frontiers in Ecology and Evolution

REVIEW
published: 19 February 2020
doi: 10.3389/fevo.2020.00022

Understanding the Effects of Sublethal Pesticide Exposure on Honey Bees: A Role for Probiotics as Mediators of Environmental Stress

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Specialty section:
This article was submitted to Conservation, a section of the journal Frontiers in Ecology and Evolution

Received: *26 August 2019*
Accepted: *27 January 2020*
Published: *19 February 2020*

Citation:
Chmiel JA, Daisley BA, Pitek AP, Thompson GJ and Reid G (2020) Understanding the Effects of Sublethal Pesticide Exposure on Honey Bees: A Role for Probiotics as Mediators of Environmental Stress. Front. Ecol. Evol. 8:22. doi: 10.3389/fevo.2020.00022

Managed populations of the European honey bee (*Apis mellifera*) support the production of a global food supply. This important role in modern agriculture has rendered honey bees vulnerable to the noxious effects of anthropogenic stressors such as pesticides. Although the deleterious outcomes of lethal pesticide exposure on honey bee health and performance are apparent, the ominous role of sublethal pesticide exposure is an emerging concern as well. Here, we use a data harvesting approach to better understand the toxicological effects of pesticide exposure across the honey bee life cycle. Through compiling adult- and larval-specific median lethal dose (LD_{50}) values from 93 published data sources, LD_{50} estimates for insecticides, herbicides, acaricides, and fungicides are highly variable across studies, especially for herbicides and fungicides, which are underrepresented in the meta-data set. Alongside major discrepancies in these reported values, further examination of the compiled data suggested that LD_{50} may not be an ideal metric for honey bee risk assessment. We also discuss how sublethal effects of pesticide exposure, which are not typically measured in LD_{50} studies, can diminish honey bee reproduction, immunity, cognition, and overall physiological functioning, leading to suboptimal honey bee performance and population reduction. In consideration of actionable solutions to mitigate the effects of sublethal pesticide exposure, we have identified the potential for probiotic supplementation as a promising strategy that can be easily incorporated alongside current agricultural infrastructure and apicultural management practices. Probiotic supplementation is regularly employed in apiculture but the potential for evidence-based targeted approaches has not yet been fully explored within a formal toxicological context. We discuss the benefits, practical considerations, and limitations for the use and delivery of probiotics to hives. Ultimately, by subverting the sublethal effects of pesticides we can help improve the long-term survival of these critical pollinators.

Keywords: honey bee, pesticide, toxicology, immune, development, cognition, probiotic, LD_{50} (median lethal dose)

INTRODUCTION

Popular interest in the biology of the common European honey bee (*Apis mellifera*) has surged in recent years due to the stark population decline of this important pollinator (Goulson et al., 2015). Managed colonies of *Apis mellifera*, strictly speaking, are an invasive insect species to the Americas (Whitfield et al., 2006), but contribute to the production of roughly a third (∼35%) of the global food supply (Blacquière et al., 2012). In Canada, this single insect species is tied to a ∼$2.5 billion (CAD) industry of pollination services, whereby colonies are strategically situated in orchards and fields to promote farmer yields via the cross-fertilization of flowering crops (Mukezangango and Page, 2017). In the United States, the value of bee-mediated pollination is even larger (Calderone, 2012). Despite the value of honey bees to the agri-food industry, we have yet to fully understand how their populations cope with natural- and agriculture-induced stress, or to what extent this stress explains recent increases in reported mortalities (Goulson et al., 2015).

Though no single factor can provide a universal explanation for the apparent decline of honey bee populations, one overriding theme to emerge from the front lines of a global research effort is that more than one factor combines to overwhelm bee health. Among them, pesticide exposure (Sanchez-Bayo and Goka, 2014; Zhu et al., 2014), pathogens (Evans and Schwarz, 2011), and habitat loss (Clermont et al., 2015; Youngsteadt et al., 2015) are prime factors that disproportionately contribute to the decline. In particular, sublethal pesticide exposure has been a popular focus of political discussion, which has highlighted the potential conflict between parties that rely on the production and use of commercial pesticides and those who advocate for their regulation and alternative means of crop pest control. Moreover, the risk of pesticides to honey bees is especially alarming due to their long half-lives (Bonmatin et al., 2015) and presence in food (Lu et al., 2018) and honey (Mitchell et al., 2017).

Herbivorous pest insects are the intended target of systemic application of agriculture insecticides. Nonetheless, honey bees are insects just the same and thus cannot help but to be vulnerable through incidental exposure. These pesticides are applied to crops in two main ways: spraying and seed coating, both of which have effects on honey bee exposure. Spraying is typically accomplished through aerial application, but some use vehicle-based sprayers or manual units. These are effective as a means of pest control but can inadvertently affect honey bees through direct topical contact or through secondary exposure via bee consumption of contaminated pollen, nectar, or water (Fairbrother et al., 2014; Poquet et al., 2014; Park et al., 2015; Zhu et al., 2015). Furthermore, spray-based application allows pesticides to disseminate into the broader environment and contaminate surrounding habitats, including orchards and fields that are not intentionally sprayed (McArt et al., 2017; Simon-Delso et al., 2017). As an alternative to sprays, seed coatings can avoid some off-site targets by more carefully controlling pesticide delivery to its intended crop. However, seed coatings can also cause collateral damage because some pesticides remain active in plant tissue, including nectar and pollen (Krupke et al., 2012; Goulson, 2013; Alburaki et al., 2015; Samson-Robert et al., 2017).

In addition to inadvertent exposure from modern agricultural practices, honey bees can be deliberately exposed to miticides and fungicides by beekeepers through basic hive management practices that aim to combat pests and pathogens. Though best practice for beekeepers is intended to augment the bee's own defences, their application may sometimes harm the pollinators.

In total, managed honey bee colonies can be exposed to a diverse set of pesticides, which can only be determined by detailed toxicological sampling (Tsvetkov et al., 2017). These chemicals affect bees through any combination of ingestion, contact exposure, or ambient intake through respiratory openings (spiracles). Contact exposure and ingestion as routes of contamination are well studied and reveal pesticide-specific effects on honey bee health (Villa et al., 2000; Stoner and Eitzer, 2013; Sanchez-Bayo and Goka, 2014; McArt et al., 2017). Honey bee respiration, which occurs in respiratory spiracles that are found along the thorax and abdomen of adults, is thought to only be a minor route of pesticide uptake (Geoghegan et al., 2013). Ultimately, these modes of exposure are responsible for the accumulation within individual bees, which can lead to bioaccumulation of pesticides throughout the hive (**Figure 1**).

The risk to honey bees as a result of pesticide exposure is evaluated by considering both the incidence of exposure and toxicity of pesticides used. Incidence of exposure is quantified

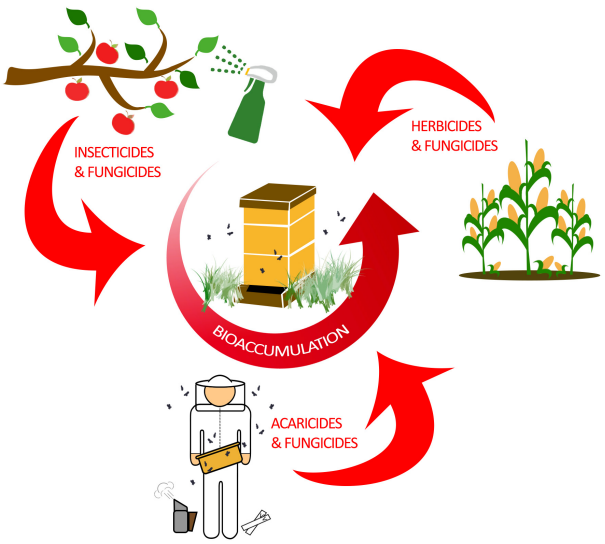

FIGURE 1 | Bioaccumulation of pesticides in a honey bee colony. Honey bees are exposed to a wide variety of pesticides through agricultural practices and modern beekeeping. Typically, farming and other agricultural practices are responsible for exposing honey bees to insecticides, herbicides, and fungicides. As honey bees forage for nectar and pollen, they are incidentally exposed to pesticides which accumulate in the hive by physically transferring the contaminated food sources to unexposed bees. However, honey bees can also be intentionally exposed to acaricides and fungicides by beekeepers in efforts to control mite burden and fungal diseases in the hive. Ultimately, pesticide bioaccumulation in the hive has the potential to negatively impact all honey bee ranks.

by examining the actual usage rates of pesticides, mode of application, and environmentally-relevant concentrations. A widely-used metric for quantifying pesticide-specific toxicity is the lethal dose (LD) at which half the population dies, or the LD_{50}. This latter metric uses acute exposure (24–96 h) of adult honey bees to predict a toxic dose. Despite its widely accepted use, reports of LD_{50} put less emphasis on larval toxicity and can also vary widely in methodology and sample size. Moreover, these LD_{50}-based risk assessments do not consider the effect that sublethal pesticide exposure can have on honey bees.

In this paper, we evaluate the role of sublethal pesticide exposure (defined herein as exposure to insecticides, acaricides, fungicides, and herbicides) in the toxicity and management of honey bees. To initiate this effort, we first collected a meta-data set of pesticide-specific LD_{50} for honey bees by analyzing published literature in PubMed, Web of Science, and Google Scholar for studies that contained the following keywords [honey bee], [LD50], and specific pesticide names. We excluded articles if (1) they did not report LD_{50} values in weight per bee (however, LD_{50} values reported as weight per mass were converted to weight per single bee, assuming that one bee is 100 mg); (2) the article was inaccessible with current access. In addition to providing both adult and larval LD_{50} values, this database also allows for the assessment of variation in reported LD_{50} estimates. We then set out to understand how sublethal pesticide exposure can harm multiple aspects of honey bee health. Again, we conducted a bibliometric search for studies that contained combinations of the following keywords: [honey bees], [pesticide], [sublethal], [development], [immune], [metabolism], [cognition], and [reproduction]. After the initial screen, we used forward and reverse citation searches of individual articles to expand our data set. With the data from these studies, we are able to construct an impartial summary of the adverse effects that sublethal pesticide exposure has on honey bees.

We close by proposing three areas of focus for future studies: expanding the knowledge of sublethal pesticide exposure to other pesticides; understanding the role for the microbiota in aiding host survival toward pesticides; and testing the ability for probiotics to mitigate the sublethal effects of pesticides.

PESTICIDE TOXICOLOGY IN HONEY BEES

The dose at which 50% of the population dies (LD_{50}) is a useful metric for quantifying pesticide-specific lethality and evaluating sublethal exposure in adult honey bees. Estimates of LD_{50} can vary by length of exposure and mode of delivery, so knowing the oral- and dermal-specific LD_{50} of individual pesticides can make a useful predictor of pesticide-associated risk. Further, by comparing LD_{50} obtained for pest and beneficial insect species, we can better assess the trade-off between intended target species and any collateral damage to pollinators. When combined with pesticide application rates, these values are useful for calculating the risk of pesticide use to pollinators. The Hazard Quotient (HQ = application rate/LD_{50}) is a viable metric to calculate field use risk of pesticide application but can be erroneous alongside variable LD_{50} values (Stoner and Eitzer, 2013).

Despite the potential of comparative analysis, the variation that is associated with published estimates of LD_{50} is substantial for both contact (**Table 1**) and oral (**Table 2**) versions of this metric, which can reduce their value in risk assessment. The seemingly high variation in LD_{50} estimates, which can range up to 500-fold, may stem in part from differences in sample size, precision of measurement, and experimental protocol. Even for toxicological studies with a high degree of statistical power, the variance associated with LD_{50} can be large (Baines et al., 2017). This suggests that the genuine effect of pesticides on insect survivorship may vary strongly between populations, regardless of how it is measured. Biological sources of variation may stem from differences in age (young, nurse-age workers versus older, foraging-age workers), genotype (natural variation as well as apicultural strains), caste (workers, queens, drones), or life stage (larvae versus adults) (Rinkevich et al., 2015; Tosi and Nieh, 2019). A cursory comparison between adult- and larval-derived LD_{50} values suggests strong biological variance from life stages (**Table 3**). A detailed dataset of all LD_{50} values collected for this manuscript can be found in **Supplementary Table S1**.

Additional sources of variation can occur from the composition of the pesticide formulations that are used. While different amounts of solvents used for toxicology analysis can affect the readout (Wilkins et al., 2013), pesticide adjuvants (other ingredients found in pesticide formulations that are thought to be inert) can also influence pesticide toxicity (Chen et al., 2019). An emerging interest is the potential for synergistic toxicity between multiple pesticides that are applied in combination. These could increase overall honey bee mortality, albeit in unpredictable ways (Johnson et al., 2013; Zhu et al., 2014; Wade et al., 2019). Despite their relevance under normal field conditions, these aspects of pesticide toxicology are often overlooked in LD_{50} studies, which typically determine the toxicity of individual pesticides in standard laboratory solvents.

PESTICIDES AFFECT DETOXIFICATION AND METABOLISM IN HONEY BEES

Like most insects, honey bees use an array of enzymes to detoxify pollutants and other harmful chemicals that they may encounter, including pesticides (Gong and Diao, 2017). However, honey bees are genetically depauperate in a number of key detoxification genes, with the remainder of relevant genes expressed at low levels (Claudianos et al., 2006). Some key detoxifying genes that appear underrepresented in the honey bee genome include many of the cytochrome P450 monooxygenases (phase I detoxification—oxidation, reduction, and hydrolysis of xenobiotics), glutathione-S-transferases (phase II detoxification—increase water solubility of xenobiotics for excretion), and carboxyl/cholinesterases (insecticide resistance) compared to the well-studied insect model, *Drosophila melanogaster* (Claudianos et al., 2006). Although honey bees possess similar amounts of detoxification genes compared to other members of the Apidae family, they

TABLE 1 | Range and median of reported contact LD_{50} values of pesticides for adult honey bees.

Pesticide Name	Contact LD_{50} (μg/bee)	Range	Quartile Coefficient of Dispersion	Number of reports
Abamectin	7.8	N/A	N/A	1
Acephate	1.2	0.019 – 1.2	0.727	3
Acetamiprid	17.045	1.69 – 276.85	0.950	6
Aldrin	0.352	0.0605 – 0.353	0.707	3
Aminocarb	0.6165	0.121 – 1.112	N/A	2
Amitraz	2.61	1.986 – 3.66	0.232	4
Carbaryl	1.1	0.055 – 26.53	0.831	15
Chlorpyrifos	0.110	0.024 – 0.320093	0.396	9
Clothianidin	0.03	0.021418 – 0.04426	0.294	5
Coumaphos	22.15	6.232 – 31.2	0.213	6
Cyfluthrin	0.0445	0.001 – 0.0677	0.783	4
DDT	5.95	0.052 – 7.378	0.235	8
Deltamethrin	0.037825	0.0015 – 112.2	1.000	4
Demeton	2.155	0.013 – 2.60	0.712	4
Diazinon	0.1675	0.0011 – 0.372	0.809	6
Dieldrin	0.136	0.0006 – 0.16	0.702	6
Dimethoate	0.16	0.0014 – 0.31	0.273	19
Dinotefuran	0.0378	0.0006 – 0.075	N/A	2
Fenitrothion	0.31	0.171 – 0.383	0.383	3
Fenpyroximate	3.99	3.00 – 6.65	0.378	3
Fipronil	0.008	0.00386 – 0.013	0.388	7
Flumethrin	0.05	N/A	N/A	1
Imidacloprid	0.04645	0.0128 – 0.19	0.515	18
λ-cyhalothrin	0.05	0.022 – 0.3	0.694	7
Malathion	0.166	0.002 – 0.726	0.699	8
Methamidophos	204.935	1.37 – 408.5	N/A	2
Methomyl	1.29	0.068 – 1.51	0.914	3
Methyl parathion	0.266	0.041 – 0.348	0.371	6
Mevinphos	0.1875	0.0013 – 0.36	0.899	4
Mexacarbate	0.061	N/A	N/A	1
Naled	0.0535	0.0008 – 0.485	0.849	6
Nitenpyram	0.138	N/A	N/A	1
Oxamyl	10.26	0.31 – 10.32	0.942	3
Oxalic acid	539.475	372.01 – 1575.85	0.273	6
Oxydemeton-methyl	2.86	0.003 – 3.00	0.834	5
Parathion	0.095	0.003 – 0.175	0.533	6
Permethrin	0.028	0.015 – 0.159	0.747	5
Phosmet	1.13	1.06 – 1.9	0.284	3
Phosphamidon	1.45	0.002 – 1.46	0.997	3
Propiconazole	>100	N/A	N/A	1
Pymetrozine	0.16	N/A	N/A	1
Pyridalyl	6.16	N/A	N/A	1
Resmethrin	0.056	0.045 – 0.076	0.468	8
Spinosad	0.058	0.0025 – 0.88	0.737	9
Tau-fluvalinate	8.78	0.448 – 65.85	0.667	9
TEPP	0.002	0.001 – 0.002	0.333	3
Thiacloprid	38.82	14.6 – 122.4	0.787	3
Thiamethoxam	0.04	0.024 – 0.124	0.529	5
Thymol	52.4	51.25 – 55.1	0.036	3
Toxaphene	0.144	N/A	N/A	1
Trichlorfon	3.053	0.024 – 5.137	0.991	3

N/A, not applicable; DDT, Dichlorodiphenyltrichloroethane; TEPP, Tetraethyl pyrophosphate.

TABLE 2 | Range and median of reported oral LD_{50} values of pesticides for adult honey bees.

Pesticide Name	Oral LD_{50} (μg/bee)	Range	Quartile Coefficient of Dispersion	Number of reports
Abamectin	0.011	N/A	N/A	1
Acetamiprid	11.815	0.0215 – 72.9	0.924	4
Amitraz	5.47	N/A	N/A	1
Carbaryl	0.125	0.11 – 0.14	N/A	2
Chlorpyrifos	0.25	0.1034 – 0.25	0.415	3
Clothianidin	0.00344	0.000000013 – 0.0269	0.711	14
Coumaphos	26.0	N/A	N/A	1
DDT	3.7	N/A	N/A	1
Deltamethrin	0.4645	0.079 – 0.85	N/A	2
Diazinon	0.2	N/A	N/A	1
Dieldrin	0.325	0.32 – 0.33	N/A	2
Dimethoate	0.14	0.1 – 0.3	0.138	20
Fenpyroximate	3.24	N/A	N/A	1
Fipronil	0.2158	0.0528 – 0.28	0.145	4
Flumethrin	0.3525	0.178 – 0.527	N/A	2
Imidacloprid	0.049	0.0000299 – 0.536	0.772	20
λ-cyhalothrin	0.9	N/A	N/A	1
Malathion	0.38	N/A	N/A	1
Methamidophos	3.7	N/A	N/A	1
Methomyl	0.23	N/A	N/A	1
Mevinphos	0.027	N/A	N/A	1
Oxamyl	0.094	N/A	N/A	1
Oxalic acid	223.2	N/A	N/A	1
Oxydemeton-methyl	0.31	N/A	N/A	1
Parathion	0.13	0.09 – 0.16	0.280	3
Permethrin	0.28	N/A	N/A	1
Propiconazole	60.55	57.25 – > 100	0.037	4
Resmethrin	0.069	N/A	N/A	1
Spinosad	0.057	0.053 – 0.063	0.086	5
Tau-fluvalinate	9.20	N/A	N/A	1
Thiacloprid	19.955	17.32 – 22.59	N/A	2
Thiamethoxam	0.004358	0.00000002 – 0.0112	0.001	10
Thymol	38.1	N/A	N/A	1

N/A, not applicable; DDT, Dichlorodiphenyltrichloroethane.

have far less compared to pest insects, thus making honey bees more susceptible to pesticide exposure (Sadd et al., 2015). The diminished repertoire of detoxifying genes in the honey bee might stem from compensatory mechanisms associated with their highly social behavior, including herd immunity (Evans et al., 2006; Cremer et al., 2007) and a 'social detoxification system,' which focuses on how hive behavioral dynamics can reduce the burden of toxin substances on the detoxification system of individual members (Berenbaum and Johnson, 2015). It is uncertain if the relatively small innate capacity of the honey bee is fully compensated by social effects or if the bees remain genetically more sensitive to the toxic effects of pesticides.

Within colonies, caste and diet can modulate the expression of detoxification-related genes. Forager bees, for example, express more detoxification genes than do nurse bees (Vannette et al., 2015), potentially owing to the heightened risk of exposure toward environmentally-derived xenobiotics. Moreover, honey bee diets with ample pollen or nectar provide some resistance toward pesticides (Schmehl et al., 2014; Tosi et al., 2017b). This may be achieved by nutrient-mediated increases in detoxification enzyme gene expression or by improving physiological resilience through ensuring adequate nutrition levels (Mao et al., 2013).

A lesser explored mechanism for detoxification is the gut microbiota—a community of microorganisms residing within the honey bee gastrointestinal tract, consisting of a core set of evolutionarily adapted bacterial species (Ellegaard et al., 2019) that compensate for insufficiencies in honey bee metabolism (Kešnerová et al., 2017; Zheng et al., 2017); thus it may have a role in detoxification. The microbiota can aid xenobiotic detoxification by *directly* detoxifying harmful substances and *indirectly* through modulating the host's detoxification response. The metagenome of the honey bee gut microbiota is enriched with carbohydrate and plant chemical metabolizing genes suggesting a direct role in detoxification (Engel et al., 2012; Kwong et al., 2014; Lee et al., 2015; Kešnerová et al., 2017).

TABLE 3 | Median larval LD_{50} values for various pesticides.

Pesticide Name	Median LD_{50} (μg/larvae)	Number of reports
Acephate	1.942	4
Amitraz	98.7185	2
Boscalid	78.78	3
Capatan	4.240	2
Carbaryl	1.466	4
Chlorpyrifos	0.209	5
Coumaphos	2.7	1
Cypermethrin	0.0405	4
Diazinon	0.00008725	4
Diflubenzuron	3.01	3
Dimethoate	1.8	18
Fluvalinate	0.83	1
Imidacloprid	2.785	2
Malathion	0.75	4
Methomyl	0.657	4
Methyl parathion	1.0145	4
Mevinphos	0.459	4
Naled	0.2405	4
Oxalic acid	44.7	1
Parathion	0.14	3
Permethrin	0.242	4
Penfluron	3.09	3
Thiamethoxam	0.1735	2
Thymol	44	1

For example, *Lactobacillus kunkeei* is able to metabolize phenolic acids found in pollen (Filannino et al., 2016), while *Gilliamella apicola* has the potential to metabolize toxic mannose constituents that are found in nectar (Barker and Lehner, 1974; Zheng et al., 2016). By directly metabolizing toxic substances, the microbiota is able to increase honey bee resistance to xenobiotics. Microbes can also regulate honey bee detoxification systems leading to *indirect* detoxication capability. For instance, the presence of certain early colonizers during development, such as *Snodgrassella alvi*, can modulate phase I detoxification pathways by affecting the expression of cytochrome P450 (CYP) enzymes (Schwarz et al., 2016) that are critical for pesticide degradation (Claudianos et al., 2006).

We do not yet know how gut colonizers influence detoxification of pesticides in honey bees under natural conditions, though findings in other insects suggest a major role for symbiont-mediated regulation of detoxification pathways (Pietri and Liang, 2018). As the gut microbiota is dependant on caste (Jones et al., 2018), diet (Maes et al., 2016; Zheng et al., 2017), and environmental factors (Jones et al., 2017), including exposure to some pesticides (Dai et al., 2018), studies should test the ability of the microbiota to either directly degrade pesticides or promote indirect detoxification of them. Caution should be exercised when investigating monoculture-based experiments because they do not account for the complexities of the entire microbiota, which include host–microbe interactions, microbe–microbe interactions, and strain-specific differences in metabolic capacities.

Pesticides themselves can also play a role in modulating metabolism and expression of detoxification genes. Boncristiani et al. (2012) showed that honey bees exposed to thymol and coumaphos have altered cytochrome P450 subfamily and protein kinase superfamily-related gene expression. These two gene families can affect resistance to insecticides like DDT (Le Goff and Hilliou, 2017) and neonicotinoids (Gong and Diao, 2017). Moreover, honey bees exposed to myclobutanil (fungicide) have impaired cytochrome P450-mediated detoxification of quercetin (flavonol phytochemical found in nectar and pollen), which is metabolized by CYP9Q1 (Mao et al., 2017). These studies reveal that synergistic effects between multiple types of pesticides can amplify the effective toxicity. As for insecticides, imidacloprid exposure has been shown to broadly up-regulate cytochrome P450 expression (Derecka et al., 2013; De Smet et al., 2017; Zhu et al., 2017), likely in response to the pesticide itself, thereby facilitating its detoxification.

Honey bees exposed separately to myclobutanil, imidacloprid, or fipronil have disrupted ATP production (Nicodemo et al., 2014; Mao et al., 2017), suggesting that exposure to these pesticides alters cellular metabolism. In particular, Nicodemo et al. (2014) demonstrated that imidacloprid and fipronil reduce oxygen consumption and impair mitochondrial function. This reduction in aerobic respiration is accompanied by an increase in glycolysis and citric acid cycle-related gene expression in exposed honey bees (Roat et al., 2014; Renzi et al., 2016a). Thus, pesticide exposure may be favouring low-efficiency means of ATP production (glycolysis and citric acid cycle) over higher efficiency means (oxidative phosphorylation). Interestingly, using near-infrared light (670 nm) to restore mitochondrial function can mitigate ATP reduction, diminish physiological impairments, and improve survival in bumblebees (*Bombus terrestris*) (Powner et al., 2016).

PESTICIDES NEGATIVELY AFFECT MOTOR FUNCTION, BEHAVIOR, AND COGNITION

Honey bees are highly social insects. They rely on individual cognition to navigate their environment and respond to changing conditions and colony needs. Forager bee cognition is demonstrated by their ability to encode memories of resources, which are typically found within a 2 – 6 km radius of the hive (Hagler et al., 2011; Couvillon et al., 2015). These memories are then transmitted through waggle dances to other foragers to encourage the process of collecting hive resources, which promotes success of a colony (Henry et al., 2012). Exposure to pesticides appears to impair the foraging response in a dose-dependent relationship.

Acute neonicotinoid exposure induces a series of symptoms that are consistent with hyper-responsive neural impairments (Suchail et al., 2001). These are observed as excitation symptoms, which include increased time in the air, increased flight distances, and an inability to right themselves when placed on their backs (Yang et al., 2008; Williamson et al., 2014; Tosi et al., 2017a). By contrast, chronic exposure induces hypo-responsive neurological

impairments (Suchail et al., 2001). These include decreased flight speed, decreased flight duration, and impaired navigation (Fischer et al., 2014; Tison et al., 2016; Tosi et al., 2017a). Thus, initial exposure to neonicotinoids can overstimulate honey bees and induce a hyper-responsiveness, which leads to exhaustion and hypo-responsiveness over continued exposure. One implication of this would be that neonicotinoid exposure drives foragers to go far distances, where they eventually become exhausted and lose their spatial awareness, thus hampering collection of resources by preventing them from returning to the hive. As a result, nurse bees may begin foraging at a younger age, thus creating a group of precocious foragers, which may reduce the number of nurse bees available for rearing brood (Robinson et al., 1989).

Honey bees likely cannot tell if food is contaminated with pesticides (Williamson et al., 2014; Kessler et al., 2015); so they are not averse to it. Fortunately, pesticide exposure reduces trophallactic transfer of food from donor to recipient (Bevk et al., 2012; Brodschneider et al., 2017). Although this may reduce the spread of pesticide-contaminated food within a colony, the change in social behavior may also compromise other forms of communication, including the waggle dance (which allows successful foragers to inform others in the colony on the direction and distance to food and water or new nesting sites) (Eiri and Nieh, 2016), or reduce larval feeding altogether (Gil and De Marco, 2005).

The most pronounced pesticide-induced cognitive impairments are on olfactory learning, visual learning, and memory. Olfactory learning occurs when honey bees learn to associate an odour with an award, which is often tested using the proboscis extension reflex (PER). Honey bees exposed to imidacloprid show reduced PER activity compared to unexposed bees (Decourtye et al., 2004; Han et al., 2010; Goñalons and Farina, 2018). Meanwhile, other pesticides show differential impairments to both short-term and long-term memory (Williamson and Wright, 2013; Wright et al., 2015). Pesticides have likewise been shown to affect visual and associative learning in honey bees (Hesselbach and Scheiner, 2018). For example, Han et al. (2010) found that using their T-tube maze, less than half of bees treated with imidacloprid were able to successfully make the correct decision in a visual learning task, suggesting that imidacloprid impaired visual learning. As this is used to remember food locations and predators, it may explain why Eastern honey bees (*Apis cerana*) exposed to sublethal imidacloprid do not show aversion to the predator hornet, *Vespa velutina* (Tan et al., 2014). Imidacloprid may reduce the visual association of the predator with the cognitive fear response. Pesticide exposure therefore appears to play a role in cognitive deficiencies, which may be mediated by direct effects of the chemicals on the brain.

On a cellular level, pesticides interfere with neuronal polarization in mushroom bodies, a segment of the honey bee brain that is associated with learning, memory, and sensory integration (Plath et al., 2017). Mushroom bodies are composed of Kenyon cells (neural cells). When these cells are exposed *in vitro* to coumaphos oxon (a metabolite of coumaphos) or imidacloprid, they show a modified synaptic profile, which is characterized by a slow depolarization, followed by increased excitability, then inhibition of the action potential (Palmer et al., 2013). These pesticides are partial agonists of nicotinic acetylcholine receptors; thus, they could be acting on these receptors and blocking a natural acetylcholine response, which will alter the neural cell action potential. The modified action potential elicited by this class of pesticides may explain some of the impairment to the aforementioned cognitive processes. In addition, there appear to be differences in the brain proteome and microRNA (miRNA) expression of bees exposed to pesticides (Roat et al., 2014; Shi et al., 2017a; Wu et al., 2017), which could lead to changes in brain development and structure that result in differential signalling. An alternate process to explain neural impairment following pesticide exposure is that pesticides may interfere with the perception of a stimulus rather than the cognition of one. Imidacloprid exposure has been shown to reduce calcium signalling in the antennal lobe in response to an odours stimulus (Andrione et al., 2016). This results in problems perceiving the stimulus as opposed to difficulty coding and recalling the stimulus (cognition). Ultimately, pesticide-induced cognitive related deficits may be a result of a combination of impairments to the honey bee brain.

PESTICIDES CAN OBSTRUCT REPRODUCTION AND DEVELOPMENT

Exposure to pesticides can slow the reproductive cycle of queens (**Figure 2**), as illustrated upon exposure to sublethal doses of thiamethoxam during development, resulting in reduced body weight and a lower probability of queen success (Gajger et al., 2017). Likewise, laboratory experiments show that queens exposed to field-realistic concentrations of neonicotinoids carry fewer viable spermatozoa and lay fewer fertilized eggs that would normally develop into diploid (female) workers (Williams et al., 2015; Chaimanee et al., 2016; Wu-Smart and Spivak, 2016). Queens that underperform are eventually targeted by workers for replacement (Sandrock et al., 2014), but in the short-term reproductive succession is costly to the colony. Furthermore, queens exposed to sublethal doses of neonicotinoids have been shown to have reduced mating compared with unexposed queens (Forfert et al., 2017).

Drones are also affected by pesticides. Sublethal concentrations of neonicotinoids and phenylpyrazoles can reduce sperm viability (Kairo et al., 2016, 2017a,b; Straub et al., 2016), which can hamper fertilization of queens and the production of diploid workers. Together, reduced sperm transfer and fertilization may limit the production of a genetically diverse workforce, which may compromise the division of labour (Jones et al., 2004) and response to disease (Sherman et al., 1988).

While pesticides are known to interfere with reproduction, they have also been implicated in changes to larval development. Honey bee larvae reared *in vitro* with thiamethoxam (1/10 of LC_{50}) show atypical progression through developmental stages, including skipping some stages, and reduced larval weight (Tavares et al., 2015). In addition, larvae exposed *in vitro* to the commonly used herbicide glyphosate show reduced weight and

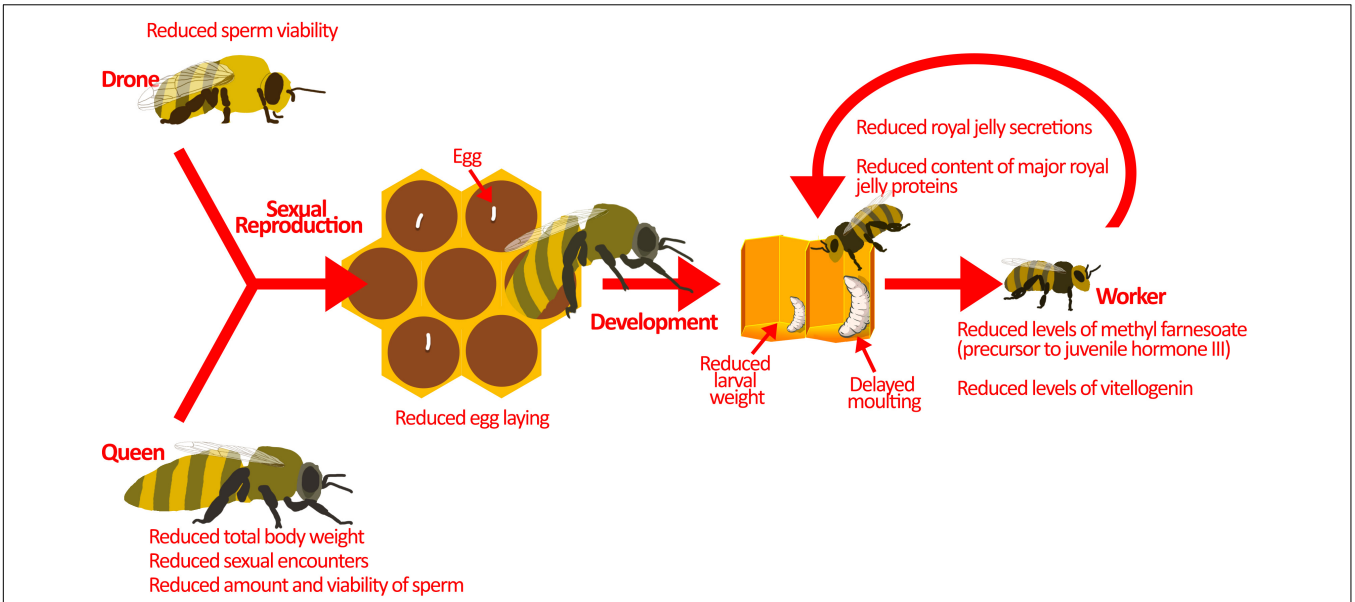

FIGURE 2 | Pesticides interfere with colony reproduction. Sexual reproduction between drones and the queen is the source of genetic diversity in the hive. This is important for pathogen resistance and colony survival. Sublethal pesticide exposure reduces sexual reproduction by affecting the drones and the queen. Drones exposed to pesticides have lower sperm viability. On the other hand, queens exposed to pesticides display reduced sexual encounters, sperm amount, and sperm viability. Moreover, pesticide exposed queens have smaller body weights, which may explain the reduction in sperm amount and egg-laying. Developing larvae are also at-risk during pesticide exposure. They demonstrate atypical progression through developmental phases, reduced larval weight, and delayed moulting. These may be a result of direct pesticide exposure, but pesticides could also be indirectly affecting larvae. Nurse bees exposed to pesticides produce reduced amounts of jelly secretions, and that which is produced has less nutritional value, potentially explaining the indirect effects of pesticides on honey bee larvae. Image of larvae in the hive is adapted from Maori et al. (2019) under Creative Commons Attribution 4.0 International (https://www.sciencedirect.com/science/article/pii/S1097276519301844).

delayed moulting (uncapping of the brood cell) (Dai et al., 2018; Vázquez et al., 2018). These laboratory studies are corroborated by field data showing similar atypical developmental progression upon pesticide exposure (Wu et al., 2011). At the molecular level, honey bees exposed to imidacloprid show changes in miRNA transcription, which are responsible for development (Derecka et al., 2013). In particular, a reduction in the miRNA, *mir-14*, has been observed (Derecka et al., 2013). Although the exact function of *mir-14* is unknown in honey bees, in *D. melanogaster* it has been shown to modulate metabolism, nutritional status, and larval survival (Varghese et al., 2010; Nelson et al., 2014). Thus, pesticide exposure impairs individual development, contributing to reduced colony strength.

Honey bee larval development is guided by hormone signalling and jelly supplementation. Honey bees treated with coumaphos and fluvalinate (acaricide) show reduced levels of methyl farnesoate, a precursor to juvenile hormone III (Schmehl et al., 2014), which is the main juvenile hormone in insects that functions to regulate honey bee caste differentiation (Nelson et al., 2007; Mutti et al., 2011) and division of labour (Fahrbach and Robinson, 1996). Exposure to neonicotinoids reduces expression of vitellogenin, another protein that is required for honey bee development (Abbo et al., 2017; Shi et al., 2017b). As brood develop, they primarily consume jelly, which is a nutritionally-rich food source produced and delivered by nurse bees. Sublethal neonicotinoids and fungicides reduce the size of the hypopharyngeal and mandibular glands where it is synthesized (Hatjina et al., 2013; Renzi et al., 2016b; Zaluski et al., 2017), which in turn decreases jelly secretions and may lead to reduced longevity and smaller honey bee populations (Yang et al., 2017). The jelly produced may further be deficient in major proteins (Wu et al., 2017) vital for honey bee development and physiology (Buttstedt et al., 2014). These changes in hormone signalling and reduced nutritional value of jelly can contribute to the atypical development of honey bee larvae exposed to pesticides. By limiting the amount of viable brood and the rate at which these few larvae develop, pesticide exposure effectively reduces the overall workforce and success of the colony.

PESTICIDES DISRUPT HONEY BEE IMMUNITY

Honey bees exposed to pesticides have increased loads of bacterial, fungal, and viral pathogens (Pettis et al., 2012; Wu et al., 2012; DeGrandi-Hoffman et al., 2013; Di Prisco et al., 2013; Alburaki et al., 2015; Doublet et al., 2015; Chaimanee et al., 2016; Fine et al., 2017). This has raised concern for the potential of synergistic interactions between pesticides and pathogens that exacerbate mortality in honey bees (Alaux et al., 2010a; Pettis et al., 2013; Paris et al., 2017; Grassl et al., 2018; O'Neal et al., 2019; Straub et al., 2019; Tesovnik et al., 2019). Vidau et al. (2011) demonstrated that honey bees previously infected with *Nosema ceranae* were more sensitive to subsequent

pesticide exposure. Parasites like *Nosema* might therefore increase pesticide-related mortality, possibly by altering the expression of detoxification enzymes. As the adult honey bee gut microbiota develops 4–6 days after eclosion and is composed of bacteria from older bees and the hive environment (Powell et al., 2014), one concern would be potential colonization with disease-causing microorganisms that could alter resistance to pesticides (Li et al., 2017; Koleoglu et al., 2018). Conversely, pesticides may cause immunosuppression in honey bees, rendering them more susceptible to pathogens. To better understand the possible synergism between pesticides and pathogens, it is essential to consider individual immunity and social immunity.

Individual honey bee immunity is divided into humoral and cellular immune responses, both of which are impaired by sublethal pesticide exposure (**Figure 3**). The humoral response is initiated by recognition of pathogen-associated molecular patterns (PAMPs), which trigger signalling through one of the four insect immune pathways: (1) the Toll pathway, (2) the Immune Deficiency (IMD) pathway, (3) the c-Jun N-terminal kinase (JNK) pathway, and (4) the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (Danihlík et al., 2015). Activation of these humoral immune pathways leads to the production of antimicrobial peptides (AMPs), which can be proteases, complement-like proteins, or broad-range microbiocidal proteins. In insects, these signalling pathways and proteins are conserved. However, honey bees harbour fewer paralogues, gene copies, and splice variants of immune genes compared to *Drosophila* and *Anopheles* (Evans et al., 2006).

Exposure to pesticides reduces global AMP generation, thus further compromising an already depauperate immune system (Garrido et al., 2013; Aufauvre et al., 2014; Tesovnik et al., 2017; Wu et al., 2017). Although the specific mechanisms by which AMP production is reduced are largely unknown, Di Prisco et al. (2013) demonstrated that honey bees exposed to clothianidin had increased expression of a leucine-rich repeat protein (*Amel/LRR*), which is similar to the *D. melanogaster* gene *CG1399*, a negative regulator of NF-κB signaling (Toll and IMD). Therefore, by increasing the expression of negative immune regulators, this pesticide acted to reduce AMP production, leading to higher infection titres of deformed wing virus (Di Prisco et al., 2013). Although this study only represents one specific mechanism for one class of pesticide, it is possible that combined exposure to multiple classes of pesticide may further dysregulate the immune response leading to drastic outcomes on pathogen load and mortality.

Activation of the cellular immune response also occurs through recognition of PAMPs, but instead triggers migration of hemocytes, which leads to encapsulation of the pathogen and activation of prophenoloxidase (PPO) to phenoloxidase (PO). Active PO catalyzes the production of a melanin polymer capsule around the pathogen (melanization response). Reactive oxygen species and nitric oxide intermediates are also created, which are important in pathogen defence (Paris et al., 2017; Walderdorff et al., 2018). Neonicotinoid exposure impairs this melanization response (Brandt et al., 2016, 2017), potentially due to reduction of PO activity (Zhu et al., 2017) or through the reduction of reactive oxygen species and nitric oxide (Paris et al., 2017; Walderdorff et al., 2018). Consequences of this would be reduced pathogen isolation and clearance, and slower wound healing, both of which could increase viral loads and systemic infections (Brandt et al., 2017).

Systemic infections in honey bees could be exacerbated by acaricide, neonicotinoid, or fungicide exposure, which reduces intestinal stem cell proliferation (Forkpah et al., 2014) and increases midgut apoptosis (Gregorc et al., 2018; Carneiro et al., 2019), potentially weakening the gut barrier. Hemocytes also function as phagocytic cells in the honey bee hemolymph; however exposure to neonicotinoids reduces hemocytes phagocytic activity (Walderdorff et al., 2018) and hemolymph antimicrobial activity (Brandt et al., 2016). These pesticide-exposed hemocytes also display altered differentiation profiles and reduced total cell counts (Brandt et al., 2016, 2017; López et al., 2017), another factor that would reduce the magnitude of the melanization response. Despite the documented consequences of neonicotinoid pesticides on hemocytes and cellular immune responses, the mechanisms remain elusive. Studies on *D. melanogaster* and *Chilo suppressalis* demonstrate that the nervous system can regulate hemocyte proliferation (Makhijani et al., 2017), and neurotransmitters have a role in modulating hemocyte phagocytosis (Wu et al., 2015; Qi et al., 2016). Thus, pesticides may act through the nervous system to dysregulate hemocytes. Future studies should explore the mechanisms of pesticide-induced impairment of hemocytes, with a focus on pesticide dysregulation of neuro-immune cell signaling.

Social immunity, where individuals contribute to group health, can arise in different ways—for example, through individual secretion of peptides that effectively sterilize the hive environment. Glucose oxidase (GOX) is secreted from the hypopharyngeal glands and catalyzes the production of hydrogen peroxide (H_2O_2) to sterilize the hive. Reeves et al. (2018) showed that GOX activity is increased in nurse bees and forager bees exposed to acaricides, tau-fluvalinate, and coumaphos. On the other hand, Alaux et al. (2010a) demonstrated that there is a synergistic interaction between imidacloprid exposure and *Nosema* infection, whereby GOX activity is reduced. Differences reported between these studies may stem from the use of dissimilar classes of pesticides or experimental methods used. For example, Alaux et al. (2010a) fed caged bees a sucrose solution, while Reeves et al. (2018) assayed bees directly collected from hives. Addressing these disparities, the latter set of bees were exposed to the natural environment, which would have allowed them access to pollen—a dietary component that increases GOX activity (Alaux et al., 2010b). Defensin 1 (*Def 1*) is a social immunity peptide that is secreted into the hive environment and is particularly effective against Gram-positive bacteria. Studies show that *Def 1* expression may increase (thiamethoxam) (Tesovnik et al., 2017), decrease (fipronil) (Aufauvre et al., 2014) or remain unchanged

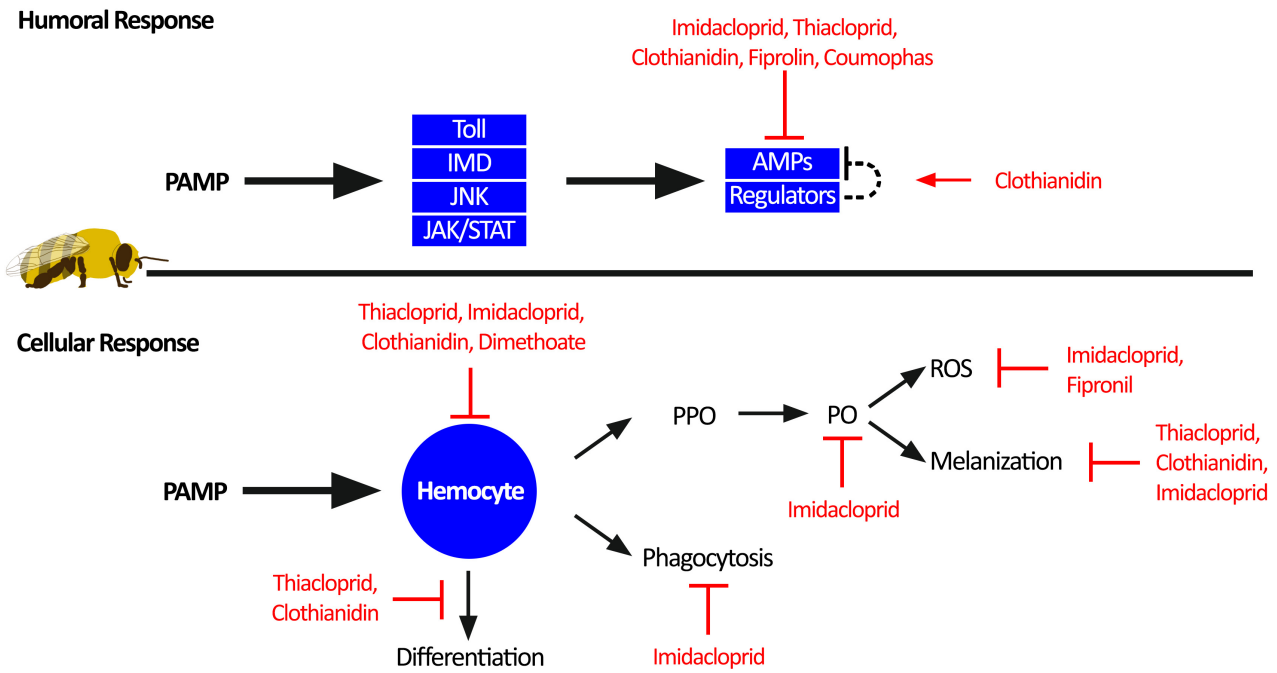

FIGURE 3 | Individual honey bee immunity impairment by pesticides. Honey bee immune response toward pathogen-associated molecular patterns (PAMPs) can be divided into humoral response and cellular response. The humoral response generates antimicrobial peptides (AMPs) through activation of the four immune pathways: Toll, immune deficiency pathway (IMD), c-Jun N-terminal kinase (JNK), and Janus kinase/signal transducers and activators of transcription (JAK/STAT). Sublethal pesticide exposure impairs the humoral immune response by reducing the production of AMPs. The cellular immune response is orchestrated through hemocyte function. Hemocytes can facilitate melanization of pathogens and wounds through activation of prophenoloxidase (PPO) to phenoloxidase (PO) and reactive oxygen species (ROS) as a by-product. In addition, hemocytes can phagocytosis and clear invading pathogens, as well as differentiation into other immune cells. Multiple aspects of the cellular immune response are impaired by sublethal pesticide exposure.

(acaricides) (Garrido et al., 2013) in response to exposure of different types of pesticides.

Honey bees also practice various hygienic behaviors that reduce pathogen load within colonies, most notably self- or mutual-grooming and removal of dead bees. Wu-Smart and Spivak (2016) found that worker bees treated chronically with imidacloprid displayed significantly reduced hygienic removal of freeze-killed brood. Likewise, de Mattos et al. (2017) showed that synthetic acaricides (coumaphos, amitraz, and tau-fluvalinate), caused workers to groom less, which led to higher *Varroa destructor* loads. Meanwhile, Williamson et al. (2013) showed that acetylcholinesterase inhibitors (coumaphos, chlorpyrifos, aldicarb, and donepezil) actually increased worker bee grooming. The discrepancy between these two studies might be a result of the methodology used. While Williamson et al. (2013) recorded grooming without a stimulus, de Mattos et al. (2017) tested grooming in the presence of a *Varroa* mite. Nonetheless, it appears that the social immune response to pesticide exposure is variable and must be further explored.

A lesser understood aspect of honey bee immunity is the role of the microbiota in both *direct* and *indirect* pathogen inhibition. It is well documented that through *direct* inhibition, microbiota constituents are able to counter pathogens via competitive inhibition, production of organic acids, and secretion of antimicrobial molecules (Evans and Armstrong, 2005, 2006; Forsgren et al., 2010; Olofsson et al., 2016; Khaled et al., 2018). Additionally, the microbiota can *indirectly* improve pathogen resistance by stimulating multiple aspects of individual immunity. In particular, constituents of the gut microbiota can activate the humoral immune system to produce AMPs, that are critical to combating pathogens (Kwong et al., 2017; Li et al., 2017). Also, organisms such as *Frischella perrara* are able to stimulate the cellular immune response and induce dark-colored 'scabs' on the epithelium of the pylorus, which is a result of melanization (Emery et al., 2017).

While it has been shown that certain neonicotinoid pesticides like imidacloprid do not alter the microbiota (Raymann et al., 2018b), a wealth of evidence suggests that exposure to a variety of other types of pesticides can alter the abundance and composition of the gut microbiota (Kakumanu et al., 2016; Dai et al., 2018; Motta et al., 2018; Blot et al., 2019; Rouzé et al., 2019). These studies reveal that pesticide exposure mainly decreases *Bifidobacterium* spp. and *Lactobacillus* spp., with some of this research (Motta et al., 2018; Blot et al., 2019; Rouzé et al., 2019) suggesting that abundances of *S. alvi* and *G. apicola* may also be affected. These alterations are associated with exacerbated pathogen mortality (Mattila et al., 2012; Maes et al., 2016; Li et al., 2017; Rubanov et al., 2019). Therefore, by altering the

microbiota of honey bees, pesticide exposure increases the insect's susceptibility to pathogens.

SOLUTIONS FOR MITIGATING THE DELETERIOUS EFFECTS OF PESTICIDES: THE POTENTIAL OF PROBIOTICS

It is quite evident that pesticides have deleterious effects on honey bees. Thus, a solution is needed to limit these off-target effects. Naturally, by completely removing pesticides, honey bees would no longer be exposed to them, but this solution may reduce crop yield and burden the food supply (Hossard et al., 2014). Another idea is to circumvent pesticide use by mandating better farming practices that reduce damage from pests (Lechenet et al., 2014). However, these methods are subject to the legislative process and competing interests, and do not empower beekeepers themselves with a means to combat the pesticide issue. As pesticides may increase the likelihood of infection, beekeepers have a few methods to reduce pathogen-associated honey bee mortality. They routinely use fungicides and acaricides to prevent *Nosema* and *Varroa* infection. However, these chemicals adversely affect the bees. Antibiotics used to combat bacterial pathogens such as *Paenibacillus larvae* (American foulbrood) and *Melissococcus plutonius* (European foulbrood), also disrupt the microbiota of honey bees, increase antibiotic resistance in pathogens, and ultimately increase mortality (Alippi et al., 2014; Krongdang et al., 2017; Li et al., 2017; Raymann et al., 2017, 2018a). All things considered, conventional solutions are not effective at combating honey bee decline and the need for new approaches is evident.

One novel concept may be through supplementation with lactic acid bacteria (LAB; such as *Lactobacillus* and *Bifidobacterium* spp.) to mitigate the harmful effects of pesticides and pathogens. The basis for this is several-fold, but the most discernible benefit of LAB supplementation is that it can reduce pesticide absorption via degradation (Islam et al., 2010; Lénárt et al., 2013; Zhang et al., 2016; Li et al., 2018) or by sequestering ingested pesticides, thereby allowing them to pass through the digestive tract rather than be absorbed (Trinder et al., 2016). In other model organisms, LAB have been shown to reduce toxicity and have a protective effect to the host (Bouhafs et al., 2015; Bagherpour Shamloo et al., 2016), thus establishing a basis for future studies to investigate this potential in honey bees.

Collectively through *direct* and *indirect* mechanisms of pathogen resistance, supplementing honey bees with beneficial bacteria is able to reduce *Nosema* spore counts (Sabaté et al., 2012; Maggi et al., 2013; Corby-Harris et al., 2016; Arredondo et al., 2018) and *P. larvae* bacterial load (Forsgren et al., 2010; Arredondo et al., 2018; Daisley et al., 2019). *In vivo* evidence from a *D. melanogaster* model of pesticide exposure has shown that supplementation with LAB improves immunity of pesticide-exposed flies via immune stimulation (Daisley et al., 2017; Chmiel et al., 2019). Likewise, LAB are able to stimulate AMP production in honey bees and improve survival during *Paenibacillus larvae* infection (Evans and Lopez, 2004; Daisley et al., 2019). Together these studies demonstrate that beneficial bacteria can *indirectly* contribute to pathogen resistance by stimulating the immune system and assisting the host in overcoming infection.

Some LAB are able to *directly* inhibit pathogens, thus enhancing overall honey bee resistance to pathogens. For example, isolates of *L. kunkeei* have been shown to inhibit *N. ceranae*, *P. larvae*, and *Serratia marcesscens* (Forsgren et al., 2010; Olofsson et al., 2016; Al-Ghamdi et al., 2017; Arredondo et al., 2018). *Lactobacillus kunkeei* also produces biofilms in honey bees, which facilitates its vertical transmission from one generation to the next (Vásquez et al., 2012). Another LAB, *Lactobacillus apis* R4B^T, can inhibit *P. larvae* and *M. pluntonius*, *in vitro* (Killer et al., 2014). Some *Bifidobacterium* species inhibit *P. larvae* and *S. marcesscens*, and when found adequately in the microbiota they are associated with reduced pathogen load (Forsgren et al., 2010; Mattila et al., 2012; Olofsson et al., 2016). Honey bee-derived *Lactobacillus johnsonii* CRL1647 is a well-documented LAB shown to reduce the abundance of *Nosema* and *Varroa* in the hive (Audisio et al., 2015). Although the mechanism for *direct* pathogen inhibition is not completely clear, it is likely a combination of the production of organic acids (Maggi et al., 2013), bacteriocins (Audisio et al., 2018), and other antimicrobial proteins (Butler et al., 2013). The net effect is that this is a viable method to mitigate the immune impairment caused by sublethal pesticide exposure, and beneficial bacterial supplementation could prove as an alternative to antibiotic use by reducing pathogen burden.

In addition, beneficial bacteria can bolster colony development, which is notably decreased by pesticide exposure. Honey bees supplemented with LAB typically produce more honey, have more pollen stores, and have increased brood counts (Audisio and Benítez-Ahrendts, 2011; Audisio et al., 2015; Alberoni et al., 2018; Fanciotti et al., 2018). For example, *L. johnsonii* CRL1647 stimulates egg-laying, which can increase the hive population (Audisio and Benítez-Ahrendts, 2011). These positive effects have been partially attributed to organic acid production (Maggi et al., 2013), but could also be attributed to microbiota restoration as 'non-thriving' hives typically have lower levels of *Lactobacillus* and *Bifidobacterium* compared to 'thriving' hives (Ribière et al., 2019).

The long-standing challenge to supplementing honey bees with beneficial bacteria is in the delivery method (**Figure 4**). A number of commercial bee supplements containing dried LAB suggest 'dusting' frames with the bacteria, which may also promote grooming. Although this application method is minimally invasive, the efficacy is not well known. Moreover, dusting is prone to uneven distribution and is negatively impacted by moisture and humidity.

More commonly, beneficial bacteria are added to sucrose-based syrup solutions. Numerous studies have demonstrated that various bacteria supplemented in this manner can reduce *Nosema ceranae* loads (Baffoni et al., 2016), improve overwintering death rates (Kuzyšinová et al., 2016), and increase brood populations and harvestable honey by ∼46% and ∼60%, respectively (Alberoni et al., 2018). However, a critical factor limiting the practicality of this method in the field is the lacklustre viability and activity of bacteria in sucrose-based solutions (>90%

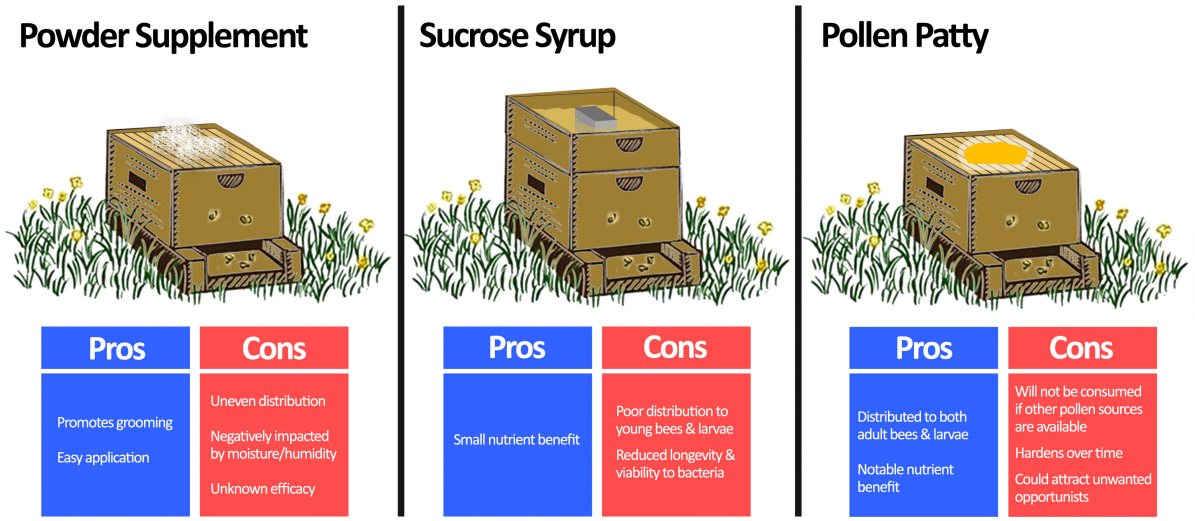

FIGURE 4 | Comparison of methods for beneficial bacteria supplementation. Beneficial bacteria are usually combined with a vehicle to supplement honey bees in one of three ways: powder supplementation, sucrose syrup, or pollen patty. Powder supplementation can be easily performed by spreading a probiotic infused dust on the beehive, which also promotes bees to groom. However, it is prone to uneven distribution, negative impacts of moisture, and unknown efficacy as an application method. Sucrose syrup supplementation can be achieved by adding probiotics directly to conventional sucrose feeders for the hive. Although this method benefits from a small nutrient enhancement, the sucrose solution is not usually distributed well to all members of the hive, and it is an unfavourable environment for the bacteria. Pollen patty supplementation involves adding beneficial bacteria directly to a traditional pollen supplement. In addition to the added nutrient benefit, pollen patty supplementation will be distributed throughout the hive to both adult bees and larvae. However, if sufficient nutrient sources already exist, then the pollen patty may be disregarded by the hive. Moreover, it is prone to hardening over time and could attract unwanted pests. Langstroth beehive image modified from Net Art under the Creative Commons Attribution 2.0 Generic License (https://netart.us/box-shaped-beehive-coloring-page/).

drop in original CFU after 96 h at 30°C) due to osmotic stress (Ptaszyńska et al., 2016b). Additionally, this method of supplementation may not transfer bacteria to younger bees and larvae (Brodschneider and Crailsheim, 2010).

Another option is to infuse beneficial bacteria into pollen-substitute patties, which have the advantage of improving honey bee nutrition. Pollen-substitute patties per se have been shown to benefit honey bee health through reducing titers of deformed wing virus (DeGrandi-Hoffman et al., 2010) and increasing hemolymph protein content (Jong et al., 2009). Evaluating pollen substitutes as a delivery method, Kaznowski et al. (2005) demonstrated that hives supplemented with probiotic-infused pollen substitutes had better overall survival, higher dry mass, and increased crude fat levels of bees when compared to groups receiving only the pollen-substitute. Another study showed that honey bees receiving probiotic bacteria delivered via pollen-substitutes have better developed peritrophic membranes (responsible for nutrient utilization and pathogen protection) compared to vehicle controls (Szymaś et al., 2012). Some points to consider are that pollen-substitute patties may attract unwanted opportunistic insects (for example the small hive beetle, *Aethina tumida*) and it may not be consumed if other pollen sources exist. Nonetheless, pollen substitutes are already used by beekeepers to ensure nutritional adequacy and can be easily supplemented with beneficial bacteria.

Along with the introduction of any live microorganism to the hive comes the risk of inducing hive microbial dysbiosis (Alberoni et al., 2016). A few documented cases exist in which negative effects were observed from supplying honey bees with supplemental bacteria. Ptaszyńska et al. (2016b) reported that supplementation with *L. rhamnosus* (no strain type provided) increased honey bee susceptibility toward nosemosis C. In the same year, Ptaszyńska et al. (2016a) also demonstrated that co-administration with three LAB (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, and *Bifidobacterium bifidum*—no strain designations provided) led to a decrease in total yeast concentrations in adult honey bee guts, but an increase in *N. ceranae* spores following infection. It is difficult to ascertain the biological relevance of these findings as crucial details are missing, including (1) strain-type information of lactobacilli used, (2) confirmation that live bacteria actually reached their target destination in the adult honey bee gut, and (3) whether or not the apparent increase in *Nosema* spp. led to any measurable changes in individual or hive-level health outcomes. Johnson et al. (2014) found no net positive or negative effect on hive health or performance following supplementation of lactobacilli in a high-fructose corn syrup vehicle. Altogether, these findings remind us to take precautions with biological agents, and stress the importance of properly considering strain type when selecting candidate lactobacilli for in-hive supplementation.

CONCLUDING REMARKS

Pesticide exposure at high doses is a notable causal factor involved in honey bee population decline; however, sublethal pesticide exposure presents inconspicuous threats to honey

bees. In particular, it negatively affects reproduction, immunity, physiology, and cognition. Beginning at the reproductive cycle, sublethal exposure to pesticides impairs sexual reproduction, reduces egg-laying, and hinders larval development.

Sublethal pesticide exposure is broadly immunosuppressive and leads to increased levels of pathogens, as well as eliciting a biphasic response on movement and flight, which is mediated by severity of exposure. Acute exposure causes hyper-responsiveness and increased movement, while chronic exposure leads to hypo-responsiveness and reduced movement. Sublethal pesticides exposure causes numerous impairments to brain function, affecting harmony and productivity in the hive, and the ability to find new resources.

Despite the extensive knowledge of the sublethal effects of neonicotinoids, research on other pesticide classes is underrepresented, with a notable absence in work on fungicides and herbicides. Future studies should aim to methodically determine and report LD_{50} values for any pesticide tested, thereby ensuring that LD_{50} values are accurate and comparable throughout the world.

Bacteria are able to directly detoxify toxins and/or stimulate host detoxification, which could be advantageous in reducing pesticide uptake in honey bees. As the microbiota is affected by diet, this may potentially lead to the development of products that reduce pathogen burden by complementing the immune system through modification of the gut microbiota. In that regard, probiotic supplementation could mitigate the sublethal effects of pesticides by reducing pesticide uptake, improving pathogen resistance, and mitigating sublethal effects on colony development. Until chemical agents are no longer used in agriculture, the ability to supplement honey bees with probiotics could help the insects fight the unintended pernicious effects.

AUTHOR CONTRIBUTIONS

JC, BD, AP, GT, and GR conceived the concept for the manuscript, contributed to the manuscript revisions, read, and approved the submitted version. JC drafted the manuscript and collected the toxicology data. BD wrote sections of the manuscript. AP generated the figures for the manuscript.

FUNDING

This work was funded by the Government of Canada Natural Sciences and Engineering Research Council of Canada (NSERC) and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00022/full#supplementary-material

TABLE S1 | Complete adult and larval LD50 values for multiple pesticides.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.