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Scott T. O'Neal
*University of Nebraska - Lincoln*, soneal3@unl.edu

Troy D. Anderson
*University of Nebraska - Lincoln*, tanderson44@unl.edu

Judy Wu-Smart
*University of Nebraska-Lincoln*, jwu-smart@unl.edu

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Interactions between pesticides and pathogen susceptibility in honey bees

Scott T. O’Neal, Troy D. Anderson, and Judy Y. Wu-Smart

Department of Entomology, University of Nebraska, Lincoln, NE, USA

Corresponding author — J. Wu-Smart, email jwu-smart@unl.edu

Abstract
There exist a variety of factors that negatively impact the health and survival of managed honey bee colonies, including the spread of parasites and pathogens, loss of habitat, reduced availability or quality of food resources, climate change, poor queen quality, changing cultural and commercial beekeeping practices, as well as exposure to agricultural and apicultural pesticides both in the field and in the hive. These factors are often closely intertwined, and it is unlikely that a single stressor is driving colony losses. There is a growing consensus, however, that increasing prevalence of parasites and pathogens are among the most significant threats to managed bee colonies. Unfortunately, improper management of hives by beekeepers may exacerbate parasite populations and disease transmission. Furthermore, research continues to accumulate that describes the complex and largely harmful interactions that exist between pesticide exposure and bee immunity. This brief review summarizes our progress in understanding the impact of pesticide exposure on bees at the individual, colony, and community level.

Introduction

Bees are important pollinators of many crops and native plants, contributing about one-third of the human diet globally and providing immeasurable ecosystem services [1–3]. There are ca. 4000 species of bees across North America, but a number of species have exhibited population declines [4, 5*], including several bumble bee species that have decreased in both abundance (up to 96%) and geographical range (23–87%) [6]. Similar declines have also been reported in solitary species, particularly with bees that are habitat and flower specialists [7]. Additionally, beekeepers have reported economically unsustainable, annual honey bee colony losses of ca. 31–46% since 2010 [8]. Research efforts are focused on the relationship between...
current agricultural practices and consistent losses of honey bee colonies. This includes large-scale conversion of natural landscapes into productive crop fields, which has led to a reduction in forage availability and malnutrition, as well as increased pesticide exposures to bees [4, 8–18, 19*, 20–22]. Other factors that affect honey bee health can include parasites and pathogens, with increased infestations and infections, respectively, in colonies with reduced immunocompetence caused by poor nutrition and exposure to pesticides [23–26, 27**].

There are multiple interacting stressors that affect honey bee colonies. For example, the ectoparasitic mite *Varroa destructor* feeds on the hemolymph of bees, resulting in physiological deficiencies that reduce overwintering success for the colony [28]. Moreover, physical damage to the bee cuticle caused by mite feeding can introduce several viruses into host bees [29, 30]. If unmanaged, *Varroa* mite infestations can increase the mortality of bees in the colony within one season [31, 32]. The lack of, or improper, *Varroa* mite management is a significant driver for losses among beginning and hobbyist beekeepers [33]. However, *Varroa* mites continue to be the major reason for the use of beekeeper-applied miticides or varroacides since their introduction to the U.S. [34, 35]. These apicultural pesticides, along with agricultural pesticides (insecticides, fungicides, herbicides) transported to the hive by foraging bees, may result in synergistic interactions that cause higher toxicity than compounds acting alone [36–40, 41*]. Pesticides may also accumulate in the hive, affect brood development, and increase selection pressure for varroacide-resistant mites [42–44]. Laboratory studies often examine individual stressors for direct evidence of their adverse effects on bees; however, complex stressor interactions and the ability for bees to socially or behaviorally defend themselves have made it difficult to understand the causes and effects of stressor interaction in the field [25, 41*, 45, 46]. This review examines the current literature focusing on pesticide exposure and pathogen impacts on honey bees, with emphasis on the interface between these stressors at different levels of biological organization (i.e., individual to colony to apiary).

**Individual-level effects**

Laboratory studies have demonstrated that exposure to sublethal doses of pesticides can negatively affect honey bee behavior [47, 48], foraging [49], longevity [43], and olfactory learning and memory [50–53]. Pesticide exposure can also impair honey bee detoxification pathways [54], and the harmful effects of interactions between multiple pesticides in bees appear to be nearly as complex as the drug interactions observed in mammals [39, 55]. More pertinent to concerns related to the increasing role of pathogens in colony decline is the impact of pesticide exposure on the immune response.
of honey bees and their ability to resist or tolerate pathogen infection. The pathogen most commonly used in laboratory studies has been the microsporidium *Nosema ceranae*, which has proven the most tractable in controlled infection studies. Significant effects on honey bee immune responsiveness to infection with *Nosema* have been observed with exposure to neonicotinoid pesticides [23, 24, 56–58, 59**, 60**], fipronil [23, 57, 61], as well as fungicides [62], in addition to altered queen physiology and survival [59**] and reduced sperm viability and gene expression [60**]. More noteworthy, given the widespread prevalence of agricultural and apicultural pesticide residues in the hive environment [42], is the finding that bees exposed to these residues in the hive also have increased susceptibility to *Nosema* [62, 63]. With regards to other honey bee pathogens, harmful interactions have been demonstrated between viral pathogenicity and exposure to the neonicotinoid pesticide clothianidin [26], as well as the pyrethroid miticide tau-fluvalinate [64]. Recent work has also employed a model insect virus [65**] to reveal that exposure to the formamidine miticide amitraz increases mortality associated with viral infections [66*]. In addition to pesticide exposure, there is also mounting evidence that organosilicone spray adjuvants used in various pesticide formulations may pose a more serious threat than previously realized, as they have been demonstrated to both impair olfactory learning [67] and increase viral pathogenicity in bees [68*]. Another exciting recent study shows a synergistic interaction when bee larvae are exposed to clothianidin or the organophosphate dimethoate in combination with *Paenibacillus larvae*, the causative agent of American foulbrood [69]. Finally, gene expression studies have also suggested that thymol, formic acid, and the phosphorothioate miticide coumaphos may suppress expression of genes related to bee immunity [70]. A number of recent reviews address in greater detail the links between pesticides and bee diseases [71**] and provide some discussion of improvements and future directions for this research [72**]. Although there exist ample correlative studies to suggest a link between pesticide exposure and the ability of bees to resist or tolerate pathogen infection, there is very little known about the mechanisms of such a connection. One outlier is a study describing a negative modulator of NF-kB activation (NF-kB function reviewed here [73]) that reduces honey bee immunocompetence when exposed to clothianidin and another neonicotinoid, imidacloprid, but not when exposed to the organophosphate chlorpyrifos [26]. Two recent studies also described an important role for the evolutionarily conserved ATP-sensitive inwardly rectifying potassium (KATP) channel in the regulation of honey bee cardiac function [65**] and antiviral immunity [74**]. This supports earlier findings that KATP channels play a role in mediating fruit fly survival during viral infections similar to that observed in mammals [75]. Although the exact mechanism has yet to be elucidated, evidence suggests that K\textsubscript{ATP} channels have a function in modulating
antiviral RNAi by facilitating tissue-specific regulation of innate immune response mechanisms by the cellular environment of the heart [76]. Taken together, these studies also support the hypothesis that disruption of cardiac function and subsequent inability to maintain homeostasis may reduce the ability of bees to tolerate infection by pathogens [66*], providing another possible mechanism by which cardioactive pesticides could reduce honey bee immunocompetence.

Colony-level effects

Pesticide effects on honey bee colonies are typically studied in the field; however, the number of interacting biotic and abiotic stressors that can affect these colonies presents variables that are difficult to manage with these studies. Additionally, social bee behaviors, such as age-based divisions of labor, can cause disparities in the evaluation of pesticide exposures, toxicities, and risks to the different castes and their roles in the colony [38, 77, 78]. For example, older forager bees are more likely to be exposed to pesticides via contact or oral exposure to contaminated nectar and water sources than younger nurse bees, and these older bees are reported to be more sensitive to these pesticide exposures [79, 80**, 81*, 82*]. By contrast, nurse bees are more likely to be exposed to pesticide-contaminated pollen than forager bees, since the nurse bees consume pollen to produce glandular secretions to feed brood and queen bees. Nurse bees infected by Varroa mites and feeding on pesticide-contaminated pollen may have higher virus titers compared to those feeding on uncontaminated pollen and, in turn, can increase the risk of transmitting viruses to the brood and queen during feeding [78, 83–85]. Additionally, young adult bees emerging from parasitized pupae may be disproportionately impacted by Varroa mites as multiple mites reproduce and feed within the developing pupal cell. Heavy parasitism alters physiological features critical for winter survival in host bees and may lead to developmental abnormalities such as malformed wings caused by Varroa-vectored deformed wing virus [86, 87]. In addition, the exposure of bees to pesticides can not only adversely affect brood care and production, but can affect other caste behaviors such as mating, egg laying, and other routine tasks that support healthy colony numbers. Forager bees exposed to certain pesticides are reported to exhibit impaired foraging behaviors and cognitive functions that not only lead to reduced food stores, lower brood production, and higher pathogen infections, but can result in increased pesticide sensitivity and disease susceptibility for malnourished colonies [49, 56, 77, 88–90]. Moreover, pesticide exposure may impair social immunity by reducing hygienic behavior, a social behavioral defense mechanism in which mite-infested or disease-infected pupae are detected and removed from the hive before mites are fully developed or
disease becomes infectious [91–93]. This is an important behavioral adaptation to suppress the transmission and infectivity of mites and pathogens in colonies. However, the over-use and unregulated use of apicultural pesticides by beekeepers to manage Varroa mites has conferred resistance in mites, further magnifying the potential for damage caused by pathogens in Varroa-parasitized colonies.

**Community-level effects**

The accumulation and persistence of pesticide residues occurs at alarmingly high levels in hive products (wax, propolis), food stores (pollen, honey), and bees [42, 44]. As biological indicators of the environment, honey bee exposure to pesticides likely reflects the complex array of pesticide exposures wild bees and other pollinators are experiencing; however, more research is needed to support this. Furthermore, the ability of Varroa mites to vector a number of viruses allows for the transmission of viruses to occur via the phoretic movement of mites among honey bees from different colonies or apiaries [94]. Pathogens and pesticide residues may also be taken or robbed from weaker colonies by neighboring bees and brought back to different hives, further distributing diseases and contaminants [95]. Beekeepers also contribute to this issue through the common practice of moving or exchanging hive components (wax, honey, pollen, and bees) from one colony to another. This redistribution of pathogens and contaminants is a particular concern when commercially-managed colonies are maintained at high density to meet pollination service demands, as is the case for almond pollination. In 2016, roughly 1.7 million colonies were transported to California to pollinate 971 400 acres of almonds, contributing $280 million in the total value of the pollination services provided by managed bees for this crop alone [96]. When managed bees are introduced to new areas, pathogens such as Nosema spp. and viruses may be transmitted among different bee species when infected bees visit common foraging sites [97–99]. Given similar population declines observed in wild bee communities and the prevalence of pesticides and pathogens in the environment globally, interactions between these two stressors should be a critical research focus. Pathogen spillover from managed bees into wild bee communities has been well documented, however, the implications of this are still not well understood. Additionally, more research to examine the relationships between pathogens and pesticide exposure is clearly needed.

**Concluding remarks**

Our review has focused on the interactions between pesticides and pathogens and their effects on bees across multiple levels of biological
organization. Although significant advances have been made in identifying interactions at the individual level, there is still considerable progress to be made in understanding the physiological mechanisms that drive pesticide-induced immunocompetence in bees. Furthermore, there exist few explanations for why many of these interactions observed at the individual level fail to translate into quantifiable effects at the colony level. Synthesizing data collected from laboratory studies on individual bees and field studies on whole colonies with 50,000 or more individuals is a critical consideration for assessing risk of pesticides with ecological relevance [100]. Finally, the impact of these interactions at the community level has proven even more challenging to describe, and presents considerable opportunities for future research.

References and recommended reading

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


19.* Smart M, Pettis J, Rice N, Browning Z, Spivak M: Linking measures of colony and individual honey bee health to survival among apiaries exposed to varying agricultural land use. *PLoS ONE* 2016, 11:e0152685. — This study examined the effect of land use on honey bee colony health and individual bee nutrition and immunity. Authors modelled physiological responses in individual honey bees simultaneously with colony health markers to predict colony survival.


27. Goulson D, Nicholls E, Botias C, Rotheray EL: Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 2015, 347:1255957. — This review article examines the complexity of challenges and the relationships between multiple stressors that drive bee health decline and highlights the need to improved regulatory assessments and policies to better conserve bee communities.


41. Rinkevich FD, Margotta JW, Pittman JM, Danka RG, Tarver MR, Ottea JA, Healy KB: Genetics, synergists, and age affect insecticide sensitivity of the honey bee, *Apis mellifera*. *PLoS ONE* 2015, 10:e0139841. — *This study examined the influence of genetic stock and age in bees on insecticide sensitivity and found Apis mellifera ligustica, one of the most commonly managed stocks in the US, is more sensitive to insecticides, however, sensitivity was highly dependent on the class of insecticide. Authors also reported increased sensitivity to insecticides as a result of physiological transitions associated with the aging process.*


60. Chaimanee V, Evans JD, Chen Y, Jackson C, Pettis JS: Sperm viability and gene expression in honey bee queens (*Apis mellifera*) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate acaricide
coumaphos. J Insect Physiol 2016, 89:1-8. — This paper, along with [59 **], provide much-needed insight into the effects of pesticide exposure on honey bee queens. Here, the neonicotinoid imidacloprid was found to reduce the viability of stored sperm, and both imidacloprid and the organophosphate acaricide coumaphos were found to decrease expression of genes related to antioxidation, immunity and development in queens.


65. ** O’Neal ST, Swale DR, Bloomquist JR, Anderson TD: ATP-sensitive inwardly rectifying potassium channel modulators alter cardiac function in honey bees. J Insect Physiol 2017, 99:95-100. — This paper demonstrated for the first time in a hymenopteran an evolutionarily-conserved role for ATP-sensitive inwardly rectifying potassium channels in the regulation of cardiac function similar to that described in mammals. Along with [74 **], this paper also suggests a physiological mechanism by which exposure to environmental stressors may be translated into disruption of cardiac function and other associated processes.

66. * O’Neal ST, Brewster CC, Bloomquist JR, Anderson TD: Amitraz and its metabolite modulate honey bee cardiac function and tolerance to viral infection. J Invertebr Pathol 2017, 149:119-126. — This paper demonstrates for the first time a negative interaction between exposure to the beekeeper-applied formamidine acaricide amitraz and viral infection in honey bees.


68. * Fine JD, Cox-Foster DL, Mullin CA: An inert pesticide adjuvant synergizes viral pathogenicity and mortality in honey bee larvae. Sci Rep 2017, 7:40499. — This article demonstrates synergistic interactions between organosilicone surfactant adjuvants and exogenous viruses on larval honey bees. Larvae exposed to ‘inert’ adjuvants exhibited higher viral titers and lower expression of immune genes associated with viral defense. This is a novel examination of the role inert formulation additives play in bee health decline.


71. ** Collison E, Hird H, Cresswell J, Tyler C: Interactive effects of pesticide exposure and pathogen infection on bee health — a critical analysis. Biol Rev 2016, 91:1006-1019. — This review provides an in-depth look at recent findings on pesticide/disease interactions and makes an argument that the trend towards laboratory-based assays do not reflect field-realistic scenarios, with the result that colony-level implications of pesticide effects the interaction between pathogens and bee immunity remain unclear.

72. ** Benuszak J, Laurent M, Chauzat M-P: The exposure of honey bees (Apis mellifera; Hymenoptera: Apidae) to pesticides: Room for improvement in research. Sci Total Environ 2017, 587-588:423-438. — This review provides an overview of the sampling methods and analytical approaches of a wide range of papers reporting the effects of pesticide exposure on honey bees, with emphasis on the lack of information on exposure via wax and beebread, as well as the pointed criticism that too many studies focus on active ingredients alone, rather than in formulation.


74. ** O’Neal ST, Swale DR, Anderson TD: ATP-sensitive inwardly rectifying potassium channel regulation of viral infections in honey bees. Sci Rep 2017, 7:8668. — This paper demonstrates the first use of the entomopathogenic flock house virus as a model virus for use in studying host/pathogen interactions and viral replication dynamics in honey bees. Furthermore, this paper demonstrates a novel role for ATP-sensitive inwardly rectifying potassium channels in the regulation of honey bee antiviral immunity.


80. ** Mullin CA, Chen J, Fine JD, Frazier MT, Frazier JL: The formulation makes the honey bee poison. Pesticide Biochem Physiol 2015, 120:27-35. — This article
reviews the potential impact of pesticide formulation additives that are generally thought of as inert. Authors highlight the challenges with assessing the environmental impacts of agrochemicals on bees when formulation additives are so widely used but tolerances have not been set, and residues of inerts are not monitored.

81. * Long EY, Krupke CH: Non-cultivated plants present a season-long route of pesticide exposure for honey bees. Nat Commun 2016, 7:11629. — This study reported chronic exposure to multiple pesticides on bees in agroecosystems and through analyses of bee-collected pollen, determined that the majority of pollen was not collected from field crops but rather from uncultivated plant sources.

82. * Mogren CL, Lundgren JG: Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status. Sci Rep 2016, 6:29608. — This study illustrated the widespread distribution of neonicotinoids and reported residues even in organic fields. Authors examined the potential impacts of neonicotinoid contamination in pollen and nectar and related residue concentration levels to nutritional status of colonies using glycogen, lipid, and protein level measures.


91. Rothenbuhler WC: Behavior genetics of nest cleaning in honey bees. IV. Responses of F1 and backcross generations to disease-killed brood. Am Zool 1964, 4:111-123.


