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Microbiome Innovation in Agriculture: Development of Microbial Based Tools for Insect Pest Management

*Masroor Qadri[1], Sierra Short[1], Kalani Gast[1], Jordan Hernandez[1] and Adam Chun-Nin Wong[1,2]**

[1] *Entomology and Nematology Department, University of Florida, Gainesville, FL, United States,* [2] *Genetics Institute, University of Florida, Gainesville, FL, United States*

This review emphasizes the potential and use of microbes in sustainable insect pest management. We first review the diverse insect traits shaped by insect-microbe associations that span nutrition, immunity, ecological interactions with natural enemy, insecticide resistance, and behavior. This is followed by discussing different microbiome manipulation approaches to alter pest traits, describing some of the opportunities and obstacles for each approach. We then highlight microbiomes as untapped chemical inventories to discover novel biopesticides, including plant-incorporated protectants and semiochemicals. The last topic covered is the use of beneficial microbes to improve mass-reared insects' performance for autocidal programs, including sterile insect technique and incompatible insect technique, in which we identify topics where data are limited or inconclusive, for future research.

Keywords: invasive species, agroecosystems, omics, pest management, microbiome

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***Correspondence:**
Adam Chun-Nin Wong
adamcnwong@ufl.edu

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INTRODUCTION

According to the Entomological Society of America's (ESA) recent position statement, invasive insects incur control costs of over \$2.5 billion and cause economic damages to crops, lawns, forests, and pastures totaling \$18 billion per year (The Not-So-Hidden Dangers of Invasive Species., 2018). The threat of invasive crop pests to food security continues to be driven by the complex dynamics of human movements, global trade activities, climate change, and changing agricultural practices. Some of these factors also facilitate the dramatic expansion of urban pests and disease vector insects. Chemical insecticides have been central to insect pest control. However, a growing demand for reduced agricultural chemical use due to environmental and human health concerns in addition to pesticide resistance issues are fueling interests in innovative approaches to manage insect pests. For decades, the role of microbes in pest management has been largely confined to using entomopathogens, with only a handful of microbial species being developed into bioinsecticides. The paradigm is shifting owing to the advent of high-throughput sequencing, functional omics, and gene editing technologies, which significantly accelerate microbial discovery, plus a better understanding of microbial functions in complex communities across biological systems. There is also overwhelming evidence that symbiotic microbes play pivotal roles in shaping various insect traits. The collection of microbes associated with a given environment (both biotic and abiotic) and their collective genetic materials is termed the microbiome. Research in the microbiomes of insects, plants, and natural resources could be leveraged to develop novel management tools for insect pests. **Figure 1** provides a graphical summary of the potential microbiome-based insect management strategies discussed in this review.

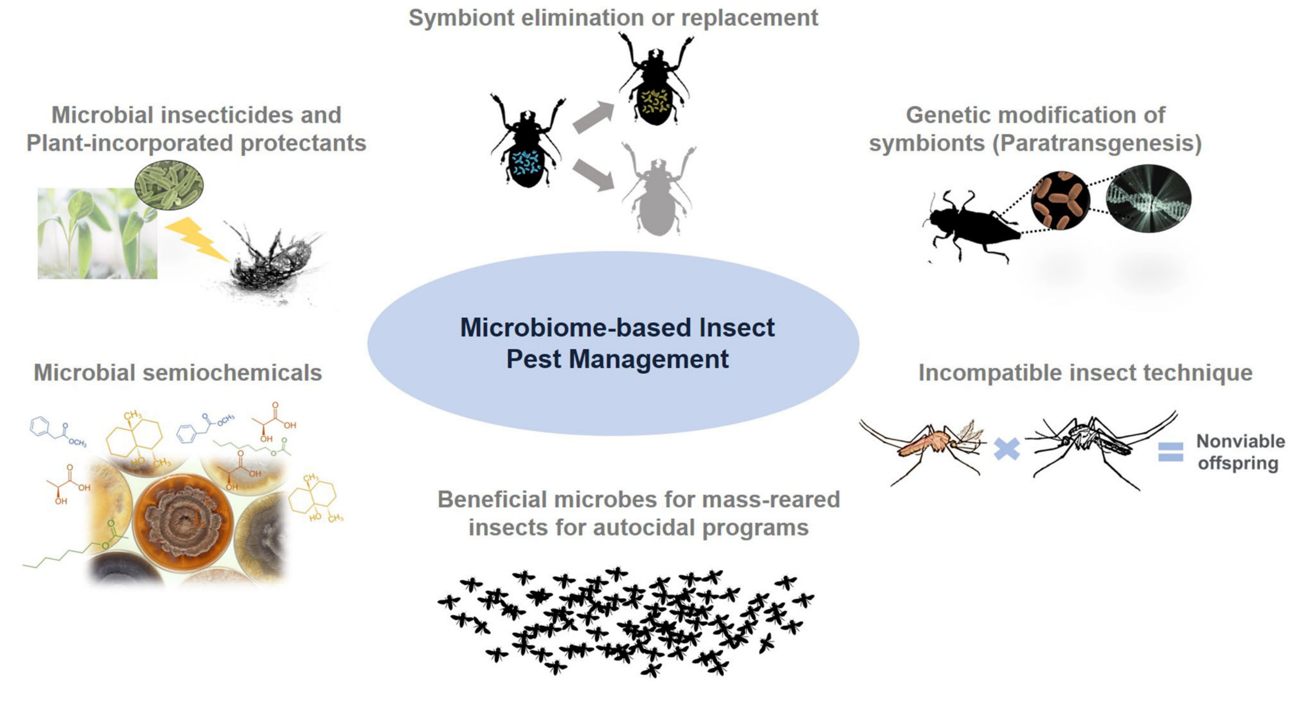

FIGURE 1 | Microbiome-based insect pest management approaches and tools.

MICROBIAL INFLUENCE ON DIVERSE INSECT TRAITS

Insects are associated with diverse microbial communities and in many cases, these associations are crucial for insect survival and development. Symbiotic microbes in the gut, hemolymph, as well as in specialized cells, carry an arsenal of enzymes that provide specialized services to the insect hosts (Blow and Douglas, 2019). Supplies of essential nutrients (particularly amino acids and B vitamins) by endosymbionts have been well documented in a number of crop pests, particularly plant sap-sucking Hemipteran insects such as aphids, whiteflies, and psyllids (Douglas, 1998; Thao et al., 2000; Thao and Baumann, 2004; Luan et al., 2015), and in human disease vectors and urban pests such as tsetse flies in the genus *Glossina* and the common bed bug (*Cimex lectularius*). Some symbionts can degrade complex polysaccharides or recycle nitrogen for insects, such as the termites (Raychoudhury et al., 2013; Brune, 2014) and cockroaches (Berlanga et al., 2016). The production of antimicrobials by symbionts aids the immune system to fight against invading pathogens, as was shown in the beewolf digger wasps (Kroiss et al., 2010) and cotton leafworm (Shao et al., 2017).

Beside nutritional and immune services, symbionts can shape the ecological interactions between insects and their natural enemies. For instance, the secondary symbiont of aphids *Hamiltonella defensa* increased the chance of host survival from parasitoid wasp attacks by disrupting wasp embryogenesis, mediated by its bacteriophage-encoded toxins (Oliver et al., 2003, 2005; Moran et al., 2005; Vorburger et al., 2009; Schmid et al., 2012; Brandt et al., 2017). *H. defensa* was also shown to attenuate volatile release in aphid-infested plants, thus reducing parasitic wasp recruitment (Frago et al., 2012, 2017). Similarly, symbiont manipulation of plant physiology that facilitates insect colonization was observed in whiteflies and the Colorado potato beetle (Chung et al., 2013; Su et al., 2015). Modification of body color by facultative symbionts may determine aphid susceptibility to predation or parasitism (Xu et al., 2009). In particular, *Rickettisella* infection in the pea aphid *Acyrthosiphon pisum* increased the synthesis of blue-green polycyclic quinone pigments, turning the host from red to green. This symbiont-dependent color variation is believed to affect the aphid's relative risks between predation and parasitism, as their predators such as the ladybird beetles preferentially prey on the red morphs, while parasitoids preferentially attack the green morphs (Libbrecht et al., 2007; Tsuchida et al., 2010, 2014).

Symbionts have also been shown to influence insecticide resistance in various insect pests. In the bean bug (*Riptortus pedestris*) and the oriental fruit fly (*Bactrocera dorsalis*), resistance to organophosphorous pesticides has been attributed to direct detoxification by their symbionts (Kikuchi et al., 2012; Cheng et al., 2017). The insecticidal activity of *Bacillus thuringiensis* (Bt) in the gypsy moth (*Lymantria dispar*) larvae was shown to be dependent on the presence of symbiotic midgut bacteria (Broderick et al., 2006). Microbiome surveys from field-collected samples indicated that Bt resistance in cotton bollworm (*Helicoverpa armigera*) was associated with distinct microbiome compositions (Paramasiva et al., 2014; Visweshwar et al., 2015). Xia et al. observed varied levels of susceptibility to chlorpyrifos in the diamondback moth (*Plutella xylostella*) treated with antibiotics and then recolonized with different gut-associated bacteria (Xia et al., 2013, 2018). The contribution of gut bacteria to insecticide resistance was also reported in mosquitoes that showed reduced mortality to insecticides when fed with different gut commensal bacteria (Barnard et al., 2019).

Accumulating evidences have suggested that microbes play a more prominent role in shaping insect behavior than previously thought (McFall-Ngai et al., 2013). Insect behaviors that have been shown to be affected by microbes include long-term dispersal, oviposition, mating, host searching, and kin recognition (Ezenwa et al., 2012). Studies also suggest that microbiomes can influence host behavior via production of metabolites acting on host neuro-endocrine circuits (Adamo, 2013; Hemarajata and Versalovic, 2013; Wang and Kasper, 2014), a phenomenon termed the "gut-brain axis." There is a plethora of research on the gut-brain axis, a majority of which has been focused on mammalian systems. However, this subject is still in infancy. One example is a recent study suggesting that the microbiome of *Drosophila melanogaster* influences host olfactory-guided foraging preferences toward foods of different microbial content (Wong et al., 2017). Similar microbiome-priming effects on fly behaviors were found in subsequent studies by Farine et al. (2017) and Qiao et al. (2019).

Advances in high throughput sequencing and functional genomics have enabled scientists to accomplish two milestones in insect microbiome research: 1. Explore previously uncharacterized microbiomes in a greater variety of insects, leading to a better understanding of host and environmental factors that shape insect microbiome diversity and composition. Some examples include microbial communities associated with Drosophilid and Tephritid fruit flies (Behar et al., 2008b; Wong et al., 2011, 2013; Ras et al., 2017), ants (Ramalho et al., 2017; Shin et al., 2017), bees (Engel et al., 2016), mosquitoes (Minard et al., 2013; Novakova et al., 2017), ticks (Narasimhan and Fikrig, 2015), beetles (Hulcr et al., 2012), and midges (Halpern and Senderovich, 2015), among others. 2. Attribute microbiome functions to specific microbial taxa or consortia. While the diversity and stability of microbiomes vary by insects, microbial influence on insect invasive traits is widely supported. For example, the invasiveness of the sweet potato whitefly (*Bemisia tabaci*) was promoted by a recent sweep of a *Rickettsia* sp. into the pest population, from 1% infected in 2000 to 97% by 2006) which led to faster development, higher survival rate to adulthood, and higher fecundity of the host (Himler et al., 2011). Studies on the powerful lab model *Drosophila melanogaster* showed that its microbiome, consisting of 5–20 microbial species, accelerates larval development, affects host foraging preference and reproduction (Ridley et al., 2012, 2013; Wong et al., 2014, 2017; Morimoto et al., 2017), which are all important parameters for invasiveness. Its close relative, *Drosophila suzukii*, or spotted wing drosophila (SWD), a significant pest of small fruits, relies on the microbiome to develop in ripening fruits (Bing et al., 2018). Among the highly destructive crop pests in the family Tephritidae, microbial symbiosis was first described in the olive fruit fly *Bactrocera oleae* (Petri, 1909, 1910). Unlike other fruit feeding *Bactrocera* species, *B. oleae* is associated with an obligate bacterial symbiont (*Candidatus* Erwinia dacicola) maintained in larval midgut caeca. The symbiont is currently uncultured but studies have shown that it facilitates fly development and reproduction by providing essential amino acids and metabolizing urea from various sources such as bird droppings, making nitrogen available to adult flies (Capuzzo et al., 2005; Ben-Yosef et al., 2014). It also helps the larvae develop in unripe olives by counteracting a plant defensive metabolite, oleuropein (Capuzzo et al., 2005; Estes et al., 2009). Interestingly, the symbiont has been found to be absent in domesticated *B. oleae* reared on artificial media containing antibiotics, demonstrating the impact of rearing on symbiont selection (Estes et al., 2012; Sacchetti et al., 2019). Other bacterial species associated with the guts of *B. oleae* include *Pantoea* sp. and *Burkholderia* sp., although their nutritional role is unclear (Ras et al., 2017). Microbiome-dependent larval development has also been seen in the medfly (*Ceratitis capitata*) and apple maggot fly (*Rhagoletis pomonella*). In medfly, microbial nutrient provisioning involves diazotrophs that express nitrogen reductase gene (nifH) in the gut (Behar et al., 2005). These medfly-associated bacteria include the genera *Citrobacter*, *Klebsiella*, *Pectobacteria*, *Enterobacter*, and *Pantoea* (Behar et al., 2008b). The community has been shown to support fly development, reproduction, and extend longevity when administered as probiotics (Behar et al., 2008b), and increase male copulatory success (Ben-Yosef et al., 2008). In addition to the dominant Enterobacteriaceae, a minor but stable community associated with medfly gut includes *Pseudomonas* spp. (Behar et al., 2008b). Using a metabarcoding approach, differences in microbial community was observed at different instar stages of medfly (Malacrinò et al., 2018). *Burkholderia* was found to be dominant in early instars and adults and may play a role in nitrogen fixation, as suggested in *Tetraponera* ants (Borm et al., 2002). Similarly, bacteria belonging to the genera *Sphingomonas*, *Pseudomonas* and an unidentified bacterium belonging to family Methylobacteraceae were found more abundant in late instars of medfly, whereas *Leuconostoc*, *Weissella*, *Acetobacter*, *Gluconobacter* and an unidentified bacterium belonging to family Xanthomonadaceae were more abundant at pupal stage (Malacrinò et al., 2018). In addition, variable microbial community was observed in medfly fed on different host plants. For example, medfly larvae feeding on *Ficus carica* (common fig) fruits were found to harbor *Acinetobacter* and *Gluconobacter*, while *Acetobacter* and *Leuconostoc* were found to be more abundant when fed on *Prunus persica* (peaches) (Malacrinò et al., 2018). *Acinetobacter* and *Gluconostoc* have been suggested to play a role in detoxifying phenolic glycosides (Mason et al., 2014). Similarly, apple maggot flies are associated with a diverse range of Enterobacteria in their gut, such as *Pantoea*, *Klebsiella*, and *Enterobacter* (Behar et al., 2009). The bacteria are deposited into the fruit during oviposition, thereby providing essential nutrients and proteins required for larval development (Lauzon, 2003; Behar et al., 2008a). In some Tephritids of the sub-families Dacinae and Trypetinae, symbiotic bacteria support larval development by metabolizing sugars, increasing the level of organic nitrogen, and synthesizing vitamins (Lauzon, 2003; Behar et al., 2009; Hamden et al., 2013). However, their functions in adult flies are unclear (Ben-Yosef et al., 2014).

MANIPULATION OF INSECT-ASSOCIATED MICROBIOMES FOR PEST MANAGEMENT

The contribution of microbiomes to diverse insect invasiveness-related traits represents a rich array of resources that could be targeted for pest management. A simple strategy is to eliminate or disrupt insect symbiosis using biochemicals (Baumann, 2005). For example, ingestion of antibiotics such as tetracycline and penicillin has been shown to render tsetse flies sterile by affecting the obligate mutualist *Wigglesworthia,* impede the development of immature ticks and diminish reproduction of adult ticks by reducing their symbiont load (Zhong et al., 2007). The use of antimicrobial peptides (AMPs) has also been explored to manipulate insect symbionts, though more commonly used to control human or plant pathogens vectored by insects (Carter et al., 2013). AMPs (including diverse amphiphilic and cationic oligopeptides) are an integral part of insect innate immunity. They confer protection against a variety of microbes, including bacteria, fungi and viruses. Cecropin was the first AMP isolated from the pupae of *Hylaophora cecropia* (Hultmark et al., 1980; Steiner et al., 1981) and since then, about 150 AMPs have been purified from insects (Yi et al., 2014). On the basis of amino acid sequences and structures they have been classified into four groups: the α-helical peptides (e.g., moricin and cecropin), cysteine-rich peptides (e.g., defensin and drosomycin), proline-rich peptides (e.g., apidaecin, drosocin and lebocin), and glycine-rich proteins (e.g., attacin and gloverin). Some of the AMPs (e.g., defensins, cecropins, attacins, and proline-rich peptides) are present across insect orders, whereas others (e.g., moricin and gloverin) are restricted only to certain orders (e.g. Lepidoptera). The antimicrobial activity of AMPs is attributed to their positively charged surface which enables them to bind negatively charged microbial surface via charge-charge interactions, thereby interfering with the integrity of bacterial cell wall (Wu et al., 2018). AMPs have been engineered in plants and some insects to confer resistance to bacterial, fungal, and some eukaryotic parasites. For example, insect defensins (gallerimycin from Galleria mellonella) and cecropin (sarcotoxin-IA from Sarcophaga peregrina) have been shown to confer resistance to pathogenic fungi in transgenic tobacco (Mitsuhara et al., 2000). Similarly, transgenic expression of cecropins has also been shown in conferring resistance to fungal and bacterial pathogens in rice and tomato plants (Sharma et al., 2000; Jan et al., 2010). Cecropins also show activity against protozoan parasites, such as Plasmodium and Trypanosoma (Rodriguez et al., 1995; Fieck et al., 2010). Transgenic expression of cecropin in Anopheles gambiae, a vector for human parasite has been shown to reduce the number of Plasmodium berghei oocysts by 60% (Kim et al., 2004). In addition, transgenic co-expression of defensin-A and cecropin-A in Aedes aegypti has been shown to cooperatively block the transmission of Plasmodium parasite (Kokoza et al., 2010).

A key limitation of using antibiotics or AMPs is the possibility of non-target effects, which can lead to disruption of the native microbiome in non-target insects. Other limitations of using AMPs include low bioavailability, instability, and emergence of antimicrobial resistance (Shen et al., 2018). Nonetheless, AMPs with enhanced specificities can be generated by methods such as fusion with antibody fragments with affinity toward specific antigens (Peschen et al., 2004), protein engineering and synthetic biology techniques (e.g., substitution of amino acids, chemical modifications) (Cao et al., 2018). Improved delivery of AMPs to different living systems using nanotechnology is also being actively researched (Biswaro et al., 2018).

Introducing a foreign microbe to insects or replacing a symbiont with another microbe is also a viable strategy for manipulating insect traits. Experimental replacement of specific cultured and uncultured insect symbionts has been demonstrated in stink bugs and aphids. Hosokawa et al. (2007) showed that the swapping of the *Ishikawaella* symbionts between stink bug *Megacopta punctatissima*, a regular pest of soybean and other legumes, and a closely related non-pest species, *Megacopta cribraria*, resulted in poor *M. punctatissima* egg hatching on the plants. Experimental replacement of the primary symbiont *Buchnera* with a different genotype by microinjection in the pea aphid has also been shown to alter pest thermal tolerance (Moran and Yun, 2015).

Incompatible Insect Technique (IIT)

Multiple protocols have been established to generate gnotobiotic insects (i.e., insects with experimentally controlled microbiomes) in the lab, such as *Drosophila* (Ridley et al., 2013), mosquitoes (Coon et al., 2014), and honey bees (Kešnerová et al., 2017). The protocol often involved cleansing the insect eggs to remove maternally deposited microbes on the surface, or treating larval or adult insects with antibiotics, followed by feeding on food seeded with cultured microbes or microbe-laden materials (e.g., fecal transplantation). While the gnotobiotic approach has been instrumental in the discoveries of microbiome impact on insect traits such as development, physiology, behavior, and insecticide resistance (Jaenike et al., 2010; Ridley et al., 2012; Coon et al., 2014; Wong et al., 2017; Xia et al., 2018), its use in pest management remains largely conceptual. A major exception is the rapidly growing interest in utilizing *Wolbachia* to control mosquitoes and mosquito-borne infectious agents. *Wolbachia* is a widely distributed, vertically transmitted endosymbiont in arthropods, estimated to infect over 60% of all insects (Hilgenboecker et al., 2008). The bacterium is known primarily for its ability to manipulate host reproduction through induction of cytoplasmic incompatibility (CI), feminization, parthenogenesis, and male killing (Stouthamer et al., 1999). By distorting sex ratios toward females, *Wolbachia*-infected females have a reproductive advantage over uninfected females, facilitating their propagation in a population. The incompatible insect technique (IIT) employs *Wolbachia*-induced CI as a strategy to control mosquitoes and other insect pests (Werren, 1997; Stouthamer et al., 1999). CI results in embryonic lethality (Bourtzis et al., 1998) and can be induced unidirectionally in crosses between *Wolbachia*-infected males and uninfected females, or bidirectionally in crosses between infected individuals bearing different strains of *Wolbachia* (Saridaki and Bourtzis, 2010). *Wolbachia*-induced sterility does not compromise the male mating competitiveness and their survival, as observed in *Aedes aegypti* (Segoli et al., 2014). In IIT, *Wolbachia*-infected males are repeatedly released to compete with the wild populations (Zabalou et al., 2004, 2009; Bourtzis, 2008; Berasategui et al., 2016). Extensive research has been carried out to use IIT against several insect pests including Rhagoletis cerasi, Ceratitits capitata, the tsetse fly, and disease vectors including Culex pipiens, Aedes albopictus and Culex quinquefasciatus (Zabalou et al., 2004, 2009; Alam et al., 2011; Bourtzis et al., 2014; Zhang et al., 2015; Atyame et al., 2016). *Wolbachia* can be transinfected into a novel host which are not naturally infected by *Wolbachia* strains that can induce CI (Zabalou et al., 2004, 2008; O'Connor et al., 2012; Zhang et al., 2015). For example, *Wolbachia* strain *w*Suz naturally infects *D. suzukii* but it does not induce CI (Ometto et al., 2013; Siozios et al., 2013; Hamm et al., 2014; Cattel et al., 2016). Two CI-inducing *Wolbachia* strains (*w*Ha and *w*Tei) from other *Drosophila* species have been identified and successfully introduced to *D. suzukii*, setting up the stage for IIT for this pest (Cattel et al., 2018; Nikolouli et al., 2018). However, the stability of the association, consequences of *Wolbachia* transinfection on host fitness including mating competitiveness need to be critically assessed before field applications can be exercised (O'Connor et al., 2012). In addition, the genotype of IIT insects should be considered since host genotype plays an important role in *Wolbachia* density and phenotypic expression of infection in hosts, including CI (Mouton et al., 2007).

Recently, significant progress has been made in identifying the molecular factors for *Wolbachia*-mediated CI. LePage et al. (2017) identified two differentially transcribed wMel genes, cifA and cifB (encoded by WO prophage), that functionally reiterated CI when expressed dually in uninfected males. Both genes are incapable of inducing CI independently. Further studies demonstrated that transgenic expression of cifA gene independently rescues CI and abolishes the embryonic lethality caused by *w*Mel *Wolbachia* in *Drosophila* (Shropshire et al., 2018). In another study, Beckmann et al. (2017) demonstrated that interaction of Wolbachia deubiquitylating enzymes (DUB) cidA and cidB induces CI in transgenic *Drosophila*.

In addition to CI, another important trait of *Wolbachia* for mosquito control concerns its pathogen-blocking ability. *Aedes aegypti,* the vector for many clinically important arboviruses, do not generally bear *Wolbachia* in nature, but when transinfected with *Wolbachia* derived from *Drosophila* or other mosquitoes (e.g., *Aedes albopictus* and *Culex quinquefasciatus*), display drastically reduced competence for dengue, chikungunya, yellow fever, and Zika viruses (Bian et al., 2010; Van den Hurk et al., 2012; Aliota et al., 2016; Carrington et al., 2018), as well as *Plasmodium* and filarial nematodes (Kambris et al., 2009; Moreira et al., 2009). The exact mechanism of *Wolbachia*-mediated pathogen blocking is under investigation. Several hypotheses have been proposed, including priming of the immune system, changes in cholesterol and lipid droplets production and trafficking (Geoghegan et al., 2017), and (viral) RNA degradation (Thomas et al., 2018). In 2011, *Ae. aegypti* carrying the *w*Mel strain were released into the wild near Cairns, Australia, marking the first trial of microbiome manipulation of a wild insect population for the purpose of reducing vector competence (Hoffmann et al., 2011). A follow-up investigation 2 years later suggested that the *Wolbachia* infection has stably established in the mosquito population (Hoffmann et al., 2014). More importantly, the release effectively stopped dengue transmission in Cairns and surrounding locations in northern Queensland, Australia. As of late 2019, mean *Wolbachia* frequencies in the original Cairns populations remains around 95%, with a 96% reduction in dengue cases. Subsequent releases have established *Wolbachia* throughout northern Queensland, as well as Yogyakarta, Indonesia, and Kuala Lumpur, Malaysia (Nazni et al., 2019; Ryan et al., 2020; Tantowijoyo et al., 2020).

Paratransgenesis

A related strategy gaining traction in recent years is to genetically modify microbes to express desired effects in insects, known as paratransgenesis (Aksoy et al., 2008; Coutinho-Abreu et al., 2010; Caragata and Walker, 2012). Instead of transforming the insects (i.e., transgenesis), paratransgenesis bypasses the disadvantages of fitness cost associated with introducing a transgene into the insects and transgene instability in insect genomes. This approach is particularly suited for microbes that can be cultured, transformed, and readily reintroduced into the insect hosts. Paratransgenesis was proposed back in the early 90's, but most of the research progress has been focusing on human disease vectors and a few Hemipteran crop pests. Beard et al. (1992, 1993, 2000) demonstrated that the gut symbiont *Rhodococcus rhodnii* of the triatomine bug (*Rhodnius prolixus*) can be genetically modified to express effector molecules (cecropin A and related pore-forming molecules) against the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease. The symbiont can be introduced to insect progeny by inoculating eggshells or food with feces seeded with the engineered symbiont. Durvasula et al. (1999) also transformed the symbiont with an anti-trypanosome single-chain antibody and showed significant reduction in parasite load. The promising results from laboratory studies led to subsequent field trials in testing the transmission efficiency of engineered *R. rhodnii* to the triatomine bug using simulated triatomine-fecal materials called CRUZIGARD which consisted of an inert guar gum matrix dyed with India ink (Hurwitz et al., 2011a,b). More recently, a study has integrated paratransgenesis with RNA interference (RNAi) technology to control *Rhodnius prolixus*. Oral administration of an *Escherichia coli* strain HT115 or *R. rhodnii* engineered to express dsRNA targeting the antioxidant genes-heme-binding protein (RHBP) and catalase (CAT) genes in *R. prolixus* was shown to trigger systemic RNAi to silence these genes, resulting in poor development of nymphs and reduced fecundity of females (Taracena et al., 2015). Similar paratransgenic approaches have been tested on tsetse flies, using engineered symbiont *Sodalis glossinidius* that expressed antigen binding molecules targeting *Trypanosoma brucei*, the causative agent of sleeping sickness. *Sodalis* is found in the hemolymph, midgut, and milk gland and can be transmitted vertically through the milk glands (De Vooght et al., 2012, 2014). In mosquitoes, several paratransgenic strategies have been explored to prevent the transmission of malaria-causing *Plasmodium* parasites, using bacteria and fungi isolated from the mosquito midguts and ovaries. The Gram-negative *Asaia bogorensis* was selected for paratransgenesis against *Plasmodium berghei* since it was shown to persist in the midguts of mosquitoes and can spread quickly within a population, both vertically and horizontally (Bisi and Lampe, 2011; Dinparast Djadid et al., 2011). Genetically modified *Asaia* strains were constructed by fusing the siderophore receptor gene with anti-plasmodial effector genes. These genes included the scorpine antimicrobial peptide and a synthetic anti-Pbs21 scFv-Shiva1 immunotoxin composed of a single chain antibody (scFv) against *P. berghei* ookinete surface protein 21-Shiva1 fusion protein. Parasite development was significantly inhibited after *Anopheles stephensi* mosquitoes were fed with the transformed *Asaia* and challenged with *P. berghei* infected blood (Bongio and Lampe, 2015). Previously, a common symbiotic bacterium of mosquitoes, *Pantoea agglomerans*, was engineered to express anti-*Plasmodium* effector proteins using Type I hemolysin secretion system derived from *E. coli*. These engineered *P. agglomerans* strains were found to inhibit the development of *P. falciparum* and *P. berghei* in the midgut of *Anopheles* mosquitoes (Wang et al., 2012). An entomopathogenic fungus, *Metarhizium anisopliae*, has also been manipulated to secrete the antimicrobial scorpine and anti-plasmodial peptide SM1 capable of inhibiting the development of *Plasmodium* parasite (Fang et al., 2011). A study by Wang et al. (2017) discovered that gut and ovaries of *A. stephensi* are colonized by a novel *Serratia* sp. AS1. The AS1 strain was both sexually and vertically transmitted, persisting for at least three generations. Mosquitoes infected with an engineered *Serratia* AS1 containing five different anti-plasmodium effector molecules (Shiva1, a cecropin-like synthetic antimicrobial peptide; MP2, midgut peptide 2; EPIP, enolase-plasminogen interaction peptide (lysine-rich enolase peptide); scorpine, scorpion *Pandinus imperator* venom antimicrobial peptide; and mPLA2, inactive bee venom phospholipase A2) displayed a reduction in the oocyte load by 93% (Wang et al., 2017).

The potential of paratransgenesis in crop protection against insect pests or insect-vectored pathogens is demonstrated in the Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*. GWSS is a vector of *Xylella fastidiosa*, the bacterial pathogen that causes Pierce's disease in grapes by producing exopolysaccharides, which helps them colonize the xylem of its host plant, blocking the xylem flow (Hopkins and Purcell, 2002; Hackett, 2003; Bextine et al., 2004; Killiny et al., 2013). Among the different bacterial species isolated from GWSS, *Alcaligenes xylosoxidans var. denitrificans* (Axd), was chosen as a candidate bacterium for genetic manipulation. It has been consistently found in the xylem of host plants; the same niche occupied by the pathogen. Genetically modified Axd containing a DsRed fluorescent protein gene was successful delivered from injected stems to GWSS. It was found to colonize the insect foregut, suggesting a paratransgenic approach to clear *X. fastidiosa* from GWSS is feasible (Bextine et al., 2004). However, the use of transformed Axd in plants has potential drawbacks since this genus of bacteria has been reported as nosocomial human pathogen implicated in causing lung infection in cystic fibrosis patients (Krzewinski et al., 2001; Saiman et al., 2001). To ease the safety concern, an endophytic bacteria of grapes *Pantoea agglomerans* E325 (an EPA-approved agent for managing fire blight in pears and apples) was genetically engineered to express anti-*Xylella* effector proteins melittin and a scorpine-like AMP, and successfully colonized in the foregut of GWSS through an artificial feeding system (AFS) (Arora et al., 2015). Furthermore, targeted delivery of recombinant *Pantoea agglomerans* E325 to the gut of GWSS using microencapsulation strategy has been established to control Pierce's disease under simulated field conditions. Microencapsulation strategy may be useful for field application as it could prevent the environmental spread of foreign genetic material (Arora et al., 2015).

More recently, Leonard et al. (2020) developed a paratransgenic approach in honeybees and showed that it improved bee survival against viral infection and varroa mites in the lab. The authors engineered a symbiotic gut bacterium *Snodgrassella alvi*, expressing dsRNA specifically targeted to interfere with bee, viral or mite genes.

Successful application of gnotobiotic or paratransgenic approaches to control insect pests largely depends on the choice of microbes, the genetic design, and implementation of the treated insects. The microbes should ideally be specific to target insects or harmless to non-target hosts, another obstacle concerns the stability of the association. Persistent association ensures microbial-mediated impacts on host insects will be lasting, but some microbes may be "lost" from the insects due to environmental selective pressure or antagonistic effects with other microbes. Despite these caveats, it is anticipated that research and development on gnotobiotic and paratransgenic insects will continue to grow.

EXPLOITING THE CHEMICAL INVENTORIES OF MICROBIOMES TO DEVELOP NEW BIOPESTICIDES

A key challenge to crop protection concerns the emergence of insecticide resistance, and for many insect pests there are no alternative control methods. Development of new synthetic pesticides has become increasingly costly and challenging, partly due to the difficulty of converting a lead compound into a product that can pass through strict environmental and safety regulations. A recent report estimated that in the US, the development of a synthetic pesticide now costs over $300 million and takes nearly 12 years (McDougall, 2016). The need to overcome resistance problems and to promote sustainable agriculture necessitates the discovery of new insecticidal agents.

In recent years, the push to develop novel insecticides with minimal environmental impact has led to a resurgence of interest in biopesticides (i.e., pesticides based on living organisms or their natural products, including their genes and metabolites). Currently, the global market for biopesticides is valued at $3 billion and is expected to grow by 15% in the next 4 years, outpacing the market growth of synthetics by 10-fold (Damalas and Koutroubas, 2018). Biopesticides fall into one of the three main categories: biochemical pesticides, which are naturally occurring chemicals that can alter pest behavior or physiology; microbial pesticides, which use pathogenic or toxic microbes as the active ingredient, and plant-incorporated protectants (PIPs), transgenic plants that produce pesticidal compounds (USEPAO, 2015).

Microbial Insecticides and Plant-Incorporated Protectants

Historically, microbial insecticides have taken the form of living microbes or spores applied directly to the field. Among the most common microbial insecticides are the entomopathogenic fungi, or EPFs, which are used in half of all classical biological control programs (Hajek and Delalibera, 2010). Several attributes of EPFs make them ideal candidates for biological control agents: many can be mass cultured *in vitro*; fungal spores have a long shelf life compared to other biological control agents; and they are often capable of persisting in the host population without repeated introductions (Hajek and Delalibera, 2010). The majority of economically significant species are soil inhabiting Hypocreales, including *Metarhizium anisopliae*, *Beauveria bassiana*, and *Entomophaga maimaiga*. Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae, and their respective bacterial symbionts *Xenorhabdus* and *Photorhabdus*, are also important biological control agents of insects. EPNs have a broad host range, kill rapidly, and actively search for hosts to infect. However, they have a short shelf-life and are sensitive to environmental conditions such as low moisture levels and UV exposure (Lacey and Georgis, 2012).

Entomopathogens as biological control agents have several advantages over applying conventional insecticides: they are often host-specific, reducing impact on non-target species, and usually harmless to humans; they can be used in organic farming and Integrated Pest Management Programs; and are less susceptible to the pest resistance issues of conventional products. However, they can be difficult and expensive to mass-produce, with many requiring a host to complete their life cycle; they are also sensitive to environmental conditions such as sun exposure and humidity; and tend to have a short shelf life. A list of entomopathogens (bacteria, fungi, protozoa, and viruses) is listed in **Table 1**.

Most of the bacterial insecticidal products are derived from a small number of species, more significantly *Bacillus thuringenesis* (Bt), a Gram-positive, spore-forming species common in soils. Bt was first discovered in 1901, as the causal agent of sotto disease in silkworms, *Bombyx mori* (Ibrahim et al., 2010). However, the mechanism of Bt-induced insect mortality was not understood until the 1950's, when it was discovered the Cry proteins of certain strains are toxic to insects (Schnepf et al., 1998; Bravo et al., 2007).

Bt remained a niche product used primarily in forestry and organic farming until 1995, when transgenic crops expressing Cry proteins became commercially available. Development of Bt transgenic crops completely transformed Bt application: the toxin becomes constitutively expressed, covers all tissues, and non-target effects were minimized, as only insects that consume the plants are affected (Sanchis, 2011). Today, Bt varieties of maize, cotton, soy, and eggplant are widely grown, and new Bt varieties are constantly being developed (ISAAA's GM Approval Database, 2019). In the United States, more than 80% of maize and cotton acreage is planted with Bt varieties (Wechsler, 2018). Furthermore, over 300 distinct forms of the *cry* gene have been identified so far, with specificity against Lepidopteran, Dipteran, Coleopteran, or nematode hosts (Ibrahim et al., 2010). However, in recent years, the emergence of Bt resistance threatens the efficacy of Bt crops, underlining the importance of insect resistance management (IRM) practice and the need to search for new microbial insecticides.

Spinosad is another successful commercial insecticide derived from microbial natural products (NPs). It is the result of a natural product discovery program, conducted in the mid-1980's by Dow Agrosciences. The program involved exposing larval mosquitoes and Lepidoptera to fermentation broths derived from soil samples. One particular soil sample collected at a Virgin Islands rum distillery showed insecticidal activity toward both taxa, which was determined to be caused by secondary metabolites produced by an unknown actinomycete, described later as *Saccharopolyspora spinosa* (Mertz and Yao, 1990). These metabolites, the spinosyns, were found to be broad-spectrum insecticides with low toxicity to vertebrates and a novel mode of action targeting nicotinic acetylcholine and γ-aminobutyric acid (GABA) receptors. Spinosad, a formulation combining spinosyn A and spinosyn D in an approximately 5:1 ratio, first received regulatory approval in South Korea and the United States in the late 1900's, and was approved in the US for over 150 conventional and organic crops by 2004 (Racke, 2006).

This relatively low number of microbial insecticides is likely non-representative of the true diversity of microbial insecticidal products. Screens of microbial NPs for insecticidal properties are rare in the literature; the majority of NP discovery efforts focus on potential pharmaceuticals. Additionally, in the past 30 years, identifying novel NPs using traditional, culture-dependent screenings has become increasingly difficult, due to the high rate of rediscovery of known compounds (Katz et al., 2016). However, considering the percentage of successfully cultured microbial taxa is frequently estimated to be 1% or less, it is likely that many bioactive microbial NPs have yet to be discovered (Hofer, 2018).

The development of culture-independent, high-throughput metagenomics techniques has begun a renaissance in the field of NP discovery. Microbial toxins could be "mined" by extracting DNA from environmental samples, cloning into plasmids and expressing in recombinant expression systems. However, this method assumes the expression system possesses the molecular machinery necessary for heterologous expression and folding of the desired gene products, including regulatory elements (such as promoters and ribosomal binding sites), chemical substrates and biosynthetic precursors, an assumption which does not always hold. For example, in an experiment by Gabor et al. (2004), shotgun cloning of environmental microbial DNA recovered only ∼40% of enzyme activity in the *E. coli* vector. This issue can be mitigated by conducting functional screenings in a broad range of expression systems, typically through the use of a specially constructed shuttle vector (Katz et al., 2016).

TABLE 1 | List of known bacterial, fungal, protozoa, and viral entomopathogens.

	Pathogen	Target pest(s)	Mechanism	Virulence factor(s)	Commercial application(s)	Citation(s)
Bacteria	*Bacilius thuringenesis* various strains (Bacillaceae)	Lepidoptera, Coleoptera, and Diptera	Colonization and perforation of midgut epithelium	Cry proteins, Vegatative Insecticidal Proteins (VIP)	Spore suspension (liquid and granular), Cry protein expression in GM crops	Sanchis, 2011; Lacey et al., 2015
	Serratia entomophila (Enterobacteriaceae)	New Zealand grass grub (*Costelytra giveni*)	Colonization of foregut and cessation of feeding	sepA, sepB, and sepC on pADAP	Soil-applied granules	Hurst et al., 2000; Wright et al., 2017
	Xenorhabdus nematophila	Lepidoptera, Coleoptera, Diptera	Symbiont in entomopathogenic nematode, *Steinernema carpocapsae.*	Interferes with host AMPs, insecticidal toxins complex (Tc)	*Steinernema-Xenorhabdus* association	Ji et al., 2004; Stilwell et al., 2018
	Bacillus popilliae, Bacillus lentimorbus	Larvae (grubs) of Japanese beetle (*Popillia japonica*)	Spores germinate in the larval gut, vegetative cells penetrate into the haemocoel leading to death.		Soil-applied spores	Rippere et al., 1998
Fungi	*Beauveria bassiana* (Cordycipitaceae)	Aphids, fungus gnats, mealy bugs, mites, thrips, whiteflies	Conidia land on host and germinate into cuticle; mortality caused by fungal toxicity and colonization of vital tissues		Spore suspension, promotion of existing natural populations	Lacey et al., 2015
	Metarhizium anisopliae	termites, mosquitoes (*Aedes* spp., *Anopheles* spp. and *Culex* spp.), and cattle ticks; Various ticks and beetles; root weevils, flies, gnats, thrips.	Conidia land on host and germinate into cuticle; mortality caused by fungal toxicity and colonization of vital tissues		Spore suspension, promotion of existing natural populations	Lacey et al., 2015; Aw and Hue, 2017
	Verticillim lecanii	Greenhouse whitefly (*Trialeurodes vaporariourum*), aphids (Hemiptera: Aphididae)	Conidia land on host and germinate into cuticle; mortality caused by fungal toxicity and colonization of vital tissues		Spore suspension	Sinha et al., 2016
	Lagenidium giganteum	Mosquito (larvae)	Biflagellate zoospores attach to larval cuticle, proliferates in host.		Spore suspension	Suh and Axtell, 1999
Protozoa	*Nosema locustae*	European cornborer caterpillars, grasshoppers and mormon crickets	Infects fat body tissues; disrupting host metabolism and energy storage		Spores applied to bait	Solter et al., 2012
	Nosema pyrausta	European corn borer (*Ostrinia nubilalis*)	Spore is consumed by larva, the spore's polar filament is extruded, penetrating a midgut cell and inoculating it with sporoplasm. Disrupts larval development, pupation, adult longevity, oviposition and fecundity.		Spore suspension applied to bait	Gassman and Clifton, 2017
	Vairimorpha necatrix	Armyworm (Noctuidae)	Spore consumed by larva, penetrating a midgut cell, and inoculating it with sporoplasm.		Spores applied to bait	Solter et al., 2012
Viruses	Cd GV *(Cydia pommonella* granulosis virus)	Codling moth (*Cydia pomonella*)			Suspension concentration of virus	Sauer et al., 2017
	Nuclear polyhedrosis viruses (NPV)	Lepidoptera	Capsid dissolves in the alkaline midgut of Lepidoptera to release the virus particle causing cell lysis.	Polyhedral capsid	Suspension concentration of virus	Chiu et al., 2012
	Ns NPV *(Neodiprion sertifer* nuclear polyhedrosis virus)	Pine sawfly	Capsid dissolves in the alkaline midgut to release the virus particle causing cell lysis.	Polyhedral capsid	Suspension concentration of virus	Podgwaite et al., 1984; Chiu et al., 2012
	Ag NPV *(Anticarsia gemmatalis* nuclear polyhedrosis virus)	Velvet bean caterpillar	Capsid dissolves in the alkaline midgut to release the virus particle causing cell lysis.	Polyhedral capsid	Suspension concentration of virus	Castro et al., 1997; Chiu et al., 2012

Microbial Semiochemicals

Insects rely on chemical communication to adjust their behaviors in response to the environment. These behaviors include foraging, mating, hazard avoidance, kin recognition, and social interactions. Chemicals that convey signals between organisms leading to changes in the behavior of recipient organisms are referred to as semiochemicals. A sustainable approach to pest management is to harness these semiochemicals to manipulate insect behavior. Insect pheromones (defined as semiochemicals that mediate interspecific interactions) constitute a large market share of attractants for plant and urban pests. Pheromone-baited traps are used in both monitoring and control of a variety of agricultural, forestry, and urban pests, such as the cotton boll weevil *Anthonomus grandis*, the gypsy moth *Lymantria dispar,* and the house fly *Musca domestica* (Witzgall et al., 2010). Field application of synthetic sex pheromones has also been shown to effectively control multiple Lepidopteran pests, including codling moth *Cydia pomonella*, pink bollworm *Pectinophora gossypiella*, and diamondback moth, by inhibiting their ability to find mates, a process known as mating disruption (Cardé and Minks, 1995).

Many commercially used insect semiochemicals are not insect pheromones, but rather, plant or microbial volatile organic compounds (VOCs) (Davis et al., 2013). VOCs are a large class of low-molecular weight compounds signified by high volatility and low vapor pressure. VOCs can be produced through different metabolic pathways, such as fermentation, amino acid catabolism, sulfur reduction, and terpenoid biosynthesis (Lemfack et al., 2017; Choudoir et al., 2019). Many VOCs are effective semiochemicals, requiring only small quantities to elicit responses from insects, which tend to have highly sensitive chemosensory (especially olfactory) systems. VOCs permit ranged communication by traveling through the air and serve as important means of environmental perception.

Recently, there is a growing interest in the discovery and implementation of microbial VOCs (mVOCs) as pest management tools. Multiple factors have contributed to this trend. First, according to mVOC 2.0, an online database of over 2,000 microbial volatiles from nearly 1,000 microbial species (Lemfack et al., 2017), <10% of known mVOCs are studied or assigned with functions. Given that an estimated 10^{18} microbial species are expected to exist on earth, many mVOCs have yet to be identified, Second, there is an increasing demand for natural products in agricultural pest management, as such products are generally better perceived by the public than synthetic chemicals in terms of safety and environmental sustainability.

Microbes have existed for over three billion years and through the processes of evolution, their metabolism is extremely diverse and versatile. The metabolic capabilities of microbiomes often far exceed than those of their eukaryotic hosts. Additionally, microbial metabolism can be augmented by genetic engineering using recombinant and synthetic techniques. This implies that microbial production of desired metabolites (including mVOCs) can be readily scaled up to meet the commercial needs. Advances in "-omics" techniques and computational biology to study the microbial metabolism can also help to accelerate the discoveries of mVOCs in a number of ways. Notably, high-throughput sequencing is illuminating the biosynthetic potential of microbiomes that extend beyond from cultured microbes, to uncultured ones found in plants, soils, and other natural resources. Computational tools are also becoming available to assemble complex metagenomes and metatranscriptomes, and to predict biosynthetic gene clusters from mining big data sets of DNA/RNA sequences (Medema et al., 2011; Weber et al., 2015). Although the vast majority of microbes on earth are yet to be cultured such that they are not readily amenable to conventional culture-dependent approaches, biologically-active natural products can be obtained from uncultured microbes by cloning environmental DNA into plasmid vectors and expressing in recombinant systems, such as *E. coli*, yeast, or baculovirus (Rappé and Giovannoni, 2003; Hofer, 2018). This approach has been actively pursued in screening for new antibiotics and therapeutic drugs in recent years, and in principle can be applied to the discoveries of microbial-based compounds for agricultural uses, including novel semiochemicals as well as biopesticides (Clardy et al., 2006).

Besides acting on insects directly, microbes can influence insect behavioral interactions with plants by altering plant VOC emission profiles. Ballhorn et al. (2013) showed that lima bean plants colonized with *Rhizobia* emitted a different VOC profile from the non-colonized plant in response to stimulation by jasmonic acid (JA), an important plant hormone involved in anti-pest defense, growth, and development. The distinct VOC profile from colonized plants corresponds to significantly greater *Epilachna varivestis* (Mexican bean beetle) repellency and was attributed to increased indole secretion (Ballhorn et al., 2013). Additionally, several symbiotic fungi, including endophytic fungi and arbuscular mycorrhizas, were shown to modify plant volatile emissions and consequently plant susceptibility to various insect pests (Fontana et al., 2009). These results hint at indirect beneficial effects of symbiotic microbes in plant defense that can be developed into probiotics for plants. On the other hand, some pathogenic microbes are known to manipulate plant volatile emissions to attract pests and potential vectors. Examples include the bacterium *Candidatus* Liberibacter asiasticus that infects citrus plants and causes Huanglongbing (HLB), also known as citrus greening disease. *Candidatus* L. asiaticus infection of citrus plants increased methyl salicylate levels, which was believed to promote attraction of its vector insect, the Asian citrus psyllid (*Diaphorina citri*) (Li et al., 2017). A number of plant viruses such as the Cucumber mosaic virus and Barley yellow dwarf luteovirus were also found to modify plant volatile emission to attract its vector insects (aphids) to improve their transmission (Sharifi et al., 2018).

Applications of microbial-based attractants or repellents in pest management have been backed by laboratory studies and field trials. For example, the invasive *Drosophila suzukii* is a major pest of small fruits and distinct from other non-pest *Drosophila* species in that it attacks intact, ripe, and ripening fruits (Keesey et al., 2015). Research has shown that *Drosophila* species are strongly attracted to fermentation products (Keesey et al., 2015), and by adding fermenting sugar-yeast mixture to a commonly used non-microbial trap bait (apple cider vinegar + 10% ethanol), *D. suzukii* capture rate increased by up to 15-fold in both laboratory and field conditions (Lasa et al., 2017).

Huang et al. (2017) also showed combination baits of yeast-wine-vinegar-sugar mixtures are superior attractants compared to wine-vinegar alone. Ishii et al. (2015) genetically modified the acetic acid bacterium (AAB) *Komagataeibacter europaeus* with increased acetoin (a key ingredient in vinegar) production and was able to trap significantly more *Drosophila* using the mutant strain compared to other strains loaded on sticky traps. Similarly, microbial semiochemicals have been shown to attract tephritid fruit flies. For instance, a set of 21 compounds including alcohols, ketones, pyrazines, phenols and acids were identified from fermented broth of *Klebsiella pneumoniae* that attracted the Mexican fruit fly (*Anastrepha ludens)* (Lee et al., 1995). The Caribbean fruit fly (*Anastrepha suspensa)* females were shown to be attracted to VOCs 3-methyl-1-butanol and ammonia emitted from *Enterobacter agglomerans*, a bacterium isolated from larvae infested fruits and fly adults (Epsky et al., 1998). Some mVOCs, especially those produced by fungi, have potential to be developed into insect repellents. Geosmin was first described to be emitted by mold, sensed and avoided by *Drosophila melanogaster* (Stensmyr et al., 2012). It was later shown to induce aversive behaviors in SWD and may serve as an oviposition deterrent on crops (Stensmyr et al., 2012; Wallingford et al., 2016). The establishment of certain fungal species (*Phoma* spp., *Fusarium* spp., or *Rhizopus* spp.) on chicken feces also significantly reduced oviposition by the housefly, *Musca domestica*, and the effect was associated with emission of dimethyl trisulfide and 2-phenylethanol (Lam et al., 2010). Plant-associated symbionts could be another source of VOCs. For instance, *Muscodor vitigenus*, an endophyte of the tropical liana *Paullina paullinoides*, produces naphthalene and is strongly repelled by insects (Daisy et al., 2002). Microbial catalysis of repellent chemicals has been demonstrated in fungi including *Penicillium*, *Aspergillus*, and *Fusarium* that can convert JA derivatives dihydrojasmone and cis-jasmone to bioactive compounds such as (+)-(R)-4-hydroxydihydrojasmone and (−)-4-hydroxyjasmone (Gliszczyńska et al., 2015). These compounds were then found to repel aphids in a food-choice behavioral assay. In a study by Skrobiszewski et al. (2018) (±)-β-aryl-γ-ethylidene-γ-lactones (compounds which have previously been determined to have phagodetterent, antifungal, antibacterial, and anticancer activity) were enantioselectively hydrolyzed by a strain of *Aspergillus ochraceus* to form (−)-(*S*)-γ-ethylidene-γ-lactones and (+)-(*R*)-γ-ketoacids (Skrobiszewski et al., 2018). The phagodeterrent/repellent properties of these derived chemicals were then demonstrated against the lesser mealworm *Alphitobius diaperinus*. Many entomopathogenic bacteria, such as *Bacillus thuringiensis* (Bt), *Pseudomonas entomophila*, and nematode-associated species in the genus of *Xenorhabdus* and *Photorhabdus* have also been shown to have insect repellent properties (Bode, 2009; Kajla et al., 2019). For example, a recent study showed that a cocktail of bioactive secondary metabolites (primarily fabclavines) isolated from *Xenorhabdus budapestensis* exhibited greater repellency against *Aedes aegypti* than DEET and required a smaller concentration to achieve the same repellent effect (Kajla et al., 2019). More examples of microbial semiochemicals and their interaction with insects has been described by Leroy et al. (2011).

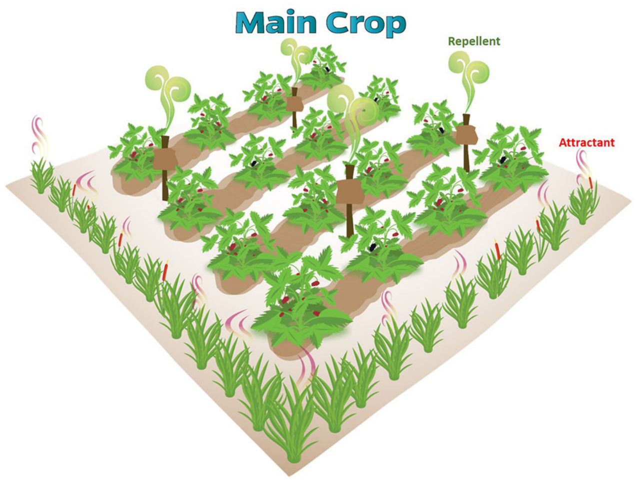

FIGURE 2 | A generic pull-push system for agricultural pest management. Microbial-based semiochemicals can replace or synergize non-microbial attractants and repellents in the pull-push system.

By identifying the different pathways from large-scale omics data, scientists will be able to make accurate inferences of microbial regulatory networks in mVOC production. Another emerging field is to dissect synergistic multi-species interactions (i.e., consortia of microbes) in metabolite production (Schulz-Bohm et al., 2015; Kai et al., 2018). Together, these studies hold promise that microbial-based insect attractants or repellents produce more diverse, effective, and versatile products for agricultural applications. One of the applications is to incorporate microbial based semiochemicals into push-pull strategies (**Figure 2**). In a typical push-pull system, a target pest is repelled or deterred away ("push") from a protected source (a valuable crop or farm animal) using stimuli that make it unattractive. In parallel, the pest is diverted to attractive stimuli ("pull") placed outside of the protected source. Stimuli commonly in use include different crop plants (intercrops or trap crops), visual signs, pheromones, synthetic volatiles, phagostimulants/repellents, and antifeedants (Cook et al., 2006). Microbial products can replace or be used in conjunction to these stimuli to improve the efficacy of future push-pull strategies.

Combining Microbial Based Biopesticides With Nanotechnologies

Advances in nanotechnology are promoting effective and sustainable use of biopesticides in field applications. For instance, the potency of entomopathogenic bacteria, including Bt and *Photorhabdus luminescens,* increased significantly when they were applied to insect pests in the form of nanoparticles (Kim and Je, 2012; Kulkarni et al., 2017). Murthy et al. (2014) demonstrated that larvae subjected to Bt nanoparticles resulted in a faster and higher mortality as compared to unhomogenized Bt powder, owing to increased solubility of the Cry toxin in the alkaline midguts. Additionally, the development of nanoformulation delivery systems, such as nanoemulsion, nanocapsule, and nanosuspension, enhances the durability and stability of biopesticides under variable environmental conditions including exposure to UV light and humidity (Li et al., 2015; Sabbour and Singer, 2016; Zhang et al., 2016; Damalas and Koutroubas, 2018; Vassilev et al., 2020). These delivery systems are ecofriendly since they are often made of biodegradable natural polymers.

An emerging theme led from the research on nanoformulation is smart nanopesticides, in which the active ingredients are encapsulated in stimuli-responsive carriers such that their release can be controlled by stimuli such as light, temperature, humidity, or pH (Khot et al., 2012; Camara et al., 2019; Kumar et al., 2019). The stimuli-responsive delivery approach enables more precise spatial delivery while reduces the dosage and frequency of pesticide applications, therefore minimizing the impacts on the environment. In case of VOCs, nanoformulations have the potential to fine-tune the thermal stability of the compounds, leading to optimal release and longevity of the semiochemicals. Comprehensive reviews on different types of nanopesticides and stimuli-responsive delivery systems have been published by Huang et al. (2018), Camara et al. (2019).

Currently, the use of nanotechnology in agrochemical applications is still at an early stage. However, commercial products in this niche are beginning to be introduced to the market. An example is Seltima®, a fungicide launched by BASF in 2016 to protect rice crops. Seltima uses humidity responsive polymers to encapsulate pyraclostrobin, a fungicide chemical that is highly toxic to aquatic organisms. This encapsulation is resistant to water, thus allowing controlled release of the fungicide to the rice leaf surface and minimizing contamination of the surrounding aquatic ecosystems.

Based on the expectation that new microbes and microbiome-derived chemicals are being discovered exponentially, there will be unforeseen obstacles in the handling, storage, and delivery of these products for agricultural uses. Integration of microbial products with nanotechnology offers versatile means to improve their efficacy and stability, while controlling their environmental dispersal. Together, the combined efforts of microbiome mining and nanotechnology offers great potential in generating transformative bioinsecticide applications in near future.

MICROBIAL INTERVENTIONS TO IMPROVE FITNESS OF MASS-REARED INSECTS FOR AUTOCIDAL PROGRAMS

Probiotics strategies to promote populations of beneficial insects (e.g., pollinators, natural enemies of pests) has been described by Engel and Moran (2013). Here, we focus on microbial strategies to promote insect rearing for autocidal programs, such as, the incompatible insect technique (IIT, described earlier in the section "Manipulation of insect-associated microbiomes for pest management") and the sterile insect technique (SIT).

SIT is an environmentally-friendly insect pest control method involving mass-rearing of the target pests, where sterile male insects are generated by exposure to non-lethal-doses of ionizing radiation (Dyck et al., 2006; Nikolouli et al., 2018). Large numbers of fully sterile males are then released to mate with wild females, resulting in suppression or eradication of the pest population (Knipling, 1979; Dyck et al., 2006). A derivative of the SIT termed inherited sterility (IS) has also been developed for insect pests, mainly lepidopterans, that require high radiation dose to achieve complete sterility. In IS, partially sterile males are generated by an exposure to sub-sterilizing doses of radiation and then mated with wild females, resulting in reduced egg viability and highly sterile offspring that are predominately male (Vreysen et al., 2010). This approach avoids deleterious effects triggered by high-dose radiation on the insects. Studies have shown that males with inherited sterility suppress wild populations more effectively than fully sterile males for several lepidopteran (moth) species.

Over the past decades, SIT has been used successfully to control several high-profile pests, including the primary screwworm (*Cochliomyia hominivorax*), medfly (*Ceratitis capitata*), the Mexican fruit fly (*Anastrepha ludens*), various Lepidoptera (moths), and tsetse flies (Pereira et al., 2013; Calla et al., 2014; Vreysen et al., 2014; Barnes et al., 2015; Lees et al., 2015; Zhang et al., 2015; Bourtzis et al., 2016; Munhenga et al., 2016). However, a major setback for pest control through autocidal programs is the cost, because large numbers of treated insects are required to be released to compete with wild males and sometimes repeated releases are necessary. For SIT, a ratio up to 100 sterile insects for each wild insect is required. Less data is available for IIT, but a study on *Wolbachia*-infected mosquitoes showed that a release at 10:1 ratio of sterile to wild males was sufficient to suppress local mosquito population (Harris et al., 2012). Additionally, irradiation and handling in mass rearing facilities compromise the survival and performance of insects, which also contributes to the high cost of autocidal programs. Empirical evidence suggested that reduced performance, such as competence in attracting and mating with wild females, of mass-reared and irradiated males could be linked to disruption of their gut microbiomes. Agricultural pests and disease vectors reared under laboratory conditions tend to have fewer gut microbial taxa than their wild counterparts (Xiang et al., 2006; Rani et al., 2009; Rinke et al., 2011; Wang et al., 2011; Morrow et al., 2015; Liu et al., 2016; Waltmann et al., 2019; Raza et al., 2020). Such reduction in species richness or diversity can be due to limited microbial exposure, as insects are believed to acquire their gut microbes from their natural habitats and food sources but these microbes are mostly absent from the artificial environment and diets (Drew and Yuval, 2000; Wong et al., 2017; Stockton et al., 2019). Additionally, artificial rearing exerts distinct selection pressure on the insect gut microbiome composition. For instance, insect diets in the laboratory are commonly added with antimicrobials as preservatives. These antimicrobials could wipe out most of the microbes naturally associated with the insects, as shown in the *B. oleae* example discussed earlier. Considering the microbiome as an ecosystem, losses in species richness or diversity may lead to poor host outcomes, especially if the microbes provide specialized services to the host.

While a considerable body of literature exists on the impact of artificial rearing on the microbiomes of different insects, very little is known about how irradiation alters the insect microbiome. Most of the research has been conducted on tephritid fruit flies because several significant pests belonging to this group have been the foci of SIT applications. In medfly, newly eclosed irradiated males had lower levels of several dominant gut bacteria (especially *Klebsiella* sp.) but higher level of *Pseudomonas* sp. that was not presented in wild flies (Ami et al., 2010) However, some of these bacteria appeared to recover in abundance after a few days post irradiation. Similarly, in the oriental fruit fly, irradiation treatment selectively increased the abundance of *Lactococcus* and *Orbus* but diminished *Lactobacillus*, members of the Orbacecae family, and *Morganella* (Stathopoulou et al., 2019). More research is needed to define how these transient changes in the gut microbiome influence fly recovery from irradiation, and their long-term effects on fly fitness.

Based on the assumption that reversing the microbiome changes associated with artificial rearing or irradiation treatment can improve the fitness of the insects, scientists have been investigating the effects of dietary supplementation with bacteria on the performance of irradiated tephritid fruit flies. A summary of the current findings is shown in **Table 2**, showing that probiotic effects were reported in many, but not all studies. The discrepancies of results among these studies highlight that different parameters likely affect the outcome of probiotic treatment on insects, such as host age, genotype, microbial strain, diet and rearing condition. It is also worth noting that studies done so far have only tested single bacterium or cocktails of a few bacteria as supplement. Other approaches to manipulate the microbiomes of artificially reared or irradiated insects remain to be tested. One approach would be microbiome transplantation, either by rearing the treated insects on diet seeded with feces from freshly collected wild insects, or co-housing treated insects with wild insects, although this may be challenging to scale up for mass rearing. Another option would be to modify the diet recipe to stimulate the growth of beneficial microbes in the insect gut ("prebiotics"), which includes optimizing the nutrient composition and possibly incorporating natural food substances into their diets.

Clearly, more work is still needed to define how probiotics improve the fitness of artificially reared, sterile insects, and to identify treatment parameters that affect insect outcomes in response to the different microbial intervention approaches mentioned above. From a basic research angle, insects are excellent models to tease apart the complexities of microbial interactions and mechanisms, since their microbiomes are generally less diverse than in the mammalian systems, where most probiotics research has been conducted.

CONCLUDING REMARKS

The ongoing advances and reduced cost of high throughput sequencing and omics technologies have enabled scientists

TABLE 2 | Effects of dietary bacterial supplementation on the performance of tephritid fruit flies.

Target insect	Insect life stage	Bacterial supplementation	Beneficial effects	Citation(s)
Ceratitis capitata (Vienna 8 strain)	Larvae	*Klebsiella pneumoniae*, *Enterobacter* sp. and *Citrobacter freundii*	Reduced the number of potentially pathogenic *Pseudomonas* spp., improved male mating competitiveness, increased adult size	Hamden et al., 2013
Ceratitis capitata (Vienna 8 strain)	Larvae	*Enterobacter* sp. AA26	Faster larval and pupal development	Augustinos et al., 2015
Ceratitis capitata (Vienna 8 strain)	Adult	*Klebsiella oxytoca*	Reduced the number of potentially pathogenic *Pseudomonas*, *Morganella* and *Providencia* spp., shortened the mating latency of the sterile males	Ami et al., 2010
Ceratitis capitata	Adult	*Klebsiella oxytoca*	Improved male mating competitiveness, reduced female remating, increases survival under starvation conditions	Gavriel et al., 2011
Ceratitis capitata	Adult	*Enterobacter agglomerans* and *K. pneumoniae* in a yeast-enhanced agar	Improved mating competitiveness	Niyazi et al., 2004
Bactrocera oleae	Adult	*Pseudomonas putida*	Increased female fecundity	Sacchetti et al., 2014
Bactrocera dorsalis	Adult	*Klebsiella oxytoca* BD177	Improved male mating competitiveness, increased survival rate and life span of irradiated males, improved food intake, sugar and amino acid levels in the haemolymph of Irradiated flies	Cai et al., 2018
Bactrocera cucurbitae	Larvae	*Enterobacter* sp.	Increased pupal weight and adult survival rate	Yao et al., 2017
Bactrocera tryoni (Froggatt)	Larvae	*Enterobacter* sp. and *Asaia* sp.	Reduced larval development time	Shuttleworth et al., 2019
		Leuconostoc and cocktail of *Asaia*, *Enterobacter*, *Lactobacillus* and *Leuconostoc*	Reduced mean time from egg hatch to adult eclosion	

to conduct in-depth analyses of microbiomes in various agroecosystems, including insects, plants, and other natural resources. It signifies an exciting era to discover new microbes or microbiome functions with potentials to be applied for insect pest management. Progresses in cutting-edge gene editing, microbial engineering, and nanotechnologies allow scientists to continue to refine procedures to extract bioactive products from uncultured microbes and apply them into pest management. These approaches will prove to be highly valuable for agricultural innovations.

AUTHOR CONTRIBUTIONS

AW conceived of the article and generated **Figure 1**. AW, MQ, SS, KG, and JH conducted the literature search, writing, and editing of the manuscript. MQ, SS, and KG generated **Table 1**. MQ generated **Table 2**. All authors have approved the final version of the manuscript.

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