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SCIENTIFIC NOTE

INSECTICIDE RESISTANCE TESTING OF *CULEX QUINQUEFASCIATUS* AND *AEDES ALBOPICTUS* FROM MISSISSIPPI

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ABSTRACT. Data on insecticide resistance in mosquitoes from Mississippi are reported. Mosquitoes were collected from June through October 2017 from 28 cities in Mississippi and included wild-caught *Culex quinquefasciatus* Say obtained as egg rafts (reared to adults), and *Aedes albopictus* (Skuse) collected as larvae (reared to adults). The Centers for Disease Control and Prevention (CDC) bottle bioassay protocol was utilized. Thirty-six populations of *Cx. quinquefasciatus* were tested for malathion resistance from 24 cities; 22 of them (61.1%) met the World Health Organization criteria as resistant, and the remaining 14 (38.9%) were classified suspected resistant. Forty-one populations of *Cx. quinquefasciatus* were also tested from 24 cities for resistance to permethrin, and of these, 18 populations (43.9%) were susceptible, 13 (31.7%) were resistant, and 10 (24.4%) were suspected resistance. Eighteen populations of *Ae. albopictus* were collected from 10 cities in Mississippi for resistance testing to malathion; 5 populations were susceptible (27.8%), 5 were resistant (27.8%), and 8 (44.4%) suspected resistant. Sixteen populations of *Ae. albopictus* were tested from 10 cities for resistance to permethrin and none were resistant.

KEY WORDS Mosquitoes, mosquito control, pesticides, resistance, surveys, Mississippi

Insecticides are important tools for controlling mosquito-borne illnesses worldwide, but their effectiveness is reduced by development of resistance (Hemingway and Ranson 2000). Insecticide resistance in arthropod vectors is rapidly increasing (Ash 2018, Rosenberg et al. 2018), and testing for that characteristic in mosquito populations is an important component of efficient mosquito control programs (Xu et al. 2005, Marcombe et al. 2014). There is a lack of published data on mosquito insecticide resistance worldwide, especially in the United States (Ranson et al. 2008), and none from the state of Mississippi (Liu et al. 2003). Such information is needed for increased mosquito control efficacy in the state, and may shed light on insecticide resistance in medically important mosquitoes for other geographic locations as well. Therefore, the purpose of this study was to ascertain the level of insecticide resistance in Mississippi populations of *Culex quinquefasciatus* Say and *Aedes albopictus* (Skuse), particularly, resistance to pyrethroids and organophosphates (in this case permethrin and malathion). These 2 mosquito species were chosen for testing because of their medical importance: *Cx. quinquefasciatus* is a vector of several disease agents, including West Nile virus, St. Louis encephalitis, and dog heartworm (Villavaso and Steelman 1970, Monath 1980, Wasay et al. 2000, Campbell et al. 2002, Xu et al. 2005), and *Ae. albopictus* is a vector of disease agents such as that of dengue, chikungunya, and possibly West Nile virus (Rosen et al. 1983, Gratz 2004, Delatte et al. 2008, Goddard et al. 2016). In addition, recent studies have shown that *Ae. albopictus* can be a competent vector of Zika virus in laboratory settings (Chouin-Carneiro et al. 2016, Di Luca et al. 2016).

This research was conducted using the Centers for Disease Control and Prevention (CDC) bottle bioassay protocol as previously described (Brogdon and Chan 2016), which was designed to determine if a formulation of an insecticide is able to control a vector at a specific location and time. The protocol relies on time mortality data, which is a measure of time required for an insecticide to penetrate the exoskeleton, cross through various tissues, and act on the target site. Anything that prevents or delays this process may contribute to resistance (Brogdon and Chan 2016)

Each test consisted of 1 control and 3 treatments, with each bottle containing approximately 25 mosquitoes of the same age. Control bottles were coated with acetone, and treatments contained acetone and either technical grade, 99% pure malathion (400 μg/bottle) or technical grade, 98% pure permethrin (43 μg/bottle; both insecticides from Sigma-Aldrich, St. Louis, MO). These pesticides were chosen because of their widespread use for mosquito control in Mississippi. The procedure, diagnostic doses, and exposure times were followed according to the published protocol (Brogdon and Chan 2016). As for resistance status, we used the World Health Organization scheme: 98–100% mortality of insects

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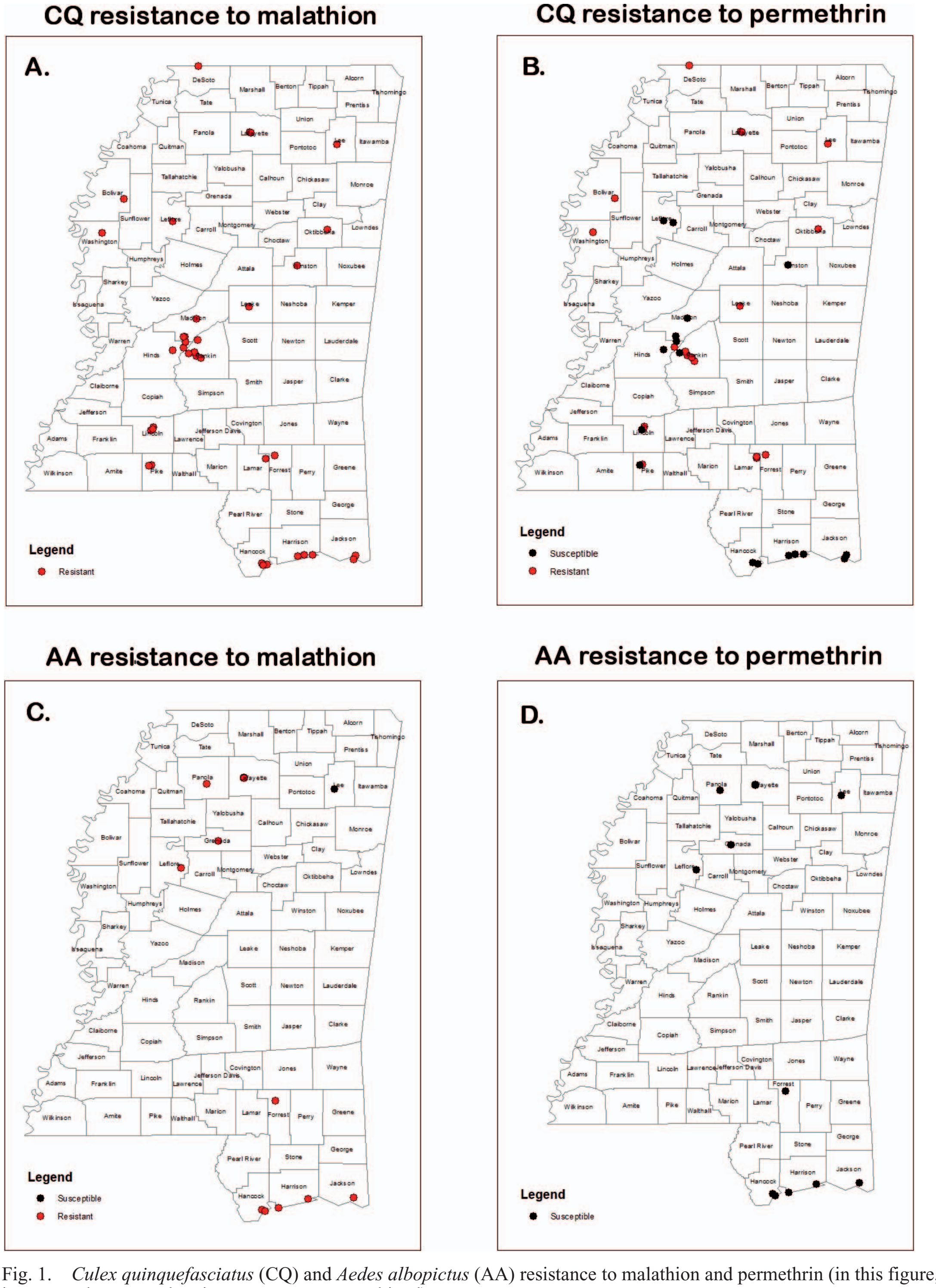

Fig. 1. *Culex quinquefasciatus* (CQ) and *Aedes albopictus* (AA) resistance to malathion and permethrin (in this figure, resistance and suspected resistance status are combined).

at the recommended diagnostic time indicates susceptible, 80–97% mortality indicates suspected resistant that may need to be confirmed, and <80% mortality indicates definite resistant (Brogdon and Chan 2016). Note: In this study we made no effort to assess the various mechanisms responsible for insecticide resistance.

Overall, mosquitoes for this study were collected in 28 cities throughout Mississippi—11 in northern, 9 in central, and 10 in southern Mississippi—and sometimes multiple samples were taken from a location. *Culex quinquefasciatus* were obtained by using a mixture of fish meal and water (1 tablespoon per gallon of water, aged 3 days) set out in shallow pans for 24 h, after which egg rafts were collected from the surface. *Culex quinquefasciatus* egg rafts were collected from 40 locations in 25 cities throughout Mississippi (Fig. 1A, 1B). *Aedes albopictus* were collected as larvae from discarded tires or by setting out containers of oak-infused water, where larvae were collected within a 14-day period. Larvae were collected from 12 locations in 10 cities primarily in north and south Mississippi (Fig. 1C, 1D). Collections of both species were made monthly from June to October 2017, and brought back to the lab, where they were reared to the adult stage. Only the F1 generation were used in resistance testing to ensure wild-type genetics.

Thirty-six populations of *Cx. quinquefasciatus* were tested for malathion resistance from 24 cities throughout Mississippi, and every one of them were either resistant (22/36 or 61.1%) or suspected resistant (14/36 or 38.9%) to malathion (Fig. 1A). Some populations from the same location had varying resistance status. Forty-one populations of *Cx. quinquefasciatus* were tested from 24 cities in Mississippi for resistance to permethrin (Fig. 1B). Of these, 18 populations (43.9%) were susceptible, 13 (31.7%) were resistant, and 10 (24.4%) showed signs of suspected resistance. Again, sometimes samples from the same location showed varying resistance status. Eighteen populations of *Ae. albopictus* were collected from 10 cities in Mississippi for resistance testing to malathion (Fig. 1C). Five populations were susceptible (27.8%), 5 were resistant (27.8%), and 8 (44.4%) showed suspected resistance. As seen in the other cases, some populations showed varying resistance within the same city and/or the same collection site. Sixteen populations of *Ae. albopictus* were collected from 10 cities to test for resistance to permethrin and none were resistant (Fig. 1D).

The overall pattern for both mosquito species showed resistance occurring statewide, with a slight preponderance in north and central Mississippi. Mosquitoes found to be resistant in west Mississippi (Leflore, Bolivar, and Washington Counties) were collected from the Delta, which is primarily an agricultural area with intense use of pesticides to control agricultural pests. This may have contributed to mosquito insecticide resistance in that area. Interestingly, 35 populations (45.5%) of *Cx. quinquefasciatus* from Mississippi showed resistance to both malathion and permethrin.

Every single sample of *Cx. quinquefasciatus* we tested ($n = 36$) from 24 cities in Mississippi was resistant to malathion (Fig. 1). This may be because of historical widespread agricultural use and mosquito control in the state using malathion. The second author (JG) reports that during the first half of his tenure at the Mississippi Department of Health (1989–1999), cities, towns, and counties predominately used 97% malathion ultra-low volume (ULV) in their spray programs. Approximately half the samples of *Cx. quinquefasciatus* we tested from Mississippi were resistant to permethrin. This variability has been noted in other surveys in other states. One study showed high levels of resistance of *Cx. quinquefasciatus* to permethrin in 2 locations in Alabama, but low levels of resistance to permethrin from a location in Florida (Xu et al. 2005). The authors noted that the Alabama mosquitoes had been exposed to resmethrin for over 15 years. In our study, we found rather widespread resistance of *Ae. albopictus* to malathion, but none to permethrin. These data support previous studies showing *Ae. albopictus* resistance to malathion in Florida, New Jersey, Texas, Illinois, and Alabama (Khoo et al. 1988, Marcombe et al. 2014). Susceptibility of *Ae. albopictus* to permethrin products in Mississippi is often anecdotally reported to the authors by mosquito control personnel.

Knowing the resistance status of mosquito populations is important in choosing appropriate mosquito control interventions. This information can be used to select pesticides from other chemical chemistries or to adjust application rates or dilutions. Continued insecticide resistance testing is important to manage mosquito populations effectively, reduce the amount of chemicals applied, prevent potential environmental contamination, and decrease overall expenses. Mosquito abatement strategies need to be altered in areas where there is known resistance by including more surveillance, detecting and monitoring resistance, and changing pesticide chemistries where needed (Brogdon and McAllister 1998, Rose 2001).

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