27 ± 2°C and 50–60% relative humidity. When sufficient numbers of viable eggs were collected (50 or more), they were hatched in 40.6 × 15.4 × 6.4 cm enamel pans containing approximately 2 liters of water at a density of 250 viable eggs per rearing tray. Egg papers were submerged and larval diet of 1:1 by weight of lactalbumin and Brewer’s yeast was added to the water. Larval development was monitored, and larval diet was added ad libitum. Pupae originating from field-collected eggs were transferred from larval rearing trays to ‘mosquito breeders’ (Bioquip, Rancho Dominguez, CA) where they were allowed to emerge. Adults were sight identified to species, aspirated, placed in a 30.5 × 30.5 × 30.5 cm adult rearing cage (Bioquip), and provided with a cotton ball soaked with 10% sucrose solution.

To increase mosquito numbers for each population, 3- to 5-d-old mosquitoes were provided a bloodmeal from a live chicken (IACUC Protocol # 201807682) and were allowed to feed for 45 min. Eggs were collected 2–3 d after the bloodmeal by placing a cup with moist germination paper inside the cage. Germination paper was replaced every 2 d until females were no longer ovipositing. Populations were not continued beyond the F2 generation (F0 = field-collected eggs).

Populations and Insecticide Susceptibility Assay

This insecticide susceptibility assay utilized was originally described by Brogdon and McAllister (1998), and protocols provided by the Centers for Disease Control and Prevention (CDC 2013, 2019a,b) were followed. Technical grade AIs used to create stock solutions were obtained from ChemService (West Chester, PA). AIs were diluted to the appropriate concentration using acetone (Thermo Fisher Scientific, Waltham, MA). Diagnostic doses used were those suggested by the CDC (Table 1). Diagnostic times were determined by conducting the CDC bottle bioassay with a known susceptible population of *Ae. aegypti* (ORL 1952 strain) previously described (Pridgeon et al. 2008) and insecticide susceptible *Ae. albopictus* (ATM-NJ95 strain, BEI Resources, Manassas, VA). The time point at which 100% mortality was achieved for the known susceptible population was considered the diagnostic time. CDC bottle bioassays were also conducted using a pyrethroid-resistant strain of *Ae. aegypti* from Puerto Rico (PR strain) previously described (Estep et al. 2017). Test solutions used in the CDC bottle bioassay were remixed every 3 mo, and the diagnostic time was validated with a susceptible population each time the test solution was reformulated.

Briefly, 250-ml glass bottles (DWK Life Sciences, Millville, NJ) were treated with the stock solution of AI and 1 ml of acetone. The entire interior of the bottle was coated by tilting the bottle to either side so the AI could be evenly spread in the bottle. After coating, bottles were uncapped and rolled on a table for 2–3 min or until dry. Uncapped bottles were stored in a dark room for at least 1 h before being used for the bottle bioassay. Control bottles were treated similarly but were treated with acetone only. All assays were completed within 24 h of the bottles being treated.

For each bioassay, unfed 3- to 7-d-old mosquitoes were used. Up to nine AIs were evaluated against each population of *Ae. aegypti* and *Ae. albopictus.* Fifteen to 25 mosquitoes were introduced into four treated bottles and one control bottle. Mortality was recorded at 0, 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min. A mosquito was considered dead if it could no longer stand or fly (CDC 2013). If greater than 10% mortality was observed in the control, the bioassay results were discarded. Mortality in treated bottles was corrected using Abbott's formula prior to designating susceptibility status (Abbott 1925).

Susceptibility status was determined by the percent mortality of the field population at the diagnostic time and was classified according to CDC definitions (CDC 2019b). Mosquito populations were considered susceptible if they suffered greater than 97% mortality at the diagnostic time, developing resistance if mortality was 90–97%, and resistant if mortality was less than 90%.

Results

A total of 37 unique *Ae. aegypti* and 42 unique *Ae. albopictus* populations from 30 Florida counties were evaluated (Table 2), resulting in a total of 512 bottle bioassays. Collections of these field populations spanned much of the state of Florida (Fig. 1), and in some locations, *Ae. aegypti* and *Ae. albopictus* were collected from the same site.

Different insecticide susceptibility trends were observed between *Ae. aegypti* and *Ae. albopictus* and in *Ae. aegyptis* responses to pyrethroids and organophosphates. Of all bottle bioassays completed using a pyrethroid AI against *Ae. aegypti*, 95% were classified as resistant, 4% as developing resistance, and 1% as susceptible. In contrast, only 31.5% of assays conducted with an organophosphate AI resulted in a resistant outcome with 7.6% classified as developing resistance and 60.9% as susceptible. Additionally, the percentage of assays that resulted in >97% mortality by the end of the 2-h assay was only 38.3% for pyrethroid AIs and 98.9% for organophosphate AIs. Of those assays conducted with *Ae. aegypti* populations, 47.1% had survivors at the conclusion of the assay.

For the organophosphate AIs evaluated against *Ae. aegypti*, the highest percentage of susceptible outcomes was observed in response to naled (78%), followed by malathion (59%), and chlorpyrifos (39%) (Fig. 2). For the pyrethroid AIs, lambda-cyhalothrin was the only pyrethroid AI that resulted in any susceptible populations (5%), with 28% classified as developing resistance. Three percent of assays conducted with deltamethrin were classified as developing resistance. The response of mosquitoes to cypermethrin, etofenprox, permethrin, and sumithrin all resulted in 100% of populations being classified as resistant (Fig. 2). The response of each field population to each AI that was tested is detailed in Table 3.

Florida populations of *Ae. albopictus* demonstrated more variability than *Ae. aegypti* in insecticide susceptibility to both pyrethroid and organophosphate AIs. Of all the bottle bioassays completed with *Ae. albopictus*, there was a nearly equal distribution of resistance classifications between pyrethroids and organophosphates. The response of *Ae. albopictus* to pyrethroid AIs resulted in 52% of populations being classified as susceptible, 17% as developing resistance, and 31% as resistant. For organophosphates, 50% were classified as susceptible, 19% as developing resistance, and 31% as resistant. The percentage of assays conducted with *Ae. albopictus* that reached >97% mortality by the end of the 2-h assay was 92.5% for pyrethroid AIs and 98% for organophosphate AIs. Of those assays conducted with *Ae. albopictus* populations, 13% had survivors at the conclusion of the assay.

For organophosphate AIs evaluated against *Ae. albopictus*, the highest percentage of susceptible outcomes was in response to naled (85%), followed by chlorpyrifos (57%), and malathion (15%) (Fig. 3). Greater susceptibility to pyrethroids was observed for *Ae. albopictus* in comparison to *Ae. aegypti.* The highest percentage of assays resulting in a susceptible outcome was in response to lambda-cyhalothrin (74%), followed by etofenprox

(68%), sumithrin (61%), cypermethrin (58%), deltamethrin (34%), and permethrin (32%) (Fig. 3). The response of each *Ae. albopictus* population to each AI that was tested is detailed in Table 4.

For both *Ae. aegypti* and *Ae. albopictus*, the highest percentage of susceptible outcomes was achieved with naled. Lambda-cyhalothrin was the only pyrethroid to result in a susceptible classification for *Ae. aegypti* populations and was also the pyrethroid with the highest percentage of susceptible outcomes for *Ae. albopictus*. The highest susceptibility for *Ae. aegypti* was observed in response to organophosphates, but this was more varied in *Ae. albopictus.* However, a greater percentage of assays reached >97% mortality for *Ae. albopictus* in comparison to *Ae. aegypti*.

Discussion

Estep et al. (2018) make the point that resistance in Florida was largely unquantified prior to their paper. In that study, the pyrethroid susceptibility of Florida populations of *Ae. aegypti* and *Ae. albopictus* was characterized using topical assays and genetic markers. Widespread pyrethroid resistance was detected in *Ae. aegypti*, but not as markedly in *Ae. albopictus* (Estep et al. 2018). In studies conducted by Richards et al. (2017, 2018), the insecticide susceptibility of four *Ae. albopictus* populations was evaluated. These studies only detected resistance to deltamethrin, etofenprox, and malathion. Similar to the results presented here, the response of *Ae. albopictus* to pyrethroid and organophosphate AIs was highly variable. We were able to build on this knowledge base in Florida and test an additional 79 *Aedes* populations. Our study supports and further expands on this trend of widespread pyrethroid resistance in *Ae. aegypti* in Florida using a different phenotypic resistance detection method. Our study provides previously lacking information on the susceptibility of these two species to organophosphate AIs.

Due to the genetic and phenotypic changes that can occur in insects when they are colonized in a laboratory (Mason et al. 1987), we made efforts to test populations as close to F0 as possible. There are fitness costs associated with insecticide resistance in insects (Rivero et al. 2011, Kliot and Ghanim 2012), and therefore, the absence of insecticides may allow for a reversion to an insecticide susceptible status. For this reason, studies on insecticide resistance should strive to keep the tested populations as close to the field generation (F0) as possible. Insecticide resistance studies on Florida populations of *Ae. aegypti* and *Ae. albopictus* have primarily been conducted with populations between F0 and F5 (Richards et al. 2017, 2018; Estep et al. 2018). Additionally, the CDC MosquitoNet system (https://wwwn.cdc.gov/Arbonet/MosquitoNET/) does not accept resistance data collected on populations greater than F2. It is highly recommended that populations be within two generations of the field, when possible, and the results should be considered in context of the generational age of the population being tested.

Aedes aegypti and *Ae. albopictus* populations collected from the same location did not always exhibit the same trends in resistance. The Indian River–1 and Indian River–2 populations of *Ae. aegypti* and *Ae. albopictus* were collected from the same site but demonstrate different responses to AIs (Tables 3 and 4). Indian River–2 *Ae. aegypti* were classified as resistant to all pyrethroid AIs and susceptible to organophosphate AIs. In contrast, *Ae. albopictus* was classified as susceptible to etofenprox and developing resistance to multiple pyrethroid AIs. This is despite being exposed to the same environmental and chemical pressures. This highlights an important point that resistance observed in a particular species does not necessarily indicate the resistance profile of all species from that location. Estep et al. (2018) also observed this and points out resistance development in *Ae. albopictus* is likely more difficult due to genetic differences in the voltage-gated sodium channel. However, it is feasible that there are behavioral differences in the two species that result in differing times and amount of exposure. The timing of adulticide sprays and the peak activity of a mosquito species directly influences their level of insecticide exposure, which in turn influences resistance. Therefore, the control strategy utilized by a mosquito control program (targeting nuisance vs vectors) can influence the level of exposure mosquitoes have to insecticides.

Although statewide characterization of Florida *Ae. aegypti* and *Ae. albopictus* populations was lacking until recently (Estep et al. 2018), resistance has been documented for these two species in other regions. A study conducted on Venezuelan populations of *Ae. aegypti* found sevenfold resistance to the pyrethroids, permethrin and lambda-cyhalothrin and the organophosphate, chlorpyrifos (Mazzarri and Georghiou 1995), although the resistance profile of these populations is likely very different today than it was when the study was published. The response of *Ae. aegypti* from Papua New Guinea to lambda-cyhalothrin and deltamethrin indicated high levels of pyrethroid resistance when using the WHO bioassay with only 7–33% mortality at 24 h. In contrast, *Ae. albopictus* from the same region achieved 92–100% mortality to the same Als (Demok et al. 2019). Similarly, the resistance ratio for 50% of the lethal concentration (RR50) was calculated for *Ae. aegypti* and *Ae. albopictus* populations. The RR50 to permethrin for *Ae. aegypti* was 12.9 and only 1.8 for *Ae. albopictus* (Ping et al. 2001). Liu et al. (2004) found resistance in *Ae. albopictus* strains from Florida and Alabama to deltamethrin (RR = 3–21) and chlorpyrifos (RR = 13–42), but no resistance to the other AIs evaluated.

The trend of resistance in Florida characterized here and by Estep et al. (2018) appears to match a global trend where pyrethroid resistance in *Ae. aegypti* is more common than it is in *Ae. albopictus* (Mazzarri and Georghiou 1995, Liu et al. 2004, Demok et al. 2019), which may be due to genetic differences between these populations (Estep et al. 2018). In Florida, pyrethroids have been widely used since the 1960s, and in 2014 made up over 90% of ground-applied sprays (Lloyd et al. 2018). Prolonged use of pyrethroids in Florida could contribute to the frequency of resistance observed in *Ae. aegypti.* Additionally, cross-resistance between DDT and pyrethroids has been documented and is attributable to mutations in the voltage-gated sodium channel (Brengues et al. 2003). Therefore, the widespread use of DDT in Florida prior to the introduction of pyrethroids could have allowed for the evolution of resistance which has been maintained by pyrethroids that are still in use today. In the Cayman Islands, cross-resistance is implicated in maintaining resistance to DDT in *Ae. aegypti* (Harris et al. 2010). However, the extent to which resistance detected using laboratory techniques relates to field failure of an adulticide treatment has been largely unexplored, with the exception of Cornel et al. (2016) where bottle bioassays and field trials were both used to determine insecticide susceptibility in California *Ae. aegypti*.

Although the mechanism conferring resistance was not assessed in our study, the detection of pyrethroid resistance in *Ae. aegypti* coupled with evidence from Estep et al. (2018) suggests that knockdown resistance (kdr) is playing a role. However, it is likely that metabolic resistance is also playing a role in the presence of resistance in both *Ae. aegypti* and *Ae. albopictus*. The presence of both target-site mutations and metabolic resistance mechanisms has been previously detected (Aponte et al. 2013, Edi et al. 2014) and could explain the detection of both pyrethroid and organophosphate resistance. Although kdr mutations do not play a role in organophosphate resistance, metabolic mechanisms do (Nauen 2007). In Florida, metabolic mechanisms have previously been detected and implicated in conferring resistance to organophosphates for *Ae. albopictus* (Marcombe et al. 2014). Therefore, the pyrethroid resistance reported in our study could be the result of both target-site and metabolic mechanisms, with the organophosphate resistance largely due to metabolic mechanisms.

The results presented here paint a very concerning picture of the status of chemical tools available for use against adult *Ae. aegypti*. It is important to note that in the presented study, resistance was detected to every AI by both *Ae. aegypti* and *Ae. albopictus* (Figs. 2 and 3). Therefore, resistance monitoring is and should remain a critical component of any public health vector control program. Presently, pyrethroids and organophosphates are the only two classes of chemical adulticides available for public health vector control use in Florida. The high frequency of statewide pyrethroid resistance puts Florida in a place of vulnerability during local transmission of pathogens. This was highlighted during local transmission of Zika virus in 2016. The aggressive mosquito-control effort that included a combination of ground pyrethroid, aerial organophosphate, and larvicide applications was likely the reason transmission decreased (Likos et al. 2016).

The CDC bottle bioassay is a tool used to monitor the presence of phenotypic resistance in different mosquito populations but does not necessarily indicate a field failure due to the lack of relationship between the bottle concentration and the field concentration utilized (Bagi et al. 2015). However, results from these assays should still be used to inform long-term use of various AIs in an operational mosquito-control setting. When resistance is detected in the CDC bottle bioassay, this should serve as a warning sign to a mosquito-control program. Routine resistance surveillance allows programs to monitor long-term trends in resistance, including return to susceptibility when resistance management strategies are implemented. Identification of resistant populations should drive subsequent investigations (identification of resistance mechanism, resistance intensity testing, field trial) that further characterize the resistance profile of that population (CDC 2016). Additionally, detection of resistance should trigger considerations on how reliance on that chemical class can be reduced. Decreased reliance on one chemical class, coupled with increased pressure through other control measures (a different chemistry, larviciding, source reduction, etc.), is a recommended strategy for slowing the occurrence of resistance in public health vectors (Dusfour et al. 2019).

Chemical rotation is a critical component of insecticide resistance management; however, ‘rotation’ with subgroups within a chemical class is not a recommended strategy as the subgroups act on the same target (Sparks and Nauen 2015). With only two chemical classes available for adulticide use in the continental United States for vector control, loss of either class would make rotation impossible. The integrity of public health vector control efforts is therefore reliant on proactive insecticide resistance management strategies and the preservation of the existing chemical classes available for use.

Knowledge of the state and direction of resistance to adulticides in Florida should 1) promote the practice of chemical rotation to maximize the longevity of the existing products currently available, 2) encourage the development of new insecticides, especially those in new chemical classes and those that challenge resistance development in mosquitoes, and 3) highlight the need for integrated mosquito management that controls mosquitoes by a combination of physical, biological, and chemical means. It is important to note that the development of new insecticides is challenged by the increased amount of time it takes to bring a new product to market and increased costs associated with product development and registration (McDougall 2016).

The importance of understanding and combatting insecticide resistance in mosquitoes is clearly illustrated by the more than 1,500 published works on the subject (Liu 2015). Insecticide resistance has been implicated as an important factor in resurgence of some vector-borne diseases and has been documented in all of the major vector species (Brown 1986). Understanding the resistance of local/field mosquito populations, developing a management plan that prevents and combats insecticide resistance, and employing this resistance management program continually will drastically improve our ability to protect public health.

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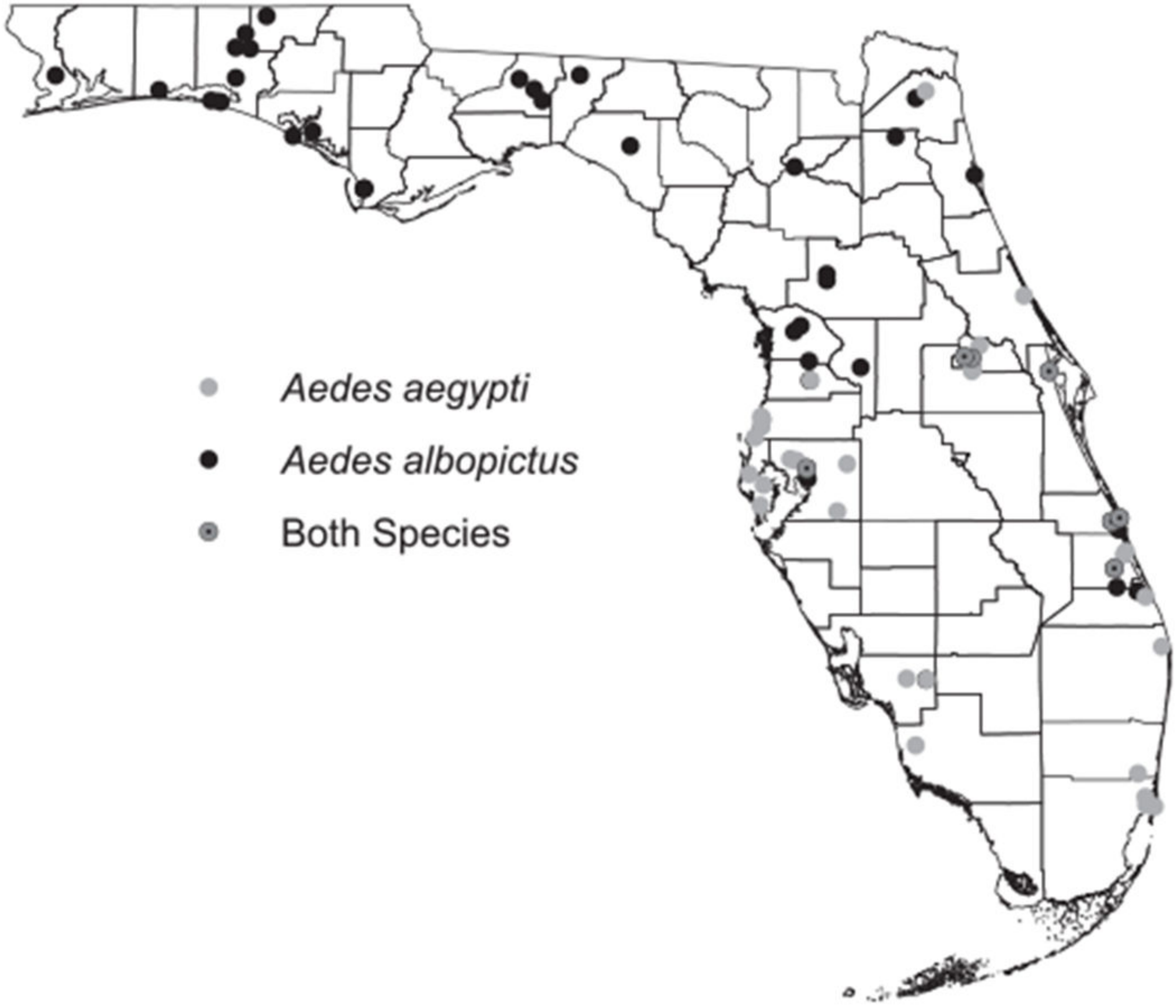

Fig. 1.

Collection locations of field populations of *Aedes aegypti* and *Aedes albopictus* used in CDC bottle bioassays.

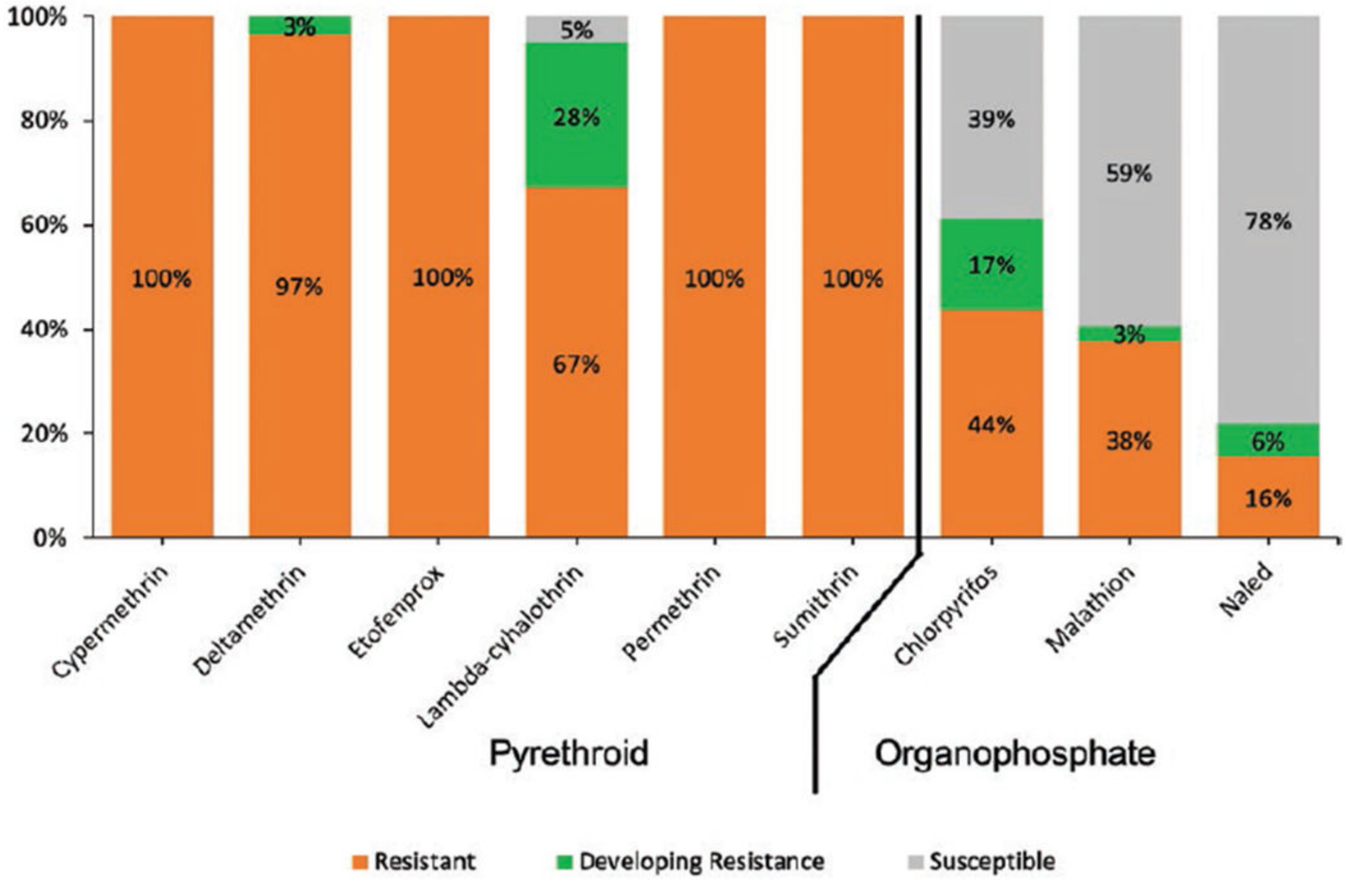

Fig. 2.

Response of *Aedes aegypti* populations to nine active ingredients. Insecticide susceptibility classification is made based on the percent mortality at the diagnostic time. Greater than 97% mortality is classified as susceptible; 90–97% is classified as developing resistance; and less than 90% is classified as resistant.

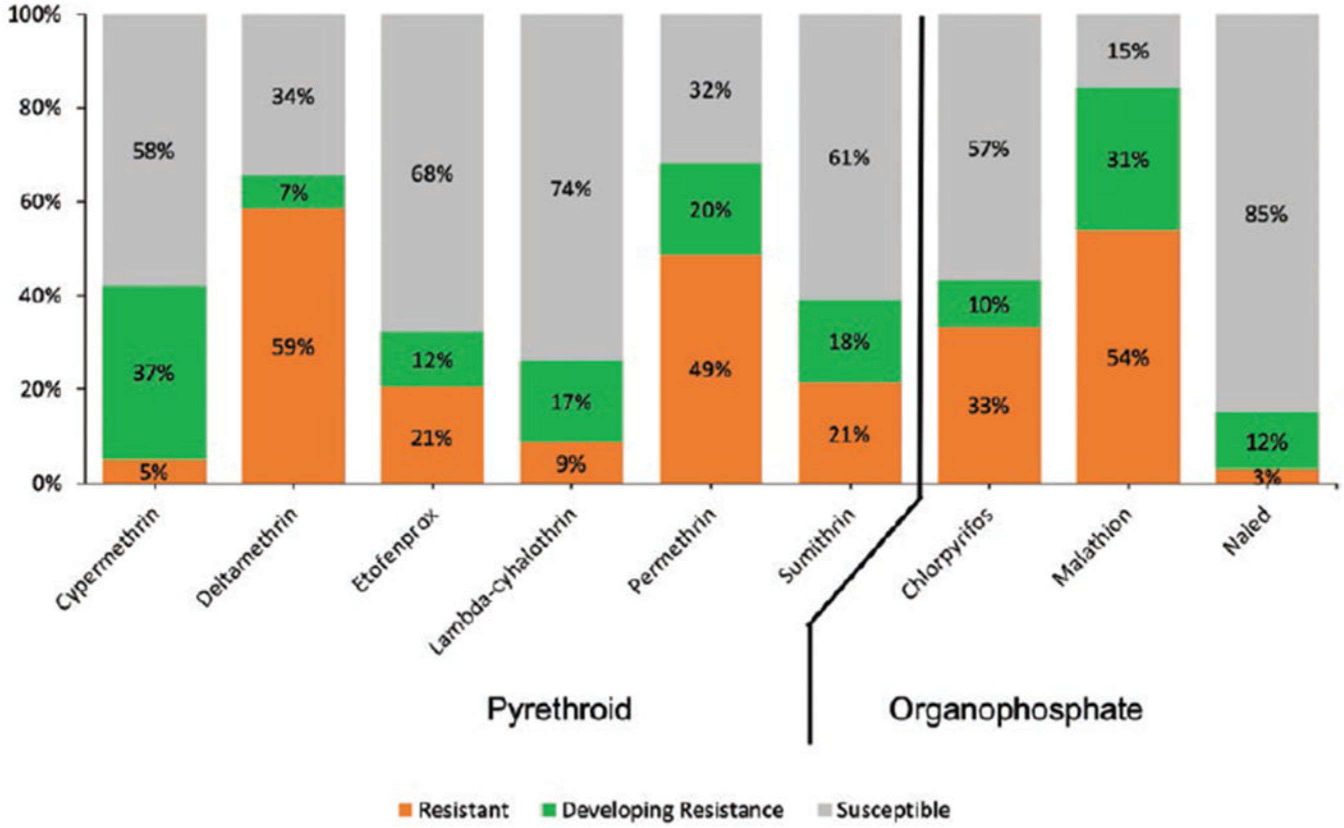

Fig. 3.

Response of *Aedes albopictus* populations to nine active ingredients. Insecticide susceptibility classification is made based on the percent mortality at the diagnostic time. Greater than 97% mortality is classified as susceptible; 90–97% is classified as developing resistance; and less than 90% is classified as resistant.

Table 1.

Chemical class, diagnostic dose, and diagnostic time for *Aedes aegypti*, percent mortality of PR strain at the *Ae. aegypti* diagnostic time, and diagnostic time for *Aedes albopictus* for the nine active ingredients used in the bottle bioassay

Active ingredient	**Chemical class**	**Diagnostic dose (μg per bottle)**	**Diagnostic Time *Ae. Aegypti* (min)**	**% Mortality at diagnostic time by PR *Ae. aegypti***	**Diagnostic time *Ae. albopictus* (min)**
Cypermethrin	Pyrethroid	10	30	3	30
Deltamethrin	Pyrethroid	0.75	30	49.5	30
Etofenprox	Pyrethroid	12.5	15	18.2	30
Lambda-cyhalothrin	Pyrethroid	10	30	11.8	30
Permethrin	Pyrethroid	43	10	1	10
Sumithrin	Pyrethroid	20	10	0	45
Chlorpyrifos	Organophosphate	20	45	96.4	45
Malathion	Organophosphate	400	15	91.4	30
Naled	Organophosphate	2.25	30	93.5	30

Table 2.

Collection information for mosquito populations evaluated for insecticide susceptibility using the CDC bottle bioassays

Species	Generation tested	County/population	Collection date	Collector	No. of AIs tested
Ae. aegypti	F1	Brevard	Sept. 2016	Brevard Co. MC	1
Ae. aegypti	F2	Broward	Aug. 2016	FMEL	5
Ae. aegypti	F2	Collier	July 2016	Collier MC	6
Ae. aegypti	F2	Duval	June 2017	City of Jacksonville MC	8
Ae. aegypti	F1	Hernando	March 2017	Hernando Co. MC	9
Ae. aegypti	F2	Hillsborough–1	Sept. 2016	Hillsborough Co. MC	7
Ae. aegypti	F1	Hillsborough–2	June 2017	Hillsborough Co. MC	9
Ae. aegypti	F1	Hillsborough–3	July 2017	Hillsborough Co. MC	7
Ae. aegypti	F2	Hillsborough–4	July 2017	Hillsborough Co. MC	7
Ae. aegypti	F1	Hillsborough–5	June 2017	Hillsborough Co. MC	9
Ae. aegypti	F2	Indian River–1	Nov. 2016	FMEL	7
Ae. aegypti	F1	Indian River–2	Sept. 2016	FMEL	6
Ae. aegypti	F1	Lee–1	Nov. 2016	Lee Co. MC	4
Ae. aegypti	F1	Lee–2	Nov. 2016	Lee Co. MC	4
Ae. aegypti	F1	Martin	May 2017	Martin Co. MC	9
Ae. aegypti	F1	Miami-Dade–1	Nov. 2016	Miami-Dade MC	6
Ae. aegypti	F1	Miami-Dade–2	Aug. 2016	Miami-Dade MC	5
Ae. aegypti	F2	Miami-Dade–3	Aug. 2016	Miami-Dade MC	1
Ae. aegypti	F1	Orange	Nov. 2016	Orange Co. MC	7
Ae. aegypti	F2	Palm Beach	Nov. 2017	Palm Beach Co. MC	8
Ae. aegypti	F1	Pasco–1	Aug. 2016	FMEL	6
Ae. aegypti	F2	Pasco–2	July 2018	FMEL	9
Ae. aegypti	F1	Pasco–3	May 2018	FMEL	4
Ae. aegypti	F1	Pasco–4	May 2018	FMEL	6
Ae. aegypti	F1	Pasco–5	Aug. 2016	FMEL	6
Ae. aegypti	F2	Pinellas–1	April 2016	Pinellas Co. MC	2
Ae. aegypti	F2	Pinellas–2	Feb. 2017	Pinellas Co. MC	8
Ae. aegypti	F2	Pinellas–3	July 2016	Pinellas Co. MC	4

Species	Generation tested	County/population	Collection date	Collector	No. of AIs tested
Ae. aegypti	F1	Seminole–1	April 2017	Seminole Co. MC	9
Ae. aegypti	F1	Seminole–2	Oct. 2017	Seminole Co. MC	9
Ae. aegypti	F2	Seminole–3	July 2017	Seminole Co. MC	9
Ae. aegypti	F1	Seminole–4	April 2018	Seminole Co. MC	7
Ae. aegypti	F1	Seminole–5	Sept. 2017	Seminole Co. MC	8
Ae. aegypti	F1	St. Lucie–1	Sept. 2016	St. Lucie Co. MC	6
Ae. aegypti	F1	St. Lucie–2	July 2016	St. Lucie Co. MC	7
Ae. aegypti	F1	St. Lucie–3	May 2017	St. Lucie Co. MC	9
Ae. aegypti	F1	Volusia	Nov. 2016	Volusia Co. MC	7
Ae. albopictus	F1	Bay–1	Aug. 2017	Bay Co. MC	8
Ae. albopictus	F1	Bay–2	Aug. 2017	Bay Co. MC	8
Ae. albopictus	F1	Bay–3	July 2017	Bay Co. MC	8
Ae. albopictus	F1	Brevard	Sept. 2016	Brevard Co. MC	6
Ae. albopictus	F1	Citrus–1	Aug. 2017	Citrus Co. MC	8
Ae. albopictus	F1	Citrus–2	Sept. 2016	Citrus Co. MC	7
Ae. albopictus	F1	Citrus–3	May 2017	Citrus Co. MC	9
Ae. albopictus	F2	Clay	Sept. 2017	Clay Co. MC	8
Ae. albopictus	F2	Duval	July 2017	City of Jacksonville MC	8
Ae. albopictus	F1	Escambia	April 2018	Escambia Co. MC	5
Ae. albopictus	F1	Gulf–1	July 2016	Gulf Co. MC	2
Ae. albopictus	F2	Gulf–2	Aug. 2016	Gulf Co. MC	6
Ae. albopictus	F2	Gulf–3	Aug. 2018	Gulf Co. MC	3
Ae. albopictus	F2	Hernando	Mar. 2017	Hernando Co. MC	2
Ae. albopictus	F2	Hillsborough–1	Oct. 2016	Hillsborough Co. MC	6
Ae. albopictus	F2	Hillsborough–2	Sept. 2017	Hillsborough Co. MC	9
Ae. albopictus	F1	Indian River–1	Nov. 2016	FMEL	7
Ae. albopictus	F2	Indian River–2	Nov. 2016	FMEL	5
Ae. albopictus	F1	Indian River–3	Aug. 2017	FMEL	9
Ae. albopictus	F1	Jefferson	Sept. 2017	Jefferson Co. MC	8
Ae. albopictus	F2	Lee	Nov. 2016	Lee Co. MC	4
Ae. albopictus	F1	Leon–1	Sept. 2016	Leon Co. MC	4

Species	Generation tested	County/population	Collection date	Collector	No. of AIs tested
Ae. albopictus	F1	Leon–2	Sept. 2016	Leon Co. MC	8
Ae. albopictus	F1	Leon–3	Sept. 2017	Leon Co. MC	8
Ae. albopictus	F1	Marion–1	Oct. 2016	Marion County Cooperative Extension	7
Ae. albopictus	F2	Marion–2	Mar. 2017	Marion County Cooperative Extension	9
Ae. albopictus	F1	Martin	Oct. 2016	Martin Co. MC	5
Ae. albopictus	F1	Okaloosa	July 2017	Okaloosa Co. MC	9
Ae. albopictus	F1	Seminole–1	April 2017	Seminole Co. MC	9
Ae. albopictus	F1	Seminole–2	July 2017	Seminole Co. MC	2
Ae. albopictus	F1	St. Johns	July 2016	Anastasia MC	2
Ae. albopictus	F1	St. Lucie–1	Sept. 2016	St. Lucie Co. MC	1
Ae. albopictus	F2	St. Lucie–2	Nov. 2017	St. Lucie Co. MC	2
Ae. albopictus	F2	Sumter	Sept. 2016	Sumter Co. MC	8
Ae. albopictus	F2	Taylor	June 2016	Taylor Co. MC	9
Ae. albopictus	F2	Union	Aug. 2016	Union Co. Emergency Management	7
Ae. albopictus	F1	Walton–1	Oct. 2017	North Walton MC	8
Ae. albopictus	F1	Walton–2	Oct. 2017	North Walton MC	9
Ae. albopictus	F1	Walton–3	Sept. 2017	North Walton MC	9
Ae. albopictus	F1	Walton–4	Oct. 2017	North Walton MC	7
Ae. albopictus	F2	Walton–5	Aug. 2016	South Walton MC	6
Ae. albopictus	F2	Walton–6	July 2016	South Walton MC	6

MC, mosquito control; AI, active ingredient.

Table 3.

Classification of *Aedes aegypti* field populations’ insecticide susceptibility to nine active ingredients using CDC definitions (CDC 2019b)

Population	Naled	Malathion	Chlorpyrifos	Lambda-cyhalothrin	Deltamethrin	Cypermethrin	Sumithrin	Etofenprox	Permethrin
Lee–1	S	S			R				R
Lee–2	S	S			R				R
Miami-Dade–2	S	S						R	R
Pasco–3	S	S			R				R
St. Lucie–1	S	S	S				R	R	R
Palm Beach	S	R	S	S		R	R	R	R
Pasco–4	S	S		DR	R			R	R
Pasco–5	S	S	DR		R			R	R
Collier	S	R	S		R			R	R
Indian River–2	S	S			R		R	R	R
Pasco–1	S	R	S		R			R	R
St. Lucie–3	S	S	S	R	DR	R	R	R	R
Seminole–2	S	S	S	R	R	R	R	R	R
Seminole–3	S	S	S	R	R	R	R	R	R
Orange	DR	S	S		R		R	R	R
Hillsborough–3	S	S		R	R		R	R	R
Hillsborough–4	S	S		R	R		R	R	R
Seminole–4	S	S		R	R		R	R	R
Seminole–5	S	R	S	R		R	R	R	R
Hillsborough–5	S	S	DR	DR	R	R	R	R	R
Hillsborough–2	S	S	R	DR	R	R	R	R	R
Seminole–1	S	S	R	DR	R	R	R	R	R
Miami-Dade–1		S	R		R			R	R
Hillsborough–1	R	S		R	R	R		R	R
St. Lucie–2	S	R	R		R		R	R	R
Volusia	R	S	R		R		R	R	R
Pinellas–2	S	R	DR	R	R	R	R	R	
Hernando	S	R	DR	R	R	R	R	R	R

Population	Naled	Malathion	Chlorpyrifos	Lambda-cyhalothrin	Deltamethrin	Cypermethrin	Sumithrin	Etofenprox	Permethrin
Martin	S	R	R	R	R	R	R	R	R
Pasco–2	R	S	R	R	R	R	R	R	R
Miami-Dade–3		DR							
Brevard					R				
Pinellas–1		R							R
Pinellas–3	DR				R		R	R	
Broward		R	R		R			R	R
Indian River–1	R	R	R		R		R	R	R
Duval	R	R	R	R		R	R	R	R

The percentage of resistant outcomes increases from left to right, with the exception of cypermethrin, sumithrin, etofenprox, and permethrin, which all resulted in 100% resistant outcomes. R indicates a resistant classification, DR indicates a developing resistance classification, and S indicates a susceptible classification. Spaces lacking these letters indicate that the AI was not evaluated against that population.

Table 4.

Classification of *Aedes albopictus* field populations’ insecticide susceptibility status to nine active ingredients using CDC definitions (CDC 2019b)

Population	Naled	Lambda-cyhalothrin	Cypermethrin	Etofenprox	Sumithrin	Chlorpyrifos	Permethrin	Malathion	Deltamethrin
Indian River–3	S	S	S	S	S	S	S	S	S
Jefferson	S	S	S	S	S	S	S	S	
Walton–1	S	S	S	S	S	S	S	S	
Leon–3	S	S	S	S	S	S	S	R	
Bay–1	S	S	S	S	S	S	R	R	
Marion–1	S			S	S	S	S	R	R
Walton–3	S	S	S	S	S	S	R	R	DR
Walton–6	S			S		S	DR	R	S
Duval	S	S	DR	S	S	S	R	DR	
Escambia	S			R	S			DR	S
Union	DR	S	S	S		DR		S	R
Walton–4	S	S		S	DR		R	DR	S
Hillsborough–2	S	S	S	R	R	R	S	R	S
Seminole–1	S	S	DR	S	R	R	S	DR	S
Walton–2	S	S	S	DR	S	R	R	R	S
Bay–2	S	DR	DR	DR	S	S	S	R	
Brevard	R			S	S	DR	DR	S	
Citrus–1	S	DR		S	R	R	S	R	S
Hernando							R	S	
Leon–1				S			S	DR	R
Okaloosa	S	DR	S	S	S	R	DR	DR	
Sumter	S	S	DR	DR	S	S		R	R
Walton–5	S			S		S	R	DR	DR
Citrus–3	S	S	S	DR	R	R	R	R	S
Marion–2	S	S	DR	S	DR	R	S	R	R
Taylor	S	S	DR	S	DR	R	DR	R	S
Citrus–2	S			R	S	S	R	R	R
Leon–2	DR			S	S	S	R	R	R

Population	Naled	Lambda-cyhalothrin	Cypermethrin	Etofenprox	Sumithrin	Chlorpyrifos	Permethrin	Malathion	Deltamethrin
Clay	S	DR		R	S	DR	S	R	R
Gulf–2	S	DR	DR	S		R			R
Bay–3	S	R	R	R	DR	S	R	R	
Lee	S						R	DR	R
Indian River–2				S	DR		DR	DR	R
Martin	S					R	R	R	R
Hillsborough–1	DR			R		S	R	R	R
Indian River–1	DR			R	R	S	R	R	R
St. Johns				DR					R
Gulf–1							DR	DR	
Gulf–3					R		DR	R	
Seminole–2							R	DR	
St. Lucie–1									R
St. Lucie–2							R	DR	

The percentage of resistant outcomes increases from left to right. R indicates a resistant classification, DR indicates a developing resistance classification, and S indicates a susceptible classification. Spaces lacking these letters indicate that the AI was not evaluated against that population.