

Volatile Pyrethroids as a Potential Mosquito Abatement Tool: A Review of Pyrethroid-Containing Spatial Repellents

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Abstract

Ongoing difficulties with successful urban mosquito management highlights the need to find novel management strategies to reduce the impact of mosquito vectors. To date, urban mosquito management can be summarized as dependent on the theme of recruiting the public to invest in their own protection. This effort takes the form of source reduction education programs and accompanying personal protection guidelines. However, the topical repellents that form the cornerstone of our personal protection guidelines are available in equal measure with spatial repellents. Spatial repellents commonly include volatile pyrethroids as active ingredients, which easily transition into a vapor phase, that are formulated into mosquito coils, vaporizers, emanators, or heated mats. These formulations are available on the global market. Consumers frequently choose spatial repellents, and existing literature indicates this choice has merits. This predicament forces integrated vector management to adapt to and understand tools that consumers selectively employ. At present, there is little information differentiating the effects of vapor-active pyrethroids from the contact efficacies we recognize from well-utilized pyrethroids. To address this, volatile pyrethroids need to have their multiple effects identified, defined, and described to maximize their utility for preventing mosquito biting and, thus, pathogen transmission. This article reviews the known utility of volatile pyrethroids in the context of repellency, acute symptoms, toxicity, and sublethal effects, and provides an overview of the evaluation methods used with volatile pyrethroids.

Key words: pyrethroid, repellent, mosquito, spatial repellent, vector control

The global burden of arthropod-borne diseases has been summarized by Hill et al. (2005) at ~1.5 million annual deaths worldwide. This burden translates into heightened pressure on humans to protect themselves from vector contact. Traditionally, we think of this as killing the vector, but contemporary vector control weaves in the goals of long-term management, which concerns itself with sustainability, avoidance of pesticide resistance selection, and multifaceted control mechanisms. The ideology that advocates prevention and tactful, multifaceted approaches to intervention (control) to achieve long-term results is collectively referred to as integrated pest management. When this concept is applied to disease vectors, it is termed integrated vector management. When applied to mosquitoes, it is integrated mosquito management. The last two are essentially one in the same.

For integrated mosquito management, capitalizing on community buy-in of this integrated approach can be as important as the direct intervention tools available. For example, Bodner et al.

(2016) highlights that areawide operations in mosquito management are encountering a growing number of hurdles. These can include limited financial options or privatized land and other types of inaccessible properties limiting capacity for vector habitat treatment. To counteract this, the World Health Organization (WHO) has recognized the need for recruiting the general populace in vector management (WHO 2015). However, leaning on citizens to supplement vector control is not a new tactic. Vector control programs, reported by Leontsini et al. (1993), used education to recruit the citizen-base into removing mosquito habitat and used proper personal protection to combat *Aedes aegypti* (L.), which is a globally significant vector of yellow fever (YFV), dengue (DENV), Chikungunya (CHIKV), Zika (ZIKV), and other viruses (Derraik and Slaney 2015, Ngoagouni et al. 2015, Wilson and Chen 2015). Averett et al. (2005) reported that source reduction and personal protection were employed similarly against the mosquitoes that transmit West Nile virus (WNV). Recent emphasis on container-inhabiting mosquitoes

has reinforced the usage of citizen recruitment methods against *Aedes albopictus* (Skuse), which is regarded as the most invasive mosquito species in the world (Benedict et al. 2007, Bartlett-Healy et al. 2011). However, recruitment of the public into vector management is not consigned only to cultural control methods like sanitation, water awareness, and pest-proofing.

The presence of Zika virus in the United States in 2016 has led to nationwide campaigns educating risk groups on avoiding transmission hot spots and using personal protective measures. Education programs and occupational safety programs for people at risk for mosquito-borne illness were urged by the Centers for Disease Control and Prevention (CDC) to employ repellent products to supplement vector control (CDC 2016). Yet, not all products available to consumers are traditional topical repellents, which have served as a personal protection standard in vector control. Some products use volatile pyrethroid compounds, which are a subset of synthetic pyrethroids that transition easily into a gaseous state, and are delivered as spatial repellents rather than traditional adulticides. These are available to consumers in emanators, vaporizer mats, burnable coils, and other means that have been shown by Xue et al. (2012a,b) and Revay et al. (2013) to prevent mosquito biting. These tools are believed to create a vector-free area using a single product, which can protect multiple persons in one application (Cook et al. 2007). The large number of products available serve as indicators of public desire for these volatile pyrethroids. These products bear labels that suggest protection from vectors. Such labeling pairs with educational programs encouraging individuals to be responsible for protecting themselves (i.e., use a repellent).

Repellency has been studied frequently in volatile pyrethroids, including metofluthrin, transfluthrin, and prallethrin (Argueta et al. 2004, Lee 2007, Abdel-Mohdy et al. 2008, Achee et al. 2012). Specific investigation by Achee et al. (2012) found volatile pyrethroids present in a gaseous state deterred entry of mosquitoes into treated spaces. Lucas et al. (2007) documented that volatile pyrethroid compounds elicited an escape response in mosquitoes, expelling them from the affected air space. However, further evaluation by Ritchie and Devine (2013) found a disorientation present in tested mosquitoes. This disorientation provoked the idea that mosquitoes may be exiting the area owing to poor spatial awareness, rather than a truly expellant effect. This has since been supported by demonstrating that regardless of proximity to the host, a disoriented mosquito is unable to contact the host, regardless of whether the mosquito escapes successfully (Buhagiar et al. 2017a). The value of repellency on the whole has been questioned in research by Moore et al. (2007), indicating that rather than pushing vectors away from all hosts, they simply push them to the nearest unprotected host. This potentially invalidates the benefit of repellency when used on a community or population scale.

Volatile pyrethroids should not be dismissed as a subpar alternative to topical repellents. Lee (2007) reported that transfluthrin and metofluthrin repellency also yielded mortality for both volatile pyrethroids. Furthermore, metofluthrin incited not only the disorientation, as previously reported by Ritchie and Devine (2013) and Buhagiar et al. (2017a), but also the effects termed “knockdown,” with eventual mortality in *Ae. aegypti* (Lee 2007; Ritchie and Devine 2013; Buhagiar et al. 2017a,b). Investigations by Bibbs and Xue (2015) took the OFF! Clip-on (4822-542, S.C. Johnson & Son, Inc, Racine, WI) product, a spatial repellent device expressing vapor-phase metofluthrin, and observed knockdown and mortality against *Ae. aegypti* in this metofluthrin-based product formulated and sold specifically for the repellent market. Prallethrin formulated as a repellent also has been found to elicit mortality as reported by

Abdel-Mohdy et al. (2008). Therefore, repellency, or disorientation as the case may be, combined with mortality as described in multiple studies eliminates the possibility described by Moore et al. (2007) of the vector moving to a less protected host. This provides a basis to consider volatile pyrethroids as a tool that generates more than one end point to achieve the goal of bite prevention. This vein of thought highlights the need to better understand volatile pyrethroids, or “spatial repellents,” as well as to refine our concept of what this tool can offer as a bite preventative.

Repellency

The goal of a repellent is to prevent a blood-feeding arthropod from making successful contact with its host. Topical repellents are applied directly to the potential bite site, which also requires the vector to come within close proximity of the application site. Furthermore, if the application is uneven or otherwise applied incorrectly, an exploitable gap in protection may be present. It remains that interfering with contact between a vector and a host is fundamentally required to reduce infection rates (WHO 2009). Volatile compounds have been studied to provide more repellent options. Successful volatile compounds have been dubbed spatial repellents for their quality of creating an air space free of vectors, namely mosquitoes (Achee et al. 2012). Spatial repellents have the potential to remediate many concerns associated with using repellents. It is noted by Sugiharto et al. (2016) that repellent use suffers a lack of uniformity in populations. Meaning, not everyone will apply them. This returns to the argument made by Moore et al. (2007) that hosts lacking a repellent will suffer the biting pressure instead. However, spatial repellents create a more uniform usage pattern due in part that only one individual needs to use the tool to protect multiple hosts in the vicinity (Cook et al. 2007, Paz-Soldan et al. 2011). This also circumvents failures to apply the repellent to all potential bite sites or to reapply when needed, both of which are experienced with topical repellents. This incentivizes the development of volatile compounds that may offer spatial repellent benefits.

It is described by Cook et al. (2007) that a prerequisite of spatial repellents is the disruption of successful blood-feeding over a large range. This includes interfering with a vector successfully detecting, locating, and approaching the host. These principles can be employed without our complete understanding of the process. For example, DDT was shown with *Ae. aegypti* to elicit a directed movement away from the source without the mosquito needing to physically contact the chemical deposit (Grieco et al. 2007) and hence the mosquito did not die from the insecticide encounter. This falls within the developing modern concept that spatial repellents stimulate directed movement away from the source without physical contact being required. However, a contact irritant would provoke directed movement away from the source after physical contact. Conversely, a toxicant chemical would impair or kill the target after physical contact (Cook et al. 2007, Grieco et al. 2007, Achee et al. 2009). These definitions have caused compounds that may have been dismissed as toxicants to be reevaluated (i.e., contact irritants and spatial repellents). Pyrethroids, for example, are synthetic organic compounds based on pyrethrum discovered in *Chrysanthemum* sp. plants (Barnes and Verschoyle 1974). Using the ultrastructure of pyrethrum, many pyrethroid compounds with much higher activity than pyrethrum are used as toxicants in insecticides. Pyrethroids now account for as much as 17% of global insecticide sales, making them the second most utilized chemical class available in today's market (Sparks 2013), as well as a class

containing several potential spatial repellents (Argueta et al. 2004, Lee 2007, Abdel-Mohdy et al. 2008, Achee et al. 2012).

The potential for certain pyrethroids to act as spatial repellents has been quantified for metofluthrin by Lucas et al. (2007) as causing 85% of *Aedes canadensis* (Theobald), 89% of *Ae. aegypti*, and 95% of *Aedes vexans* (Meigen) to actively leave the treatment site. Furthermore, Achee et al. (2012) showed metofluthrin deterred 58% of exposed *Ae. aegypti* from entering a test room. Transfluthrin forced up to 93% of wild *Culex pipens quinquefasciatus* Say and *Anopheles gambiae* Giles s.s. away from test subjects and into nearby sentinel traps, as reported by Pates et al. (2002). This was corroborated by Ogoma et al. (2012), who reported 98% of *Anopheles gambiae arabiensis* Giles escaping transfluthrin-impregnated hessian strips by moving to a different portion of the test area and away from human subjects. This was replicated again using transfluthrin on long-lasting variants of hessian strips, and over 90% repellency of *Mansonia* spp., *Culex* spp., and *An. arabiensis* was recorded for 6 mo (Ogoma et al. 2017). Similarly, prallethrin as investigated by Abdel-Mohdy et al. (2008) yielded 100% repellency of a mixed group of mosquitoes in lab testing. Liu et al. (2009) exposed *Culex tritaeniorhynchus* Giles and *Cx. quinquefasciatus* to prallethrin and observed 80.34% repellency. Each of these volatile pyrethroids are currently used in liquid and heated emanators available on the global market to consumers (Argueta et al. 2004; Lee 2007; Abdel-Mohdy et al. 2008; Liu et al. 2009; Achee et al. 2012; Xue et al. 2012a,b; Revay et al. 2013).

The evidence on repellency derived from volatile pyrethroids is not without contradiction. In a movement study, Rapley et al. (2009) documented that *Ae. aegypti*, under the influence of metofluthrin, still entered and left the room equally. This is contrary to the idea that a spatial repellent should initiate directed movement away from the source of exposure. A more recent study by Ritchie and Devine (2013) did not observe *Ae. aegypti* to have any increase in escape response, effectively ignoring the repellent effect. This was found alongside an 87% reduction in biting noted by Rapley et al. (2009) and 100% reduction in biting noted by Ritchie and Devine (2013). This was partly attributed to a disorientation effect, which resulted in what could be interpreted as repellency when only seen as the mosquito leaving the area. However, the findings by Rapley et al. (2009) have provided an example where disorientation can lead a mosquito to possibly ignore or not recognize the stimulus as well, which may confound interpretation of repellency studies. Disorientation was documented by Kawada et al. (2006) with *Ae. aegypti*, wherein the disorientation was classified as a positive attribute, interfering with the mosquito's ability to find a host effectively. Interference with host seeking agrees with the studies in question that despite recording a variable repellent effect, a significant reduction in biting was observed. Investigation by Msangi et al. (2010) found a significant difference in repellency between different vector species. In their study, d-allethrin, a volatile pyrethroid, caused 92–98% of *Cx. quinquefasciatus* to exit the study area, but only 60–64% of *An. gambiae* exited the area under similar conditions. This same study also demonstrated a difference in reduction of actual blood feeding by the mosquitoes that did not exit the area, with *Cx. quinquefasciatus* being stopped 91% of the time as compared with 59% in *An. gambiae*.

Sathantriphop et al. (2014) expanded interspecies response comparison by testing permethrin, a Type-I pyrethroid, and deltamethrin, a Type-II pyrethroid, in noncontact assays against *Ae. aegypti*, *Ae. albopictus*, *Anopheles minimus* Theobald, and *Cx. quinquefasciatus*. In these assays, *Ae. aegypti* and *Cx. quinquefasciatus* were found to not be repelled by permethrin and deltamethrin. *Anopheles*

minimus was not repelled by deltamethrin, but was successfully repelled by permethrin. *Aedes albopictus* expressed the highest sensitivity, being consistently repelled by both permethrin and deltamethrin and an accompaniment of other test compounds. Interestingly, *Ae. aegypti* displayed low responsiveness across the nonpyrethroid compounds as well, which included DEET (N,N-Diethyl-meta-toluamide), picaridin, citronella, and several botanical oils. The other mosquito species tested were repelled by the nonpyrethroid compounds. *Culex quinquefasciatus* with low responsiveness, in contrast with studies such as in Msangi et al. (2010) with 92–98% response, could be an expression of resistance, as it responded to the nonpyrethroid compounds. *Aedes aegypti* could be an example of resistance or species-based insensitivities. Differential responses owing to either reason hamper the benefits of acute symptoms such as knockdown and mortality.

Findings by both Stanczyk et al. (2013) and Sugiharto et al. (2016) demonstrated that in repellency assays with DEET, insensitivities can develop in mosquitoes following repeated contact with a repellent chemical. This was found in mosquitoes as little as 3 h after initial exposure to DEET (Stanczyk et al. 2013). In this case, the insensitivities were attributed as a learned state, citing examples from Kelly and Thompson (2000) and Mwandawiro et al. (2000) where mosquitoes would respond differently to olfactory cues to maximize feeding success. If spatial repellents such as metofluthrin and d-allethrin have variable response in targets, this could be behaviorally mediated as well. Contemporary research by Wagman et al. (2015a) found similar insensitivities when testing transfluthrin on *Ae. aegypti*. In these assays, the behavioral plasticity of *Ae. aegypti* was deliberately measured following repeated exposures to transfluthrin volatiles, and it was found that mosquitoes exposed to the chemical in consecutive days were less likely to be repelled. Wagman et al. (2015a) also linked a predisposition for developing insensitivity to a heritable phenotype known as *kdr*.

Behaviorally overcoming a stimulus may account for some differences observed in the studies by Rapley et al. (2009) and Msangi et al. (2010); however, a key difference remains in the findings of insensitivity to DEET, a topical repellent, versus the insensitivities to transfluthrin, a spatial repellent. DEET is attributed as interfacing with mosquito targets through odorant-binding receptors (Stanczyk et al. 2013, Sugiharto et al. 2016). The disorientation effect noted by Kawada et al. (2006) and Rapley et al. (2009) demonstrates the different mode of action for spatial repellents. Ritchie and Devine (2013) tested metofluthrin against *Ae. aegypti* and found disorientation, acute paralysis, and significant mortality. The sum effect of these contributed to their recommendation that spatial repellents be used against vectors. Rapley et al. (2009) also noted that mosquitoes may have entered a treated room despite active chemical, but that knockdown afflicted over 80% of the tests subjects. Thus, although insensitivities may develop such as in Wagman et al. (2015a), the mosquito still runs high risks of neurological penalties such as disorientation, knockdown, and mortality (Buhagiar et al. 2017a). Therefore, understanding acute symptoms, even toxicity, now becomes essential for determining appropriate development of spatial repellent compounds.

Acute Symptoms and Toxicity

It should be revisited that the spatial repellents being discussed are volatile pyrethroids. They degrade quickly in the environment as well as present low mammalian toxicity in compounds we know to be potent against insects (Elliot et al. 1973, Miyamoto 1976).

These factors have contributed to the aforementioned widespread availability of the chemical class. However, their mode of action is fundamentally different from compounds like DEET, which act on odorant-binding receptors. Pyrethroids are sodium channel modulators, a neuro-toxicant which interferes with the uptake of sodium in the neuron and results in persistent depolarization of the cell membrane (Barnes and Verschoyle 1974, Miyamoto 1976). Meaning, compounds like metofluthrin, prallethrin, transfluthrin, and d-allethrin should have some expectation of being insecticidal. In fact, the depolarization of the neuron results in observed effects like hyperexcitation. The insect consequently develops ataxia, which renders it flightless and moribund. The visible effect of the insect being rendered flightless is termed knockdown or KD (Harrison 1951). It could be attributed that the behavioral avoidance of volatile pyrethroids is simply avoiding harmful stimulus. Many studies have elaborated on these other benefits of the volatile pyrethroids available to consumers.

Lee (2007) followed up their repellency assays with analysis to determine paralytic effects. Transfluthrin yielded up to 87% knockdown within 10 min of exposure to *Aedes togoi* (Theobald) and *Ae. albopictus*. Metofluthrin achieved up to 59% knockdown on the same species, within the 10-min period. Other findings examine products already available to consumers, rather than single ingredients. For example, ThermaCELL devices (MRGJ06-00, Schawbel Corporation, Bedford, MA) devices containing d-allethrin caused 80–100% knockdown within 30 min in *Aedes taeniorhynchus* (Wiedemann), *Aedes atlanticus* Dyar and Knab, *Psorophora columbiana* Dyar and Knab, and *Psorophora ferox* Humboldt (Bibbs et al. 2015). Follow-up studies using the OFF! Clip-on, which contains metofluthrin, also caused over 90% knockdown in *Ae. aegypti* (Bibbs et al. 2015). Supporting that volatile pyrethroids cause knockdown as an acute symptom is relevant to findings in previously discussed work. Returning to Rapley et al. (2009) and Ritchie and Devine (2013), *Ae. aegypti* was reported to enter a test room and, upon contact with metofluthrin volatiles, suffer from knockdown. This particular acute effect prevents not only the vector from contacting a host, but also prevents the vector's escape from the area. This leads the vector to experience sustained exposure to the volatiles. Rapley et al. (2009) quantified this effect and found that 98% of the *Ae. aegypti* that failed to escape the treatment area subsequently died from exposure. This allows the knockdown effect to synergize with the mortality outcomes, given that mosquitoes will accumulate greater doses of chemical the longer they fail to leave the area of volatilization. Here is where it is significant to evaluate tools on combined attributes, rather than one outcome. Previously, these compounds were evaluated based on repellency. For those vectors that are not repelled, the higher dose acquired by failing to avoid the area has a high probability of immobilizing the target. This stops the vector mid-flight, preventing bite contact. More importantly, the continuous inability of the vector to escape the area increases the likelihood of death, thereby eliminating any further pathogen transmission potential of that individual. Thus, the mortality in and of itself becomes an endpoint. How significant, then, is the mortality that can be expected from using these volatile pyrethroids?

The study by Bibbs et al. (2015) using d-allethrin removed the mosquitoes from the exposure area after the 30-min testing window. After removal from the chemical source, mosquitoes were held in the laboratory for 24 h postexposure, where 94–99% of all tested mosquito species were observed to have died. In a similar study by Bibbs and Xue (2015), metofluthrin-exposed mosquitoes were removed from chemical exposure at time points ranging from 5 min to 60 min. Here, nearly 30% mortality was observed in mosquitoes

removed after 15 min of exposure. Following 30- and 60-min exposures, mortality jumped to 97% and 100%, respectively. Prior work by Xue et al. (2012a) had determined dimefluthrin, meperfluthrin, and rich-d-transallethrin to be insecticidal when applied as a spatial repellent via mosquito coils. *Anopheles albimanus* Wiedemann and *Ae. albopictus* both expressed from 70% to 97% mortality across these different chemicals. *Culex quinquefasciatus* was hardier, as dimefluthrin and rich-d-transallethrin caused 60–70% mortality despite meperfluthrin causing 92% mortality. Prallethrin is commercially available in mosquito coil products. In an evaluation by Katsuda et al. (2009), prallethrin was evaluated against *Ae. aegypti* individually expressing one of the 11 genotypes, with some genotypes known to have reduced susceptibility to d-allethrin. All individuals in the 11 genotypes evaluated in a 30-min exposure window died following exposure. The time until 100% mortality ranged from 11 min to 120 min.

These studies collectively support a wide range of volatile pyrethroid compounds, generating significant mortality in vectors within a relatively short 30-min period. Although mosquitoes successfully repelled by these compounds will not necessarily perish owing to leaving the treatment cloud, individuals that succumb to knockdown will more reasonably be exposed to the lethal 30-min window. However, Katsuda et al. (2009) approaches an important consideration with their work on multiple genotypes of the same species.

Not all mosquitoes will be repelled or knocked down by these vapor-active chemicals. Katsuda et al. (2009) deliberately sampled a large area and acquired *Ae. aegypti* strains with lower susceptibility to d-allethrin, a spatial repellent with considerable repellency, knockdown, and mortality data reported (Msangi et al. 2010, Revay et al. 2013, Dame et al. 2014, Bibbs et al. 2015). The mechanism for this resistance has been documented by Wagman et al. (2015a). Resistant phenotypes develop when selective pressures are high; as pointed out with transfluthrin, a set of alleles dubbed *kdr* has been linked with a target's ability to resist or tolerate the acute symptoms of pyrethroid exposure (Harrison 1951), and even repellency (Wagman et al. 2015a).

As discussed earlier, there are multiple spatial repellent products available on the market for consumers. If increased usage results from either successful marketing or because consumers prefer them to topical repellents, then resistance development is a realistic consequence to consider. Resistance development is compounded by preexisting differences in sensitivities across species. Msangi et al. (2010) demonstrated a 30–40% lower response in *An. gambiae* in both repellency and reduction of blood feeding as compared with *Cx. quinquefasciatus* when exposed to d-allethrin. Similarly, Xue et al. (2012a) reported *Cx. quinquefasciatus* exhibited 20–30% less mortality than did *An. albimanus* and *Ae. albopictus* when exposed to dimefluthrin or rich-d-transallethrin.

Sublethal Effects

Sublethal effects are an additional layer of action against target vectors, and for the purposes of this review will include different effects of exposure to that do not result in death. For example, if mosquitoes avoid acute effects, such as through successful avoidance of the active ingredient or through mechanisms like knockdown resistance or variations in sensitivity, these will be weighed alongside effects that are harmful, such as neurological impacts or behavioral outcomes that ultimately harm the mosquito. In understanding those sublethal effects, difficulties posed by variable sensitivity or resistance can be overcome. One can examine the patterns observed

following the use of DDT, one of the flagship examples of enhanced selection pressure resulting in increased insect resistance expression (Harrison 1951). It has been argued that despite the extreme selection pressure on insects with the advent of insecticides, there are anomalous examples where resistance did not develop (Chareonviriyaphap 2012). For example, Trapido (1954) reported that *An. albimanus* in Panama had been controlled using DDT for several years, and yet were equivalently as susceptible as a colony with no prior DDT exposure. In teasing apart the cause, it was concluded that when mosquitoes were allowed adequate time between DDT exposure, the population remained susceptible (Trapido 1954, Chareonviriyaphap 2012). In another example, *Anopheles darlingi* Root was shown to have not developed insecticide resistance in Brazil when surveyed in 1984 despite an active multiyear malaria control program that relied on DDT applications (Roberts et al. 1984). Another study reported a lack of DDT resistance in western Thailand's *An. minimus* population even when routine indoor residual spray (IRS) application of DDT generated resistance in other regions of Thailand (Chareonviriyaphap et al. 1999, 2001). Behavioral avoidance of direct contact with DDT has been considered causative of this phenomenon (Chareonviriyaphap 2012). This is somewhat analogous to Wagman et al. (2015a) where an alternating test sequence of 24-h and 48-h rest periods for *Ae. aegypti* preexposed to transfluthrin supported that *Ae. aegypti* resumed typical behaviors and susceptibilities when allowed 48 h without exposure. Thanispong et al. (2009) utilized two DDT-resistant field strains of *Ae. aegypti* to determine that physiological resistance negatively correlated with sensitivity to either contact irritancy or noncontact repellency. Yet, the *Ae. aegypti* population retained a sublethal behavior to avoid contact with the insecticide. This echoes the patterns from Brazil and Thailand in that behavioral modification is a separate event from target site or metabolic resistance. This same sublethal avoidance behavior would correspondingly relax the selection pressure on resistance and allow susceptible phenotypes to reemerge (Roberts et al. 2000).

As with the above examples involving DDT, just because a physiological mechanism of repellency fails does not mean the behavioral aspect is unimportant. Outside of resistant phenotypes, though, a confounding factor in repellency has already been observed: disorientation. This disorientation is another form of sublethal effect. Recall that Wagman et al. (2015a) determined that a group of *Ae. aegypti* displayed insensitivity to the repellent action of transfluthrin. This also was linked to the *kdr* phenotype that imparts resistance to mortality (Harrison 1951, Wagman et al. 2015a). However, their method of evaluation used a high-throughput screening system that did not involve host contact as a way of measuring overall bite prevention. It is not conclusive in this study that the lack of repellent sensitivity necessarily translates to a failure of the compound.

Compare this to Rapley et al. (2009) which introduced a volunteer host into the experimental design. Rapley et al. (2009) reported that mosquitoes were as likely to enter treated rooms as exit them while a host was present. This was attributed to disorientation with support for this interpretation being the lack of affected mosquitoes attempting to find the host in either treated or untreated rooms. Instead, they would retreat to harborage. If that harborage was in a treated area, knockdown and mortality would occur as a result of lengthy exposure times. If they left the treated area entirely, Rapley et al. (2009) postulated the escaped mosquitoes abandoned the treated area despite the presence of a host. Ritchie and Devine (2013) also noted this when conducting their own treated room studies. They added that the disorientation appeared to reduce flight

speed, and again prioritized seeking harborage over host contact. Their reports included data that upon introduction of metofluthrin into the room, successful contacts with a host, even a brief landing, were negligible within the 10-min evaluation time. Those mosquitoes that found harborage outside the treatment area were noted to readily escape into traps mounted on the building windows. They terminated treatment by forcefully evacuating the metofluthrin vapors for a 2-h period after which it was noted that mosquitoes resumed landing on hosts. This suggests that this disorientation was a physiological state and not a learned state.

Interrupting blood-feeding behavior and ultimately success is another sublethal effect of chemical exposure that could factor into bite prevention. Hao et al. (2008) made the point that some compounds can alter the blood-feeding behavior of a vector. Their work with *Ae. albopictus* indicated some vapors from botanical compounds would impair the mosquitoes' ability to find a host. It also was found that mosquitoes would have increased time until probing the host, attributed to delays in orientation and activation (Hao et al. 2008). Sugiharto et al. (2016) exposed *Ae. aegypti* to DEET and observed reduced blood engorgement for the following 24 h. Pyrethroids have been shown to depress blood engorgement. Liu et al. (1986) reported that when d-phenothrin, d-allethrin, and tetramethrin were applied as spray mist droplets, *Ae. aegypti* had 50–60% reduction in bloodmeal uptake. Liu and Georghiou (1987) found trans-permethrin significantly reduced blood engorgement with topical applications to *Cx. quinquefasciatus*. Adanan et al. (2005) used vaporizer mats to administer d-allethrin and prallethrin to mosquitoes. This study reported both chemicals depressed blood engorgement of *Cx. quinquefasciatus* by 30% and *Ae. aegypti* by 70%. Ogoma et al. (2014a) evaluated volatile pyrethroids for deterrence, knockdown, mortality, and other sublethal effects. They concluded that blood-feeding inhibition was the greatest observed effect, with *An. arabiensis* and *An. gambiae* suffering from 98% depression of engorgement by transfluthrin and 93% depression by metofluthrin exposure. Ultimately, reduced contact time upon attempted blood feeding may reduce blood-borne parasite transmission.

Delaying the onset of a blood-feeding response can enhance other beneficial effects, particularly mortality, that will lower pathogen transmission risk. Ogoma et al. (2014b) reported that transfluthrin did not prevent *An. gambiae* from being attracted to the host, but >75% of mosquitoes that passed through the transfluthrin treatment had reduced blood-feeding attempts for at least 12 h. In their design, mosquitoes were removed from the testing area and offered bloodmeals in a lab setting. In more realistic applications, the delay they demonstrated provides more than enough time for sublethal effects to manifest or for the host to escape the area.

Interrupting blood feeding does translate to another benefit: reduction of oviposition success. Ogoma et al. (2014a) continued monitoring *An. gambiae* after depression of blood feeding and allowed successfully blood-fed, treated mosquitoes the opportunity to oviposit. They documented a 97% reduction in egg production related to transfluthrin and a 91% decrease related to metofluthrin exposure, as compared with unexposed mosquito treatments. This has been contested in an indoor study in Australia in which a stationary emanatory containing metofluthrin did not reduce reproductive fitness of either male or female *Ae. aegypti* (Buhagiar et al. 2017b). This too could stem from differential sensitivities among species.

Earlier work used a more direct approach to look at oviposition deterrence. A wide array of experimental and commercial skin repellent compounds have been shown to deter *Ae. albopictus* from

ovipositing in containers fitted with a repellent-treated cloth (Bar-Zeev and Ben-Tamar 1968) or repellent-contaminated water (Xue et al. 2001, 2003, 2006). Furthermore, Xue et al. (2004) found that gravid mosquitoes denied the opportunity to oviposit through repellent-treated water had reduced fecundity and increased hatching mortality. Choi et al. (2016) exposed gravid *Ae. aegypti* to transfluthrin volatiles to assess changes in attraction to experimental oviposition sites. They found that bacteria-baited oviposition cups were twice as attractive to treated cohorts. They hypothesized that transfluthrin exposure increased grooming. This may have correspondingly changed the olfactory acuity of mosquitoes. This suggests that spatial repellents may interact with olfactory sensors and alter a mosquitoes' ability to perform appropriately, if not necessarily through a mechanism of repellency as with DEET binding to odorant receptors.

Olfactory outcomes are still poorly studied for a wide range of chemicals and taxa, but it is recognized as a sublethal outcome of exposures to carbamates and organophosphates in agricultural pests (Dewar et al. 2016). For mosquitoes, prior investigations by Cohnstaedt and Allan (2011) showed that permethrin and deltamethrin both impaired host-seeking ability in *Cx. quinquefasciatus*, *An. albimanus*, and *Ae. aegypti*. They noted erratic flight patterns, decreased flight speeds, increased changing of direction, increased intensity of turns during flight, and slower initiation and termination of flights during exposure to host volatiles. This again supports the idea of disorientation in mosquitoes exposed to pyrethroid volatiles. In this case, the results were considered indicative of a compromise in the olfactory acuity of test subjects. Choi et al. (2016) suggested that an increased olfactory acuity may have resulted from excessive grooming to compensate for presence of chemical. The critical difference in Cohnstaedt and Allan (2011) was that their recording environment was restrictive, and may have resulted in differences in behavior that otherwise would have been observable in more open flight areas.

Unfortunately, interrupting or depressing a bloodmeal may not always result in a change in vectorial capacity. In the case of DEET, the observed reduction in blood engorgement was not accompanied by reduced landing and probing of hosts (Sugiharto et al. 2016). For certain pathogen transmission cycles, this may be a detrimental combination of qualities, as there is evidence that mosquitoes that take suboptimal bloodmeals are both still responsive to host cues (Klowden and Lea 1978) and more likely to engage in multiple bite feeding patterns (Edman et al. 1975), both of which are likely to inflate vectorial capacity. It has been suggested that volatile pyrethroids, like metofluthrin, reduce landing rates through disorientation, but it is not clear at this time if that is a sufficient counter-balance to the vector's refeeding possibility.

Evaluation Methods

The effectiveness of volatile pyrethroids has been assessed through a few common designs. These variations are critical in the history of their development into spatial repellents. The earliest methods replicate human structures, often dubbed a hut design. The principle involves having a point of attraction, such as a host or harborages, in an area of defined boundaries in which mosquitoes are allowed flight. The environment is freely navigable by the mosquitoes, but involves high rates of exposure to a treatment present inside the boundaries. These could take place within literal structures (Pates et al. 2002, Lee 2007, Katsuda et al. 2009, Rapley et al. 2009, Achee et al. 2012, Ritchie and Devine 2013, Ogoma et al. 2014a,

Wagman et al. 2015a) in which the space is partitioned into multiple rooms. Variations are seen where defined borders still exist, but are not a dwelling or analogue of living space (Cohnstaedt and Allan 2011; Ogoma et al. 2012, 2014b), with examples being tunnels or screened enclosures. All variations are consistent in that a known number of mosquitoes are within the defined boundaries alongside a measure of the success of insects in reaching the point of attraction, often a host. Behavioral effects are not severely limited, so factors such as avoidance of the treatment may be observed. This makes an informative design for objectives concerned with spatial components, like repellency, but poor where spatial components interrupt data collection, such as toxicity.

An alternative evaluation method is high-throughput screening. This is essentially a containerized testing environment in which the mosquito passes through or is contained within a device to expose the target to specific conditions. This began with containers composed of simple materials, such as paper, cloth, metal, and glass, where the insect is contained for maximum exposure (Roberts et al. 1984, Liu et al. 1986, Adanan et al. 2005, Abdel-Mohdy et al. 2008, Stanczyk et al. 2013, Sathantriphop et al. 2014). Contemporary work has birthed a true high-throughput design with a rather specific construction, and allows optimal control of exposure time, dosage, and the allowance of contact or noncontact variants (Achee et al. 2009, Thanispong et al. 2009, Wagman et al. 2015b). Regardless of type, these are defined by the insect's inability to avoid treatment, and often involves close observation of the signs of exposure, making them more informative for sublethal effects, but less informative when requiring environments with competing stimuli. The majority of the discussed effects from repellency, acute symptoms, and sublethal effects have been generated by hut studies and high-throughput studies. It can be gathered from their designs that it is difficult to account for competing stimuli and resilience to confounding spatial components.

Semifield and field methods are employed as a response to the shortfalls of the prior two methods. These types of test allow competing stimuli and multiple stressors, such as environmental constraints, to factor into treatment outcomes. Semifield methods are more controlled than field methods, and still allow for a known test group, approximated dosage, and are not impaired by the spatial components of testing (Bibbs et al. 2015; Bibbs and Xue 2015; Obermayr et al. 2015; Buhagiar et al. 2017a,b). Field studies are the most realistic method, but the most difficult to define. Studies often take place where a treatment is employed against known pressures, such as bite contact, and it is then assessed for its ability to impede or remediate those pressures (Xue et al. 2012a,b; Revay et al. 2013; Dame et al. 2014). Treatments in these types of studies cannot be measured directly, as there is an uncontrolled input of targets. Instead, success is measured through proxies such as trap surveillance or recording successful contacts with a volunteer. Field designs are not limited in scope. They can incorporate multitreatment evaluations and strategic implementation in informative ways.

The developmental strides made by the hut and high-throughput studies have allowed semifield and field studies to show that spatial repellents can act as barriers, by deterrence and knockdown, to mosquitoes entering structures (Achee et al. 2012, Syafruddin et al. 2014, Menger 2015, Wagman et al. 2015b). When used in tandem with traps or attractants, they can create push-pull systems by repelling mosquitoes into the traps or toward attractants, creating localized areas of mosquito absence (Revay et al. 2013, Syafruddin et al. 2014, Obermayr et al. 2015, Wagman et al. 2015b). When combined with barriers or insecticides, such as with netting or residual treatments, push-kill synergy is observed (Yuan and Huang 2014,

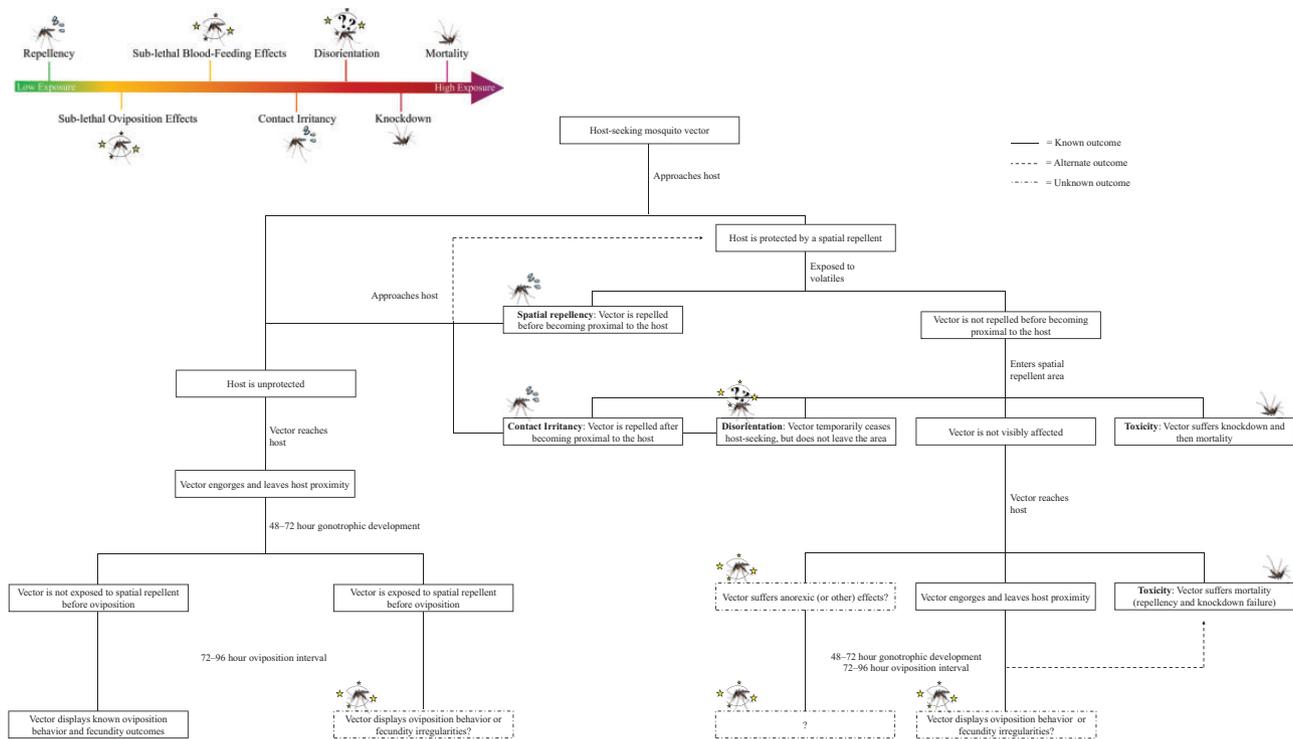


Fig. 1. Hypothetical exposure outcomes of mosquitoes versus volatile pyrethroids (spatial repellents).

Huho et al. 2015, Paliga et al. 2015). These findings demonstrate that the known effects of spatial repellents can be put to use in integrated management strategies. New strategies, or enhancements to existing strategies, could be developed around other known benefits. For example, compounds extracted from various plants have deterred *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* from laying eggs at otherwise suitable oviposition sites (Swathi et al. 2010, Yu et al. 2015). Spatial repellents have been hinted at impairing mosquito oviposition (Ogoma et al. 2014a, Choi et al. 2016). If this were reinforced with further findings, a new strategy could develop where volatile pyrethroids are used to limit access to containerized breeding sites normally occupied by *Ae. aegypti* or *Ae. albopictus*. What strategies could be created if other effects were better understood?

Conclusions

The large body of work through decades of vector management research reflects that the experimental designs across these studies, as well as which chemicals, the form of the products, the vectors chosen, and even the genotype of the targets are all variable. This makes findings difficult to compare. The differences between the Rapley et al. (2009) and Wagman et al. (2015a) studies, as discussed in the sublethal effects section above, serve to illustrate this point. These differences are further reinforced in the discussion of examples including Cohnstaedt and Allan (2011) and Choi et al. (2016) with their opposed findings regarding olfaction impacts. Without the ability to compare, it remains controversial how best to move forward. Regardless, spatial repellents have been repeatedly advocated to combat disease vectors (Xue et al. 2012a,b; Ritchie and Devine 2013; Dame et al. 2014; Bibbs et al. 2015; Bibbs and Xue

2015, 2016). This is in part because of abundant documentation supporting both simple mixtures and formulated products causing knockdown and mortality in mosquitoes. However, the efficacies presented do not qualify as a measure of lethal dose or lethal concentration. There is no concerted effort to deliberately evaluate these compounds for their toxicity because of the existing paradigm that volatile pyrethroids are repellents. Given the evidence at present, it may be better to step away from the idea of a spatial repellent and instead embrace that these are vapor-active insecticides. Aside from toxicity, many sources bring to light a suite of partially explored behavioral and sublethal effects, or those occurring at the LD_{10-25}/LC_{10-25} or lower part of the dose response. It is easy to imagine this string of potential outcomes along the dose response as a kind of spectrum that corresponds to different encounters between the mosquito and the toxicant. This concept is summarized in Fig. 1, and is a hypothetical rendering of how the attributes discussed in this review may pertain to a flowchart of potential outcomes when a mosquito encounters a spatial repellent volatile pyrethroid. It is not clear at this time whether different volatile pyrethroids can be expected to generate the same effects. Regardless, these and similar attributes represent a potential that ultimately could change how these products are developed and deployed.

In summary, volatile pyrethroids, or spatial repellents, have multiple effects on mosquito vectors, but are currently used one-dimensionally for spatial repellency. These tools occupy a unique niche of being spatial, yet both insecticidal and safe enough to use on your person. There lies in this a great potential for incorporating volatile pyrethroids into vector abatement efforts, even if only by encouraging their use by the at-risk populace. The current knowledge base does not address the broader epidemiological impacts of using spatial repellents. Furthermore, it is unclear at this time how exactly volatile pyrethroids can be used to reduce genetic resistance

to insecticides. Because of this, volatile pyrethroids need to have their multiple effects identified, defined, and described to maximize their utility for preventing pathogen transmission. We must close the knowledge gap surrounding the various outcomes when vectors are exposed to spatial repellents (Fig. 1). With this information available, we will enhance our ability to recommend spatial repellents and allow the creation of a suitable vector management paradigm that then enables us to integrate spatial repellents into vector management.

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