

Article

# Nonlethal Effects of Pesticides on Web-Building Spiders Might Account for Rapid Mosquito Population Rebound after Spray Application

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**Featured Application:** In this study, a broad-spectrum insecticide is shown to halt mosquito capture by orb-weaving spiders, even when the application does not kill the spiders. Reduced prey-capture, even temporary, can allow mosquito populations to rebound quickly. Adoption of other mosquito control methods, such as bacterial larvicides, avoids these potential problems.

**Abstract:** Spiders are important population regulators of insect pests that spread human disease and damage crops. Nonlethal pesticide exposure is known to affect behavior of arthropods. For spiders such effects include the inability to repair their webs or capture prey. In this study, nonlethal exposure of Mabel's orchard spider (*Leucauge argyroabapta*) to the synthetic pyrethroid permethrin, via web application, interfered with web reconstruction and mosquito capture ability for 1–3 days. The timing of this loss-of-predator ecosystem function corresponds to the rapid population rebound of the yellow fever mosquito (*Aedes aegypti*) following insecticide application to control arbovirus epidemics. We suggest this temporal association is functional and propose that follow-up study be conducted to evaluate its significance.

**Keywords:** *Aedes aegypti*; *Leucauge argyroabapta*; nontarget effects; permethrin; pyrethroid; Tetragnathidae



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## 1. Introduction

Non-target mosquito predators, including spiders and wasps, are often harmed by neurotoxic pesticides [1]. Orchards where conventional insecticides are used have far lower spider populations and species counts than unsprayed orchards [2]. Spiders with bodies larger than mosquitoes are not killed by ultra-low volume (ULV) spraying of insecticides such as permethrin [3]. However, studies such as these sometimes conflate survival with lack of harm, and they have not examined effects on small-bodied spiders, which do capture mosquitoes. Spiders exposed to sublethal pesticides change their behavior in ways that reduce prey capture. For instance, sublethal pyrethroid spray residues lower activity rates of spiders, even when the spiders contacted the pyrethroids on foliage 20 days after application [4]. Following nonlethal exposure to Spinosad, an acetylcholine disrupter, the orb-weaving spider *Agalenatea redii* showed irregularities in web design and lower prey capture activity [5]. An increase in spider migration was found within a large plantation where sublethal amounts of organophosphate pesticides were sprayed around its borders, suggesting spiders sense and actively avoid areas with some insecticide treatments [6].

Spider webs are particularly effective at capturing insecticide sprays, retaining an order of magnitude higher concentration than an equivalent area of paper [7]. Orb-weaving spiders recycle their webs daily, ingesting the spiral silk strands while leaving some of the radial structural strands intact as a scaffold for rebuilding [8–11]. By consuming the web, orb-weaving spiders ingest higher amounts of sprayed pesticides than would contact their bodies and legs. Araneidae typically construct orb webs in a vertical plane,

whereas Tetragnathidae typically construct webs in the horizontal plane [12]. Horizontal webs should intercept more insecticide droplets following a spray application, making tetragnathids particularly vulnerable.

Following aerial insecticide application in New Orleans, yellow fever mosquitoes (*Aedes aegypti*) rebounded faster than the population model predicted [13]. The authors attributed this rapid increase to release from larval competition, a phenomenon well-documented in the literature [14–16]. However, the data presented in that study show that the rebound occurred within the 3-day non-feeding window prior to eclosure, a period when there can be no competition for food. A similar effect could be seen in Miami's *Ae. aegypti* populations during the Zika outbreak of 2017. Adult *Ae. aegypti* in Miami's Wynwood neighborhood were virtually eliminated by aerial spraying on two occasions, only to rebound to pre-spray levels in just three days [17]. In both studies, populations leveled off after rebounding. Release from larval competition does not fit the timing window for the rapid rebound observed following aerial spraying of adulticides, but temporary release from predation might allow such a rebound to occur. Off-target effects of insecticides on mosquito predators such as spiders, even non-lethal effects, might allow greater survival of adult mosquitos during the rebound period.

Mabel's orchard orbweaver spider (*Leucauge argyrobapta*, Tetragnathidae) is found in Florida, throughout the Caribbean, and from Mexico to Brazil, with congeners common throughout the North American continent [18]. In South Florida, *L. argyrobapta* is abundant in treed areas and around residences, constructing webs in vegetation, eaves of houses, porches, and patio furnishings. Both juvenile and adult *L. argyrobapta* are capable of subduing mosquitoes. *L. argyrobapta* produces viscous horizontal orb webs, and recycles sticky spiral capture threads nightly, as is typical of tetragnathid orb-weavers [12,19].

We explored the effects of sprayed permethrin, absorbed through web contact and web recycling, on the abilities of adult *L. argyrobapta* to repair their webs and capture live adult mosquitoes. Permethrin is a type I synthetic pyrethroid used routinely to control adult mosquitoes in Miami-Dade County (FL, USA) and elsewhere. Pyrethroids bind the pore of the voltage-gated sodium channel preventing its closure, thus causing the nerves to hyperpolarize and muscle action to cease [20].

## 2. Materials and Methods

We chose permethrin as our insecticide because of its wide use in mosquito control in Miami-Dade County. The treatment solution was a 0.0368% solution of permethrin in acetone (1000× dilution of Martin's 36.8% Permethrin SFR, Control Solutions Inc., Pasadena, TX, USA) with a cis/trans ratio 42–58%. Two negative controls were (1) the solvent carrier (AU582) diluted 1000× in acetone, and (2) acetone alone. By trial and error, we determined that this permethrin concentration did not kill any *L. argyrobapta* when applied to their webs. To put this 0.0368% concentration in context, one hour of foot contact with 2.1% permethrin impregnated paper is the LC-50 concentration for local *Ae. aegypti*, and 0.1% is the highest concentration that kills none of them (P.K. Stoddard, unpublished). Acetone is recommended as a pyrethroid carrier in arthropod toxicity studies because it evaporates quickly [21] and is tolerated well by arthropods [22]. Because of acetone's high volatility and our limitation on number of available and comparable spiders for allocation to treatment groups, we considered acetone-only a reasonable negative control and omitted an unsprayed control group. The perfect hunting success of spiders recorded on acetone-treated webs supported our choice. At just 0.06% of the solution, the amount of AU582 carrier solvent in the sprayed permethrin solution was insignificant and had no measurable effect on spider behavior.

*L. argyrobapta* were tested near a residence within a 0.4 ha plot of native hardwood forest in Miami-Dade County (25°30'00.25", 80°28'03.48"). In March 2020, we removed 45 spiders from their webs, delicately so as not to damage the webs. We tore sections of the webs, both spiral mesh strands and several radii, to allow us to determine if the web had been recycled and reconstructed. We sprayed 15 webs with permethrin solution, 15

with acetone plus carrier, and 15 with acetone, dispensing of 2 mL of each solution per web using a hand sprayer. Webs receiving the different treatments were chosen at random with respect to the body size of the resident spider. We allowed webs to dry for 20 min then returned each spider to its original web. Webs were photographed 1 h after spraying and again 24 h later. We took two orthogonal measurements of web mesh diameter, both before spray application and again 24 h later, and noted whether the web had been substantially reconstructed (at least 75% replacement of spiral mesh).

In June 2020, we repeated the same web treatments, and determined the efficacy of treated webs and their resident spiders at retaining and subduing live mosquitoes, as well as whether the web had been substantially reconstructed. Ambient temperatures were higher in June than March, with overnight lows averaging 18 °C in March and 26 °C in June.

The mosquitoes we used were an even mix of wild *Ae. aegypti*, and *Wyeomyia vanduzeei*, captured locally with a BG-2 Sentinel trap (Biogents, Regensburg, Germany). Twenty-one webs were sprayed with permethrin and 16 with the acetone control. Using a mouth aspirator (John W. Hock Company, Gainesville, FL, USA), we aspirated five mosquitoes at random from the mosquito cage and propelled them toward an intact section of the web. Before conducting the trials, we practiced on untreated webs until we could reliably expel mosquitoes at the correct velocity to strike the web but not break the strands. Preliminary trials determined that undisturbed *L. argyrobapta* readily capture and consume any mosquito that sticks in the spiral web for at least three seconds (Figure 1). During the trials, we noted whether mosquitoes stuck to the web for 3+ s, whether the spider seized a stuck mosquito, and the latency for the spider to respond to the web strike.



**Figure 1.** (A) In the mosquito handling procedure, mosquitoes were propelled toward the web with a light puff of air, being careful not to blow air directly at the spider. (B) An adult female *L. argyrobapta* subdues a mosquito (*W. vanduzeei*) that had been propelled into the web.

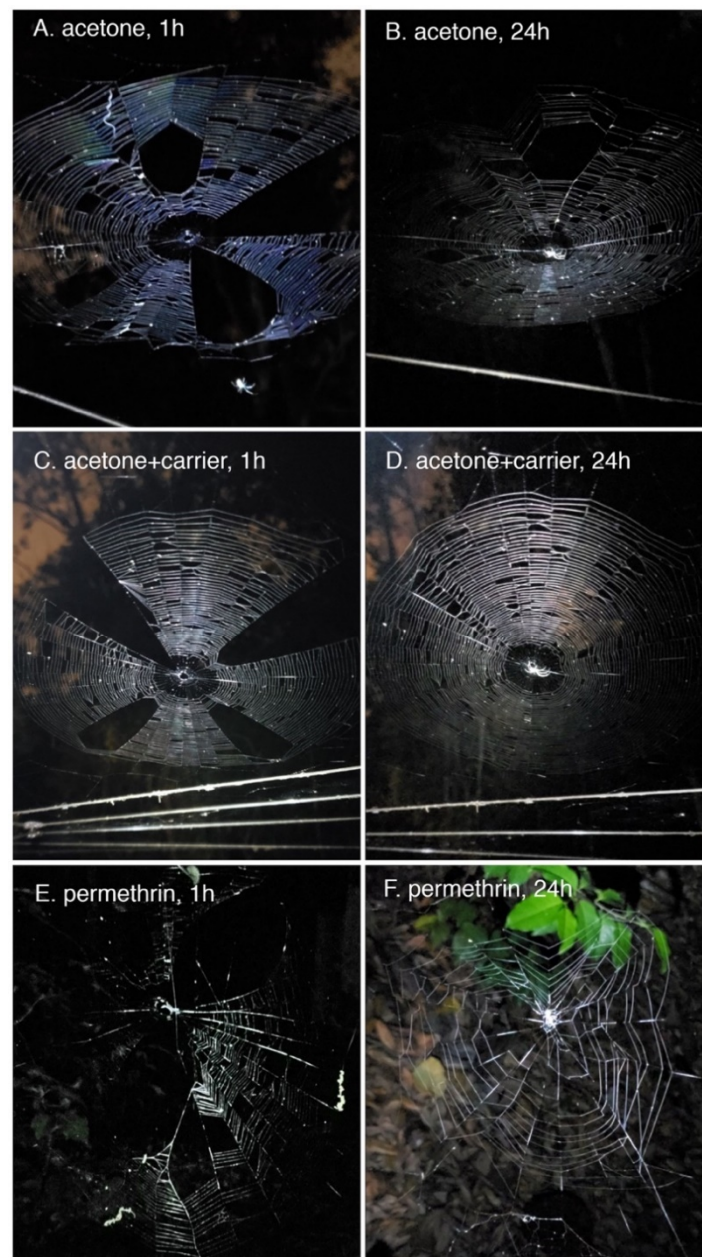
Data on frequency of web repair were analyzed by comparing repaired and non-repaired webs as categorical variables using chi-square or Fisher's Exact Test to compare the treatment groups. We calculated web area from the two diameter measurements. Diameters of reconstructed webs were identical for every spider in the two control groups but differed in the permethrin-treated group, so web areas of spiders that attempted web reconstruction were compared before and after permethrin application using a paired T-test for unequal variance. Whether at least one mosquito adhering to each web was seized by spiders in the two treatment groups was evaluated with Fisher's Exact Test. Numbers of mosquitoes captured by the two treatment groups were compared using a 2-sample *t*-test.

### 3. Results

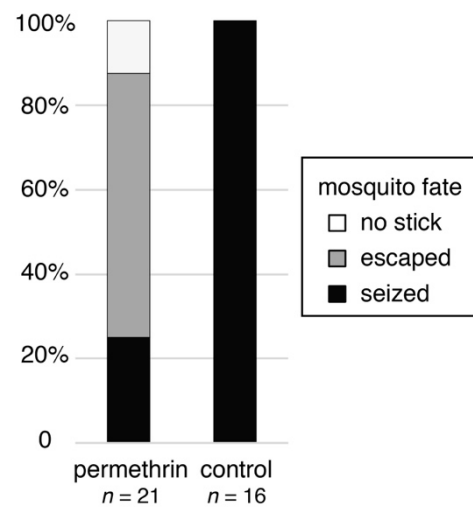
In March, all webs receiving the two control treatments had been substantially restored to their previous structure 24 h later, whereas none of the permethrin-treated webs were substantially restored ( $p = 1.69 \times 10^{-10}$ ;  $X^2 = 30$ ; 2 df). Six of the 15 permethrin-treated webs had been partially restored, and the rest were in the same torn condition as the day before. Of those webs that had been rebuilt, the mesh areas were, on average, just 42% of their original size ( $p = 0.005$ ,  $t = 4.13$ ,  $df = 6.8$ , paired T-test, unequal variance, 2-tailed). Visual inspection revealed that spacing of the adhesive spiral mesh strands was wider than on a normal web (Figure 2F). Most spiders on the permethrin-sprayed webs remained on the web, though several disappeared, having either abandoned the web or been predated.

In June, when we treated webs in preparation for mosquito capture, all control webs were substantially restored within 24 h, whereas just 29% (6 of 21) of the spiders on permethrin-treated webs had substantially restored their webs ( $p < 0.0001$ ; Fisher's Exact Test). All permethrin-treated webs had been restored after three days, showing the interference at this dose was temporary.

The day after treating the webs in June, we tested the webs and spiders for mosquito-catching ability. Permethrin reduced the number of mosquitoes captured by 71%, independent of the reduced web area (Figure 3). Permethrin-treated webs were less effective at retaining the mosquitoes that struck them than the control webs; ( $p = 0.00024$ ;  $t = 4.44$ , 15 df). Mosquitoes adhered to each of the 16 control webs for at least three seconds, and the resident spider seized a mosquito in each web. In the 21 permethrin-treated webs, we managed to stick a mosquito in what remained of 86% (18) of the webs; three webs could not retain a mosquito at all. Spiders on treated webs were less likely to seize a mosquito that did stick than spiders on control webs ( $p < 0.0001$ ; Fisher's Exact test). In 67% of the webs that held a mosquito for at least 3 sec but that had not been substantially repaired (12 of 18), none of the spiders seized the mosquito. In the 21% of webs that were substantially reconstructed, each spider seized the mosquito immediately. Mosquitoes not seized by the spiders eventually wiggled free.



**Figure 2.** Side by side comparisons of spider webs before (A,C,E) and after (B,D,F) the residing orchard spiders were exposed to treatments. The control treatments (A–D) had no adverse effect on web reconstruction. For the first day after application, permethrin treatment (E,F) interfered with the resident spider’s ability to reconstruct a normal web. The few webs that were partially reconstructed (6 of 15) had a significantly smaller web area, and irregular and wide spacing of adhesive spiral strands visible in the permethrin treatment after 24 h (F).



**Figure 3.** Spiders on permethrin-treated webs captured far fewer mosquitoes than those on control webs.

#### 4. Discussion

Permethrin treatment of webs interfered with mosquito capture by spiders in at least two ways. After consuming the webs to recycle the silk, spiders were rendered sluggish. They were delayed in reconstructing the webs and produced small webs with irregularly spaced adhesive strands. Such webs were less likely to hold mosquitoes. Further, when mosquitoes did stick in these webs, the spiders most often did not react, allowing mosquitoes to struggle free. Similar effects have been documented for another synthetic pyrethroid, alpha-cypermethrin, which interferes with web reconstruction in the araneid orbweaver *Araneus diadematus* in the same ways we found for *L. argyrobapta* [23]. In *Tenuiphantes tenuis*, a common sheet-web spider of European cereal agricultural fields, the pyrethroid cypermethrin had similar detrimental effects on web construction and activity as well as reduced reproduction and shortened lifespan [24]. Spiders can still detect prey in webs with distorted structure [25], though they take about twice as long to do so, increasing likelihood of prey escaping. Reduced disc size would reduce capture efficiency proportionally.

Spiders exposed to permethrin in our study recovered in one to three days. A third of the spiders had recovered in one day in June, but none had in March. Seasonal temperature and humidity differences may have contributed to differences in speed of recovery. In South Florida, March is dry and cool, whereas June is hot and humid. Accordingly, diurnal mosquitoes are virtually absent in March, but their populations explode in June with the onset of the rainy season, so the June data better typify encounters between mosquitoes and spiders. Elevated temperature affects the spiders' response to pyrethroids in two opposing ways, hastening absorbance, which increases toxicity, but also increasing metabolism of the pesticide, which shortens recovery time for sublethal doses [26]. Pyrethroids also interfere with water transport in arthropods, leading to dehydration [26,27], which can affect all aspects of physiology.

The rapid rebound of mosquito populations over the three days following adulticide application [13,17] matches the three-day period of spider immobility found in this study and others [23,24,27]. Recovery of spiders' web construction and prey capture abilities would correspond to the return to the lower survival rate of adult *Ae. aegypti* three days after adulticide spray application. Non-lethal effects on spiders in this study have been seen in other species and with other pesticides [1,4,5,27]. Similarly, population increase of agricultural pests following reduction of spiders through pesticide application has been documented repeatedly [28]. Because spider immobility following pesticide application has significant consequences for control of agricultural pest infestation, the possibility of harm to spiders should be taken seriously in control of arbovirus epidemics as well. For

that reason, research should be conducted to see whether nonlethal effects of pesticides on mosquito capture by spiders actually does enhance the speed of mosquito population rebound. Our study minimized environmental exposure by applying permethrin directly to the webs. A more direct comparison to typical mosquito control conditions would benefit from replicating these experiments using ULV spray applications of the pesticide of interest.

Mosquito populations are regulated by a mix of bottom-up and top-down processes including intraspecific and interspecific competition among larvae, and predation on all life stages [29–32]. Most studies of mosquito competition (bottom-up control) and predation (top-down control) focus on the larval stages. Small bats consume adults of mosquito species that fly in the open but not the urban mosquito species that stay close to dwellings and vegetation [33]. No evidence suggests bats eat enough mosquitoes to affect their populations [34]. Adult dragonflies can be seen hunting in the right places to catch mosquitoes in a variety of habitats, but, probably for logistic reasons, the literature is devoid of data on mosquito predation by adult dragonflies. Evidence for spiders as effective predators of adult mosquitoes is only slightly better. Web-building spiders are the dominant predators of flying insects in most terrestrial ecosystems [35], regulating prey populations, and, sometimes capturing far more prey in their webs than they can consume [36]. Density of pest insects is significantly lower in areas with higher densities of spiders [28]. Orb-weaving spiders, in particular, are key predators of dipterans including mosquitoes [37–39]. Accordingly, spider predation on mosquitoes is thought to have potential for control of dengue and malaria [40,41]. Our finding that spider predation of mosquitoes is temporarily compromised after pyrethroid exposure, combined with earlier findings that mosquito populations increase faster immediately following adulticide spraying [13,17] are consistent with the possibility that spiders play a significant role in suppression of urban/suburban mosquito populations. The argument thus far is correlative and would be illuminated by direct study of spider suppression of mosquitoes at the local population scale. Because spiders are so common in and around human dwellings, their potential as suppressors of adult mosquito populations warrants more direct experimental investigation.

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