Predicting Both Obvious and Obscure Effects of Pesticides on Bees

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Abstract

Pesticides are a necessary component of the monoculture-based food production system. The chemical management of pests can affect non-target organisms, including honey bees. Risk assessment is a way to evaluate the cost—benefit of pesticide use to honey bees and involves understanding the exposure routes and hazards posed by each particular pesticide. The effects of insecticides on bees are intuitively recognized, but other types of pesticides can affect honey bees too. Even "inactive" ingredients in a pesticide formulation can pose a risk to bees. Bees encounter pesticides as they forage in the environment through direct exposure to pesticide applications, and through contaminated resources such as pollen, nectar, water, comb, and propolis. Pesticides can affect bees in myriad ways. The toxicity of pesticides is highly context-specific, challenging risk assessments. Mortality is the most commonly measured effect of pesticides on bees but sublethal effects from developmental problems, reduced reproductive fitness, diminished overwintenng capacity, and numerous behavioral issues that may not kill the bee ouü•ight, but may kill the hives. The pervasiveness of pesticides in the environment means that bees cannot avoid exposure to numerous chemicals. Selecting for bees that are adapted to agrichemical-intensive landscapes may be a short-term solution, but the dynamic evolution of chemical use may prohibit long-term tolerances. Beekeepers and farmers need to work together to create and promote reduced chemical intensive food production systems. This is the only long-term answer for the survival of honey bees and biodiversity in general.

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Broad scale simplification of the landscape accompanied the rise of industrial-scale food production, and a variety of agrichemicals are used to support these monoculture-based systems. Biodiversity provides substantial resistance to the proliferation of pests in a variety of ways, but this diversity is removed from our food production systems in order to maximize short-tern production goals. In the absence of biotic resistance to pest proliferation, land managers rely on pesticides to replace the pest management function provided by diverse biological communities. The downside is these pesticides do not solve the causative problem that produced the pest. Within this context, pests become resistant, and more and new pesticides are required to maintain pests at low densities in a system that is designed for them to excel (i.e., the pesticide treadmill). This is the environment into which honey bees and other pollinators have been inserted, and pesticides inherent in these systems affect pollinators in complex ways. Pesticides are not intended to hurt bees or any other beneficial organisms, but they often do; estimating this harm is called tisk assessment.

1 Assessing Risk

Defining the non-target organism is a crucial first step for a valid risk assessment (NRC 1983; Suter 2016). First, it is important to select ecologically relevant species on which to conduct risk assessments (Carignan and Villard 2002). It is not feasible to evaluate the risk of every pesticide against every non-target organism in a habitat. For instance, there are 467 beneficial or neutral insect species in South Dakota sunflowers (Bredeson and Lundgren 2015a), 382 in SD corn (Welch and Lundgren 2016), 150+ in eastem South Dakota dung pats (Pecenka and Lundgren in press). To curtail this list, indicator species that represent certain species groups are often selected to make risk assessments more manageable (NRC 1983), and honey bees are often one of these indicator species (Duan et al. 2008; ECFR 2017). Once the species is selected, the physiological status of the organism affects the outcome of a risk assessment. Life stage, time of day, time of year, history of exposure, nutritional status, reproductive status, exposure to other stressors, social caste, etc., all can influence the perception of risk, and so the context of these risk assessments needs to be clearly defined. A pesticide may have little toxicity to a healthy bee in a Petri dish, but be very toxic to a bee that has been exposed to stressful conditions (e.g., a lack of forage, extreme temperatures, infected with disease, and exposure to other pesticides, etc.).

In its simplest form, risk is defined as hazard x exposure (NRC 1983). "Hazard" is the negative effect that you are measuring. But even the most hazardous chemical poses no risk if an organism is not exposed to it. Conversely, a fairly benign chemical can be toxic if one is exposed to too much. For example, a single sting from a honey bee is relatively harmless, but a whole hive of stings can be lethal; unless of course you are allergic. Dose often makes the poison, but how an organism is exposed also matters. Whether a substance is ingested, breathed,

or physically contacted are types of exposure that influence the risk equation (Vandenberg et al. 2012). Also, some chemicals are only toxic at low doses, while others may actually benefit the organism at low doses (Calabrese 2004; Guedes and cutler 2014). Hazards posed by pesticides might include increased mortality, reduced reproduction, foraging capability, or honey production (discussed at length later in the chapter). Defining potential hazards at the onset of a risk assessment is critical to an accurate perception of the risk involved. The trouble is, we often times cannot predict how a pesticide is going to adversely affect the environment. For example, who could have foreseen that certain herbicides would alter the sexual characteristics of frogs (Hayes et al. 2002)? The hazard is severe, but risk assessment of this hazard could not be evaluated until we observed the effect in nature. Because we cannot predict all of the risks a pesticide poses to the environment, a precautionary principle is often advocated (Kriebel et al. 2001). In this case, the precautionary principle invokes the notion that the effects of pesticides are unknown and their use unnecessarily or prophylactically should be avoided.

Risk is not unique to pesticides. There are costs and benefits to all decisions, and our cunent sociological values define how much cost within a certain set of circumstances is acceptable. As society's values, or the environment, or even our ability to characterize hazard changes, our perception of an acceptable level of risk is also altered. For example, DDT was deemed fairly safe when its evaluation was simply based on acute mammalian toxicity. Only when we could measure the widespread bioaccumulation of DDT and its metabolites did we alter our decision and recognize that DDT posed an unreasonable risk posed to the environment (Dunlap 2008; Perkins 1982). Likewise, our society often values threatened species at a higher level than common ones (Mace et al. 2008). So the risks posed by a pesticide that inadvertently kills a portion of a bee population (or a lady beetle population, or a fox population, ad infinitum) does not raise actionable concern until that population is diminished to the point where additional mortality becomes untenable. Given these complexities, it is clear that risk assessments require regular re-evaluations to ensure that risks remain acceptable.

2 Types of Pesticides

Pesticides are categorized at the highest level based on which class of organisms they are designed to control (e.g., herbicides, insecticides, acaricides, rodenticides, fungicides). Within these categories, pesticides are further subdivided based on how they kill the pest (their mode of action). There are hundreds of pesticide active ingredients that are currently registered in the US and Europe (Chauzat et al. 2009; Mullin 2015), but these products represent only a handful of modes of action (i.e., they only affect a handful of physiological targets in a pest). This broad classification system is somewhat misleading. Just because an herbicide is designed to kill plants does not mean that its effects on other groups of organisms will be negligible. Indeed we found that herbicides can be toxic to lady beetles at levels far below the

label rate (Freydier and Lundgren 2016). Morton et al. (1972) found that the arsenic herbicides Paraquat, methane arsonic acid (MAA), monosodium methanearsonate (MSMA), disodium methanearsonate (DSMA), hexaflurate, and cacodylic acid were all highly toxic to newly

emerged honey bee workers (mortality was signif_ icant at 10 ppm). Different classes of pesticides can even synergize to enhance the toxicity of an "insecticide." For instance, adding fungicides (piperonyl butoxide, triflumizole, and propiconazole) increased the oral toxicity of neonicotinoid insecticides (acetamiprid and thiacloprid) to honey bees, sometimes by as much as 1100-fold (Iwasa et al. 2004). The effects of many pesticides on bee health have been investigated, but arguably none have drawn more recent attention than neonicotinoid insecticides. This relatively new group of insecticides targets the nervous system of insects (the neonicotinoids are surrogates for the insect neurotransmitter acetylcholinesterase, to be specific), and are highly toxic to bees (sublethal effects have been observed with as little as I billionth of a gram per bee). Given their widespread use and implications in honey bee declines, substantial controversy has sunounded these chemicals (Carreck and Ratnieks 2014; Douglas and Tooker 2016). The effects of neonicotinoids on honey bees will be better explained throughout this chapter.

To complicate matters, the risk posed by a pesticide is strongly influenced by the myriad "inactive" ingredients that are included with the product. Inactive ingredients are classified as surfactants, penetrant enhancers, activators, spreaders, stickers, wetting agents, buffers, antifoaming agents, drift retardants, etc. (Mullin et al. 2015). Registered "inactive" ingredients are largely unregulated; the US Environmental Protection Agency (EPA) only requires that adjuvants submit to seven of the 20 short-tem avian and mammalian safety tests that active ingredients must address (Mullin et al. 2015). This can be problematic, as greater amounts of "inactive" ingredients are used, they can sometimes have greater impact on the pest or non-target organisms than the active ingredients within a pesticide formulation (Cox and Surgan 2008; Mann and Bidwell 1999; Surgan et al. 2010). The "inactive" ingredients may also temper the effects of the active ingredient on non-target organisms. One example comes with the herbicide Paraquat, which alone significantly reduces fat body cells (called oenocytes) in bees. But when the adjuvant N-acetylcysteine is added to the formulation, the herbicide has fewer deleterious effects on these cells (Cousin et al. 2013). This notwithstanding, most examples reported in the literature discuss synergistic or additive, deleterious effects on non-target organisms of adding adjuvants and pesticides. One penetrant enhancer, called NMP (N-methyl-2-pyrrolidone), has received recent attention for its toxicity to bees. Hundreds of millions of pounds of NMP are applied in the US alone. This chemical has demonstrated negative effects on wildlife (Mullin et al. 2015), and is itself highly toxic to honey bee larvae (Zhu et al. 2014). Some organosilicone surfactants (Dyne-Amic, Silwet, and Syltac) also have negative effects on honey bees at low concentrations, this time affecting bee learning ability (Ciarlo et al. 2011). When one combines the hundreds of potentially active ingredients with the hundreds of potential "inactive" ingredients, the number of assessments required to

understand the risks posed by agrichemicals to bees becomes rather staggering, especially when one considers that formulated products are largely unregulated.

3 Honey Bee Exposure to Pesticides

There are two sources of pesticide exposure for bees, environmental and within-hive exposures. Most of the diversity of chemistries in the hive originates from the environment. Also, the beekeeper can sometimes be his own enemy, and a major source of contaminating pesticides within the hive is those pesticides applied to protect the bees from in-hive pests. Indeed, nearly all of the hives tested in one study had coumaphos and fluvalinate, two acaricides used to manage Varroa destructor in infested hives (Mullin et al. 2010). A honey bee can have a much different exposure scenario based on its age and social caste. The oldest workers are the first to be exposed to a particular environmental pesticide, as they are the active foragers (Winston 1987). Moreover, these oldest workers are also the ones to remove the dead bees, and so their exposure could be high if there are pesticide-related deaths in the hives. Once an environmental pesticide enters the hive, middle-aged bees are likely the next to be exposed, as they accept nectar and pollen from the returning foragers (Johnson 2010). Finally, the youngest nurse bees are exposed to pesticides as they feed nectar to the developing larvae and the queen, as well as when they manipulate and build pesticide-impregnated comb. All of this is to say that younger workers may have a very different risk equation than older workers. Likewise, larvae developing in a pesticide-contaminated cell may have a different risk equation than any other age guild or caste within the hive. As such, risk assessments for honey bees are much more complicated than are typically necessary for other non-target species, and simply evaluating the toxicity of a pesticide to a random worker bee is insufficient to assess the risk of a pesticide.

Environmental exposure. The reality is that pesticide use in North America and worldwide is continuing to rise, and some level of pesticides pervade most habitats in the soil, water, and plants. Environmental samples are frequently contaminated with pesticides (Ryberg and Gilliom 2015; Toccalino et al. 2014), including plants and water sources frequented by honey bees (Botias et al. 2015; David et al. 2016; Mogren and Lundgren 2016). In the case of insecticides, fewer pounds of insecticides are applied to farms, but the area treated continues to increase (Fausti et al. 2012), and in some cases, the toxicity of insecticides has increased dramatically from earlier chemistries. In the past 10 years, neonicotinoids have become one of the most commonly used insecticides in North America, and are currently applied to nearly 13% of the land surface of the continental United States (Douglas and Tooker 2015). These neonicotinoids are 5000—10,000 times more toxic to honey bees than DDT (Pisa et al. 2015). Fungicide and herbicide application rates also continue to rise (NASS 2017).

Obvious and

Glyphosate is cunently applied to the majority of row crop acres around the world (Benbrook 2016); the active ingredient of this

herbicide has little toxicity to honey bees, but the Roundup Weathermax@ for. mulation has some deleterious effects on bees (Mullin et al. 2010).

Application technology also affects a honey bee's exposure to a pesticide, and these approaches to deploying pesticides can be generally categorized as broadcast and systemic approaches. Aerial sprays pose a threat of direct contact with the bees and thus are presumed to have the greatest impact on bee workers if applied during foraging peaks. Systemic insecticides, those that are applied to the soil or the seed and then transported throughout the treated plant, may reduce direct exposure of bees to the toxin, but this technique does not eliminate bee exposure. In the case of neonicotinoid seed treatments, very little of the active ingredient is taken up by the treated plant, and the remaining 80—98% (Sur and Stork 2003) of the active ingredient is released into the environment, possibly being transported in the soil and water (Long and Krupke 2016; Morrissey et al. 2015). These insecticides can also be inadvefiently disseminated into the environment during planting. When insecticide-coated crop seeds are planted, it accompanies a dust that falls into the sun•ounding habitats. The neonicotinoids associated with this planter dust can adversely affect honey bee hives, especially those within a certain distance of the planted field (Krupke et al. 2012; Sgolastra et al. 2012; Tapparo et al. 2012). These exposures to "dust off' can be catastrophic for a beekeeper, with a loss of nearly 100% of hives (J.G.L., personal observation). Bees flying during planting can be contaminated with high levels of the neonicotinoid; for example, some bees exposed to the dust had 1240 ng of clothianidin per bee (Tapparo et al. 2012). The systemic nature of neonicotinoid allows them to be taken up by untreated sources of bee forage in the environment (Botias et al. 2015; David et al. 2016; Krupke et al. 2012; Long and Krupke 2016; Pecenka and Lundgren 2015). Mogren and Lundgren (2016) found that untreated flowering strips planted to conserve pollinators near organic and conventional cornfields were contaminated with the neonicotinoid clothianidin, and the level of clothianidin found in the bee bread of contaminated hives was strongly and positively con-elated with nutritional stress on the bees. The end result of this substantial environmental exposure is that numerous pesticides are returned to the hive.

In-hive exposure. Pesticide contamination of the hive makes this a dangerous place to live for a honey bee. Hundreds of pesticides and their residues have been isolated from wax comb, pollen, or dead bees within bee hives (Frazier et al. 2015; Long and Krupke 2016; Mullin et al. 2010). In one of the most comprehensive examinations of hive contaminants, Mullin et al. (2010) found that all tested Florida and California hives were contaminated with pesticides and their metabolites, with an average of 6.5 pesticides per hive (118 different pesticides were identified in the study). Of these, nearly half the hives were contaminated with the systemic neonicotinoid insecticides. The majority of samples were contaminated with fluvalinate and coumaphos (two acaricides used to combat Varroa destructor), chlorpyHfos (an insecticide), and chlorothalonil (a fungicide). More often than not, multiple pesticides were found in each sample tested. Pesticide exposure within the hive is a consistent stressor on hive health. Comb and propolis, pollen, nectar,

water, and dead bees can all be a source of pesticide exposure for bees living exclusively within the hive.

comb and propolis. Many pesticides are lipophilic and can accumulate to high levels within the wax comb in which the bees spend nearly all of their lives. Once a pesticide enters the comb, it diffuses throughout the wax over a matter of weeks. The exact mechanism behind this transference is unknown. Also, the diversity and quantity of the chemistry found in the comb is subsequently correlated with the pesticides found in the nectar stored in the comb (Byrne et al. 2014) and in dead bees found outside of the hive (Mullin et al. 2010). In one study, Wu et al. (2011) found that pesticides in contaminated comb had moved to adjacent pesticide-free comb within 19 days (a typical brood cycle). Once contaminated, pesticides in the comb can persist for long periods of time. For example, some of the common acaricides used in managing Varroa mites can persist for years in the comb (Bogdanov 2004). Other pesticides (e.g., imidacloprid) may persist for much less time (Dively et al. 2015). The end result is that a tremendous diversity of pesticides and their residues are found in the wax comb of nearly all bee hives tested. Eighty-seven and 39 pesticides (or their residues) have been recovered from wax samples from active bee hives in Nonh America, with an average of 6—10 pesticides reported (Mullin et al. 2010; Wu et al. 2011). The most common pesticides found were consistently the acaricides (fluvalinate and coumaphos) used in Varroa conffol, but chlorothalonil (a fungicide) and chlorpyrifos (an insecticide) were also found in most wax samples, as were neonicotinoids. Mullin et al. (2010) found up to 39 pesticides in a single wax sample! The quantities of these pesticides in the comb can be staggering; the average amount of fluvalinate and coumaphos found in the combs were around 6700 ppb and 8300 ppb (respectively), and the maximum quantity of these pesticides found in a single wax sample was more than 22,000 ppb (Wu et al. 2011). Methods for detecting various pesticide groups in propolis have been developed (Chen et al. 2009; dos Santos et al. 2008), but more research is needed on applying these methods to hive-collected propolis samples. Coumaphos and chlorpyrifos were found in nearly all propolis samples analyzed in Uruguay (Pérez-Parada et al. 2011). The implications of pesticide-contaminated comb on beeswax that is sold commercially remains a question. If pesticides are volatilized upon burning, does this pose a health hazard? Two overarching conclusions that can be drawn here are that the bees are consistently living in a matrix of pesticide cocktails and that beekeepers that manage the in-hive pests with pesticides can be exposing their bees to relatively high levels of toxin for long periods of time.

Pollen. The major source of protein for worker bees, queens, and developing larvae is pollen. Hives consume large quantities of pollen, especially during

reproductive growth phases of the hive. One study showed that hives collect 40 kg of pollen annually (Villa et al. 2000), and complete exposure scenarios given the amount of pollen collected by typical hives are available (Halm et al. 2006; Rortais et al. 2005). Comprehensive evaluations of pesticide contaminants in pollen or bee bread suggest that this is a major source of toxins for the hive (Chauzat et al. 2006; Long and Krupke 2016). Hundreds of pesticides have been found in bee pollen.

Surveys report between 21 and 32 pesticides in a single pollen sample (Long and Krupke 2016; Mullin et al. 2010; Pettis et al. 2013). Pyrethroid insecticides were found in every pollen sample tested, organophosphates were found in of samples, and fungicides were one of the most common pesticides found (Pettis et al

2013). Pyrethroids were also the most commonly found insecticide in Indiana pollen samples (Long and Krupke 2016). An average of 7—9 pesticides was found per pollen sample in these surveys. The quantity of pesticides in a single sample is also concerning: one survey found a maximum of 29,000 ppb (an average of 4400 ppb per sample) of the fungicide chlorothalonil in the bee's pollen (Pettis et al. 2013). Neonicotinoids are also frequently found in pollen of seed-treated crops (Bredeson and Lundgren 2015b; Byrne et al. 2014; KniPke et al. 2012). Pollens from untreated wildflowers and conservation strips that are embedded in an agricultural matrix also are frequently contaminated with neonicotinoids (Botiß et al. 2015; Chauzat et al. 2006; David et al. 2016; Lu et al. 2015). Hives placed in conservation suips adjacent to comfields collected pollens that had 10 times the honey bee LD50 for clothianidin (Mogren and Lundgren 2016). Although these neonicotinoids are frequently encountered in the pollen, they usually are not the dominant pesticide encountered based on the few investigations published.

Nectar. Simple carbohydrates are a source of rapid energy used by workers and other hive members to fuel flight and basic metabolic processes. Although it con. sists primarily of simple sugars (sucrose, fructose, and glucose), nectars can have a diversity of micronutrients that influence the biology and behavior of floral visitors (Lundgren 2009). Because nectar is derived from phloem contents, any pesticides that are transported in phloem will often be present in the nectar. Indeed, insecticides can be found in flower nectar within a few days of application (Barker et al. 1980) and can persist for days or even months (Byrne et al. 2014; Waller et al. 1984). Numerous insecticides have been found in floral nectar, including dimethoate, trichlorfon, deltamethrin, Schraden, imidacloprid, clothianidin, phosphamidon, and furadan, among many others (Lundgren 2009). One older literature review found that systemic insecticides were found in floral nectar in 71% of 34 published studies (Davis et al. 1988). Uncontaminated nectar from the field that is stored in insecticide-contaminated wax can become contaminated with fairly high doses; this was observed with imidacloprid in citrus nectar (Byrne et al. 2014). Converting nectar to honey does not necessarily reduce the risk of pesticide contamination (Blasco et al. 2003; Chen et al. 2014; Rissato et al. 2007). In one recent study, 70% of Massachusetts honey samples were contaminated with neonicotinoids, with imidacloprid being particularly prevalent (Lu et al. 2015). In addition to the harm these pesticides and residues pose to the hive itself, these contaminants become problematic when marketing the honey due to food safety regulations.

Water. Honey bees require water to survive and cool the hive, and pesticides can contaminate surface and plant-based water sources at levels that may affect bee hives. Many pesticides contaminate environmental sources of surface water, including ponds, rivers, and streams (Eichelberger and Lichtenberg 1971; Martinez et al. 2000; Schwarzenbach et al. 2010). This contamination is related to both the chemistry of the pesticide itself (e.g., its water solubility, adsorption to soil

molecules, and stability in the environment) as well as the environment (proximity to the source of a pesticide, soil physical and chemical properties, biological communities within a habitat, etc.) (Arias-Estévez et al. 2008). Nevertheless, surface waters that are visited by honey bees (Butler 1940; Robinson et al. 1984) are prone to contamination with pesticides, and this exposure pathway is particularly pertinent to agricultural areas where water samples often have higher contamination levels. Foraging workers devote part of their lives to water collection, and a specific caste of workers devotes their efforts exclusively to water collection (Robinson et al. 1984). These bees return to the nest with a crop full of water that they share with other members of the hive (Visscher et al. 1996; Woyciechowski 2007). In addition to surface waters, bees also collect water from guttation fluids from insecticide-treated plants, and this may be an exposure pathway whereby systemic insecticides like neonicotinoids can affect bees (Girolami et al. 2009; Hoffman and Castle 2012; Tapparo et al. 2011). Their systemic nature does not preclude these neonicotinoids from contaminating other environmental sources of water (Main et al. 2016; Morrissey et al. 2015), but we do not entirely understand how these contaminants get from cropland to surface waters. Certainly, more risk assessments should focus on water as a relevant exposure pathway for agrichemicals to affect pollinators.

Dead bees. When bees die from pesticide exposure in the hive, the remaining nestmates may be adversely affected by pesticide residues in the bee corpses. Also, piles of dead bees in front of the hive can sometimes give an indication of an acute pesticide exposure (Frazier et al. 2015). For example, atrazine (herbicide), metolachlor (herbicide), and clothianidin were found in the corpses of bees piled in front of Indiana hives (Krupke et al. 2012). Often, analysis of dead bees reveals this type of multiple pesticide exposure; an average of 2.5 pesticides were found per bee in one study (Mullin et al. 2010). The exposure level of pesticides revealed by these dead bees can be astounding. Bees flying during corn planting were exposed to planter dust with clothianidin. Flying bees were then collected and allowed to die without further exposure. Some workers had up to 640 ng of clothianidin on their bodies (Tapparo et al. 2012). The half-lives of insecticides on and in dead bees are another consideration. In neonicotinoid-contaminated bees, the parent compound is only detectable for a few hours after exposure (Chauzat et al. 2009; Tapparo et al. 2012); this short half-life may explain why neonicotinoids are not always detected on bee corpses following a "dust off" event. Finally, acute pesticide exposure often kills the bees during foraging, and these poisoned bees never return to the hive. For this reason, it can be difficult to rank risk factors leading to hive declines because direct evidence of pesticide mortality is lacking.

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The presence of an active egg laying queen in pheromonal control of colony integrity, sufrient ratio of bees to brood to maintain population growth, relatively disease/pestfree, and adequate nutrition are principal determinants of a healthy honey bee colony. (Dively et al. 2015)

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The health of the hive is an aggregation of lethal and sublethal effects on the various life stages and social castes over time. Thus, acute toxicity of a pesticide can have prdictable effects on hive performance. But pesticides also can exact numerous sublethal effects on individual members of the hives, and these many little hammers can combine into substantial hive-level effects on hive survival under the correct circumstances. These combined lethal and sublethal effects also can increase the negative effects that other stressors such as diseases or pests have on hive health. Moreover, these more obscure sublethal effects can operate at very low doses of pesticides. For instance, locomotor activity was significantly reduced when fipronil was administered at doses 600-fold lower than the LD50 for this pesticide (Charreton et al. 2015). For these reasons, risk assessments of pesticides against honey bees can be very challenging to conduct and interpret (Mullin et al. 2015).

Honey bees may be more prone to pesticide effects than other insects. Social insects sometimes sacrifice some of their innate immunity and detoxification capabilities in favor of "social immunity"; this is the case with the honey bee. Individual bees have fewer detoxification enzymes to help nullify pesticide contaminants (Claudianos et al. 2006). But behaviors like nest cleaning and inherent aspects of the hive meant to replace this innate immunity against environmental toxicants may be less effective against pesticides. As mentioned above, comb and propolis which have antibiotic characteristics aggregate pesticides rather than reduce their exposure. One hopeful aspect is that different hive genotypes are differentially affected by pesticides (Laurino et al. 2013; Sandrock et al. 2014), which suggests that selection toward living in a matrix of pesticides should be possible, once natural selection has culled pesticide-susceptible hives.

Here, I document some of the lethal and sublethal effects of pesticides that affect hive health. It is important to note that most of these studies were conducted with a fairly narrow focus, and none consider multiple contributing mechanisms or declines based on pesticide acute or chronic toxicity on bee performance. Suffice it to say that many if not most life history parameters of honey bee hives can be affected by pesticides.

Mortality and survival. Mortality is an easily observed and oft-reported experimental endpoint in hazard assessments. Within a population, there is often a wide range of susceptibilities to even the most toxic substances, and rare resistant individuals can survive high doses. This can make determining a dose that kills 100% of a population challenging and has prompted risk assessors to instead report the doses that kill some (50%) or most (90%) of a population. These are called the LD50 and LD90 values; other values are also sometimes reported (LD80, LD99, etc.). In the cases when the ingested dose cannot be determined in an assay (for example,

when the amount of pesticide-contaminated diet ingested cannot be measured), the lethal concentrations (LC values) that the bees are exposed to are used in lieu of the LD value. The duration and frequency of exposure have great bearing on these LD assessments. Most often, risk assessments of pesticides focus on individual bee mortality under very controlled (e.g., isolated in a laboratory) conditions. One study reports the lethality of a range of pesticides relative to the organochlorine insecticide DDT (Pisa et al. 2015). Some newer formulations that have much less active

ingredient applied in the environment (like neonicotinoids) likely pose as much if not more hazard to honey bees than some earlier insecticidal chemistries, due to their lowered LD50S. Also, a particular pesticide may kill larvae and adults at different rates. For example, Wu et al. (2011) showed that larval mortality was unaffected by comb pesticides, but the longevity of workers was reduced by pesticide exposure. Yet in another study, larvae were much more susceptible to pesticides than the adults, possibly because the larvae only defecate at the end of the stage, prolonging exposure to ingested pesticides (Zhu et al. 2014). One of the most toxic pesticides to bees that I was able to find reported in the literature is Fipronil. Significant mortality was experienced at 0.1 ng/adult bee after a 7 d exposure (Aliouane et al. 2009). At some level, mortality of individual hive members will contribute to hive collapse, but this is a dynamic process that is difficult to predict. The ratio of mortalities inflicted on larvae, workers, and reproductives ultimately combine to form an aggregate risk to the hive itself. Also, contextual considerations like the condition of the hive prior to a pesticide exposure, hive age, or other stressors on the hive all could contribute to the lethality of a pesticide on the hive. Thus, the question of how much mortality is too much mortality is a challenging one to answer.

Development. Alterations in larval development can have cascading negative effects on adult bees and hive population dynamics. Pesticide exposure can slow larval development, and sometimes these effects are seen at doses much lower than LD values for adult workers (Davis et al. 1988; Wu et al. 2011). Delayed development rates could affect the duration of susceptibility to pests like Varroa destructor, as well as decrease the hive growth rate. Hive size is correlated with strength and its ability to survive other stressors. Larval development rates are infrequently reported relative to other hive fitness parameters, but warrant additional attention from researchers.

Mobility and behavior. Many insecticides function in part as neurotoxins, taking advantage of the unique characteristics of insect nervous systems to minimize acute effects on non-insect animals. Some classes of insecticides are sumgates for the insect neurotransmitter enzyme acetylcholine esterase (AChE). Their use overexcites the acetylcholine receptor by replacing the enzyme, causing the nerve cells to continually fire (these compounds do not

allow nerves to switch off). This insecticide mode of action is employed by many organophosphates, carbamates, and neonicotinoids (Barker et al. 1980; Boily et al. 2013; Iwasa et al. 2004). Many pyrethroids and organochlorines are also neurotoxins, but affect the ability of nerve cells to repolarize and effect an action potential (these pesticides switch nerves off). Placing hives near neonicotinoid-treated com fields is sufficient to alter the AChE levels in adult workers (Boily et al. 2013). Targeting these receptors can lead to other effects on the nervous system. Field-relevant doses of imidacloprid can impair the development of mushroom bodies (calyces) in the brain, which are organs with large quantities of AChE receptors (Peng and Yang 2016). These mushroom bodies are where learning occurs, which affects many other aspects of the natural history of honey bees. Impairment of nerve function affects several measurable characteristics

of honey bees, including mobility, learning, behavior, orientation, foraging, walking, and communication of the honey bee.

A common measurement of learning capacity is the proboscis extension reflex (PER) to food rewards, and this approach has been used multiple times to demonstrate how pesticides interfere with honey bee learning (El Hassani et al. 2008; Ramirez-Romero et al. 2005). It is also possible that the pesticide active ingredient and its metabolites have different effects on the PER (Guez et al. 2001). At very low doses of imidacloprid (1.5 ng per bee), the PER was increased over the control, but as dose and time went on, the bees became lethargic and unresponsive. One explanation for this may be that the metabolites of the imidacloprid are more effective at reducing learning compared to the parent compound (Lambin et al. 2001). Another explanation for the observation of increased PER response at low doses of neonicotinoids is that nerves over firing requires energy from carbohydrates; as the dose increases, it shuts down the metabolism in the insect. In this way, neurotoxin exposure can manifest itself in altered feeding behavior. Demares et al. (2016) found that thiamethoxam exposure did not affect the PER to protein-based foods, but did alter responsiveness to sucrose solution at certain pesticide and sucrose concentrations. Ability to recognize and imbibe water can also be affected after ingesting pesticides (Aliouane et al. 2009). So, the nutritional status of bees and entire hives can be compromised when learning ability is reduced by a pesticide; mobility and locomotor activity also affects the nutritional stress of the hive by altering foraging behavior.

Short- and long-distance dispersal is affected by pesticides, and sublethal effects on mobility by a pesticide can have important ramifications for the hive (Matsumoto 2013; Ramirez-Romero et al. 2005). Sublethal doses of thiamethoxam and clothianidin (5 and 2 ppb, respectively) reduced foraging success and lowered pollen and nectar collections by treated hives (Sandrock et al. 2014). More specifically, neonicotinoids disrupt navigation capabilities, and the affected bees struggle to find their way back to the nest (Fischer et al. 2014). Pesticide-treated and untreated foraging workers equally found their way to the intended floral resources, but harmonic radar attached to the honey bees revealed that treated workers were significantly less likely to remember the direction back to their nest. This inability to return to the nest appears could be related to navigation rather than on flight capability (Fischer et al. 2014; Matsumoto 2013), both of which are adversely affected by neonicotinoids (Blanken et al. 2015). Walking is another important behavior that can be affected by neurotoxic pesticides. Sublethal doses (10—50-fold lower than the LDsos) of pyrethroid and neonicotinoid insecticides reduced walking speeds and distances in adult honey bees (Charreton et al. 2015). Walking may seem trivial to a hive-dwelling insect with flight, but ability to disperse resources throughout the hive, communicate foraging sites (e.g., with the waggle dance), clean the hive, thermoregulate, etc., all depend on locomotor (i.e., walking) behavior.

Winter survival. The aggregate effects of many small detriments to hive health may manifest themselves in the overwintering success of a honey bee hive. In one study, sublethal doses of neonicotinoid insecticides were administered to hives and

their performance was compared to untreated hives. Summer performance was equivalent in the two groups, but significantly fewer treated hives survived the winter; the lack of dead bees in failed hives was reminiscent of colony collapse disorder (Lu et al. 2014). This winter mortality resulting from sublethal exposures to midaclopfid follows a standard dose-response curve (Dively et al. 2015). The stress of overwinteHng then is the final blow to a pesticide-weakened hive.

Reproduction. Queen and drone health dictate the hive growth rate. Several studies have demonstrated direct physiological effects of pesticides on the physiology and fecundity of honey bee queens. Ovarial and spermathecal development was reduced in queens that had been reared on as little as I ppb of clothianidin (or 4 ppb of thiamethoxam). Queen survival was reduced by 25%, the number of sterile females were increased and the proportion of queens laying fertilized eggs was reduced by 38%. Sperm viability, number of spermatozoa, and ovariole size were also adversely affected by the neonicotinoids (Williams et al. 2015). Drones are also adversely affected by low levels of neonicotinoids (1.5 ppb clothianidin or 4.5 ppb of thiamethoxam), where adult drone longevity and sperm viability were both reduced significantly (Straub et al. 2016). Lethal and sublethal effects can combine into outright queen failure and supersedure within the hive. For example, imidacloprid administered as 20 and 100 ppb prompted queen failure in late summer, following a broodless period (Dively et al. 2015). Similarly, field-relevant doses of clothianidin and thiamethoxam administered to larvae over two brood cycles resulted in reduced brood production, and greater queen supersedure rates in the treated hives (Sandrock et al. 2014). These effects on reproductive capacity of the hive are not restricted to neonicotinoids. The miticides fluvalinate and coumaphos increase queen mortality and coumaphos lowered queen body weight, reduced ovary size, and lowered the number of sperm in exposed relative to untreated queens (Haarmann et al. 2002).

Susceptibility to other stressors. Pesticides and other stressors interact, sometinrs in unpredictable ways. As a result, pesticide toxicity is not always well correlated with a specific response variable in a narrowly focused experimental design without considering other contributing factors to experimental outcomes. Pesticides, diseases and pests are often synergistic or additive in their effects on the toxicity of pesticides to honey bees. Nosema ceranae spore counts and impact on the hive are aggravated when bees are simultaneously exposed to one of several pesticides in the diet (Dively et al. 2015; Pettis et al. 2012, 2013) or pesticide-contaminated comb (Wu et al. 2012). Combined deleterious effects of these two stressors accrete over time, and even low doses (e.g., one-hundredth of the LD50) of a pesticide can significantly increase its lethality when combined with N. ceranae infection (Retschnig et al. 2014; Vidau et al. 2011). This is in part [b]ecause the pesticide lowers the innate and social immune responses of the honey [b]ees, making them more susceptible to pathogen infection (Alaux et al. 2010). One [b]ehavioral response that has been observed is that bees exposed to neonicotinoids [an]d N. ceranae consume more sugar resources, which then exposes them to additional pesticide (Alaux et al. 2010; Vidau et al. 2011).

Varroa mite infestations and pesticide exposures can combine to reduce hive performance more than the individual stressors. When combined, imidacloprid and Varroa mites reduced the flight distance and flight time of affected honey bees more than either stressor did alone (Blanken et al. 2015). The hives that died in spring flew significantly shorter distances than the surviving hives, suggesting that fitness reductions produced by both imidacloprid and Varroa mite effects may reduce hive survival. These effects may also be the result of suppressed anti-viral immunity in bees that were exposed to low doses (one thousandth of the of pesticides (Di Prisco et al. 2013). Deformed wing virus, a pathogen vectored by Varroa mites, replicated significantly more following the host's exposure to imidacloprid and clothianidin (but not chlorpyrifos).

5 Conclusions

If pesticides were the answer to pest problems, then we should have overcome pests decades ago. Pesticides are not the sole cause of bee declines; they are an artifact of a simplified agroecosystem. Siloing the pesticide issue will not solve the bee problem. The most effective way to reduce the impact of pesticides is to reform our food production systems in which the bees must live. Diversifying our food production system will have knock-on effects like providing additional and diversified forage that bees use to reduce nutritional stress. By improving honey bee nutrition and reducing their toxin exposure, bee immune function and resistance to pests will be promoted. Anything less than reforming this food production system will not solve the problem of bee declines. Paradigm shifts of this nature are not entirely within the beekeepers' ability to control, but there are things that beekeepers and others can do.

6 Suggestions for Beekeepers

From a research and regulatory standpoint, risk assessments of pesticides need to be conducted within a realistic context. The risk is context dependent, and risk assessments need to factor in the many stressors that affect a hive's performance when assessments are conducted. Moreover, hives are systems, and toxicological assessments on hive components (e.g., brood or worker survival) outside of the context of the hive system do not give a true perception of risk. Test scenarios that simultaneously account for numerous lethal and sublethal effects of pesticide exposure over time on hive performance may help to overcome doubt and ambiguity regarding the importance of pesticides in international bee declines. The current infrastmcture that funds science fosters doubt regarding the role that pesticides play in bee declines. Beekeepers need to fight fire with fire and fund the independent science and scientists that are willing to pursue these often

career-altering research projects into the truth underlying pesticides and bees. The money will not likely come from sources other than the beekeepers (individually and as club groups).

A research priority for beekeepers should be finding non-chemical alternatives for Varroa mite control. Beekeepers need to understand that their use of acaricides is compromising the integrity and longevity of their hives. Moreover, the acaricides persist in the hives, so decisions to apply could have long-term implications for the contamination of treated hardware. For these reasons, prophylactic applications of pesticides to control Varroa should be avoided. Also, hygienic bee lines (those bees that clean themselves of mites), organic acids, essential oils, and natural enemies of the Varroa may be acceptable alternatives to pesticides that could replace the stressors of pesticides in the hive.

The use of pesticides continues to rise, and food production systems are not going to reform overnight. By feeding and treating bee hives, beekeepers slow the adaptation of their hives to living within a pesticide-contaminated landscape matrix. Preventing short-term hive losses with interventions may well be fostering a long-tern extension of bee declines. Balancing an operation's profitability with selecting for pesticide-tolerant bee genetics is a central challenge facing the bee industry and hobbyists alike.

Pesticides are ubiquitous in the environment and in the hives, and can be highly toxic to bees; what can a beekeeper do? Most farmers do not understand that there is a better way to farm than conventional, high-input monoculture systems. The nature of beekeeping is such that beekeepers often know many of the farmers in their communities. They know the farmers that are farming ecologically and in diversified systems, and they know those who are not. Worldwide bee declines have initiated tremendous media attention, and beekeepers are frequently the stars of this attention. Beekeepers need to make the ecologically based farmers in their communities into heroes. Take the media attention that has been given and turns the stories about "the bee problem" into one about "the solution," which must come from the farmers themselves.

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