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Mechanisms of resistance to commercially relevant entomopathogenic bacteria

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Bacteria represent the most commercially successful entomopathogenic microbial group, with most commercialized insecticides containing gram-positive bacteria in the *Bacillaceae* family. Resistance to entomopathogenic bacteria threatens sustainable agriculture, and information on the mechanisms and genes involved is vital to develop management practices aimed at reducing this risk. We provide an integrative summary on mechanisms responsible for resistance to commercialized entomopathogenic bacteria, including information on resistance to transgenic crops producing insecticidal proteins from *Bacillus thuringiensis* (*Bt* crops). The available experimental evidence identifies alterations in binding of insecticidal proteins to receptors in the host as the main mechanism for high levels of resistance to entomopathogenic bacteria.

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Introduction

The use of microbial pesticides continues to grow driven by the demand for organic produce and their benefits compared to synthetic pesticides, including environmental safety, efficacy, and amenability to use in integrated pest management (IPM) programs. Among commercialized microbial pesticides, bacteria account for >76% of the biopesticide market worldwide [1], and evolution of target resistance threatens their sustainable use. Information on the mechanisms and genes involved in high levels of resistance to entomopathogenic bacteria is critical to develop effective management practices and improved products that reduce this risk in the field.

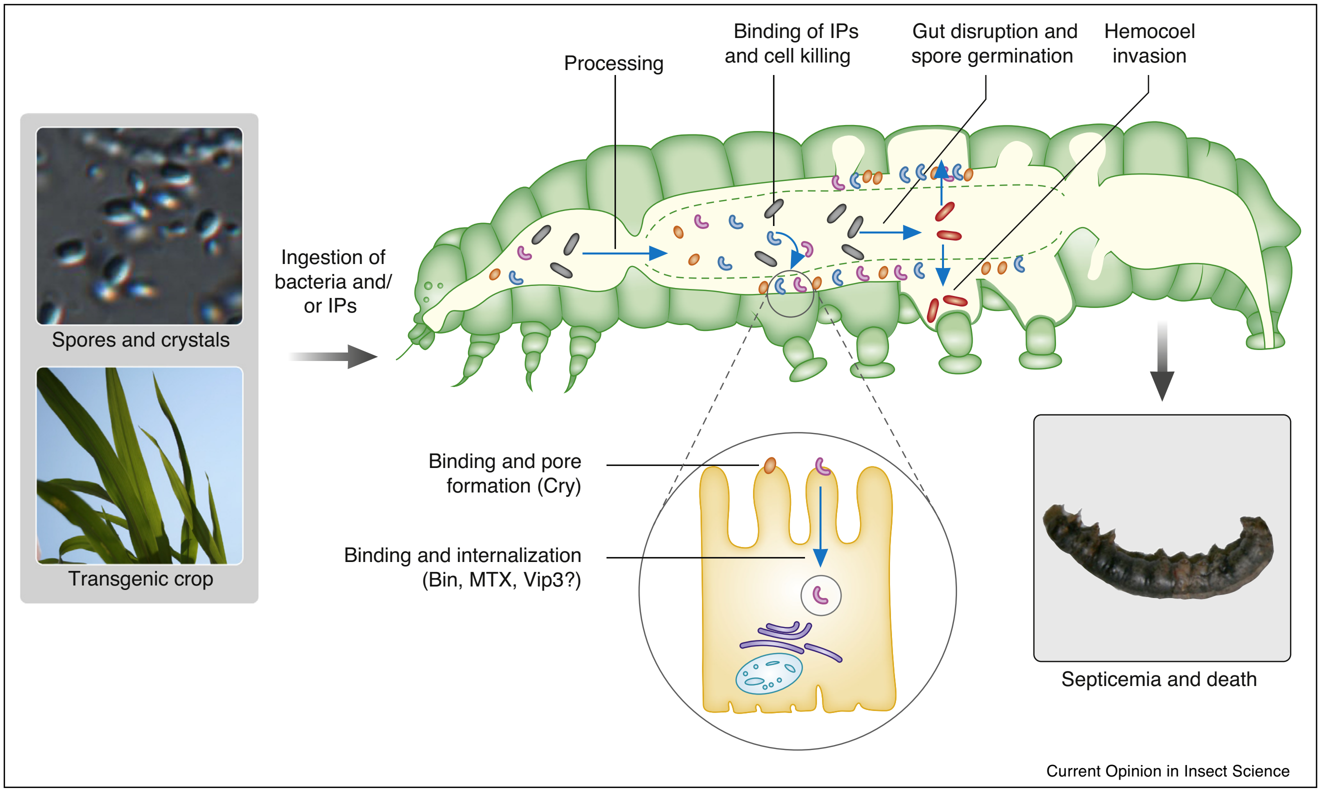
Most available information on resistance mechanisms to bacterial insecticides is limited to strains of *Bacillus thuringiensis* (*Bt*), as it accounts for about 90% of the available microbial pesticides. Insecticidal proteins (IPs) are the main virulence factor produced by *Bt*, and these IP genes have been cloned and transformed into plants (*Bt* crops) for increased efficacy and durability of control. Microbial products based on *Bt* subsp. *kurstaki* (*Btk*) and *aizawai* (*Bta*) for control of lepidopteran larvae, *Bt* subsp. *tenebrionis* (*Btt*) for control of coleopteran larvae, and *Bt* subsp. *israelensis* (*Bti*) and *Lysinibacillus sphaericus* (*Ls*, synonymous with *Bacillus sphaericus*) for control of dipteran larvae such as mosquitoes and black flies, are widely used microbial pesticides. Another commercialized gram-positive entomopathogenic bacterium is *Paenibacillus popilliae*, which was commercialized to control Japanese beetle (*Popillia japonica*). Examples of gram-negative entomopathogenic bacteria successfully developed commercially are *Serratia entomophila* strain 154 controlling coleopteran larvae [2], and *Yersinia entomophaga* which displays a broader range of activity [3]. This minireview will concentrate on resistance against *Bt* and *Ls* as models, and will include resistance to IPs produced by transgenic *Bt* crops. The goal of this work is to summarize current knowledge and identify the most relevant mechanism of resistance to entomopathogenic bacteria and *Bt* crops to guide strategies minimizing the risk of resistance evolution in the field.

Entomopathogenic bacteria mode of action

In general, many species of entomopathogenic bacteria share similar pathogenesis characterized by the production of multiple virulence factors, among which distinct insecticidal proteins (IPs) have a major role in lethality. Since commercialized entomopathogenic bacteria infect insects orally, these IPs bind to receptors on midgut cells, and are then internalized or form pores that kill the cell. Disruption of the gut epithelial barrier allows access of the bacteria to the nutrient-rich hemolymph, where they proliferate to cause host death by septicemia (Figure 1). Consequently, the IPs produced by a bacterium mostly dictate its specificity and that of the *Bt* crop producing the IP.

In the case of *Bt*, the bacterium produces IPs targeting midgut cells during the vegetative phase of growth (vegetative insecticidal proteins or Vip toxins) and as crystalline inclusions during the sporulation phase (crystal proteins or Cry toxins). Interestingly, targeting of midgut cells by Vip3 toxins seems to contradict the observation that insects are the optimal site for *Bt* spore germination. Accordingly, Vip toxins would be expected to occur in the

Figure 1



Integrative model of pathogensis for gram-positive entomopathogenic bacteria. The figure illustrates the steps in pathogenesis, detailing the interaction of insecticidal proteins (IPs) produced by the bacteria and target midgut cells. The two routes of intoxication described involving cell killing by membrane pore formation (in the case of Cry toxins) or internalization and apoptosis (Bin and MTX toxins) are shown. While resistance can emerge from alteration of any of the steps in pathogenesis, available evidence identifies prevention or reduction of IP binding to midgut receptors as the most relevant resistance mechanism to entomopathogenic bacteria, based on levels of resistance and frequency.

late stages of *Bt* pathogenesis, once the insect midgut barrier has been already disrupted by Cry toxins and the *Bt* spores have germinated to vegetative cells. Research is needed to confirm if midgut or alternative cells are the primary target of Vip toxins. To date, >700 *cry* toxin and 101 *vip3* genes, the only *vip* genes used for insect control in *Bt* crops, have been described, named and classified according to their amino acid sequence identity (*Bacillus thuringiensis* toxin nomenclature database; URL: http://www.btnomenclature.info/). Some *Bt* crystals also contain cytolytic (Cyt) proteins, which bind to midgut membrane lipids and cause detergent-like defects resulting in cytolysis.

Even though Vip3 and Cry toxins do not share sequence or three-dimensional structural features [4], they are both processed by host gut proteases, bind to distinct receptors and then form pores on the membrane of midgut cells [5,6]. A number of receptors, including aminopeptidase-N (APN), cadherin, alkaline phosphatase (ALP), ATP-binding cassette (ABC) transporters and glycolipids have been described as Cry toxin receptors [4]. Comparatively, less is known about the mode of action and receptors recognized by Vip3 toxins. Scavenger receptor-C has been described as a receptor mediating internalization of the Vip3Aa toxin [7••], which has also been shown to interact with ribosomal S2 protein [8]. The high levels of resistance and lack of cross-resistance to Cry toxins, reported for Vip3A-resistant insects [9•,10•,11], are suggestive of alterations in Vip3A toxin receptors as resistance mechanism, yet only alterations in proteolytic processing are reported for Vip3A-resistant insects [9•].

In the case of *Ls*, strains produce mosquitocidal toxins (MTX) in the vegetative phase and binary (Bin) proteins (BinA and BinB) during sporulation as main virulence factors targeting midgut cells. The mode of action of both MTX and Bin proteins involves binding to midgut cells and internalization to induce either ADP-ribosylation, as is the case of the best studied MTX1 toxin [12], or activation of caspases and apoptosis via an intrinsic or mitochondrial pathway, as described for Bin toxins [13]. Binding of Bin toxins to maltases as receptors in midgut cells is mediated by the BinB subunit [14], while the BinA subunit is traditionally considered responsible for toxicity by a yet uncharacterized mechanism. Similarly to Cyt toxins, MTX and Bin proteins synergize Cry toxicity and overcome resistance to Cry toxins [15,16]. Some *Ls* strains produce crystal inclusions containing the binary Cry48Aa/Cry49Aa toxins.

Resistance mechanisms to *Bt* and *Bt* crops in Lepidoptera

Resistance to *Bt* toxins in lepidopteran pests involves alterations in steps of the mode of action of an IP. Resistance was first described to *Btk* in the laboratory for a population of the Indian meal moth (*Plodia interpunctella*), a pyralid typically collected from storage bins [17]. In that case, resistance was associated with reduced binding of the main IP produced by *Btk* (Cry1Ab) [18]. Since then, a number of lepidopteran species especially in the family Noctuidae have shown the ability to develop resistance to diverse *Bt* toxins [19]. However, for yet unknown reasons, only the diamondback moth (*Plutella xylostella*) in the family Plutellidae has evolved resistance to *Bt* products under field conditions [20]. In contrast, three noctuids, and one species in the families Gelechiidae and Crambidae have developed field-evolved resistance to single IPs from *Bt* produced in *Bt* crops [21]. This more common type of resistance that evolves in response to *Bt* crops compared to *Bt* sprays can be explained by the presence of multiple IPs and virulence factors (including bacterial spores) in *Bt* formulations that are sprayed compared with *Bt* crops producing single IPs.

In the vast majority of *Bt* resistance cases in Lepidoptera, resistance is associated with reduced binding of at least one of the Cry toxins in the *Bt* formulation [19]. This type of resistance mechanism results in the highest levels of resistance reported to particular *Bt* formulations or IPs. When observed, cross-resistance in these cases involves IPs sharing binding sites with the most active Cry toxin in the *Bt* formulation, which is the expected selecting agent. This observation strongly supports the utility of binding site models to identify IPs that may be combined to delay resistance evolution [22]. This phenotype characterized by high levels of resistance and cross-resistance associated with reduced toxin binding is the most common resistance mechanism to *Bt* formulations and *Bt* crops producing IPs from *Bt*.

In rare examples, selection with a *Bt* toxin resulted in cross-resistance to toxins not sharing binding sites with the selective toxin [23,24]. As expected, in these cases IP binding in resistant insects is not affected and resistance is explained by alteration of a common step affecting the mode of action of the toxins. Examples include alterations in gut proteolytic activity [25], toxin sequestration [26] or enhanced midgut healing [27]. While these mechanisms lead to a broad resistance phenotype, they commonly result in comparatively lower levels of resistance [19], which may explain why they are not commonly observed in the field. This is especially true for *Bt* crops, as resistant insects must survive exposure to high levels of toxins produced by the plants, and explains why reduced toxin binding is the only mechanism described for resistance to *Bt* crops [28,29].

Resistance mechanisms to *Bt* and *Bt* crops in Diptera and Coleoptera

The *Bti* bacterium harvests a megaplasmid encoding Cry (Cry11Aa, Cry4BA, Cry4Aa, Cry10A) and Cyt (Cyt1Aa, Cyt1Ca, Cyt2Ba) proteins [30]. Unfortunately, quantitative binding competition analyses and binding site models determining sharing of receptors between *Bti* toxins are not available for mosquito larvae. However, available information on functional receptors for mosquitocidal toxins [31], and cross-resistance in mosquitoes resistant to a single *Bti* toxin [32] suggest that *Bti* IPs share at least some binding sites. Consequently, one would expect resistance to multiple *Bti* toxins to evolve easily by alteration of a single receptor, yet resistance to *Bti* is rare and limited to selection in the laboratory environment. One of the reasons for this observation is the synergism of Cry toxicity by the Cyt1Aa protein serving as a surrogate receptor [33], which hinders evolution of resistance to *Bti* through reduced toxin binding. In fact, in mosquitoes and beetles, Cyt1 proteins synergize and overcome resistance to Cry toxins and are currently being explored as tools to delay resistance evolution [34,35,36••].

Pesticides based on formulations of *Btt* contain the Cry3Aa toxin, which is highly effective against larvae of selected Coleoptera. Selection with *Btt* resulted in resistance associated with reduced Cry3Aa toxin binding in Colorado potato beetle (*Leptinotarsa decemlineata*) [37]. Similarly, resistance to Cry3Aa in the cottonwood leaf beetle (*Chrysomela scripta*) was suppressed by Cyt1Aa [35], suggestive of a resistance mechanism involving reduced binding. Transgenic corn producing Cry3Bb and Cry3Aa-derived toxins, and/or the binary Cry34/Cry35 protein targets Western corn rootworm (*Diabrotica virgifera virgifera*) larvae. Field-evolved resistance and cross-resistance has been observed against Cry3Bb and Cry3A-derived toxins, but not to the binary Cry34/35 toxins produced in *Bt* corn. Since Cry3 and Cry34/35 toxins do not share binding sites, this observation suggests (although it has not been experimentally tested) that altered receptors may be involved in resistance [38••]. However, the modest levels of resistance to Bt corn (<20-fold) in rootworm compared to lepidopteran pests, may suggest receptor-independent resistance. Unfortunately, there is no available empirical evidence for reduced IP binding in resistant rootworms or for identification of the mechanism involved in rootworm resistance.

Genes involved in receptor-mediated resistance to Cry toxins

Identification of the genes and alleles responsible for reduced toxin binding has been mostly done with laboratory-selected insects. Mutations or disruptions in cadherin genes resulting in truncated cadherin proteins or reduced presence of the cadherin protein on the membrane are linked to reduced binding and resistance against Cry1Ac in laboratory-selected *Heliothis virescens* [39] and in field-derived *Helicoverpa armigera* [40,41] and *Pectinophora gossypiella* [42,43]. Alternative cadherin splicing was associated with field-evolved resistance and reduced Cry1Ac binding in *P. gossypiella* from India that were resistant to *Bt* cotton [28,44]. In all these cases, resistance was transmitted as a recessive trait, yet non-recessive resistance linked to a cadherin mutation was reported in a field-derived strain of *H. armigera*, yet it was not associated with reduced Cry1Ac binding but with altered unknown post-binding steps [45].

Mutations in ABC transporter genes are linked with high levels of resistance to Cry1Ac in *H. virescens* [46] and *H. armigera* [47], to Cry1Ab in *Bombyx mori* [48], to Cry2Ab in *H. armigera* and *Helicoverpa punctigera* [49], to *Bt* corn producing the Cry1F toxin in *Spodoptera frugiperda* from Puerto Rico [50••], and to transgenic poplar producing the Cry3Aa toxin in *Chrysomela tremulae* [51]. Resistance to Cry2Ab in *P. gossypiella* is associated to ABC transporter mis-splicing [52••]. When tested, reduced toxin binding was detected in all these cases, except in cases where alternative, putatively non-functional receptors, were available [48]. Importantly, these alterations in ABC transporters may affect their role in detoxification processes and result in increased susceptibility to alternative pesticides [53•], and unintended yet positive effect of resistance to *Bt* that could be helpful for resistance management.

Resistance to Cry1Ac in *Trichoplusia ni* and *P. xylostella*, and to Cry1Ca in *Spodoptera exigua* was mapped to an ABC transporter gene locus [54,55], although in these cases resistance implies trans-regulatory reduction in expression of APN and ALP as putative receptor genes [56–58]. Reduced levels of an ALP in the midgut were also associated to resistance against Cry1Ac in *H. virescens* [59], *Helicoverpa zea* [60], and in *S. frugiperda* with field-evolved resistance to Cry1F [61]. However, no strong genetic linkage between reduced ALP levels and resistance is observed [50••]. These observations suggest the existence of multiple genes that may have major and/or minor contributions to resistance.

While all cases discussed above involved recessive or near-recessive resistance to *Bt* toxins, a mechanism for dominant resistance to Cry1Ac has been recently reported for field-derived *H. armigera* [62••]. In this case, resistance was linked to a mutation in a tetraspanin gene, supporting the need for research to identify the role of this protein in the mode of action of Cry1Ac and potentially other *Bt* toxins.

Resistance mechanisms to *L. sphaericus*

Commonly used microbial pesticides based on *Ls* contain spores and the Bin toxin produced by the bacterium. There are examples of laboratory and field-evolved resistance to *Ls* formulations in mosquito populations worldwide [63]. This high relative propensity to resistance evolution compared to *Bti* is due to a major toxin (Bin) targeting a single receptor (maltases) being the main *Ls* virulence factor. This observation also explains why resistance to *Ls* and Bin toxin is commonly associated with reduced toxin binding and mutations affecting toxin receptors [64]. Neither Mtx nor Cry48Aa/Cry49Aa toxins recognize Bin receptors, and are active against *Ls*-resistant mosquito larvae [65,66]. As the only known exception, the mechanism of high levels of field-evolved resistance in West Mediterranean *Culex pipiens* populations is sex-linked and independent of reduced Bin binding or improper digestion of the toxin [67], indicating the existence of alternative, unknown resistance mechanisms.

Final considerations and conclusions

The body of evidence in the literature strongly supports the fact that resistance to commercialized entomopathogenic bacteria in the family *Bacillaceae* targets disruption of IP binding. Accordingly, the use of mixtures of IPs recognizing diverse receptors significantly delays the risk of high levels of resistance and is the current resistance prevention tactic for *Bt* crops. However, evidence also supports that this tactic reduces but does not completely eliminate the risk of resistance evolution by receptor-independent mechanisms. In the case of Bt toxins, development of Cyt and peptides enhancing toxin binding should contribute to delay resistance evolution. Receptor-mediated resistance is recessive and autosomal, except for very rare exceptions of dominant resistance mechanisms for which more information is urgently needed.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Kohl J, Marrone P, Morin L, Stewart A: **Have biopesticides come of age?** *Trends Biotechnol* 2012, **30**:250-258.

2. Jackson TA, Pearson JF, O'Callaghan M, Mahanty HK, Willcocks MJ: **Pathogen to product - development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological agent for the New Zealand grass grub (*Costelytra zealandica*).** In *Use of Pathogens in Scarab Pest Management.* Edited by Jackson TA, Glare TR. Intercept Press; 1992:191-198.

3. Hurst MR, Becher SA, Young SD, Nelson TL, Glare TR: ***Yersinia entomophaga*** **sp. nov., isolated from the New Zealand grass grub *Costelytra zealandica***. *Int J Syst Evol Microbiol* 2011, **61**:844-849.

4. Adang M, Crickmore N, Jurat-Fuentes JL: **Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action**. In *Advances in Insect Physiology Vol 47: Insect Midgut and Insecticidal Proteins.* Edited by Dhadialla TS, Gill S. Academic Press; 2014:39-87.

5. Yu CG, Mullins MA, Warren GW, Koziel MG, Estruch JJ: **The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects**. *Appl Environ Microbiol* 1997, **63**:532-536.

6. Lee MK, Walters FS, Hart H, Palekar N, Chen JS: **The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab delta-endotoxin**. *Appl Environ Microbiol* 2003, **69**:4648-4657.

7. •• Jiang K, Hou X-y, Tan T-t, Cao Z-l, Mei S-q, Yan B, Chang J, Han L, Zhao D, Cai J: **Scavenger receptor-C acts as a receptor for *Bacillus thuringiensis* vegetative insecticidal protein Vip3Aa and mediates the internalization of Vip3Aa via endocytosis**. *PLoS Pathog* 2018, **14**:e1007347.

Presents strong experimental evidence, including gene silencing and expression in transgenic *Drosophila*, identifying a functional receptor for Vip3Aa in *Spodoptera frugiperda*. It also provides evidence for internalization as relevant to Vip3Aa mode of action.

8. Singh G, Sachdev B, Sharma N, Seth R, Bhatnagar RK: **Interaction of *Bacillus thuringiensis* vegetative insecticidal protein with ribosomal S2 protein triggers larvicidal activity in *Spodoptera frugiperda***. *Appl Environ Microbiol* 2010, **76**:7202-7209.

9. • Chakroun M, Banyuls N, Walsh T, Downes S, James B, Ferre J: **Characterization of the resistance to Vip3Aa in *Helicoverpa armigera* from Australia and the role of midgut processing and receptor binding**. *Sci Rep* 2016, **6**:24311.

It describes isolation of Vip3Aa-resistance allele from the field and presents evidence supporting that reduced toxin binding is not involved in resistance. Presents preliminary evidence that slower processing of the toxin may be involved in resistance.

10. • Yang F, Morsello S, Head GP, Sansone C, Huang F, Gilreath RT, Kerns DL: **F2 screen, inheritance and cross-resistance of field-derived Vip3A resistance in Spodoptera frugiperda (Lepidoptera: Noctuidae) collected from Louisiana, USA**. *Pest Manag Sci* 2018, **74**:1769-1778.

This work presents data supporting that despite lack of documented resistance cases, alleles for high levels of resistance to Vip3A in fall armyworm are present in southern USA and may be detected using an F2 screen.

11. Pickett BR, Gulzar A, Ferre J, Wright DJ: ***Bacillus thuringiensis*** **Vip3Aa toxin resistance in *Heliothis virescens* (Lepidoptera: Noctuidae)**. *Appl Environ Microbiol* 2017, **83**.

12. Thanabalu T, Berry C, Hindley J: **Cytotoxicity and ADP-ribosylating activity of the mosquitocidal toxin from *Bacillus sphaericus* SSII-1: possible roles of the 27- and 70-kilodalton peptides**. *J Bacteriol* 1993, **175**:2314-2320.

13. Tangsongcharoen C, Chomanee N, Promdonkoy B, Boonserm P: ***Lysinibacillus sphaericus*** **binary toxin induces apoptosis in susceptible *Culex quinquefasciatus* larvae**. *J Invertebr Pathol* 2015, **128**:57-63.

14. Darboux I, Nielsen-Leroux C, Charles J-F, Pauron D: **The receptor of *Bacillus sphaericus* binary toxin in *Culex pipiens* (Diptera: Culicidae) midgut: molecular cloning and expression**. *Insect Biochem Mol Biol* 2001, **31**:981-990.

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

16. Wirth MC, Jiannino JA, Federici BA, Walton WE: **Synergy between toxins of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus***. *J Med Entomol* 2004, **41**:935-941.

17. McGaughey WH: **Insect resistance to the biological insecticide *Bacillus thuringiensis***. *Science* 1985, **229**:193-195.

18. Van Rie J, McGaughey WH, Johnson DE, Barnett BD, Van Mellaert H: **Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis***. *Science* 1990, **247**:72-74.

19. Ferré J, Van Rie J: **Biochemistry and genetics of insect resistance to *Bacillus thuringiensis***. *Annu Rev Entomol* 2002, **47**:501-533.

20. Tabashnik BE, Groeters FR, Finson N, Liu Y-B, Johnson MW, Heckel DG, Luo K, Adang MJ: **Resistance to *Bacillus thuringiensis* in *Plutella xylostella*: the moth heard round the world**. In *Molecular Genetics and Evolution of Pesticide Resistance.* Edited by Brown TM. American Chemical Society; 1996:130-140. ACS Symposium Series 645.

21. Tabashnik BE, Carriere Y: **Surge in insect resistance to transgenic crops and prospects for sustainability**. *Nat Biotechnol* 2017, **35**:926-935.

22. Jakka SR, Ferré J, Jurat-Fuentes JL: **Cry toxin binding sites and their use in strategies to delay resistance evolution**. In *Bt Resistance - Characterization and Strategies for GM Crops Producing Bacillus thuringiensis Toxins.* Edited by Soberón M, Gao Y, Bravo A. CAB International; 2015.

23. Moar WJ, Pusztai-Carey M, Van Fassen H, Bosch D, Frutos R, Rang C, Luo K, Adang MJ: **Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae)**. *Appl Environ Microbiol* 1995, **61**:2086-2092.

24. Gould F, Martinez-Ramirez A, Anderson A, Ferre J, Silva FJ, Moar WJ: **Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens***. *Proc Natl Acad Sci U S A* 1992, **89**:7986-7990.

25. Oppert B, Kramer KJ, Beeman RW, Johnson D, McGaughey WH: **Proteinase-mediated insect resistance to *Bacillus thuringiensis* toxins**. *J Biol Chem* 1997, **272**:23473-23476.

26. Gunning RV, Dang HT, Kemp FC, Nicholson IC, Moores GD: **New resistance mechanism in *Helicoverpa armigera* threatens transgenic crops expressing *Bacillus thuringiensis* Cry1Ac toxin**. *Appl Environ Microbiol* 2005, **71**:2558-2563.

27. Forcada C, Alcacer E, Garcera MD, Tato A, Martinez R: **Resistance to *Bacillus thuringiensis* Cry1Ac toxin in three strains of *Heliothis virescens*: proteolytic and SEM study of the larval midgut**. *Arch Insect Biochem Physiol* 1999, **42**:51-63.

28. Ojha A, Sree KS, Sachdev B, Rashmi MA, Ravi KC, Suresh PJ, Mohan KS, Bhatnagar RK: **Analysis of resistance to Cry1Ac in field-collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera:Gelechiidae), populations**. *GM Crops Food* 2014, **5**:280-286.

29. Jakka SR, Gong L, Hasler J, Banerjee R, Sheets JJ, Narva K, Blanco CA, Jurat-Fuentes JL: **Field-evolved Mode 1 resistance of the fall armyworm to transgenic Cry1Fa-expressing corn associated with reduced Cry1Fa toxin binding and midgut alkaline phosphatase expression**. *Appl Environ Microbiol* 2015, **82**:1023-1034.

30. Berry C, O'Neil S, Ben-Dov E, Jones AF, Murphy L, Quail MA, Holden MT, Harris D, Zaritsky A, Parkhill J: **Complete sequence and organization of pBtoxis, the toxin-coding plasmid of *Bacillus thuringiensis* subsp. *israelensis***. *Appl Environ Microbiol* 2002, **68**:5082-5095.

31. Zhang Q, Hua G, Adang MJ: **Effects and mechanisms of *Bacillus thuringiensis* crystal toxins for mosquito larvae**. *Insect Sci* 2017, **24**:714-729.

32. Lee SB, Aimanova KG, Gill SS: **Alkaline phosphatases and aminopeptidases are altered in a Cry11Aa resistant strain of *Aedes aegypti***. *Insect Biochem Mol Biol* 2014, **54**:112-121.

33. Pérez C, Fernandez LE, Sun J, Folch JL, Gill SS, Soberón M, Bravo A: ***Bacillus thuringiensis*** **subsp. *israelensis* Cyt1Aa synergizes Cry11Aa toxin by functioning as a membrane-bound receptor**. *Proc Natl Acad Sci U S A* 2005, **102**:18303-18308.

34. Wirth MC, Georghiou GP, Federici BA: **CytA enables CryIV endotoxins of *Bacillus thuringiensis* to overcome high levels

of CryIV resistance in the mosquito, *Culex quinquefasciatus*. *Proc Natl Acad Sci U S A* 1997, **94**:10536-10540.

35. Federici BA, Bauer LS: **Cyt1Aa protein of *Bacillus thuringiensis* is toxic to the cottonwood leaf beetle, *Chrysomela scripta*, and suppresses high levels of resistance to Cry3Aa**. *Appl Environ Microbiol* 1998, **64**:4368-4371.

36. •• Bravo A, Lopez-Diaz JA, Yamamoto T, Harding K, Zhao JZ, Mendoza G, Onofre J, Torres-Quintero MC, Nelson ME, Wu G *et al.*: **Susceptible and mCry3A resistant corn rootworm larvae killed by a non-hemolytic *Bacillus thuringiensis* Cyt1Aa mutant**. *Sci Rep* 2018, **8**:17805.

Describes development of Cyt1Aa mutants with increased activity against mosquito and rootworm larvae. Importantly, it demonstrates that insecticidal and hemolytic activities in Cyt1Aa are independent, highlighting the potential for using non-hemolytic Cyt1Aa for insect control, including mCry3A-resistant rootworms.

37. Whalon ME, Miller DL, Hollingworth RM, Grafius EJ, Miller JR: **Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis***. *J Econ Entomol* 1993, **86**:226-233.

38. •• Jakka SR, Shrestha RB, Gassmann AJ: **Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*)**. *Sci Rep* 2016, **6**:27860.

Presents evidence of field-evolved broad-spectrum resistance in rootworm to all except for one of the Cry toxins produced by Bt corn, raising concerns on the durability of Bt corn for rootworm management.

39. Gahan LJ, Gould F, Heckel DG: **Identification of a gene associated with Bt resistance in *Heliothis virescens***. *Science* 2001, **293**:857-860.

40. Xu X, Yu L, Wu Y: **Disruption of a cadherin gene associated with resistance to Cry1Ac d-endotoxin of *Bacillus thuringiensis* in *Helicoverpa armigera***. *Appl Environ Microbiol* 2005, **71**:948-954.

41. Yang Y, Chen H, Wu Y, Yang Y, Wu S: **Mutated cadherin alleles from a field population of *Helicoverpa armigera* confer resistance to *Bacillus thuringiensis* toxin Cry1Ac**. *Appl Environ Microbiol* 2007, **73**:6939-6944.

42. Wang L, Ma Y, Wan P, Liu K, Xiao Y, Wang J, Cong S, Xu D, Wu K, Fabrick JA *et al.*: **Resistance to *Bacillus thuringiensis* linked with a cadherin transmembrane mutation affecting cellular trafficking in pink bollworm from China**. *Insect Biochem Mol Biol* 2018, **94**:28-35.

43. Morin S, Biggs RW, Sisterson MS, Shriver L, Ellers-Kirk C, Higginson D, Holley D, Gahan LJ, Heckel DG, Carrière Y *et al.*: **Three cadherin alleles associated with resistance to *Bacillus thuringiensis* in pink bollworm**. *Proc Natl Acad Sci U S A* 2003, **100**:5004-5009.

44. Fabrick JA, Ponnuraj J, Singh A, Tanwar RK, Unnithan GC, Yelich AJ, Li X, Carriere Y, Tabashnik BE: **Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to Bt cotton in India**. *PLoS One* 2014, **9**:e97900.

45. Zhang H, Wu S, Yang Y, Tabashnik BE, Wu Y: **Non-recessive Bt toxin resistance conferred by an intracellular cadherin mutation in field-selected populations of cotton bollworm**. *PLoS One* 2012, **7**:e53418.

46. Gahan LJ, Pauchet Y, Vogel H, Heckel DG: **An ABC transporter mutation is correlated with insect resistance to *Bacillus thuringiensis* Cry1Ac toxin**. *PLoS Genet* 2010, **6**:e1001248.

47. Xiao Y, Zhang T, Liu C, Heckel DG, Li X, Tabashnik BE, Wu K: **Mis-splicing of the ABCC2 gene linked with Bt toxin resistance in *Helicoverpa armigera***. *Sci Rep* 2014, **4**:6184.

48. Atsumi S, Miyamoto K, Yamamoto K, Narukawa J, Kawai S, Sezutsu H, Kobayashi I, Uchino K, Tamura T, Mita K *et al.*: **Single amino acid mutation in an ATP-binding cassette transporter gene causes resistance to Bt toxin Cry1Ab in the silkworm, *Bombyx mori***. *Proc Natl Acad Sci U S A* 2012, **109**:E1591-E1598.

49. Tay WT, Mahon RJ, Heckel DG, Walsh TK, Downes S, James WJ, Lee SF, Reineke A, Williams AK, Gordon KH: **Insect resistance to *Bacillus thuringiensis* toxin Cry2Ab is conferred by mutations in an ABC transporter subfamily A protein**. *PLoS Genet* 2015, **11**:e1005534.

50. •• Banerjee R, Hasler J, Meagher R, Nagoshi R, Hietala L, Huang F, Narva K, Jurat-Fuentes JL: **Mechanism and DNA-based detection of field-evolved resistance to transgenic Bt corn in fall armyworm (*Spodoptera frugiperda*)**. *Sci Rep* 2017, **7**:10877.

First mechanistic description of an allele responsible for high levels of field-evolved resistance to a transgenic *Bt* crop that led to voluntary withdrawal from the market. It also presents the first successful demonstration of using DNA-based screening to monitor the frequency of alleles for resistance to a *Bt* toxin, in this case at migratory *S. frugiperda* destinations.

51. Pauchet Y, Bretschneider A, Agustin S, Heckel DG: **A p-glycoprotein is linked to resistance to the *Bacillus thuringiensis* Cry3Aa toxin in a leaf beetle**. *Toxins (Basel)* 2016, **8**:362.

52. •• Mathew LG, Ponnuraj J, Mallappa B, Chowdary LR, Zhang J, Tay WT, Walsh TK, Gordon KHJ, Heckel DG, Downes S *et al.*: **ABC transporter mis-splicing associated with resistance to Bt toxin Cry2Ab in laboratory- and field-selected pink bollworm**. *Sci Rep* 2018, **8**:13531.

Identifies ABCA2 genes as responsible for laboratory-selected resistance in US and field-evolved resistance in India to Cry2Ab in pink bollworm. This information facilitates DNA-based monitoring for resistance to Cry2Ab in India, where pink bollworm resistance to Cry1Ac and Cry2Ab cotton has already resulted in severe economic losses.

53. • Xiao Y, Liu K, Zhang D, Gong L, He F, Soberon M, Bravo A, Tabashnik BE, Wu K: **Resistance to *Bacillus thuringiensis* mediated by an ABC transporter mutation increases susceptibility to toxins from other Bacteria in an invasive insect**. *PLoS Pathog* 2016, **12**:e1005450.

Presents evidence of negative cross-resistance between Cry1Ac and pesticides from alternative bacteria, which may be used for resistance management.

54. Baxter SW, Badenes-Perez FR, Morrison A, Vogel H, Crickmore N, Kain W, Wang P, Heckel DG, Jiggins CD: **Parallel evolution of *Bacillus thuringiensis* toxin resistance in Lepidoptera**. *Genetics* 2011, **189**:675-679.

55. Park Y, Gonzalez-Martinez RM, Navarro-Cerrillo G, Chakroun M, Kim Y, Ziarsolo P, Blanca J, Canizares J, Ferre J, Herrero S: **ABCC transporters mediate insect resistance to multiple Bt toxins revealed by bulk segregant analysis**. *BMC Biol* 2014, **12**:46.

56. Guo Z, Kang S, Chen D, Wu Q, Wang S, Xie W, Zhu X, Baxter SW, Zhou X, Jurat-Fuentes JL *et al.*: **MAPK signaling pathway alters expression of midgut ALP and ABCC genes and causes resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamondback moth**. *PLoS Genet* 2015, **11**:e1005124.

57. Tiewsiri K, Wang P: **Differential alteration of two aminopeptidases N associated with resistance to *Bacillus thuringiensis* toxin Cry1Ac in cabbage looper**. *Proc Natl Acad Sci U S A* 2011, **108**:14037-14042.

58. Herrero S, Gechev T, Bakker PL, Moar WJ, de Maagd RA: ***Bacillus thuringiensis* Cry1Ca-resistant *Spodoptera exigua* lacks expression of one of four aminopeptidase N genes**. *BMC Genomics* 2005, **6**.

59. Jurat-Fuentes JL, Karumbaiah L, Jakka SR, Ning C, Liu C, Wu K, Jackson J, Gould F, Blanco C, Portilla M *et al.*: **Reduced levels of membrane-bound alkaline phosphatase are common to lepidopteran strains resistant to Cry toxins from *Bacillus thuringiensis***. *PLoS One* 2011, **6**:e17606.

60. Caccia S, Moar WJ, Chandrashekhar J, Oppert C, Anilkumar KJ, Jurat-Fuentes JL, Ferre J: **Association of Cry1Ac toxin resistance in *Helicoverpa zea* (Boddie) with increased alkaline phosphatase levels in the midgut lumen**. *Appl Environ Microbiol* 2012, **78**:5690-5698.

61. Jakka SRK, Gong L, Hasler J, Banerjee R, Sheets JJ, Narva K, Blanco CA, Jurat-Fuentes JL: **Field-evolved Mode 1 resistance of the fall armyworm to transgenic Cry1Fa-expressing corn associated with reduced Cry1Fa toxin binding and midgut alkaline phosphatase expression**. *Appl Environ Microbiol* 2016, **82**:1023-1034.

62. •• Jin L, Wang J, Guan F, Zhang J, Yu S, Liu S, Xue Y, Li L, Wu S, Wang X *et al.*: **Dominant point mutation in a tetraspanin gene associated with field-evolved resistance of cotton bollworm**

to transgenic Bt cotton. *Proc Natl Acad Sci U S A* 2018, **115**:11760-11765.

First identification of a field-derived dominant resistance allele to a *Bt* toxin. This work further demonstrates the power of using genomic analysis combined with gene editing and DNA-based field monitoring to identify and track resistance alleles, providing a serious warning for resistance evolution and spread in China. It also represents the first description of tetraspanin as a gene involved in Cry1Ac mode of action, making the case for additional research to understand the role of tetraspanin in Cry1Ac intoxication.

63. Silva Filha MHNL, Berry C, Regis L: **Chapter three - Lysinibacillus sphaericus: toxins and mode of action, applications for mosquito control and resistance management**. In *Advances in Insect Physiology*, , vol 47. Edited by Dhadialla TS, Gill SS. Academic Press; 2014:89-176.

64. Ferreira LM, Silva-Filha MHNL: **Bacterial larvicides for vector control: mode of action of toxins and implications for resistance**. *Biocontrol Sci Technol* 2013, **23**:1137-1168.

65. Wei SZ, Cai QX, Cai YJ, Yuan ZM: **Lack of cross-resistance to Mtx1 from *Bacillus sphaericus* in *B. sphaericus*-resistant *Culex quinquefasciatus* (Diptera: Culicidae)**. *Pest Manage Sci* 2007, **63**:190-193.

66. de Melo JV, Jones GW, Berry C, Vasconcelos RH, de Oliveira CM, Furtado AF, Peixoto CA, Silva-Filha MH: **Cytopathological effects of *Bacillus sphaericus* Cry48Aa/Cry49Aa toxin on binary toxin-susceptible and -resistant *Culex quinquefasciatus* larvae**. *Appl Environ Microbiol* 2009, **75**:4782-4789.

67. Nielsen-Leroux C, Pasquier F, Charles JF, Sinegre G, Gaven B, Pasteur N: **Resistance to *Bacillus sphaericus* involves different mechanisms in *Culex pipiens* (Diptera:Culicidae) larvae**. *J Med Entomol* 1997, **34**:321-327.