RESISTANCE AND RESISTANCE MANAGEMENT OF BIORATIONAL LARVICIDES FOR MOSQUITO CONTROL

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ABSTRACT

Mosquitoes and mosquito-borne diseases remain a significant threat to public health and the well-being of humans and animals. Often mosquito control is the only feasible way to combat mosquito-borne diseases. Biorational mosquito larvicides based on microbials and insect growth regulators (IGR) have been playing an irreplaceable role in integrated mosquito control worldwide. While the relative target specificity, non-target safety and environmentally friendly profile are well recognized in biorational larvicides, their risk of resistance and cross resistance must be taken into consideration in mosquito control operations. This paper provides a review of the resistance risk, historical and current status, and management tactics for the commonly used mosquito larvicides such as *Bacillus thuringiensis* subsp. *israelensis (Bti)*, *Bacillus sphaericus*, spinosad, methoprene, pyriproxyfen, and diflubenzuron. *Bti* poses the lowest risk of resistance and plays a unique role in resistance management. Various levels of resistance to *B. sphaericus* have been reported in both laboratory and field populations during the past decades worldwide. High level of resistance to spinosad has been documented recently in laboratory populations of *Culex quinquefasciatus*, followed by preliminary report from field populations of *Cx. pipiens*. As to resistance to IGRs, documentations on laboratory and/or field populations have become available since the early 1970s for methoprene and the 1990s for pyriproxyfen. The most recent report on resistance to diflubenzuron reconfirmed the earlier studies. The tactics to prevent resistance and restore the susceptibility in mosquitoes to these biorational larvicides have been developed and implemented in some cases, which is crucial to sustainable integrated mosquito management.

Key Words: Microbial larvicides; Insect growth regulators; Mosquito control; Resistance; Resistance management

INTRODUCTION

Mosquitoes and mosquito-borne diseases pose a significant public health threat and economic burdens worldwide, particularly to the countries in tropical and subtropical regions. Upon globalization, demographic growth, and subsequently environmental impact, public health concerns created by mosquitoes have been on the rise despite diligent efforts of integrated mosquito control programs. Often, mosquito control is the only effective and feasible way to combat mosquito-borne diseases, where larviciding to target aquatic immature stages is often the primary intervention. However, the availability of effective, environmentally friendly, and non-target safe and affordable larvicides is very limited today. Thís situation has been worsened by strict regulations, high cost in development and registration, narrow market niche of products, emergence, or resurgence of new vector species and associated diseases and lastly, development of resistance. To achieve sustainability in mosquito control, resistance management with the limited available control tools must be integrated by mosquito control operations. The current paper is considerably concentrated and updated from the previously published book chapters to facilitate the need of field mosquito control professionals The audience who are interested in details of this topic can refer to Su (2016a,b).

BACILLUS THURINGIENSIS SUBSP. *ISRAELENSIS (BTI)*

The entomopathogenic *Bacillus* was identified in 1901 from silkworm that suffered the sotto disease and was named *Bacillus sotto*. However, the finding of this *bacillus* in 1911 from Mediterranean flour moth *Anagasta kuehniella* caterpillars lead to the official name of *Bacillus thuringiensis* (Roh et al. 2007). To date, at least 70 serotypes, with more than 80 subspecies have been identified, among which 14 serotypes and 16 subspecies show lethal activities against mosquito larvae. *Bacillus thuringiensis* subsp. *israelensis* (*Bti*), serotype H-14, was discovered in Israel in 1976 (Goldberg and Margalit 1977, Margalit and Dean 1985). Four endotoxins including cytolytic toxin Cyt 1A and crystal toxins of Cry4A, Cry4B, Cry 11A are produced during sporulation (Tabashnik 1992, Wirth et al. 2004), which are activated by enzymatic proteolysis at a high pH environment in the mosquito midgut. *Bti* is categorized as a Group 11 pesticide, i.e., microbial disruptor of insect midgut membranes by Insect Resistance Action Committee (IRAC) (Su 2016a). *Bti* is registered as biopesticide by the

US Environmental Protection Agency (US EPA) in 1982 (Wang et al. 2018a).

Numerous studies have been attempted and published about induction of larval resistance to *Bti* in *Culex pipiens* complex or *Aedes aegypti* since 1983. Response to sublethal exposure for numerous generations, tolerance or very low, unstable resistance was developed (Vasquez-Gomez 1983, Goldman et al. 1986, Saleh et al. 2003, Mittal 2005, Su 2016a). However, the cryptic *Bti* resistance in field *Aedes* populations was detected to crystal toxins in response to previous exposures to whole *Bti* when tolerance or low-level resistance has developed (Tetreau et al. 2012, 2013). In field populations, the risk of resistance development to wild type *Bti*, i.e., the intact toxin complex, is very low. The extensive use of *Bti* products to control floodwater mosquitoes *Ae. vexans* over an area of approximately 500 km^2 for more than 36 years in the Rhine River area in Germany has been systematically documented, no noticeable reduction in susceptibility was detected (Becker et al. 2018). Low levels of resistance were noticed in *Cx. pipiens* complex populations in different geographical locations where *Bti* products were used for different periods of time (Wirth et al. 2001, Vasquez et al. 2009), but these levels of resistance did not cause much concern. One field study however, reported that collections from Syracuse and Albany, New York showed 33-41- and 6-14-fold resistance, respectively, the test material was laboratory cultured strain ISP-80 (Paul et al. 2005). It is worthwhile to follow the resistance status in these populations. Exposures to individual crystal toxins of *Bti* are conducive to resistance and cross resistance development among the toxins, in the absence of Cyt1A toxin, highlighting the importance of the full combination of toxins found in wild *Bti* in resistance management (Georghiou et al. 1997, Wirth et al. 1997). Cyt1A from *Bti* does not possess significant larvicidal activity alone, but plays a critical role in overcoming, preventing, and delaying resistance development to Cry toxins, partially since Cyt1A functions as a receptor to enhance the binding of the crystal toxins (Chueng et al. 1987, Pérez et al. 2007).

BACILLUS SPHAERICUS

To date, over 300 strains of *B. sphaericus* belonging to 49 serotypes have been identified, among which 16 strains, 9 serotypes showed various levels of activity against mosquito larvae. The following strains possess high mosquitocidal activity - 2362, 1597, 2297, C3-41 and IAB-59, among which the strain 2362 was isolated from adult blackfly *Simulium damnosum* in Nigeria in 1984 and was extensively studied and developed. Active strains produce parasporal inclusions during sporulation, which contains crystal binary toxins. Some strains also synthesize non-crystal mosquitocidal toxins (Mtx) during the vegetative growth phase. The mode of action of the binary toxins is somewhat similar to *Bti* toxins. The receptor of the binary toxins is a 60 kDa α-glucosidase, which is anchored in the mosquito midgut membrane via a glycosyl-phosphatidylinositol (GPI) anchor. While belonging to the same IRAC group as *Bti*, *B. sphaericus* has a narrower species spectrum. Some *Aedes* spp., for example *Ae. aegypti* and *Ae. Albopictus*, are much less susceptible than *Culex* spp. to this microbial agent (Su 2016b). *Bacillus sphaericus* strain 2362 was registered as biopesticide by the US EPA in 2000 (Wang et al. 2018a).

Various levels of resistance to *B. sphaericus*, mostly strain 2362, in laboratory colonies of *Cx. pipiens* complex, has been reported in different countries since 1994 as a result to sublethal exposure for different periods of time (Rodcharoen and Mulla 1994, Wirth et al. 2000, Pei et al. 2002, Amorim et al. 2007, Zahiri et al. 2002, Zahiri and Mulla 2003). It appeared that the resistance evolution to *B. sphaericus* in response to laboratory selection depends on genetic background, selection procedures, and other unknown factors. Resistance level is also dependent on the susceptibility of the reference population tested. The resistance to *B. sphaericus* is stable in absence of selection pressure (Amorim et al. 2010). As to the cross resistance among different strains, once mosquitoes develop resistance to a given strain of *B. sphaericus*, they are also often resistant to other strains because of the similarity of the binary toxins in most strains. Fortunately, mosquitoes that have developed resistance to various strains of *B. sphaericus* remain susceptible to *Bti* (Wirth 2010, Su 2016a). The cross resistance among different strains is mild between the strains that also produce the Mtx (Yuan et al. 2003). The Mtx from some *B. sphaericus* strains not only enhance the larvicidal activity of *Bti* Cry toxins, but also mitigate resistance development to Cry toxins (Wirth et al. 2014). These results indicated the potential role of Mtx in resistance management to *Bti* and *B. sphaericus*.

The earliest resistance to *B. sphaericus* in field populations was reported in *Cx. pipiens* in southern France where the resistance ratio at LC_{50} was 70-fold because of extensive field applications (Sinègre et al. 1994). Numerous reports on resistance have been published since then in the *Cx. pipiens* complex from different countries (Su 2016a). The highest level of resistance was documented in a *Cx. quiquefasciatus* population in Thailand, where *B. sphaericus* was used for only 4 months with 5 treatments (Mulla et al. 2003). The resistance levels at LC_{50}, depending on reference colonies, were 21,100-28,100-fold against commercial product or > 125,000-200,000-fold against technical-grade material (Su and Mulla 2004). Two cases

on high levels of resistance to *B. sphaericus* in the USA, where *B. sphaericus* products-based strain 2362 have been applied for various time, were reported in wild populations of *Cx. pipiens* in California and Utah (Su et al. 2018, 2019). In the *B. sphaericus*-resistant population from California, various levels of resistance or tolerance were also noticed to abamectin, pyriproxyfen, permethrin and indoxacarb. However, it would not be feasible to determine they are cross- or independent multiple resistance due to unknown field exposures (Su et al. 2018). The resistance evolution in response to field application of *B. sphaericus* products varies greatly, depending on exposure to naturally existing strains, population genetic background, gene exchange with untreated populations, as well as product application strategies. As to the mechanism of resistance to *B. sphaericus*, it is mostly believed that recessive genes are involved. Although various theories have been proposed, lack of specific binding of binary toxins to α-glucosidase receptors in the midgut appeared the main reason, which is due to the partial deletions of the gene that encodes the receptor (Su 2016a).

Beside conventional practice for resistance management, *Bti* can be used as a powerful tool to mitigate resistance to *B. sphaericus*. Before it occurs, resistance to *B. sphaericus* can be delayed or prevented by the mixture of *Bti* and *B. sphaericus* because of the synergistic action among total 6 toxins (Cyt 1A, Cry4A, Cry4B, Cry 11A from *Bti* and binary toxins from *B. sphaericus*), particularly the presence of Cyt1A (Wirth 2010). While rotation of two pesticides with different modes of action can be commonly used for resistance prevention, the rotation of *B. sphaericus* and *Bti* surprisingly resulted in much higher levels and faster emergence of resistance as compared with *B. sphaericus* alone for the unknown reasons. However, selection with mixtures of *Bti* and *B. sphaericus* almost negated emergence of resistance to *B. sphaericus* (Zahiri and Mulla 2003). Recently, the recombinant that produces toxins from both *Bti* and *B. sphaericus* provides another path for not only mitigation of resistance also enhancement of lavicidal activity and efficacy. Combination of *B. sphaericus* with botanical pesticides such as azadirachtin also provided a potential to mitigate resistance development to *B. sphaericus* (Poopathi et al. 2002). The susceptibility to *B. sphaericus* in a resistant colony was partially restored by *Bti*, and rotation or mixture of *Bti* and *B. sphaericus* (Zahiri et al. 2002). In field operations, highly *B. sphaericus*-resistant mosquitoes can be effectively controlled by *Bti* alone or through a combination of *Bti* and *B. sphaericus*. At the same time, the lost susceptibility to *B. sphaericus* can be restored upon time by new interventions applied (Yuan et al. 2000, Mulla et al. 2003, Su et al. 2018, 2019b). The *B. sphaericus* resistant mosquitoes might carry some fitness disadvantages, but there seemed not to be any difficulties in sustaining the population integrity (Rodcharoen and Mulla 1997, Amorim et al. 2010).

SPINOSYNS

Spinosad, consisting of spinosyn A ($C_{41}H_{65}NO_{10}$) and D ($C_{42}H_{67}NO_{10}$) in the ratio of 85% and 15% respectively, is produced by a naturally occurring, soil-dwelling actinomycete, *Saccharopolyspora spinosa*, which acts as a nicotinic acetylcholine receptor (nAChR) allosteric modulator. Spinosad, along with spinetoram that consists of spinosyn J and L, is categorized as a Group 5 insecticide by IRAC, and registered as an organophosphate alternative/reduced risk pesticide by the US EPA in 1997 (Wang et al. 2018b).

Spinosyns exert pesticidal activity after ingestion and cuticle absorption against a broad spectrum of susceptible insect species by stimulating nACh and γ-aminobutyric acid (GABA) receptors and causing rapid excitation of the insect nervous system. As a relatively new product for mosquito control, studies to evaluate resistance development risk and resistance management strategies for spinosyns are rather rare. The first attempt was made for *Cx. quinquefasciatus* where a selection pressure was applied at $LC_{70\text{-}90}$ levels to late 3rd and early 4th instar larvae in each generation in a laboratory colony. Resistance increased gradually to 1,415.3- to 2,229.9-fold at LC_{50} and 9,613.1- to 17,062.6-fold at LC_{90} at after selection for 45 generations. The exponential elevation of resistance levels throughout selection indicated that a recessive mechanism might have been involved during resistance development to spinosad (Su and Cheng 2012, 2014a). This "recessive mechanism" was indicated later by a two-way cross test between males and females of the resistant and susceptible populations, where high levels of resistance disappeared at F_1 (Su et al. unpublished). Regardless of the high-level resistance, the bio-fitness cost seemed very minimum as the colony has propagated well under standard maintenance protocols. The resistance to spinosad tended to decline in the absence of selection pressure and more so if with simultaneous infusion of susceptible individuals. The resistance declined faster when existing resistance was at the lower levels than at the higher levels (Su et al. unpublished).

There was a lack of cross resistance to the following pesticides in this highly spinosad-resistant *Cx. quinquefasciatus*: *B.t.i.*, a combination of *B.t.i.* and *B. sphaericus*, methoprene, pyriproxyfen, diflubenzuron, novaluron, temephos or imidacloprid. However, it did show various levels of cross resistance to *B. sphaericus*, spinetoram, abamectin and fipronil. On the other hand, a long-term laboratory colony of *Cx. quinquefasciatus* that

is highly resistant to *B. sphaericus* (Wirth et al. 2000), was as susceptible as a laboratory reference colony to spinosad and spinetoram, indicating a one-way cross resistance from spinosad to *B. sphaericus*. Field-collected and laboratory-selected *Cx. quinquefasciatus* that were resistant to methoprene, did not show cross resistance to spinosad and spinetoram (Su and Cheng 2014b). Currently, there is a lack of research on resistance management strategies pertinent to spinosad. Preliminary studies indicated that *Bti* plays a unique role in spinosad resistance management. Treatment by *Bti* for 15 generations almost completely restored the susceptibility to spinosad in a highly spinosad-resistant laboratory population (Su et al. unpublished).

As to the field monitoring on resistance in mosquitoes to spinosad, data is quite meager. Recent report has indicated the occurrence of spinoad resistance in field populations of *Cx. pipiens* in urban northern California (Wheeler et al. 2022). Further monitoring is hence highly recommended.

INSECT GROWTH REGULATORS

Methoprene, a true juvenile hormone analog, interrupts juvenile hormone balance during the transition from late 4th instar larvae to pupae and adults. Most mortality occurs at pupal stage or incompletely emerged adults. This synthetic compound was categorized as Group 7A by IRAC and registered as biopesticide by the US EPA in 1975 (Wang et al. 2018a). The earliest laboratory studies on resistance development in mosquitoes dates back to early 1970s, when the collective results indicated low risk of resistance development (Su 2016a). One recent study showed that the resistance level was significantly elevated by continuously exposing field collected *Cx. quinquefasciatus* that had low level of existing resistance to methoprene for 30 generations. At this time, various levels of cross resistance to other commonly used pesticides were revealed in the selected population. Cross resistance to *B. sphaericus* was the most profound, amounting to 77.50- to 220.50-fold. This cross resistance seemed only one-way from methoprene to *B. sphaericus*, as *B. sphaericus*-resistant mosquitoes remained susceptible to methoprene (Su et al 2018, 2019b).

As to resistance development in wild populations of mosquitoes, data are quite limited mostly due to lack of monitoring. The first report in this regard was published in 1998, when an *Ae. taeniorhynchus* population in Florida showed 15-fold resistance after applications of a methoprence product during 1989 to 1994 (Dame et al. 1998). Methoprene tolerance in *Ae. nigromaculis* was discovered in central California after 20 years of treatment by methoprene products, followed by a control failure during 1998-1999 (Cornel et al. 2000, 2002). The documented resistance seemed not related to the metabolic detoxification by P450 monooxygenase and carboxylesterase, and treatments using *Bti* partially and gradually restored the susceptibility to methoprene (Cornel et al. 2002). Other reports on field populations showed varying and moderate levels of resistance, such as 4.7-16-fold in *Cx. pipiens* in Cypress (Vasquez et al. 2009), 9-54-fold in *Cx. quinquefasciatus* in southern California (Su and Cheng 2014, Su et al. 2021), and elevated resistance levels in *Cx. pipiens* in northern California (Wheeler et al. 2022).

The juvenile hormone analog mimic pyriproxyfen was synthesized in the early 1970s, the IRG activity of which is much higher than methoprene (Su and Cheng 2014, Su et al. 2018, 2019a, b). Pyriproxyfen has the identical activity to juvenile hormone III (JH III) in mosquitoes as does methroprene, but is not structurally related to JH III, which is the opposite of methoprene. This compound was categorized as Group 7C by IRAC and registered as organophosphate alternative/reduced risk pesticide by the US EPA in 1998 (Wang et al. 2018b). Limited data showed very low risk of resistance in mosquitoes (Schaefer et al. 1991) until one report was published (Su et al. 2019a) that showed a noticeable level of resistance in a field population of *Ae. aegypti* in southern California. It is unlikely that this field-occurred resistance is caused by public health applications, as there was no record of such application up to collections of samples for testing. This pyriproxyfen-resistant *Ae. aegypti* did concurrently show low level resistance to methoprene which possesses the similar mode of action (Su et al. 2019a). Assuming that this low-level methoprene resistance is caused by exposure to pyriproxyfen, there might be a two-way low level cross resistance between methoprene and pyriproxyfen, when connecting this finding with the cross-resistance profile in methoprene-resistant *Cx. quinquefasciatus* populations (Su et al. 2021).

Chitin synthesis inhibitors such as diflubenzuron have a very limited use in the USA. This compound is a non-selective chitin synthesis inhibitor which interrupts formation of the exoskeleton, interferes with integrity of cuticle, and leads leakage of body fluid and ultimately mortality of a wide variety of target organisms. It acts on the entire life cycle, particularly younger larvae which show higher susceptibility than other stages. This compound was categorized to Group 15 (Inhibitors of chitin biosynthesis affecting CHS1) by IRAC and registered as organophosphate alternative/reduced risk pesticide by the US EPA in 1998 (Wang et al. 2018b). To date, most studies on resistance management are limited to laboratory populations and results point to

low risk of resistance (Su 2016a). However, high levels of resistance to diflubenzuron were identified very recently in *Cx. pipiens* populations from Italy (Grigoraki et al. 2017, Porretta et al. 2019) and Turkey (Guz et al. 2020). This resistance was associated with mutations at amino acid I1043 (I1043F, I1043M, and I1043L) of the chitin synthase gene. The contribution of these mutations to diflubenzuron resistance was validated by introducing them to the *Drosophila melanogaster* chitin synthase gene, where I→M mutation results in a >2,900-fold and the I→L mutation a >20-fold resistance (Grigoraki et al. 2017, Porretta et al. 2019, Fotakis et al. 2020, Mastrantonio et al. 2021).

CONCLUSIONS

In summary, while the need for mosquito larvicides is on the rise due to the emergence and resurgence of vectors and vector-borne diseases, their availability unfortunately is at the lowest point for numerous reasons. Resistance to the limitedly available larvicides creates further challenges for mosquito control operations. Among the advantages of *Bti*, minimum risk of resistance evolution due to the intact endotoxin complex, synergism among individual toxins and presence of Cyt1A, make this microbial agent a unique tool in controlling mosquitoes, blackflies, and midges. More importantly, *Bti* seems to be a critical tool in resistance mitigation to other biorational larvicides, including delaying resistance evolution before the fact and restoring susceptibility after the fact. While appreciating the values of *B. sphaericus*, its toxin simplicity, along with previous exposure to wild strains in nature and the genetic background of larval populations, collectively lead to a noticeable level of risk in resistance development. Combining *Bti* and *B. sphaericus* deems many benefits in resistance management and efficacy enhancement. Based on limited data, it is not recommended to rotate *Bti* and *B. spahaericus* to delay resistance development to *B. sphaericus*, although more studies are needed to elucidate the unknown mechanism. Larval mosquitoes develop resistance to spinosad quickly if resistance management tactics are not implemented strategically, largely due to the mode of action of these neurotoxins and chances of sub-lethal exposures, which has been well documented in agricultural pests. Tactics to prevent, or at least delay resistance development, and to restore spinosad susceptibility after resistance development in mosquitoes, should be developed and implemented. The overall risk of resistance development to methoprene is low when one reviews the historical cases over decades of applications. However, due to the narrow window of susceptibility, i.e., the transition period from late 4th instar larvae to pupae and adult emergence, sublethal exposure, the leading cause of resistance development, is unavoidable when treating larval populations with mixed stages, as young larvae have a high lethal level as compared to older ones. It is generally believed that pyriproxyfen has low resistance risk because of its strong growth regulation and other activities against various life stages. However, its persistence in the environment could lead to sublethal exposure, hence development of tolerance and resistance. It is a surprise to see the recent documentation of resistance to diflubenzuron in *Cx. pipiens*. As a chitin synthesis inhibitor with a broad activity window as compared with juvenile hormone analog or mimic, diflubenzuron is obviously still not resistance proof. Another important point is that mosquitoes have specific exposures of *Bti* and *B. sphaericus* from public health applications only, while the exposures to other larvicides such as spinosad, methoprene, pyriproxyfen, and diflubenzuron, can be undocumented and quite broad from urban, horticulture and agriculture applications. Although often there is a bio-fitness cost in resistant mosquitoes which may bring negative impacts on life events and vectorial capacity of mosquitoes (Su 2016a), the consequences of resistance evolution remain costly.

Considering the widespread occurrence of pyrethroid resistance detected in adult mosquito populations, resistance to biorational larvicides must be monitored and mitigation measures must be implemented to ensure their availability in mosquito management programs.

REFRENCES CITED

Amorim LB, Oliveira CMF, Rios EM, Regis L, Silva-Filha MHNL. 2007. Development of *Culex quinquefasciatus* resistance to *Bacillus sphaericus* strain IAB59 needs long term selection pressure. *Biol Control* 42:155-160.

Amorim LB, de Barros RA, Chalegre KD, de Oliveira CM, Regis LN, Silva-Filha MH. 2010. Stability of *Culex quinquefasciatus* resistance to *Bacillus sphaericus* evaluated by molecular tools. *Insect Biochem Mol Biol* 40:311-316. doi: 10.1016/j.ibmb.2010.02.002

Becker N, Ludwig M, Su T. 2018. Lack of resistance in *Aedes vexans* field populations after 36 years of *Bacillus thuringiensis* subsp. *israelensis* application in the upper Rhine Valley, Germany. *J Am Mosq Control Assoc* 34: 154-157.

Chueng, PYK, Buster D, Hammock BD. 1987. Lack of mosquitocidal activity by the cytolytic protein of the *Bacillus thuringiensis* subsp. *israelensis* parasporal crystal. *Current Microbiol* 15:21-23.

Cornel AJ, Stanich MA, Farley D, Mulligan FS III, Byde G. 2000. Methoprene tolerance in *Aedes nigromaculis* in Fresno County, California. *J Am Mosq Control Assoc* 16:223-238.

Cornel AJ, M. Stanich A, McAbee RD, Mulligan FS III. 2002. High level methoprene resistance in the mosquito *Ochlerotatus nigromaculis* (Ludlow) in central California. *Pest Manag Sci* 58:791-798.

Dame DA, Wichterman GJ, Hornby JA. 1998. Mosquito (*Aedes taeniorhynchus*) resistance to methoprene in an isolated habitat. *J Am Mosq Control Assoc* 14:200-203.

Fotakis EA, Mastrantonio V, Grigoraki L, Porretta D. 2020. Identification and detection of a novel point mutation in the chitin synthase gene of *Culex pipiens* associated with diflubenzuron resistance. *PLoS Neg Trop Dis* 14(5):e0008284. DOI:10.1371/journal.pntd.0008284

Georghiou GP, Wirth MC. 1997. Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* var. *israelensis* on the development of resistance in the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). *Appl Environ Microbiol* 63:1095-1101.

Goldberg LJ, Margalit JA. 1977. Bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univitattus*, *Aedes aegypti* and *Culex pipiens*. *Mosq News* 37:355-358.

Goldman I, Arnold J, Carlton BC. 1986. Selection for resistance to *Bacillus thuringiensis* subspecies *israelensis* in field and laboratory populations of the mosquito *Aedes aegypti*. *J Invert Pathol* 47:317-324.

Grigoraki L, Puggioli A, Mavridis K, Douris V, Montanari M, Bellini R, Vontas J. 2017. Striking diflubenzuron resistance in *Culex pipiens*, the prime vector of West Nile Virus. *Scientific Report* 7, Article number: 11699

Güz N, Çağatay N.S, Fotakis E.A, Durmuşoğlu E, Vontas J. 2020. Detection of diflubenzuron and pyrethroid resistance mutations in *Culex pipiens* from Muğla, Turkey. *Acta Trop* 203:105294 10.1016/j.actatropica.2019.105294

Margalit J, Dean D. 1985. The story of *Bacillus thuringiensis* var. *israelensis* (B.t.i.). *J Am Mosq Control Assoc* 1:1-7.

Mastrantonio V, Porretta D, Lucchesi V, Güz N, Çağatay NS, Bellini R, Vontas J, Urbanelli S. 2021. Evolution of adaptive variation in the mosquito *Culex pipiens*: Multiple independent origins of insecticide resistance mutations. *Insects* 12:676

Mittal P. 2005. Laboratory selection to investigate the development of resistance to *Bacillus thuringiensis* var. *israelensis* H-14 in *Culex quinquefasciatus* Say (Diptera: Culicidae). *Nat Acad Lett India* 28:281-283.

Mulla MS, Thavara U, Tawatsin A, Chomposri J, Su T. 2003. Emergence of resistance and resistance management in field populations of tropical *Culex quinquefasciatus* to the microbial control agent *Bacillus sphaericus*. *J Am Mosq Control Assoc* 19:39-46.

Paul A, Harrington LC, Zhang L, Scott JG. 2005. Insecticide resistance in *Culex pipiens* in New York. *J Am Mosq Control Assoc* 21:305-309.

Pei G, Oliveira CMF, Yuan Z, Nielsen-LeRoux C, Silva-Filha MH, Yan J, Regis L. 2002. A strain of *Bacillus sphaericus* causes slower development of resistance in *Culex quinquefasciatus*. *Appl Environ Microbiol* 88:3003-3009.

Pérez C, Munoz-Garcia C, Portugal LC, Sanchez J, Gill SS, Soberón M, Bravo A. 2007. *Bacillus thuringiensis* subsp. *israelensis* Cyt1Aa enhances activity of Cry11Aa toxin by facilitating the formation of a pre-pore oligomeric structure. *Cell Microbiol* 9:2931-2937.

Poopathi S, Mani TR, Raghunatha RD, Baskaran G, Kabilan L. 2002. Evaluation of synergistic interaction between *Bacillus sphaericus* and a neem based biopesticide against *Culex quinquefasciatus* larvae susceptible to *Bacillus sphaericus*. 1593M. *Insect Sci App* 22:303-306.

Porretta R, Fotakis R, Mastrantonio R, Chaskopoulou R, Michaelakis M, Kioulos I, Weill M, Urbanelli S, Vontas J, Bellini R. 2019. Focal distribution of diflubenzuron resistance mutations in *Culex pipiens* mosquitoes from Northern Italy. *Acta Trop* 193: 106-112.

Rodcharoen J, Mulla MS. 1994. Resistance development in *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus*. *J Econ Entomol* 87:1133-1140.

Rodcharoen J, Mulla MS. 1997. Biological fitness of *Culex quinquefasciatus* (Diptera; Culicidae) susceptible and resistant to *Bacillus sphaericus*. *J Med Entomol* 34:5-10.

Roh JY, Choi JY, Li MS, Jin BR, Je YH. 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. *J Microbiol Biotechnol* 17:547–559.

Saleh MS, El-Meniawi FA, Kelada NL, Zahran HM. 2003. Resistance development in mosquito larvae *Culex pipiens* to the bacterial agent *Bacillus thuringiensis* var. *israelensis*. *J Appl Entomol* 127:29-32.

Schaefer CH, Mulligan FS III. 1991. Potential for resistance to pyriproxyfen: a promising new mosquito larvicide. *J Am Mosq Control Assoc* 7:409-411.

Sinègre G, Babinot M, Quermel JM, Gavon B. 1994. First field occurrence of *Culex pipiens* resistance to *Bacillus sphaericus* in southern France. *Proc 8th European Meet Soc Vector Ecol* P17. September 5-8, 1994; Barcelona, Spain. Society for Vector Ecology, Santa Ana, California.

Su T. 2016a. Resistance and its management to microbial and insect growth regulator larvicides in mosquitoes. In: Trdan, S. (Ed.), Insecticides Resistance, InTech Europe, Rijeka, Croatia. PP. 135-154.

Su T. 2016b. Microbial control of pest and vector mosquitoes in North America north of Mexico. *In*: Microbial Control of Insect and Mite Pests (Ed. L. Lacey). Academic Press. PP. 393-407.

Su T, Cheng ML. 2012. Resistance development in *Culex quinquefasciatus* to spinosad: A preliminary report. *J Am Mosq Control Assoc* 28:263-267.

Su T, Cheng ML. 2014a. Laboratory selection of resistance to spinosad in *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 51:421-427.

Su T, Cheng ML. 2014b. Cross resistances in spinosad – resistant *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 51:428-435.

Su T, Mulla MS. 2004. Documentation of high-level *Bacillus sphaericus*-resistance in tropical *Culex quinquefasciatus* populations from Thailand. *J Am Mosq Control Assoc* 20:405-411.

Su T., Thieme J, Cummings R, Cheng ML, Brown MQ. 2021. Cross resistance in *s*-methoprene-resistant *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* DOI: 10.1093/jme/tjaa182

Su T., Thieme J, Lura T, Cheng ML, Brown MQ. 2019a. Susceptibility profile of *Aedes aegypti* L. (Diptera: Culicidae) from Montclair, California, to commonly used pesticides, with note on resistance to pyriproxyfen. *J Med. Entomol* 56: 1047-1054. https://doi.org/10.1093/jme/tjz019.

Su T., J. Thieme, C. Ocegueda, M. Ball, M. L. Cheng. 2018. Resistance to *Lysinibacillus sphaericus* and other commonly used pesticides in *Culex pipiens* (Diptera: Culicidae) from Chico, California. *J Med Entomol* 55: 423-428. doi: 10.1093/jme/tjx235

Su T., J. Thieme, G.S. White, T. Lura, N. Mayerle, A. Faraji, M.L. Cheng, and M.Q. Brown. 2019b. Resistance to *Lysinibacillus sphaericus* and susceptibility to other common pesticides in *Culex pipiens* (Diptera: Culicidae) from Salt Lake City, Utah. *J Med Entomol* 56: 506-513. PMID:30383248. DOI: 10.1093/jme/tjy193

Tabashnik BE. 1992. Evaluation of synergism among *Bacillus thuringiensis* toxins. *Appl Environ Microbiol* 58:3343-3346.

Tetreau G, Bayyareddy K, Jones CM, Stalinski R, Riaz, MA, Paris, M David, JP, Adang MJ, Després L. 2012. Larval midgut modifications associated with *B.t.i* resistance in the yellow fever mosquito using proteomic and transcriptomic approaches. *BMC Genomics* 13:248 doi:10.1186/1471-2164-13-248.

Tetreau G, Stalinski R, David JP, Després L. 2013. Monitoring__resistance to *Bacillus thuringiensis* subsp. *israelensis* in the field by performing bioassays with each Cry toxin separately. *Mem Inst Oswaldo Cruz* 108:894-900.

Vasquez MI, Violaris M, Hadjivassilis A, Wirth MC. 2009. Susceptibility of *Culex pipiens* (Diptera: Culicidae) field populations in Cyprus to conventional organic insecticides, *Bacillus thuringiensis* subsp. *israelensis*, and methoprene. *J Med Entomol* 46:881-887.

Vasquez-Gomez M. 1983. Investigations of the possibility of resistance to *Bacillus thuringiensis* ser. H-14 in *Culex quinquefasciatus* through accelerated selection pressure in the laboratory. Riverside: University of California.

Wang, YY., Yuan SK, Zhong XZ, Su T. 2018a. Biopesticide ingredients registered in US-EPA in 2017. *World Pesticides* 40(1): 40-49, 52.

Wang, YY., Zhao YH, Su T, Zhang ZJ, Jv YH, Zhao ZT. 2018b. List of registered active ingredients and application range of reduced risk pesticides by US EPA. *World Pesticides* 40(3): 5-13.

Wheeler. S, Wagner R, Reed M. 2022. Detection and management of larval insecticide resistance in catch basin habitats. 88th Ann Meet Am Mosq Control Assoc https://web.cvent.com/event/7d5f0e74-2023-4bd6-bcee-aa8d4f9687a4/websitePage:645d57e4-75eb-4769-b2c0-f201a0bfc6ce (accessed: January 22, 2022).

Wirth MC. 2010. Mosquito resistance to bacterial larvicidal toxins. *Open Toxinol J* 3:126-140. [DOI: 10.2174/1875414701003010126]

Wirth MC, Berry C, Walton WE, Federici BA. 2014. Mtx toxins from *Lysinibacillus sphaericus* enhance mosquitocidal cry-toxin activity and suppress cry-resistance in *Culex quinquefasciatus*. *J Insect Pathol* 115:62-67.

Wirth MC, Ferrari JA, Georghiou GP. 2001. Baseline susceptibility to bacterial insecticides in populations of *Culex pipiens* complex (Diptera: Culicidae) from California and from the Mediterranean Island of Cyprus. *J Med Entomol* 94:920-928.

Wirth MC, Georghiou GP. 1997. Cross-resistance among CryIV toxins of *Bacillus thuringiensis* subsp. *israelensis* in *Culex quinquefasciatus* (Diptera: Culicidae). *J Econ Entomol* 90:1471-1477.

Wirth MC, Georghiou GP, Malik JI, Hussain G. 2000. Laboratory selection for resistance to *Bacillus sphaericus* in *Culex quinquefasciatus* (Diptera: Culicidae) from California, USA. *J Med Entomol* 37:534-540.

Wirth MC, Jiannino JJ, Federici BA, Walton WE. 2004. Synergy between toxins of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*. *J Med Entomol* 41:935-41.

Yuan ZM, Pei GF, Regis L, Nielsen-LeRoux C, Cai QX. 2003. Cross resistance between strains of *Bacillus sphaericus* but not *B. thuringiensis israelensis* in colonies of the mosquito *Culex quinquefasciatus*. *Med Vet Entomol* 17:251-256.

Yuan Z, Zhang Y, Cai Q, Liu EY. 2000. High-level field resistance to *Bacillus sphaericus* C3-41 in *Culex quinquefasciatus* from southern China. *Biocon Sci Technol* 10:41-49.

Zahiri NS, Mulla MS. 2003. Susceptibility profile of *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis*. *J Med Entomol* 40:672-677.

Zahiri NS, Su T, Mulla MS. 2002. Strategies for the management of resistance in mosquito to the microbial control agent *Bacillus sphaericus*. *J Med Entomol* 39:513-520.

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