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The Influence of Ambient Temperature on the Susceptibility of Aedes aegypti (Diptera: Culicidae) to the Pyrethroid Insecticide Permethrin

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Abstract

Insecticides are the most common strategy used for the management of mosquitoes. Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms. Studies show organophosphate insecticides exhibit a positive correlation between ambient temperature and mortality for many insect species, and carbamate insecticides exhibit a slightly negative correlation between ambient temperature and mortality. Pyrethroid insecticides exhibit a distinctly negative correlation between increasing ambient temperature and mortality for insects. However, this relationship has not been systematically studied for adult mosquitoes. Therefore, we examined the influence of temperature on the susceptibility of adult Aedes aegypti L. (Diptera: Culicidae) when exposed to permethrin. The median lethal concentration, LC$_{50}$, was estimated for adult Ae. aegypti when exposed to eight concentrations of permethrin (ranging from 0.06–0.58 ng/cm$^2$) at each of the following temperatures—16, 23, 26, 30, 32, and 34°C—for 24 h in bottle assays. The estimated LC$_{50}$ for each temperature was 0.26, 0.36, 0.36, 0.45, 0.27, and 0.31 ng/cm$^2$, respectively. Results indicated a negative correlation between temperature and mortality from 16 to 30°C, a positive correlation between temperature and mortality from 30 to 32°C, and a negative correlation between temperature and mortality from 32 to 34°C.

If mosquito populations are expanding in space and time because of increased ambient temperatures and cannot be managed as effectively with pyrethroids, the spread of mosquito-borne diseases may pose considerable additional risk to public health.

Aedes aegypti L. (Diptera: Culicidae), which vectors the viruses that cause dengue (family Flaviviridae, genus Flavivirus, DENV), chikungunya (family Togaviridae, genus Alphavirus, CHIKV), and yellow fever (family Flaviviridae, genus Flavivirus, YFV) (Kuno 2010), can be found throughout the tropics and subtropics worldwide (Vontas et al. 2012). Ae. aegypti thrive in environments closely associated with humans; the majority of their breeding sites result from outdoor pots and containers around human dwellings (Vontas et al. 2012). Insecticides are the most common strategy used for mosquito management (Alves et al. 2002, Swain et al. 2009). Over the past decade, the use of synthetic pyrethroid insecticides for the control of vectors has increased (Gratz 2004, Vontas et al. 2012). The use of synthetic pyrethroids rapidly increased during the 1970s (Schleier III and Peterson 2011), as they have a quick knockdown effect, high insecticidal potency, low mammalian toxicity, and do not bioaccumulate (Swain et al. 2009, Casida 2010).

Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms (Andersson and Forlin 1992). The temperature-dependence relationship in insecticides is of great importance, as from 1948 to 1991 there was a shift to warmer temperatures in the evening hours when compared with other parts of the day for all seasons of the year in the United States (Knappenberger et al. 1996). Globally, it is projected that by the year 2100 there will be approximately a 1.0 to 3.5°C increase in the annual mean surface temperature (U.S. Global Change Research Program [USGCRP] 2009).

The majority of current mosquito management strategies utilize insecticides in environments shown to have increasing annual minimum, maximum, and late afternoon temperatures (Knappenberger et al. 1996, Vose et al. 2005). Increased temperature has also been shown to positively affect mosquito population development and survival, the biting rate of mosquitoes, and the incubation or replication time of a virus within mosquitoes (Moudy et al. 2007, Kilpatrick et al. 2008, Johnson and Sukhdeo 2013, Anyamba et al. 2014). All of the above-mentioned scenarios may pose considerable risk to public health and the spread of mosquito-borne diseases.

Changes in ambient temperature can alter the rate of chemical uptake, metabolism, depuration, and pesticide toxicity in many
permethrin. We hypothesize that mortality of adult *Ae. aegypti* will be positively correlated with mortality for many insect species, and carbamates are known to exhibit a slightly negative correlation between ambient temperature and mortality for many insect species (Devries and Georghiou 1979). Pyrethroids are known to exhibit a clearly negative correlation between increasing ambient temperature and mortality for many insect species.

The few studies that have characterized the influence of temperature on insecticide toxicity in mosquitoes have focused on the aquatic, immature stages. Species studies include *Anopheles quadrinaculatus* Say, *Anopheles albimanus* Wiedemann, *Aedes triseriatus* Say, *Culex restuans* Theobald, and *Culex salinarius* Coquillett (Diptera: Culicidae) (Swain et al. 2008). To our knowledge, only two studies have been conducted to test the effects of temperature on insecticide toxicity to adult mosquitoes (Hadaway and Barlow 1963, Hodjati and Curtis 1999). Hodjati and Curtis (1999) observed a positive temperature coefficient between 16 and 22°C for susceptible and resistant strains of adult *Anopheles gambiae* Giles (Diptera: Culicidae) exposed to permethrin. In contrast, Hadaway and Barlow (1963) observed a negative temperature coefficient between 20 and 30°C when adult *Anopheles stephensi* Liston (Diptera: Culicidae) were exposed to DDT, an insecticide known to have a similar toxicity-temperature relationship as pyrethroid insecticides (Devries and Georghiou 1979). As a result of the equivocal findings presented in the literature for adult mosquitoes, our study characterizes the influence of temperature on the susceptibility of adult *Ae. aegypti* when exposed to the Type I pyrethroid insecticide, permethrin. We hypothesize that mortality of adult *Ae. aegypti* will decrease with increasing ambient temperature.

**Materials and Methods**

**Insects**

Eggs of *Ae. aegypti* from the susceptible New Orleans (NO) strain were provided by East Baton Rouge Mosquito Abatement and Rodent Control (EBRMARC, Baton Rouge, LA). The EBRMARC colony, which was started in 1995, originates from the New Orleans Mosquito, Termite, and Rodent Control Board colony (New Orleans, LA), and has been in culture for >20 yr with no wild supplements.

**Insect Rearing**

Eggs were shipped (USDA APHIS Permit Number 123889) overnight on egg papers in secure packaging from EBRMARC to Montana State University (MSU) and immediately placed in a rearing room (7.65 m²) with temperature of 27 ± 2°C, relative humidity (RH) 60–80%, and a photoperiod of 14:10 (L:D) h. The eggs were housed in plastic containers (27.94 by 16.83 by 6.99 cm³ or 35.56 by 22.23 by 10.16 cm³; Ziploc brand Containers with One Press Seal, SC Johnson, Racine, WI) containing 27 ± 2°C deionized water. On day three and day six of rearing (days counted from time the eggs were placed in plastic containers), *Ae. aegypti* larvae were fed 1.25 g of ground Wardley Pond Pellets fish food (The Hartz Mountain Corporation, Secaucus, NJ). At pupation, they were transferred to 27 ± 2°C deionized water in 29.57 ml graduated containers. Each 29.57 ml container was placed in a 2.36 liter cylindrical holding container with a netted top. The cylindrical holding containers were placed in small rearing cages (31.12 by 31.12 by 31.12 cm) until adult eclosion. After adult eclosion, 125–150 adult female mosquitoes were placed in 946 ml plastic holding containers with netted tops and provided a 10% sucrose solution. The mosquitoes were transferred to the plastic holding containers via a mouth aspirator. After 5–7 d, a 946 ml container, housing 125–150 adult females, was randomly selected and used for a Wheaton bottle bioassay replication.

**Chemicals**

Permethrin was the active ingredient for all experiments because it is the most commonly used insecticide for adult mosquito control in the United States (U.S. Environmental Protection Agency [USEPA] 2009a). Technical-grade permethrin (98% purity, 50:50 mixture of the toxicologically active cis and trans isomers) was obtained from Sigma-Aldrich (St. Louis, MO), and stock solutions were prepared in high-pressure liquid chromatography acetone (99.7% purity; EMD Chemicals, Gibbstown, NJ).

**Bioassays at Different Temperatures**

The CDC bottle bioassay protocol (4.3.3 CDC Bottle Bioassays) was modified and used for all experimental replications at different temperatures (Brogdon and McAllister 1998a,b). Modifications to the CDC bottle bioassay include observing mortality after 24 h of exposure to the permethrin solution, including six test temperatures, and including a range of concentrations to determine the median lethal concentration at each test temperature. Percival growth chambers (model E30B, Percival Scientific, Inc., Perry, IA) in the MSU Plant Growth Center (PGC) were randomly set to temperatures 16, 23, 26, 30, 32, and 34 ± 0.5°C with 60–80% RH and a photoperiod of 14:10 (L:D) h.

Two sets of 250 ml glass Wheaton bottles (A. Daigger & Company, Inc., Vernon Hills, IL) were dosed with a 2 ml aliquot of permethrin solution at one of eight different concentrations (0.06, 0.13, 0.19, 0.22, 0.26, 0.29, 0.39, and 0.58 ng/cm²) or an acetone control (2 ml). The Wheaton bottles were placed on mechanical rollers (model HDR-565, The Helman Group, Ltd., Oxnard, CA) and rotated until the acetone dried and the permethrin evenly coated each bottle. Treated bottles and caps were placed in the rearing room (27 ± 2°C, 60–80% RH, and a photoperiod of 14:10 [L:D] h) overnight and covered to avoid light exposure.

The next morning, two 946 ml plastic holding containers with mosquitoes and the 18 treated bottles were transported to the PGC in a large weatherproof plastic container. Each holding container with mosquitoes was placed in a randomly selected temperature unit or environmental growth chamber and allowed to acclimate for 2 h. After 1.5 h of female mosquito acclimation, the exposed Wheaton bottles were introduced into the environmental growth chambers and allowed to acclimate to the respective temperature for 30 min. Then, 10–12 adult females (5–7 d old) were introduced to each Wheaton bottle and covered with a treated cap. After 24 h, the number of alive and dead mosquitoes was assessed. If control mortality was found to be greater than 20%, the experiment was discarded and conducted again. Individuals that lacked movement when stimulated by shaking the holding bottle were considered dead.

**Experimental Design**

The experimental design was a split-plot design. The whole plots were the temperature units or environmental growth chambers, and the split plots were the eight concentrations and acetone control within the temperature units. A minimum of seven replications were conducted at each temperature.
Statistical Analysis

Treatment mortality was corrected using Abbott’s formula (Abbott 1925). At each temperature (16, 23, 26, 30, 32, and 34°C), the data were pooled and analyzed by probit analysis using Polo Plus (LeOra 2002). The LC_{50} and associated 95% confidence interval was calculated at each temperature. Differences between LC_{50} values for the temperatures were considered insignificant if the 95% confidence intervals overlapped.

The temperature coefficients, which represent how changes in temperature affect the toxicity of a compound at the LC_{50}, were calculated for each temperature (Toth and Sparks 1988, Alzogaray et al. 1998). A temperature coefficient was designated negative if the LC_{50} value at the higher temperature was larger than the LC_{50} at the lower temperature and designated positive if the opposite occurred (Alzogaray et al. 1998).

Results and Discussion

The toxicity of permethrin in adult female Ae. aegypti was estimated at 16, 23, 26, 30, 32, and 34°C. The estimated LC_{50} for each temperature was 0.26, 0.36, 0.36, 0.45, 0.27, and 0.31 ng/cm², respectively. Significantly different LC_{50} estimates were observed between 16°C (LC_{50} = 0.26, 95% CI = 0.20–0.35) and 30°C (LC_{50} = 0.45, 95% CI = 0.37–0.64), and 30°C (LC_{50} = 0.45, 95% CI = 0.37–0.64) and 32°C (LC_{50} = 0.27, 95% CI = 0.22–0.35), as the confidence intervals did not overlap (Table 1).

When compared with the LC_{50} at 16°C, all temperature coefficients were negative (Table 2). As suspected, the largest negative temperature coefficient was observed at 30°C (Table 2). Interestingly, the negative temperature coefficient was lower at 32°C than at 34°C (Table 2). As a result, there was a negative correlation between temperature and toxicity from 16 to 30°C, a positive correlation between temperature and toxicity from 30 to 32°C, and a negative correlation between temperature and toxicity from 32 to 34°C (Table 2).

Since their introduction in the 1970s, pyrethroids have become the most widely used class of insecticides for agricultural and public health practices (Yu 2008, Schleier III and Peterson 2011). Pyrethroids conventionally are classified by a negative temperature coefficient for toxicity (Toth and Sparks 1988). However, not all insects display a negative temperature coefficient for toxicity when exposed to pyrethroids (Toth and Sparks 1988, Alzogaray et al. 1998, Ma et al. 2012). For example, Boina et al. (2009) reported a negative temperature coefficient for toxicity when adult Diaphorina citri Kuwayama (Hemiptera: Psyllidae) were exposed to the Type I pyrethroid bifenthrin between 17 and 27°C, but a positive temperature coefficient for toxicity was observed between 27 and 37°C (Boina et al. 2009). Likewise, Toth and Sparks (1988) reported a small positive temperature coefficient for toxicity between 26.7 and 37.8°C when cis and trans permethrin were topicaly applied to third-instar Trichoplusia ni (Hübner) (Lepidoptera: Noctuidae). Toth and Sparks (1988) also reported a small positive temperature coefficient for toxicity between 15.6 and 26.7°C when cis permethrin was incorporated into the diet of T. ni.

When resistant adult An. stephensi were exposed to permethrin for 5 h, a negative temperature coefficient for toxicity was observed between 16 and 22°C (Hodjati and Curtis 1999). However, as a part of the same study, a positive temperature coefficient for toxicity was observed between 28 and 37°C when resistant An. stephensi were exposed to permethrin for 5 h. Although our exposure time was 24 h, we also observed varying temperature coefficients for toxicity when adult Ae. aegypti were exposed to permethrin at different temperatures. Negative temperature coefficients for toxicity were observed between 16 and 23°C, 26 and 30°C, and between 32 and 34°C. A positive temperature coefficient for toxicity was observed between 30 and 32°C. Our largest negative temperature coefficient for toxicity (−1.73) was observed at 30°C when compared with our lowest temperature, 16°C. Similarly, the largest negative temperature coefficient for toxicity reported by Ma et al. (2012) occurred at 30°C when Apolygus lucorum (Hemiptera: Miridae) were exposed to β-cypermethrin for 48 h. These studies and our results suggest pyrethroids often display negative temperature coefficients for toxicity, but for a given range of

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Unit increase (°C)</th>
<th>LC_{50} (ng/cm²)</th>
<th>Temp. coefficient b</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0</td>
<td>0.26</td>
<td>−1.38</td>
</tr>
<tr>
<td>23</td>
<td>7</td>
<td>0.36</td>
<td>−1.38</td>
</tr>
<tr>
<td>26</td>
<td>10</td>
<td>0.36</td>
<td>−1.25</td>
</tr>
<tr>
<td>30</td>
<td>14</td>
<td>0.45</td>
<td>−1.73</td>
</tr>
<tr>
<td>32</td>
<td>16</td>
<td>0.27</td>
<td>−1.03</td>
</tr>
<tr>
<td>34</td>
<td>18</td>
<td>0.31</td>
<td>−1.19</td>
</tr>
</tbody>
</table>

Table 2. Temperature coefficient estimates for adult female Ae. aegypti after 24-h exposure to permethrin

a Values to the left are the unit increase (°C) from the baseline temperature (16°C). Values to the right are the unit increase (°C) between the two temperatures.

b Values to the left are for temperature coefficients calculated using the 16°C baseline temperature. Values to the right are the temperature coefficients calculated between two temperatures.

Table 1. Influence of temperature on permethrin toxicity to adult female Ae. aegypti

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>n a</th>
<th>Slope (SE)</th>
<th>LC_{50} b (ng/cm²)</th>
<th>95% CI (ng/cm²)</th>
<th>LC_{95} b (ng/cm²)</th>
<th>95% CI (ng/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>612</td>
<td>1.82 (0.21)</td>
<td>0.26 d</td>
<td>0.20–0.35</td>
<td>2.09</td>
<td>1.07–9.95</td>
</tr>
<tr>
<td>23</td>
<td>788</td>
<td>2.04 (0.21)</td>
<td>0.36</td>
<td>0.30–0.48</td>
<td>2.35</td>
<td>1.34–6.90</td>
</tr>
<tr>
<td>26</td>
<td>710</td>
<td>2.69 (0.24)</td>
<td>0.36</td>
<td>0.29–0.47</td>
<td>1.46</td>
<td>0.91–3.97</td>
</tr>
<tr>
<td>30</td>
<td>703</td>
<td>2.98 (0.28)</td>
<td>0.45 d e</td>
<td>0.37–0.64</td>
<td>1.60</td>
<td>0.97–4.70</td>
</tr>
<tr>
<td>32</td>
<td>699</td>
<td>2.52 (0.22)</td>
<td>0.27 e</td>
<td>0.22–0.35</td>
<td>1.22</td>
<td>0.74–3.72</td>
</tr>
<tr>
<td>34</td>
<td>774</td>
<td>2.46 (0.21)</td>
<td>0.31</td>
<td>0.26–0.39</td>
<td>1.44</td>
<td>0.90–3.70</td>
</tr>
</tbody>
</table>

a Number of female mosquitoes tested.

b Concentration lethal to 50% of the test population.

c Concentration lethal to 95% of the test population.

d LC_{50} values without overlapped 95% confidence intervals were considered significantly different.
temperatures the temperature coefficient can alternate from a negative to positive temperature coefficient for toxicity.

Changes in ambient temperature affect the rate of chemical uptake, binding affinity, metabolism, and excretion of insecticides for insects, as they are ectothermic organisms (Osterauer and Kohler 2008, Harwood et al. 2009, Weston et al. 2009, Laetz et al. 2014). Previous studies have suggested different mechanisms for the decrease in toxicity often associated with pyrethroid insecticides as temperature increases (Devlies and Georgiou 1979, Toth and Sparks 1988, Alzogaray et al. 1998, Ma et al. 2012). Weston et al. (2009) reported greater uptake of permethrin at 23°C when compared with 13°C for Chironomus dilutus (Diptera: Chironomidae). As a result, we suspect that at lower temperatures there is less penetration of the parent compound and less metabolite formation in insects. This is supported by the decreased rate of parent compound biotransformed to the less toxic metabolites at 23°C compared with 13°C when C. dilutus were exposed to permethrin.

Insecticide metabolism is temperature dependent, and at lower temperatures the more toxic parent compound persists in insects. Buildup of the more toxic parent compound at lower temperatures causes changes in the voltage-gated sodium channels, and for type I pyrethroids, such as permethrin, result in repetitive nerve firing (Khan and Akram 2014). Likewise, decreased temperature may also increase the susceptibility of neurons to pyrethroid insecticides making them more sensitive to excitation (Salgado et al. 1989).

Although the precise mechanism was not determined, each of these provide likely mechanisms for the lower LC50 values observed at lower temperatures. Temperature affects the excretion of parent compound, but does not affect the excretion of metabolites (Weston et al. 2009). Parent compound excretion increases as temperature increases from 13 to 23°C for C. dilutus (Weston et al. 2009). If the rate of parent compound metabolism and parent compound elimination constantly increases as temperature increases, it is possible the decreased mortality we observed as temperature increased up to 30°C could be linked to increased excretion due to temperature.

Although studies suggest the negative temperature coefficient observed at lower temperatures is associated with decreased metabolism of the parent compound or decreased penetration, other studies suggest the positive temperature coefficient seen at higher temperatures is associated with heat stress (Grafius 1986, Toth and Sparks 1988). We observed a positive temperature coefficient for toxicity from 30 to 32°C. However, the positive temperature coefficient for toxicity was not observed between 32 and 34°C. To determine if heat stress influenced the positive temperature coefficient seen between 30 and 32°C, molecular tests on heat shock gene expression are needed. The expression of heat shock protein genes, in particular, hsp70 genes are induced by a number of stressors including increased temperature (Gross et al. 2009). Our hypothesis that stress was related to the positive temperature coefficient for toxicity between 30 and 32°C is supported by an increase in observed control mortality seen at 32°C (mortality ranged from 33 to 81% at 32°C). In particular, there were three replications with greater than 20% control mortality at 32°C. These three replications were discarded and conducted again.

Pyrethroids are relatively photostable insecticides and, in particular, permethrin has a foliar half-life of 35 d (USEPA 2009b). Therefore, chemical degradation as a possible cause of the decreased mortality seen at 30°C is not likely. Although we suspect the increase in mortality observed between 30 and 32°C is associated with heat stress, the increased mortality at 32°C could also provide evidence against chemical degradation at higher temperatures.

According to Knappenberger et al. (1996), temperatures in the late afternoon have become increasingly warmer when compared with other parts of the day for all seasons of the year in the United States. Given our results, pyrethroid insecticides may not be as effective for the control of adult mosquitoes when applied at dusk. In particular, the monthly high for average air temperature from 1961 to 1990 was 27.5°C for Jacksonville, FL, 27.7°C for New Orleans, LA, 29.1°C for Brownsville, TX, 27.5°C for Charleston, SC, and 28.1°C Memphis, TN (Eisen et al. 2014). Consequently, environmental conditions and timing of application should be considered to optimize the effectiveness of outdoor mosquito management strategies.

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