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Current Opinion in Insect Science

Microbiome toxicology — bacterial activation and detoxification of insecticidal compounds

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Insect gut bacteria have been implicated in a myriad of physiological processes from nutrient supplementation to pathogen protection. In fact, symbiont-mediated insecticide degradation has helped explain sudden control failure in the field to a range of active ingredients. The mechanisms behind the loss of susceptibility are varied based on host, symbiont, and insecticide identity. However, while some symbionts directly break down pesticides, others modulate endogenous host detoxification pathways or involve reciprocal degradation of insecticidal and bactericidal compounds both inspiring new questions and requiring the reexamination of past conclusions. Good steward of the chemical pesticide arsenal requires consideration of these ecological interactions from development to deployment.

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Introduction

Insecticide resistance is a perennial concern. Dependence on relatively few active ingredients, poor pest management strategies, laborious processes for development, and approval of new chemistries creates the ideal conditions for increasingly tolerant target insects. However, the targeted insects are not the only organisms exposed to these compounds. Insect symbionts are subjected to the environment their hosts inhabit and the environments their hosts create in their guts. In the last decade, the appreciation for symbiont-mediated pesticide tolerance has revealed that bacteria are often mediating the resistance phenotypes [1–4].

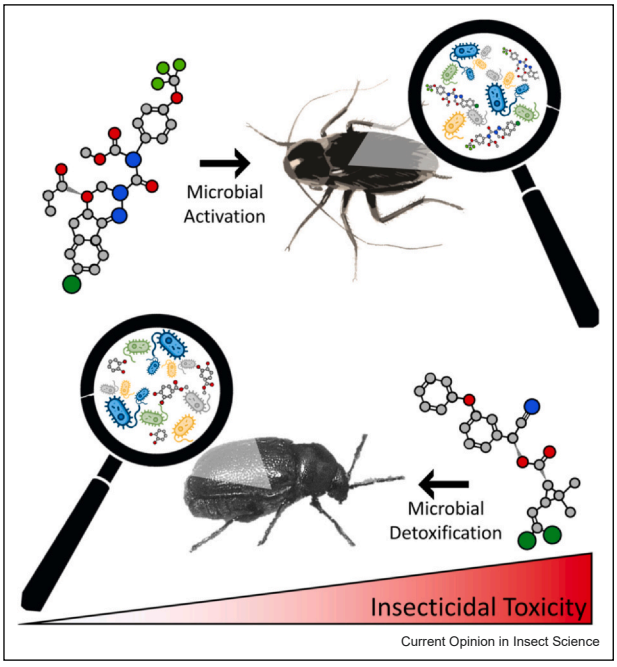
Bacteria often interact directly with pesticides intended to target their host insects. Some bacterial symbionts enzymatically transform insecticidal compounds often using them as a carbon source. This biotransformation typically works in one of two directions: neutralization (the pesticide is detoxified and can be excreted) [5–7] or enhanced toxicity (the by-products are more toxic than the parent compound) [8–13] (Figure 1). An insect symbiont may be solely responsible for the metabolism of a pesticide, but in many cases, the resistance phenotype is the result of an interaction between host and symbiont [14,15]. As with all ecological interactions, this is an oversimplification based on observable phenotypes. Additionally, there is evidence that gut bacteria can modulate host-endogenous detoxification mechanisms [16,17]. Independently, each mechanism expands our understanding of the ways microbiota influence the dynamics between insect and insecticide.

Herein, I will highlight the ways in which insect-associated microbes have been documented to influence pesticide action. Specifically, this paper will explore ways that symbionts influence the outcomes of their hosts' contact with pesticides, including direct and indirect detoxification, activation, and outcomes dependent on condition. The goal is to articulate the ways bacterial symbionts interfere and have the potential to interfere with insecticide activity.

Direct insecticidal detoxification by gut bacteria

Several recent reviews have highlighted the impact of symbiont-mediated insecticide detoxification across insects and the direct role that bacteria play in mitigating the toxic effect of these chemicals [1–4]. Since the discovery of a fenitrothion-degrading symbiont in the bean bug [18], a global pattern has emerged where insecticide-resistant crop pests are associated with individuals or communities of bacteria that degrade various active ingredients. The ability to identify and cultivate specific bacteria taxa responsible for the biotransformation of insecticidal compounds is challenging. In one such study, gut bacteria isolated from the stored product pests *Sitophilus oryzae*, *Cryptolestes ferrugineus*, and *Rhyzopertha dominica* demonstrated insecticide tolerance to the same three active ingredients as their hosts [7]. When each host was monoassociated with individual isolates of bacteria, their insecticidal tolerance was either completely restored (similar to control animals) or intermediate to control and

Figure 1



Gut microbes associated with target insects can interact with insecticides leading to a variety of outcomes. Top: Indoxacarb appears to be activated by microbes in the German cockroach gut likely leading to the formation of metabolites with increased insecticidal activity, such as ring-open indoxacarb and DCJW. Bottom: In the leaf beetle, beta-cypermethrin is degraded into less-toxic metabolites, catechol and protocatechuate, by gut bacteria.

gnotobiotic animals [7]. While not feasible in all systems, this reinoculation approach is a powerful tool to demonstrate which bacterial degradation capabilities are performed independent of their host and how much insecticide tolerance can be attributed to individual isolates within the gut community.

When investigating symbiont-mediated pesticide detoxification, it is important to consider what is known about the role for the symbiont in that system. Many bacterial symbionts are important for nutrient supplementation and the removal of the symbiont even in the absence of pesticide can have deleterious effects. This is the case in bedbugs, where their symbiotic bacteria are important for vitamin-B biosynthesis. Therefore, the careful experimental approach taken by Soh and Singham is laudable [5]. *Cimex hemipterus* treated with antibiotics (and supplemented with vitamin B) demonstrated a loss of tolerance to fenitrothion and imidacloprid that was rescued through reinoculation [5].

Host-symbiont collaboration yields a resistant phenotype

In several studies, antibiotic treatments correlate the presence of gut microbiota with insecticide resistance, leading to the conclusion that microbes are directly degrading these chemicals. However, a growing body of work suggests that symbioses may mask the physiological complexity and cross-talk necessary for pesticide detoxification. In several systems, the gut symbionts may indirectly contribute to pesticide degradation by regulating host-endogenous detoxification mechanisms, such as cytochrome oxidase P450s (P450s) [16,17,19,20]. This masks the direct mechanism of degradation and may manifest as variation across populations given microbiome variability [19].

A simpler of this is found in the vector mosquito, *Aedes albopictus*. At first glance, resistance to deltamethrin is linked to relative abundance of a gut bacterium *Serratia oryzae*. High titers of this bacterium yield a resistant phenotype, and *in vitro*, this bacterium is capable of pesticide turnover. However, with closer examination, Wang and colleagues connected the abundance of this bacterium with an upregulation of a suite of detoxification genes, including cytochrome oxidase P450s (P450s), glutathione-S-transferases (GSTs), and carboxyltransferases (CarEs) [21].

Even still, enzymatic collaboration may underlie insecticide degradation. In *Drosophila melanogaster*, the degradation of imidacloprid is partitioned with the by-product of nitroreduction being produced by bacteria and by-products of oxidation being produced by endogenous P450s [22]. The degradation of beta-cypermethrin is similarly partitioned by the leaf beetle and bacteria in its gut. Endogenous leaf beetle CarEs and P450s join forces with bacterially contributed GSTs and catalases to form intermediates that are subsequently further cleaved [15].

In a similar vein, it seems that the association with certain bacteria can yield beneficial phenotypes and modulate host metabolism. *Tribolium castaneum*, the stored product pest, is another model insect able to be reared in sterile and gnotobiotic conditions. One study showed that *T. castaneum* associated with a gut isolate of *Bacillus cereus* and *Achromobacter xylosoxidans* was more tolerant to three active ingredients, malathion, pirimiphosmethyl, and deltamethrin. Additionally, these isolates conferred fitness benefits to their hosts compared with sterile *T. castaneum* [23]. This study also investigated the expression of several host detoxification pathways, including P450s, GSTs, and carboxylesterases across normally faunated, sterile, and monoassociated red flour beetles. Of special note, *T. castaneum* adults monoassociated with *B. cereus* often had higher expression of host-endogenous detoxification pathways, P450s and CarEs, than the normally faunated control, indicating cross-talk between a single symbiont and its insect host [23].

One quintessential example of symbiont-mediated pesticide biotransformation occurs in the bean bug. Historically,

we assumed this a simple example of degradation of the insecticide fenitrothion by *Burkholderia* residing in the crypts of the host [18]. While the bacterial partner does neutralize fenitrothion's insecticidal activity, the metabolite 3-methyl-4-nitrophenol is highly bactericidal; in turn, the insect host rapidly excretes 3-methyl-4-nitrophenol [14]. Therefore, the resistance phenotype requires host and symbiont to synchronize enzymatically. Considering that many of the bug-*Burkholderia* symbioses are facultative and environmentally acquired, this phenomenon is nothing short of remarkable. It is noteworthy, and that the ease with which these symbioses, and no doubt others, can be acquired means that an insect population can shift from susceptible to resistant in a single-field season after associating with a soil microbe [24].

Bacterial activation increases the toxicity of insecticides

Bacterial metabolism of insecticides does not always benefit the host. In some cases, the metabolites generated in the breakdown of a pesticide may be just as toxic or more toxic than the parent compound. The best-known insecticidal protoxin is likely *Cry* toxin that has been bioengineered into crops such as corn and cotton. *Cry* is a pore-forming insecticide activated in the midgut naturally produced by the entomopathogenic bacterium *Bacillus thuringiensis*. Resident gut bacteria have been implicated in increased efficacy of *B. thuringiensis* or *Cry* toxin in the diamondback moth, Colorado potato beetle, Asiatic rice borer, and willow leaf beetle [10–13]. While these studies all implicate different symbiotic taxa in other examples, a pattern emerges where *Cry* susceptibility is specifically linked to the presence of *Enterobacter* species in the gut of the insect target [24–26]. Reaffirming a role for bacteria in *Cry* activation, western corn rootworm resistance has been correlated with reduced numbers of *Citrobacter*, *Serratia*, *Klebsiella*, and *Acinetobacter* [27].

Apart from natural protoxins, there have been efforts made to develop synthetic protoxins to control insects and result in off-target impacts. Indoxacarb (DPX-JW062) is one such engineered proinsecticide. The metabolite *N*-decarbomethoxylated JW062 (DCJW) is more insecticidal than indoxacarb itself. Indoxacarb is an important pesticide used in the control of the German cockroach, *Blattella germanica*. Recently, a role for bacterial activation of indoxacarb was illuminated using antibiotic treatment. Wolfe and Scharf found that regardless of initial strain susceptibility, reduction of the gut bacteria with antibiotic treatment increased indoxacarb tolerance [8]. Reduction in gut bacteria was correlated with reduced hydrolase activity and decreased DCJW content in frass, suggesting that bacteria were responsible for this biotransformation [8]. Independently, copper and zinc oxide nanoparticles, which are supposed to have antibacterial properties, did not reduce bacterial load in *B. germanica* [28]. Additionally, the presence of these nanoparticles, Cu and ZnO, was correlated with an increase in cockroach resistance to indoxacarb [28]. The authors posit that these nanoparticles must interfere with other metabolic processes and given that both copper and zinc oxide are known to have antibacterial properties, perhaps these effects are inhibiting a process of bacteria-derived pesticide activation. If true, this would also support the microbe-mediated activation hypothesis. Though these studies are in contrast to earlier work that presented correlative evidence that a reduction in gut bacterial diversity led to increased susceptibility to indoxacarb [29]. Even still, it would be interesting to see if host–microbe cross-talk is responsible for indoxacarb resistance in *B. germanica* given evidence of both endogenous mechanisms and microbe-mediated toxin activation [8,30].

The organophosphate chlorpyrifos, while not classified as a proinsecticide, shares this trait. The metabolite chlorpyrifos oxon is more toxic than chlorpyrifos and is preferentially produced by the gut bacterium *Lactobacillus plantarum* in its fruit fly host [9].

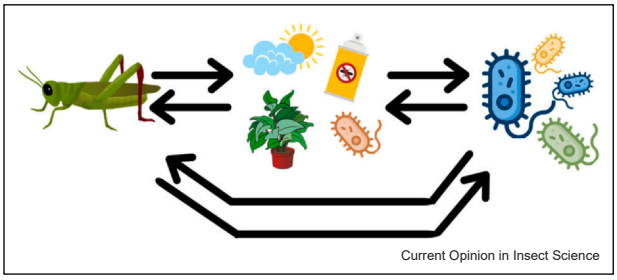
Insect–microbe–insecticide interactions are context-dependent

Many examples of symbiont-mediated pesticide degradation are plastic. Colonization of a host insect by a symbiont capable of conferring tolerance does not necessarily result in a protective phenotype. This is an ecological relationship and, as such, other biotic and abiotic factors can modulate the outcome of insect–microbe–insecticide interactions (Figure 2).

Temperature is one such abiotic variable that is of particular interest with global climate that continues to change. In the planthopper *Nilaparvata lugens*, a temperature-sensitive *Wolbachia* isolate has been implicated in imidacloprid detoxification [17]. Imidacloprid susceptibility could be restored by either antibiotic or high-temperature exposure before contact with the insecticide [17]. Interestingly, in field populations, *Wolbachia* was not linked to resistant phenotypes of *N. lugens*, but abiotic factors such as geography (latitude/longitude), precipitation, and temperature impacted the composition of the gut community and the resulting host insecticide susceptibility [19].

Host diet is a factor frequently linked to the abundance and composition of gut bacterial communities. The nutritional composition of this diet affects not only the host, but also the microbes in association with it. In the polyphagous herbivore *Spodoptera litura*, increased host plant nitrogen content was linked to higher insecticide-degrading bacterial load [31].

Figure 2



The interactions between insect host and microbe are ecological interactions. This means that the outcomes of these interactions are context-dependent. Biotic and abiotic factors modulate the outcomes of these interactions.

Another ecological factor of these interactions to consider is the composition of an insect's microbial community. The relative abundances of taxa in the insect host may impact the propensity of that host to succumb when challenged with a given pesticide. For example, increased abundances of *Ochrobactrum*, *Lysinibacillus*, and *Stenotrophomonas* were correlated with deltamethrin tolerance and decline with age in adult *Anopheles gambiae* [32]. Similarly, in the example mentioned earlier, the abundance of *Serratia oryzae* in the mosquito *Aedes albopictus* predicts expression of host detoxification metabolism and thus deltamethrin susceptibility [21].

Important considerations warranting further exploration

Despite the amount of work focused on symbiont-mediated pesticide tolerance in the last decade, the depth of our understanding around this topic is piecemeal. Frequently, the gut bacteria community rather than individual isolates is associated with a host resistance phenotype. This makes it impossible to parse whether the interplay between members of the community, between an individual symbiont and host, or both is responsible. Additionally, changes to the environment [19], host genotype [33], or pesticide concentration [34] can restore insecticide susceptibility. Making pest management recommendations based on these data, then, is challenging at best.

Most of the studies highlighted herein have focused on bacteria associated with insecticide-resistant insects. While a logical approach, it seems that a lack of a resistance phenotype does not mean an insect is devoid of bacteria capable of conferring such a trait. A *Chryseobacterium* isolated from insecticide-naive termites demonstrated not only an inherent tolerance for imidacloprid, but also rapid adaptation to tolerate higher rates [35]. This example underscores the importance of integrated pest management strategies that consider the whole environment, inside and outside of the target species. As with the previous example, bacterial potential explored *in vitro* may be worthwhile as a mechanism for generating hypotheses. Nonpathogenic *Escherichia coli* is a common commensal microbe in animal guts. The interaction between this bacterium and fipronil varies. At high concentration, the bacterium dies, but at low concentration, the bacterium has been found to remediate fipronil. More concerningly, it has also been found to bioaccumulate fipronil rapidly, accumulating 9-ppm fipronil after only 1 hour of exposure to media containing 21-ppm fipronil [36]. This has important implications for the role of environmental and symbiotic bacteria in off-target toxicity. For these reasons, basic, *in vitro* microbiological techniques have value in furthering research in this field. Questions regarding the potential interactions of symbionts and chemistries of interest can be explored in the laboratory in a cost-effective and productive way.

An important caveat to much of the work in the insect–microbe–pesticide world is that the antimicrobial treatments have unintended consequences. Not only are many insects associated with obligate symbionts, but there is evidence that antimicrobial compounds can cause deleterious effects on insects' development, fitness, and survival [37–39]. It is important to note that these impacts are detectable even in insects that are secondarily encountering antibiotics by way of a human host through a blood meal [37] or postmortem [40]. Antibiotics also affect more than just bacteria; they can impact the abundances of other microbes associated with the insect host such as fungi [39,41] and protists [42]. Given these off-target effects the use of antibiotics may result in, researchers explore other methods as we saw in the German cockroach [8,28,29]. To address this, two emerging methods show promise.

First, rather than perturb a normally colonized insect host, researchers are working to rear sterile or gnotobiotic animals [43,44]. This approach can mitigate the detriment fitness effects observed when obligate or mutualistic microbes collaterally eliminated by antibiotic treatment [5,37,39,40]. As previously discussed, inoculating an insect host with one or a few candidate pesticide-degrading bacteria is a more powerful, mechanistic approach than the correlative antibiotic studies [7,23,43,44]. The advantages of gnotobiotic techniques are clear, but they are not feasible or practical in all insect systems. While better-studied systems have clearly defined relationships with their obligate symbionts and vitamin [5] or amino acid biosynthesis [45], in other taxa, we can only vaguely link microbes to development and fitness without a clear mechanism [46,47]. Importantly, these insects do not live in a gnotobiotic or sterile world, and so these studies albeit valuable to linking individual taxa to mechanisms, may have limited ecological relevance.

In cases where lab-rearing or sustained microbiome manipulation has not been successful, the progress made in high-throughput 'omic approaches has made exploring host–microbe–pesticide interactions more feasible without manipulation. These methods come with their own caveats and must be guided with a solid question or hypothesis (as cautioned by Prosser [48]) due to the sheer amount of data they have the potential to produce, but they also allow for microbiota to be sampled with less-artificial manipulation. We can see differences in bacterial diversity between susceptible and resistance strains [32], measure the upregulation of microbial genes in the presence and absence of insecticide [15], assay the influence of abiotic factors on microbial community composition [19], and the like with more streamlined experimental designs.

Both gnotobiotic manipulations and 'omic approaches-associated straightforward research questions allow for more direct conclusions, something that microbial ecology often struggles to deliver. Correlative data are often the first step in a new line of investigation, but we must not be satisfied or limited to these methods, ideas, or ways of thinking.

Conclusions

While the appreciation for insect–microbe interactions has grown with the advent, accessibility, and affordability of culture-independent techniques, we continue to discover novel roles for bacterial symbionts in association with their insect hosts. Given the diversity of bacteria with demonstrated insecticide-related physiology, we must think more broadly about how to use these chemical tools. It will be imperative to consider symbioses when developing, testing, and deploying insecticides. As important as microbes may control efficacy, we should also invest in understanding how these organisms may contribute to the environmental fate of pesticides. This includes both lingering effects in the food chain and natural resources.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of interest

I affirm that there the content of this review was not influenced by financial, commercial, legal, or professional interest.

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. Blanton AG, Peterson BF: **Symbiont-mediated insecticide detoxification as an emerging problem in insect pests**. *Front Microbiol* 2020, **11**:547108.

2. Zhao M, Lin X, Guo X: **The role of insect symbiotic bacteria in metabolizing phytochemicals and agrochemicals**. *Insects* 2022, **13**:583.

3. Siddiqui JA, Fan R, Naz H, Bamisile BS, Hafeez M, Ghani MI, Wei Y, Xu Y, Chen X: **Insights into insecticide-resistance mechanisms in invasive species: challenges and control strategies**. *Front Physiol* 2023, **13**:1112278.

4. Chaitra HS: ***Gut symbionts: hidden players of pesticide resistance in insects***. *ije* 2022, **84**:997-1002, https://doi.org/10.55446/IJE.2021.70

5. Soh L-S, Veera Singham G: **Bacterial symbionts influence host susceptibility to fenitrothion and imidacloprid in the obligate hematophagous bed bug, *Cimex hemipterus***. *Sci Rep* 2022, **12**:4919.

6. Lv N, Li R, Cheng S, Zhang L, Liang P, Gao X: **The gut symbiont *Sphingomonas* mediates imidacloprid resistance in the important agricultural insect pest *Aphis gossypii* Glover**. *BMC Biol* 2023, **21**:86.

7. Wang Z, Wang W, Lu Y: **Biodegradation of insecticides by gut bacteria isolated from stored grain beetles and its implication in host insecticide resistance**. *J Stored Prod Res* 2022, **96**:101943.

8. •• Wolfe Z, Scharf M: **Microbe-mediated activation of indoxacarb in German cockroach (*Blattella germanica* L.)**. *Pestic Biochem Physiol* 2022, **188**:105234.
Gut symbionts' metabolism of the insecticide indoxacarb increases host susceptibility.

9. Daisley BA, Trinder M, McDowell TW, Collins SL, Sumarah MW, Reid G: **Microbiota-mediated modulation of organophosphate insecticide toxicity by species-dependent interactions with *Lactobacilli* in a *Drosophila melanogaster* insect model**. *Appl Environ Microbiol* 2018, **84**:e02820-17.

10. Polenogova OV, Noskov YA, Artemchenko AS, Zhangissina S, Klementeva TN, Yaroslavtseva ON, Khodyrev VP, Kruykova NA, Glupov VV: ***Citrobacter freundii,* a natural associate of the Colorado potato beetle, increases larval susceptibility to *Bacillus thuringiensis***. *Pest Manag Sci* 2022, **78**:3823-3835.

11. Chen G, Li Q, Yang X, Li Y, Liu W, Chen F, Han L: **Comparison of the co-occurrence patterns of the gut microbial community between Bt-susceptible and Bt-resistant strains of the rice stem borer, *Chilo suppressalis***. *J Pest Sci* 2023, **96**:299-315.

12. Lei X, Zhang F, Zhang J: **Gut microbiota accelerate the insecticidal activity of plastid-expressed *Bacillus thuringiensis* Cry3Bb to a leaf beetle, *Plagiodera versicolora***. *Microbiol Spectr* 2023, **11**:e05049-22.

13. Li S, Xu X, De Mandal S, Shakeel M, Hua Y, Shoukat RF, Fu D, Jin F: **Gut microbiota mediate *Plutella xylostella* susceptibility to Bt Cry1Ac protoxin is associated with host immune response**. *Environ Pollut* 2021, **271**:116271.

14. •• Sato Y, Jang S, Takeshita K, Itoh H, Koike H, Tago K, Hayatsu M, Hori T, Kikuchi Y: **Insecticide resistance by a host-symbiont reciprocal detoxification**. *Nat Commun* 2021, **12**:6432.
Insect host and bacterial symbiont collaborate to protect each other from an insecticide and its bactericidal metabolites to confer a pesticide resistance phenotype *in vivo*.

15. Bai L, Cai X-L, Li M-Y, Wang F-X, Zou C-S, Zhang J-S, Xie M-Y, Qi F-H, Jing T-Z: **Countering beta-cypermethrin: partitioning roles of the insect gut and its bacteria**. *J Pest Sci* 2023, **96**:1243-1255.

16. Wu Y, Zheng Y, Chen Y, Wang S, Chen Y, Hu F, Zheng H: **Honey bee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract**. *Microb Biotechnol* 2020, **13**:1201-1212.

17. • Zhang Y, Cai T, Ren Z, Liu Y, Yuan M, Cai Y, Yu C, Shu R, He S, Li J, *et al*.: **Decline in symbiont-dependent host detoxification metabolism contributes to increased insecticide susceptibility of insects under high temperature**. *ISME J* 2021, **15**:3693-3703.
Environmental conditions change the efficacy of symbiont-mediated pesticide metabolism.

18. Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, Fukatsu T: **Symbiont-mediated insecticide resistance**. *PNAS* 2012, **109**:8618-8622.

19. Zhang Y, Cai T, Yuan M, Li Z, Jin R, Ren Z, Qin Y, Yu C, Cai Y, Shu R, *et al*.: **Microbiome variation correlates with the insecticide susceptibility in different geographic strains of a significant agricultural pest, *Nilaparvata lugens***. *npj Biofilms Micro* 2023, **9**:1-8.

20. Tang T, Zhang Y, Cai T, Deng X, Liu C, Li J, He S, Li J, Wan H: **Antibiotics increased host insecticide susceptibility via collapsed bacterial symbionts reducing detoxification metabolism in the brown planthopper, *Nilaparvata lugens***. *J Pest Sci* 2021, **94**:757-767.

21. Wang H, Liu H, Peng H, Wang Y, Zhang C, Guo X, Wang H, Liu L, Lv W, Cheng P, *et al*.: **A symbiotic gut bacterium enhances *Aedes albopictus* resistance to insecticide**. *PLoS Negl Trop Dis* 2022, **16**:e0010208.

22. Fusetto R, Denecke S, Perry T, O'Hair RAJ, Batterham P: **Partitioning the roles of CYP6G1 and gut microbes in the metabolism of the insecticide imidacloprid in *Drosophila melanogaster***. *Sci Rep* 2017, **7**:11339.

23. Wang Z, Zhao Y, Yong H, Liu Z, Wang W, Lu Y: **The contribution of gut bacteria to pesticide resistance of *Tribolium castaneum* (Herbst)**. *J Stored Prod Res* 2023, **103**:102160.

24. Polenogova OV, Noskov YA, Yaroslavtseva ON, Kryukova NA, Alikina T, Klementeva TN, Andrejeva J, Khodyrev VP, Kabilov MR, Kryukov VY, *et al*.: **Influence of *Bacillus thuringiensis* and avermectins on gut physiology and microbiota in Colorado potato beetle: Impact of enterobacteria on susceptibility to insecticides**. *PLoS One* 2021, **16**:e0248704.

25. Xu C, Luo J, Wang L, Zhu X, Xue H, Huangfu N, Gao X, Li D, Zhang K, Chen R, *et al*.: **Gutbacterial community and gene expression alterations induced by transgenic Btmaize contribute to insecticidal activity against *Mythimna separata***. *J Pest Sci* 2023, **97**:685-700, https://doi.org/10.1007/s10340-023-01671-z

26. Xu T, Wang Y, Wang Y, Bi S, Hu B, Hu F, Xu L: **Comparison of gut microbial community between Bt-resistant and susceptible strains of *Ostrinia furnacalis***. *Agronomy* 2023, **13**:1923.

27. Paddock KJ, Pereira AE, Finke DL, Ericsson AC, Hibbard BE, Shelby KS: **Host resistance to *Bacillus thuringiensis* is linked to altered bacterial community within a specialist insect herbivore**. *Mol Ecol* 2021, **30**:5438-5453.

28. Zha C, Turner M, Ray R, Liang D, Pietri JE: **Effects of copper and zinc oxide nanoparticles on German cockroach development, indoxacarb resistance, and bacterial load**. *Pest Manag Sci* 2023, **79**:2944-2950.

29. Pietri JE, Tiffany C, Liang D: **Disruption of the microbiota affects physiological and evolutionary aspects of insecticide resistance in the German cockroach, an important urban pest**. *PLoS One* 2018, **13**:e0207985.

30. Gondhalekar AD, Nakayasu ES, Silva I, Cooper B, Scharf ME: **Indoxacarb biotransformation in the German cockroach**. *Pestic Biochem Physiol* 2016, **134**:14-23.

31. • Hu L, Sun Z, Xu C, Wang J, Mallik AU, Gu C, Chen D, Lu L, Zeng R, Song Y: **High nitrogen in maize enriches gut microbiota conferring insecticide tolerance in lepidopteran pest *Spodoptera litura***. *iScience* 2022, **25**:103726.
Host diet directly impacts gut bacterial community composition which can shirt pesticide susceptibility.

32. Pelloquin B, Kristan M, Edi C, Meiwald A, Clark E, Jeffries CL, Walker T, Dada N, Messenger LA: **Overabundance of Asaia and Serratia bacteria is associated with deltamethrin insecticide susceptibility in *Anopheles coluzzii* from Agboville, Côte d'Ivoire**. *Microbiol Spectr* 2021, **9**:e00157-21.

33. Li Y, Liu X, Wang N, Zhang Y, Hoffmann AA, Guo H: **Background-dependent *Wolbachia*-mediated insecticide resistance in *Laodelphax striatellus***. *Environ Microbiol* 2020, **22**:2653-2663.

34. Li Q, Sun J, Qin Y, Fan J, Zhang Y, Tan X, Hou M, Chen J: **Reduced insecticide susceptibility of the wheat aphid *Sitobion miscanthi* after infection by the secondary bacterial symbiont *Hamiltonella defensa***. *Pest Manag Sci* 2021, **77**:1936-1944.

35. • Blanton AG, Perkins S, Peterson BF: ***In vitro* assays reveal inherently insecticide-tolerant termite symbionts**. *Front Physiol* 2023, **14**:1134936.
Naïve insect hosts can harbor pesticide-tolerant bacterial symbionts.

36. •• Bhatti S, Satyanarayana GNV, Patel DK, Satish A: **Bioaccumulation, biotransformation and toxic effect of fipronil in *Escherichia coli***. *Chemosphere* 2019, **231**:207-215.
Demonstration of bacterial sequestration of an insecticide with implications for bioaccumulation and ramifications up the food chain.

37. Pietri JE, Liang D: **Insecticidal activity of doxycycline against the common bedbug**. *Antimicrob Agents Chemother* 2020, **64**:5, https://doi.org/10.1128/aac.00005-20

38. Ho LK, Daniel-Ivad M, Jeedigunta SP, Li J, Iliadi KG, Boulianne GL, Hurd TR, Smibert CA, Nodwell JR: **Chemical entrapment and killing of insects by bacteria**. *Nat Commun* 2020, **11**:4608.

39. He W, Pan L, Han W, Wang X: **Isothiazolinones as novel candidate insecticides for the control of Hemipteran insects**. *Antibiotics* 2021, **10**:436.

40. Preußer D, Bröring U, Fischer T, Juretzek T: **Effects of antibiotics ceftriaxone and levofloxacin on the growth of *Calliphora vomitoria* L. (Diptera: Calliphoridae) and effects on the determination of the post-mortem interval**. *J Forensic Leg Med* 2021, **81**:102207.

41. Li Y, Schal C, Pan X, Huang Y, Zhang F: **Effects of antibiotics on the dynamic balance of bacteria and fungi in the gut of the German cockroach**. *J Econ Entomol* 2020, **113**:2666-2678.

42. Sapkota R, Nakatsu CH, Scharf ME: **Regulation of host phenotypic plasticity by gut symbiont communities in the eastern subterranean termite (*Reticulitermes flavipes*)**. *J Exp Biol* 2021, **224**:jeb242553.

43. Wu J, Wang Q, Wang D, Wong ACN, Wang G-H: **Axenic and gnotobiotic insect technologies in research on host–microbiota interactions**. *Trends Microbiol* 2023, **31**:858-871.

44. Steven B, Hyde J, LaReau JC, Brackney DE: **The axenic and gnotobiotic mosquito: emerging models for microbiome host interactions**. *Front Microbiol* 2021, **12**:714222.

45. Michalik A, Bauer E, Szklarzewicz T, Kaltenpoth M: **Nutrient supplementation by genome-eroded *Burkholderia* symbionts of scale insects**. *ISME J* 2023, **17**:2221-2231.

46. Gilliland CA, Patel V, McCormick AC, Mackett BM, Vogel KJ: **Using axenic and gnotobiotic insects to examine the role of different microbes on the development and reproduction of the kissing bug *Rhodnius prolixus* (Hemiptera: Reduviidae)**. *Mol Ecol* 2023, **32**:920-935.

47. Coon KL, Valzania L, Brown MR, Strand MR: **Predaceous Toxorhynchites mosquitoes require a living gut microbiota to develop**. *Proc R Soc B: Biol Sci* 2020, **287**:20192705.

48. Prosser JI: **How and why in microbial ecology: an appeal for scientific aims, questions, hypotheses and theories**. *Environ Microbiol* 2022, **24**:4973-4980.