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Chapter 8

Resistance and Its Management to Microbial and Insect Growth Regulator Larvicides in Mosquitoes

Tianyun Su

Additional information is available at the end of the chapter

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Abstract

Mosquito larvicides derived from microbial organisms and insect growth regulators have been increasingly used to control mosquito larvae worldwide. Their relative target specificity, nontarget safety, and environmentally friendly profile have been well documented. The current chapter was intended to review and analyze the relevant information regarding resistance development and resistance management tactics. *Bacillus thuringiensis israelensis* de Bajac (*B.t.i.*) is a quick-acting and highly target-specific biopesticide against mosquitoes, blackflies, and other nematoceran species. Resistance development toward intact complementary toxin complex of *B.t.i.* was rare; however, low to high levels of resistance to individual toxins have occurred in laboratory mosquito populations. The toxins from bacterium *Bacillus sphaericus* Neide (recently renamed *Lysinibacillus sphaericus* Meyer and Neide) is another highly active larvicide against mosquitoes, toward which low to high levels of resistance have occurred in both laboratory and field mosquito populations. The Cyt1A toxin from *B.t.i.* and Mtx toxin from certain strains of B. *sphaericus* are the key components in resistance management to *B.t.i.* and *B. sphaericus*. The resistance management strategies have been well developed and implemented. Spinosad derived from *Saccharopolyspora spinosa* Mertz and Yao has been recently used for mosquito control; high levels of resistance and cross-resistance have occurred in laboratory mosquito populations and no management tactics have ever been developed. Methoprene has been used to control mosquitoes for decades, and low to high levels of resistance have been occasionally reported in both laboratory and field mosquito populations. Studies on mechanism and management of methoprene resistance are quite meager. Very little attention has been paid to the resistance management in mosquitoes to other insect growth regulators such as pyriproxyfen and diflubenzuron. The prevention of resistance and restoration of susceptibility in mosquitoes to these biorational larvicides are crucial to the success of sustainable integrated mosquito management.

Keywords: Microbial larvicides, Insect growth regulators, Mosquito control, Resistance, Resistance management

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1. Introduction

Mosquitoes and mosquito-borne diseases remain one of the leading public health concerns and socioeconomic burdens of mankind globally, particularly in tropical and subtropical regions. Nowadays, human and animal population movement, freight exchange, fast demographic growth, economic development, and subsequent environmental impact further elevate the scope and magnitude of the problem. Mosquito control is often the only or most effective way of the integrated management to combat mosquito-borne illnesses. Considering the strict governmental regulations, high environmental vulnerability, and increasing demand of mosquito control upon emergence, and spreading of mosquito-borne diseases, ecologically friendly management approaches based on microbial and insect growth regulator larvicides have been the great promise for their high activity and efficacy, target specificity, and environmental and nontarget safety profile. However, the development of resistance in the mosquito populations to these biorational larvicides has been reported since the past decades. In order to maintain the sustainability of mosquito control, susceptibility monitoring and resistance management tactics toward these available control tools must be developed and implemented.

2. *Bacillus thuringiensis* subsp *israelensis* (*B.t.i.*)

The entomopathogenic *Bacillus* was first discovered in 1901 by Japanese biologist Ishiwata Shigetane, who was investigating the cause of the sotto disease (sudden-collapse disease) that was killing large populations of silkworms. Shigetane named the bacterium *Bacillus sotto*, but the name was later ruled invalid. In 1911, this pathogen was rediscovered in Germany by Ernst Berliner, who isolated it from Mediterranean flour moth *Anagasta kuehniella* (Zeller, 1879) caterpillars that suffered *Schlaffsucht.* He named it *Bacillus thuringiensis*, after the German town Thuringia where the moth was found [1]. Up to date, at least 70 serotypes, more than 80 subspecies have been identified, among which 14 serotypes and 16 subspecies show lethal activities against mosquito larvae. *Bacilllus thuringiensis israelensis* (*B.t.i.*), a subspecies belonging to serotype H-14, was discovered from a natural mosquito habitat located in Israel desert in 1976 [2, 3]. Four synergistic endotoxins including Cry4A, Cry4B, Cry 11A, and Cyt1A are produced during sporulation of *B.t.i.* [4–7]. These protoxins are activated by enzymatic proteolysis activities under high pH environment in mosquito midgut. The activated toxins combine with the receptors located at the epithelium cells in midgut brush border and cause subsequent pathological consequences and death of the target species. Toxins of *B.t.i.* are toxic to species in nematoceran group including culicidae, simuliidae [8, 9], chironomidae [10–12], and some fungus gnats. *B.t.i.* is categorized as Group 11 pesticide, i.e., microbial disruptor of insect midgut membranes by the Insect Resistance Action Committee (IRAC). During the past 35 years or so, *B.t.i.* products have been extensively used to control mosquitoes and blackflies and occasionally chironomid midges worldwide.

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2.1. Field occurrence

Generally, the risk of resistance development to wild-type *B.t.i.*, i.e., the natural toxin complex, is very low. For example, *B.t.i.* products were widely used to control floodwater mosquitoes *Aedes vexans* (Meigen) over an area of approximately 500 km^2 for more than 10 years in Rhine River area in Germany; no reduction in susceptibility was noticed [13]. One report, however, from New York, USA, showed low-level resistance in wild population of *Culex pipiens* L. Briefly, collections from Syracuse and Albany showed 33- to 41-fold and 6- to 14-fold resistance to *B.t.i.* Based on the considerable difference in resistance levels between the populations from Syracuse and Albany, it seems that there was lack of gene flow between these populations of *Cx. pipiens*, resulting in aggregation of resistant individuals [14]. *Culex pipiens* populations from Cyprus (2002–2008) showed dose–response values ranging approximately 8-fold to *B.t.i.*, but no resistance was detected after years of application [15]. Between 1990 and 1993, the susceptibility of *Cx. pipiens* complex to *B.t.i.* was determined in 31 collections from California, USA. The samples were collected before the widespread use in California. Seven collections from the Mediterranean island of Cyprus, where no microbial insecticides have ever been used, also were tested. The collections from California during 1990–1991 exhibited 3- to 4-fold variations in susceptibility at LC_{50} and LC_{95}. The collections from Cyprus in 1993 exhibited both higher mean LC values, and greater variability in those values, than the California collections. No significant geographic variation in susceptibility was observed among regions within California [16].

2.2. Laboratory studies

Multiple attempts to select resistance in laboratory colonies of *Cx. pipiens* complex or *Aedes aegypti* L. for various generations only resulted in low-level and unstable resistance. Four populations of *Cx. quinquefasciatus* Say collected from Southern California were subjected to different levels of selection pressure for 11–60 generations; 4.1- to 16.5-fold resistance was achieved. The resistance tended to decline in absence of selection pressure [17]. One laboratory and two wild populations of *Ae. aegypti* were used in selection for resistance to *B.t.i.* After 14 generations of selection at LC_{50}, a statistically significant decline (2-fold) in susceptibility was observed in the F_{15} of one wild strain only. Regression lines, LC_{50} values, and slopes of parental populations between strains did not differ significantly [18]. When larvae of *Cx. pipiens* were subjected repeatedly to selection pressure with *B.t.i.* in the laboratory, only 2.78-fold tolerance was induced as a result of 20 generations of selection, which decreased by about 58% after the selection was withdrawn for 3 generations. Larval selection with *B.t.i.* caused a reduction in the reproductive potential in resulted survivors [19]. Another study revealed 2- to 3-fold increase in the LC_{50} or LC_{90} values to *B.t.i.* preparation in the larvae of *Cx. quinquefasciatus* after 20 generations of selection, and these values fluctuated in different generations during the selection [20]. The laboratory selection with field-persistent *B.t.i.* toxins led to a 3.5-fold resistance to VectoBac WG in *Ae. aegypti* after 30 generations, but to relatively high levels of resistance to individual Cry toxins. Bioassay procedure was developed using each Cry toxin to detect cryptic *B.t.i.* resistance that evolved in field mosquito populations. Although no resistance to *B.t.i.* was detected in three *Aedes* mosquito species tested, an increased tolerance

to Cry4Aa (3.5-fold) and Cry11Aa toxins (8-fold) was found in one *Ae. sticticus* population. This study facilitated information of susceptibility status to individual Cry toxins of *B.t.i*. Bioassays with individual Cry toxins allow a more sensitive monitoring of *B.t.i.* resistance in the field [21]. When individual Cry toxins were tested against the LiTOX strain that carried low-level resistance to *B.t.i.*, increased resistance of 68-, 9-, and 9-fold to Cry4Aa, Cry4Ba, and Cry11Aa protoxins, respectively was revealed [22].

Exposures to individual toxins of *B.t.i.* are conducive to resistance development, where *Cx. quinquefasciatus* developed high resistance to individual toxins in absence of Cyt1A toxin. Resistance became evident in the mosquitoes that were selected with a single toxin (CryIVD), reaching the highest level of 913-fold. Resistance developed at a slower pace and reached a lower level when being selected with CryIVA + CryIVB. Resistance levels were further suppressed when being selected with CryIVA + CryIVB + CryIVD or full combination of all four toxins. These results highlighted the importance of the full combination of toxins found in wild *B.t.i.* in resistance management [23]. This fact, i.e., target species rapidly develops resistance to individual toxins but not to the toxin complex from the wild strain, also exists in other *B.t.* subspecies studied, for instance, *B. thuringiensis* subsp *jegathesan* [24]. Cross-resistance occurs among the Cry toxin of *B.t.i*. Resistance was generally highest toward the toxin(s) that were used in the selections. The strain that was selected with CryIVD demonstrated significant cross-resistance to CryIVA + CryIVB, and vice versa. Strain that was selected with naturally occurring insecticidal toxin mixture in *B.t.i.* only showed a low level of resistance to this mixture, but much higher levels of resistance occurred to individual CryIV toxins and also to combinations of 2 or 3 CryIV toxins. The fact that all of the selected populations stayed susceptible to the natural toxin mixture in *B.t.i.* suggested that the CytA toxin with different sequence and mode of action from the CryIV toxins plays an important role in suppressing resistance to CryIV toxins [25]. Cross-resistance among Cry toxins can be extended to other *B.t.* subspecies. *Cx. quinquefasciatus* that are resistant to *B.t.i.* Cry toxins also show cross-resistance to Cry11B from *B.t. jegathesan* [26], but not to Cry19A from the same species [27].

Cyt1A, a cytolytic endotoxin of *B. thuringiensis*, does not possess significant larvicidal activity alone [28]. However, it plays a critical role in overcoming, preventing, and delaying resistance development to Cry toxins.

The high levels of resistance to CryIV in *Cx. quinquefasciatus* was reduced remarkably by combining CryIV with sublethal quantities of CytA. A 127-fold resistance to a combination of CryIVA, B, and D was completely suppressed by combining CytA in a 1:3 ratio with CryIVA, CryIVB, and CryIVD. Combining the CytA with CryIVA and CryIVB also completely suppressed 217-fold resistance to the latter toxins, whereas the combination of CytA with CryIVD reduced resistance in a CryIVD-selected mosquito strain from >1000-fold to < 8-fold [29]. The same species of mosquitoes were subjected to selection for 20 generations using the recombinant strains of *B.t.* that produced Cyt1Aa, Cry11Aa, or a 1:3 mixture. The resistance in the Cry11Aa strain- and Cyt1Aa strain-selected populations reached 1,237-fold and 242-fold, respectively. On the other hand, the resistance only reached 8-fold in the population that was selected with the Cyt1Aa and Cry11Aa (1:3) strain. Mosquitoes that were selected by Cyt1Aa-Cry11Aa for 48 generations only developed 9.3-fold resistance to Cry11Aa. Obviously, resistance to Cry11Aa developed at a much slower pace in the presence than in the absence of

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Cyt1Aa [30]. Studies on the mechanism of activity enhancement and resistance prevention of Cry toxins by Cyt1A indicated that Cyt1A functions as a receptor of Cry11A. Cyt1Aa binding to *Ae. aegypti* brush border membrane vesicles enhanced the binding of Cry11Aa. Two exposed regions in Cyt1Aa, namely, loop beta6-alphaE and part of beta7, bind Cry11Aa. On the other side, Cry11Aa binds Cyt1Aa proteins by means of domain II-loop alpha8 and beta-4, which are also involved in midgut receptor interaction. The key residues involved in the interaction and synergism between Cry11Aa and Cyt1Aa were S259 and E266 in Cry11Aa and K198, E204 and K225 in Cyt1Aa [31]. Further studies revealed that binding of Cry11A to Cyt1A facilitates the formation of a Cry11A prepore oligomeric structure that is capable of forming pores in membrane vesicles [32].

It was discovered recently that the mosquitocidal toxins (Mtx) from some *B. sphaericus* strains not only enhance the larvicidal activity of *B.t.i*. Cry toxins but also mitigate resistance development to Cry toxins. Mtx-1 and Mtx-2 were active against mosquitoes that were susceptible or resistant to Cry toxins. A mixture of Mtx-1 or Mtx-2 with different Cry toxins in the ratio of 1:1 showed moderate synergism. Some combinations of Mtx and Cry toxins were highly active to kill resistant larvae and also suppressed resistance to Cry toxins [33]. There is a lack of cross-resistance to the wild type of other *B.t.* subspecies such as *B.t. jegathesan*, *B.t. kyushuensis*, and *B.t. fukuokuensis* in *Cx. quinquefasciatus* that are resistant to individual toxins from *B.t.i.* [34]. It is advised not just to express Cry toxins of *B.t.i.* in transgenic microbial organisms or algae from the perspectives of resistance prevention in target species.

3. *Bacillus sphaericus*

The mosquitocidal activity of some strains of *B. sphaericus* has long been recognized. Up to date, 49 serotypes over 300 strains of *B. sphaericus* have been identified, among which 9 serotypes 16 strains showed activity against mosquito larvae at different extent. Strains that possess high mosquitocidal activity are 2362, 1597, 2297, C3-41, and IAB-59. The mostly studied and developed strain 2362 was isolated in 1984 from adult blackfly *Simulium damnosum* (Diptera: Simuliidae) in Nigeria. Recently, *B. sphaericus* was renamed as *Lysinibacillus sphaericus* Meyer and Neide [35]. Active strains produce parasporal inclusions during sporulation, which contains crystal binary toxins. Some strains also synthesize noncrystal Mtx. The mode of action of the binary toxins is somewhat similar in general to *B.t.i.* toxins with detail differences at molecular levels. The receptor of the binary toxins is a 60-kDa alpha-glucosidase, which is anchored in the mosquito midgut membrane via a glycosyl-phosphatidylinositol (GPI) anchor. As *B.t.i.*, *B. sphaericus* also belongs to IRAC Group 11. Compared with *B.t.i.*, the target species spectrum is narrower, some *Aedes* spp., for example, *Ae. aegypti* is much less susceptible than *Culex* spp. to *B. sphaericus*. During the past decades, numerous products have been developed using various strains and applied to control *Culex* spp. worldwide.

3.1. Field occurrence

The earliest resistance in field populations was reported in *Cx. pipiens* in southern France where the resistance ratio at LC_{50} was 70-fold as a result of extended field applications [36]. A field-

collected population of *Cx. quinquefasciatus* larvae from an urban area of Recife in Brazil, which has been treated for 2 years with *B. sphaericus*, was found to be about 10-fold less susceptible than the untreated control field populations [37]. The field resistance to strain 1593 was reported in Kochi, India, in the same year. The larvae of *Cx. quinquefasciatus* from the sprayed area showed LC_{50} and LC_{90} values that were 146 and 180 times higher than corresponding values for a susceptible strain from an unsprayed area after 35 rounds of application over 2 years. The subsequent laboratory selection of the collection from the treated area resulted in much higher levels of resistance, 6,223- and 31,325-fold at LC_{50} and LC_{90}, respectively [38]. The similar situation also happened to strain B101 in the same mosquito species where low levels of resistance occurred in response to field applications; the population reached 52,000-fold resistance after selection for 6 generations in the laboratory [39]. Field *Cx. pipiens* mosquitoes that were collected after a control failure with Spherimos in southern France developed high resistance (>10,000-fold) after <8 generations of laboratory selection [40]. In southern China, a flowable formulation of strain C3-41 was continuously used for 8 years to control *Cx. quinquefasciatus* larvae. The resistance of field-collected larvae at LC_{50} was 22,672-fold [41]. More occurrences on resistance to strain 2362 were reported later in France (5,958-fold) [42] and Tunisia (750-fold) [43]. Declined efficacy and control failure of *B. sphaericus* was noticed within 4 months after 5 treatments using VectoLex WDG at the dosages of 50–200 mg/m^2 to control *Cx. quinquefasciatus* in Thailand [44]. A high level of *B. sphaericus* resistance was documented in this population. The resistance levels at LC_{50}, depending on reference colonies, were 21,100- to 28,100-fold against VectoLex WDG (650 ITU/mg) or >125,000- to 200,000-fold against *B. sphaericus* technical-grade material (2000 ITU/mg) [45]. Between 1990 and 1993, the susceptibility of *Cx. pipiens* complex to *B. sphaericus* was determined in 31 collections from California, before the registration of this agent. Variation was about 5-fold at the LC_{50} and LC_{95} [16]. Similar results were obtained for *Culex* spp. breeding in dairy lagoons in southern California soon after *B. sphaericus* was registered and applied in California [46]. No case on resistance to *B. sphaericus* in the USA has ever been reported in wild mosquito populations thus far regardless of the substantial amount of *B. sphaericus* products that has been applied, particularly since the invasion of the West Nile virus. The resistance development in response to the field application of *B. sphaericus* products varies greatly, depending on prior exposure to naturally existing strains, population genetic background, and gene exchange with untreated populations, as well as product application strategies.

3.2. Laboratory studies

Resistance to *B. sphaericus* in laboratory colonies of *Cx. pipiens* complex has been reported in different countries since 1994. Larvae of laboratory colony and field-collected (southern California) *Cx. quinquefasciatus* developed moderate level of resistance to strain 2362 (27- to 37-fold) in response to selection at LC_{80} for 80 generations [47]. This moderate level of resistance to strain 2362 in laboratory colony of the same species was reconfirmed by later studies on resistance management tactics [48, 49]. A previously untreated field population of *Cx. quinquefasciatus*, collected near Bakersfield, California, survived the LC_{50} of *B. sphaericus* that was 7,000 times higher than in the susceptible reference colony after 12 generations of selection at LC_{95}. Late and early instar larvae in this study showed the similar levels of resistance [50].

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After 13 and 18 generations of exposure to high concentrations of C3-41 and IAB59, a field-collected low-level-resistant colony of *Cx. quinquefasciatus* developed >144,000-fold and 46.3-fold resistance, respectively. A field-collected susceptible colony was selected with strain 2362 and IAB59 for 46 and 12 generations and reached >162,000-fold and 5.7-fold resistance to the two agents, respectively. The slower evolution of resistance against strain IAB59 may be attributable to the presence of another larvicidal factor [51]. However, in another study, selection by treating about 15,000 of 3rd and 4th instar larvae at each generation at LC_{70} of IAB59 resulted in 40,000-fold resistance after a much longer selection for 72 generations [52]. The resistance development to selection depends on genetic background, size of population used, selection pressure, length of selection, etc. Resistance ratio is also dependent on the susceptibility levels of the reference population. The resistance to *B. sphaericus* is fairly stable in absence of selection pressure. For instance, bioassays showed that the frequency of resistant larvae did not decrease throughout 11 generations after interruption of selection, and it was associated with a similar frequency of larvae lacking the Cqm1alpha-glucosidase receptor. The frequency of the cqm1 (REC) allele remained stable throughout 11 generations without further selection [53].

Furthermore, 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 *B.t.i.* [38, 39, 44, 45, 48–52, 55–59]. The cross-resistance among different strains is mild between the strains that also produce Mtx toxins. For example, cross-resistance in strain 2362-resistant *Cx. quinquefasciatus* was detected toward strains 1593 and 2297, but little or no cross-resistance was observed toward strains IAB59 or ISPC5 [50]. The resistant colonies resulted from the selection with C3-41 or 2362 showed very high levels of cross-resistance to strains 2362, C3-41, 1593, and 2297 but only low-level cross-resistance to IAB59, LP1-G, and 47-6B, which all produce a major 49-kDa protein, another mosquitocidal factor. On the other hand, the IAB59-selected colonies showed high cross-resistance to both strains C3-41 and 2362 [57].

3.3. Resistance mechanism

It is mostly believed that recessive genetic mechanism is involved in resistance to *B. sphaericus* [20, 42, 43, 50, 52, 59]. Among the multiple theories, the predominant one is the lack of specific binding of binary toxins to alpha-glucosidase, which act as midgut receptors [37, 53, 59–63]. The main reason leading the lack of specific binding is related to the deletions of gene encoding the receptor alpha-glucosidase [64–66], where the integrity of the receptor is compromised. However, the resistance in field *Cx. pipiens* mosquitoes after a control failure of Spherimos in southern France is not associated with any loss of binding affinity between brush border membrane fractions and toxins [40]. The similar results were also seen in Brazil, with additional findings of slight declined receptor density [37]. Behavioral modifications such as reduced ingestion on toxins [67] and other unknown mechanisms [40, 42] are also involved.

3.4. Resistance management

B.t.i. can be used as a powerful tool to mitigate resistance to *B. sphaericus* in mosquitoes. The susceptibility to *B. sphaericus* was partially restored by the selection of previously resistant

colony with *B.t.i.* alone for 10 generations. After this colony was reexposed to *B. sphaericus* for 20 generations, resistance to *B. sphaericus* surged back to a stable level. Selections of *B. sphaericus*-resistant colonies with *B.t.i.* and *B. sphaericus* in rotation or mixture lead to steady decline of resistance over 30 generations [48]. Resistance to *B. sphaericus* can be delayed or prevented by the mixture of *B.t.i.* and *B. sphaericus* because of the synergistic action among 4 toxins, particularly the presence of Cyt1A [49, 68–71]. While *B. sphaericus* resistance increased after F_{15} in response to the selection using *B. sphaericus* alone, the rotation of *B. sphaericus* and *B.t.i.* surprisingly resulted in much higher level and faster emergence of resistance to *B. sphaericus*. However, selection with mixtures of *B.t.i.* and *B. sphaericus* for 36 generations showed no emergence of resistance to *B. sphaericus* [49]. Recently, the recombinant that produces toxins from both *B.t.i.* and *B. sphaericus*, even at greater amount than the wild type of bacteria [72–74], provides another path for not only mitigation of resistance but also enhancement of larvicidal activity and efficacy. The combination of *B. sphaericus* with botanical pesticides such as azadirachtin from the neem oil is also considered as an alternative to mitigate resistance development to *B. sphaericus* in mosquitoes [75].

Efforts were made to find practical strategies for controlling resistant mosquitoes and to prevent or delay the development of resistance in wild mosquito populations. In Nonthaburi Province, Thailand, the larvae of *Cx. quinquefasciatus* that were highly resistant (>125,000-fold) to *B. sphaericus* strain 2362 were successfully controlled with applications of *B.t.i.* alone or in combination with *B. sphaericus*. In order to elucidate resistance management strategy in the field, one selected site was treated with *B. sphaericus* 2362 alone and the other treated with a mixture of *B. sphaericus* 2362 and *B.t.i.* Moderate resistance was detected after the 9th treatment and almost complete control failure occurred by the 17th treatment in the site that was treated with *B. sphaericus* 2362 alone. However, no noticeable change in susceptibility to *B. sphaericus* was detected after 9 treatments with the mixture at another site. During this period, the site treated with *B. sphaericus* alone required 19 treatments, whereas the site treated with mixtures only took 9 treatments because of comparatively slower resurgence of larval populations [44]. In this resistance population, the resistance levels to the mixtures of *B. sphaericus* + *B.t.i.* increased steadily upon the increase of *B. sphaericus* ratios in the mixtures from 50%, 75%, 90%, 95%, to 99%. The resistance levels to the mixtures with various ratios of *B. sphaericus* and *B.t.i.*, however, were substantially lower than that in *B. sphaericus* alone, suggesting that the addition of *B.t.i.* to *B. sphaericus* enhanced the mosquitocidal activity (synergism) against these highly *B. sphaericus*-resistant *Cx. quinquefasciatus*. Moderate tolerance and low levels of resistance to *B. sphaericus*/*B.t.i.* recombinant (RR 7.29–12.75 at LC_{50} and 5.15–13.40 at LC_{90}) were also noted in this *B. sphaericus*-resistant population [45]. The similar success was achieved in southern China. After 6 months of treatment with *B.t.i.* in the *B. sphaericus*-resistant populations, their susceptibility to *B. sphaericus* C3-41 recovered, with the resistance ratio of field-collected larvae declining from 22,672-fold to 5.67-fold [41]; the gene exchange with populations in surrounding untreated areas may also have contributed to the rapid decline of resistance levels. There is a lack of cross-resistance between binary toxins and Mtx toxins [76], indicating that Mtx could be a potential tool to manage resistance to binary toxins in the future. It was suggested that once resistance to *B. sphaericus* is detected in the field, its use should be discontinued until the mosquito population becomes susceptible again because of the decline in number of resistant individuals [77].

3.5. Fitness cost of resistance

In a laboratory studies [77], the resistant strains showed some disadvantages such as lower fecundity and fertility, but higher survival rates were observed at the same time. The immature stages of the females from the resistant population developed slightly faster as compared with those of the susceptible strains, which could result in a shorter generation time. The similar findings are that the resistant colony showed lower fecundity and fertility and slower development than the susceptible colony [78]. However, the opposite results were achieved in another study where the resistant colony did not display biological costs regarding fecundity, fertility, and pupal weight [53].

4. Spinosyns

Spinosad, a biopesticide consisting of spinosyn A ($C_{41}H_{65}NO_{10}$) and D ($C_{42}H_{67}NO_{10}$), is produced by a naturally occurring, soil-dwelling bacterium, *Saccharopolyspora spinosa* Mertz and Yao. As a new class of polyketide-macrolide insecticide that acts as nicotinic acetylcholine receptor (nAChR) allosteric modulator, spinosad is categorized as Group 5 insecticide by IRAC. Spinosad exerts 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. The application of spinosad products for mosquito control is relatively new; studies to evaluate resistance development risk and resistance management strategy are rather rare. The first attempt was made in *Cx. quinquefasciatus*. Surviving late instars and pupae were collected from a semifield evaluation on Natular® XRG (2.5% spinosad), and a laboratory colony was established. Selection pressure was applied at LC_{70-90} levels to 10,000–15,000 of the late 3rd and the early 4th instar larvae of each generation after initial lethal levels of Natular XRG against this colony were determined. Susceptibility changes upon selection were determined every other generation. The susceptibility to spinosad in this selected population gradually and steadily declined from generation F_1 to F_{35}. From generations F_{37} to F_{45}, the susceptibility decreased at a much faster pace. For reference purposes, the susceptibility of freshly collected wild populations as well as a laboratory reference colony of the same species was also determined concurrently. By comparing with the wild populations and laboratory reference colony for resistance ratio calculation, spinosad tolerance was observed from the first 9 generations. Resistance increased gradually from generations F_{11} to F_{35} and elevated significantly from generations F_{37} to F_{45}, when resistance ratios reached 1415.3- to 2229.9-fold at LC_{50} and 9,613.1- to 17,062.6-fold at LC_{90}. The exponential elevation of resistance levels throughout selection indicated that a recessive mechanism might have been involved during resistance development to spinosad [79, 80]. The spinosad-resistant *Cx. quinquefasciatus* with various levels of resistance was found not to be cross-resistant to *B.t.i.*, a combination of *B.t.i.* and *B. sphaericus*, methoprene, pyriproxyfen, diflubenzuron, novaluron, temephos, or imidacloprid. However, it showed 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* [50] was as susceptible as laboratory reference colony to spinosad and spinetoram.

Field-collected and laboratory-selected *Cx. quinquefasciatus* that were resistant to methoprene did not show cross-resistance to spinosad and spinetoram. The presence and absence of cross-resistance to other pesticides in spinosad-resistant mosquitoes seemed to be related to their modes of actions [81].

5. Insect growth regulators

5.1. Juvenile hormone analogs (methoprene and pyriproxyfen)

Methoprene, hydroprene, kinoprene, and triprene were synthesized in 1960s. These insect growth regulators interrupt juvenile hormone balance during the transition from the late 4th instar larvae to pupae and adults. Most mortality occurs at pupal stage or incompletely emerged adults. Another juvenile hormone analog pyriproxyfen was synthesized in 1970s, the IRG activity of which is much higher than methoprene [80]. The earliest experimental studies on potential of resistance development in mosquitoes to juvenile hormone analogs were in 1973 [82]. The collective results indicated low risk of resistance development [82–86]. For example, the selection of *Cx. quinquefasciatus* by methoprene for 10 generations only lead 3.9- to 21.3-fold of resistance [86], while the selection of *Cx. pipiens* for 8 generations only resulted in 8- to 13-fold resistance to methoprene and cross-resistance to triprene [83]. Higher levels of resistance to methoprene did not necessarily occur in response to longer period of selection. *Culex tarsalis* Coquillett developed 86-fold resistance after 62 generations of selection [84], while 218-fold of resistance was achieved in *Cx. pipiens* after 40 generations of selection. In the latter case, selected mosquitoes were also cross-resistant to hydroprene and triprene, but not to diflubenzuron [85]. Rapid discharge and reduced detention of methoprene in mosquito tissue played an important role during entire process of resistance development, while metabolic detoxification seemed related to development and maintenance of high level resistance [87, 88].

Data are meager with regard to resistance development in wild populations of mosquitoes. *Aedes taeniorhynchus* (Wiedemann) in Florida, USA, showed 15-fold resistance after applications of methoprene product during 1989 to 1994 [89]. Methoprene tolerance in *Aedes nigromaculis* (Ludlow) was discovered in central California, USA, after 20 years of treatment. Control failure was encountered during 1998–1999 [90], where resistance levels reached thousands of fold [91]. The documented resistance seemed not related to the metabolic detoxification by P450 monooxygenase and carboxylesterase, and treatments using *B.t.i.* partially restored the susceptibility to methoprene [91]. Another reports in wild populations showed that 4.7- to 16-fold in *Cx. pipiens* in Cypress [15] and 9- to 54-fold in *Cx. quinquefasciatus* in southern California [81]. Limited data showed very low risk of resistance to pyriproxyfen in mosquitoes [92].

5.2. Chitin synthesis inhibitor (diflubenzuron)

Diflubenzuron was synthesized in mid 1970s by Philips-Duphar B.V. This compound is a nonselective chitin synthesis inhibitor that interrupts formation of exoskeleton, interferes with

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integrity of cuticle, and causes leakage of body fluid and ultimately mortality of target organisms. Diflubenzuron acts on all stages of the mosquito life cycle, larval stages in particular, younger larvae show higher susceptibility. Up to date, studies on resistance management are limited to laboratory populations. For instance, *Cx. pipiens* developed 7-fold resistance to diflubenzuron in response to selection for 5 generations [85]. *Culex quinquefasciatus* collected from the east coastal area in Dar es Salaam, Tanzania, developed 2.4- to 6.6-fold resistance after 10 generations of selection [86]. *Aedes aegypti* developed 3.3-fold resistance after 10 generations of selection, of which the resistance level increased to 8- to 20-fold after this population was hybridized with a mixing collection from 35 locations and then selected for 5 generations [93]. In general, the risk of resistance development to diflubenzuron in mosquitoes is relatively low.

6. Conclusions

This chapter reviewed and analyzed historical data of resistance and resistance management in mosquitoes to biorational larvicides with microbial and IGR origins. Bacterial larvicide *B.t.i.* possesses the lowest risk of resistance development, which depends on the intact endotoxin complex and synergism among individual toxins, particularly the presence of Cyt1A. More importantly, *B.t.i.* plays a critical role in mitigation of resistance development and susceptibility restoration and maintenance in other biorational larvicides. The binary toxins from *B. sphaericus* have numerous advantages in controlling mosquito larvae; the resistance development risk is somewhat difficult to determine, as many factors are attributable to the ultimate outcome of the scope and magnitude of resistance. Based on available data from laboratory and field studies worldwide, the combination of *B.t.i.* with *B. sphaericus*, through biofuse technology or genetic engineering, is the best choice to enhance the larvicidal activity and efficacy, to prevent resistance development, and to restore susceptibility to *B. sphaericus*. It seems that larval mosquitoes develop resistance to spinosad fairly fast if resistance management tactics are not implemented strategically, which can be attributed to the mode of action, i.e., the activation of nACh receptors in competition with acetylcholine, and chances of sublethal exposures. Strategies to prevent resistance development and to restore spinosad susceptibility after resistance development in mosquitoes should be developed and implemented urgently. As to the resistance development to IGRs, the overall risk is low. However, it must be pointed out that juvenile hormone analogs such as methoprene and pyriproxyfen act at the transition from the late 4th instar larvae to pupae and adults; the activity mostly depends on the internal juvenile hormone level. Individuals with lower internal juvenile hormone titer such as the late 4th instar larvae and pupae are more susceptible to the analogs. In wild immature mosquito populations, different instars coexist in the aquatic habitats, of which the internal juvenile hormone levels vary greatly. This phenomenon would lead to sublethal exposures and subsequently tolerance even resistance development.

There is no doubt about the consequence resulted from occurrence and spread of resistance, such as cost increase of control operations, outbreak of vector populations, and vector-borne diseases. On the other hand, there are some negative impacts of resistance development on mosquito biological fitness, such as shortened longevity and reduced fecundity [77, 78, 94],

which may lower the vectorial capacity [95–97]. Therefore, evaluation on the exact impact of vector resistance to pesticides on the epidemiology of vector-borne diseases can be complicated. During the past decades, pesticide resistance development and spread promoted banning or limited applications of nonselective, long-lasting synthetic pesticides. At the same time, this situation also advanced toxicological studies and detection technology of resistance, as well as the research, development, and application of biorational pesticides, and other mosquito control techniques.

It must be emphasized that the occurrence of resistance to pesticides in mosquitoes has been on the rise, including cases to the biorational pesticides discussed in this chapter. For long-term benefits, susceptibility monitoring by standardized protocols must be implemented at the same time when a pesticide is introduced to the control operations. The collaboration among academic research, industrial development, and field application and evaluation is crucial to prolong the life and enhance efficacy of pesticides, as well as protect the environment and nontarget organisms.

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Author details

Tianyun Su*

Address all correspondence to: tyccsu@hotmail.com

West Valley Mosquito and Vector Control District, Ontario, CA, USA

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