



Abiotic and biotic factors affecting the replication and pathogenicity of bee viruses

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Bees are important pollinators of plants in both agricultural and non-agricultural landscapes. Recent losses of both managed and wild bee species have negative impacts on crop production and ecosystem diversity. Therefore, in order to mitigate bee losses, it is important to identify the factors most responsible. Multiple factors including pathogens, agrochemical exposure, lack of quality forage, and reduced habitat affect bee health. Pathogen prevalence is one factor that has been associated with colony losses. Numerous pathogens infect bees including fungi, protists, bacteria, and viruses, the majority of which are RNA viruses including several that infect multiple bee species. RNA viruses readily infect bees, yet there is limited understanding of their impacts on bee health, particularly in the context of other stressors. Herein we review the influence environmental factors have on the replication and pathogenicity of bee viruses and identify research areas that require further investigation.

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Current Opinion in Insect Science 2016, 16:14–21

This review comes from a themed issue on **Vectors and medical and veterinary entomology**

Edited by **Zach Adelman** and **Kevin Myles**

<http://dx.doi.org/10.1016/j.cois.2016.04.009>

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Introduction

Honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.), and other insects play a vital role in ecosystems as plant pollinators. The annual estimated value of crops directly dependent on insect pollination worldwide is \$175 billion [1*] and approximately \$17–18 billion in both North America

and the European Union [2,3]. Wild, native, and managed bee species perform the majority of pollination services in both agricultural and non-agricultural landscapes. Bumble bees are the primary pollinators of some crops (e.g., tomatoes) and augment pollination of other crops [4]. In large-scale crop (e.g., almond, apple, cherry) production honey bees are the primary pollinators, since they forage over large distances and can be maintained in transportable hives. Honey bees were introduced to North America in the early 1600s as a managed species kept by beekeepers primarily for honey production [5]. Today, the majority of US honey bee colonies are maintained by commercial beekeeping operations. Colonies managed by small-scale beekeepers and feral (or unmanaged) colonies make up the remaining population.

High annual losses of managed honey bees and population declines of wild bumble bees are of great concern since bee pollinators are important for plant reproduction and crop production [6,7,8]. In some regions of the US, bumble bees have experienced between 23% and 86% range reduction [7,8] and annual losses of US honey bee colonies have averaged 33% since 2006 (reviewed in [9*]). Several studies have focused on assessing the relationship between colony health and the effects of multiple biotic (e.g., pathogens, bee genetics, and queen longevity) and abiotic factors (e.g., agrochemical exposure, weather, and management practices) [7,10,11,12,13,14]. These studies indicate that pathogens, agrochemical exposure, and lack of quality forage and habitat all contribute to bee losses, though investigating the relative role of these factors is an active area of research. Pathogens, including the microsporidia *Nosema ceranae*, trypanosomatids, viruses, and the ectoparasitic mite *Varroa destructor*, contribute to honey bee colony losses [15,16,17*,18*,19*,20,21,22,23,24,25] (reviewed in [11,26,27,28*,29]), and the microsporidia *Nosema bombi* is associated with declining bumble bee populations in the US [7,8].

The largest class of honey bee infecting pathogens are positive-sense single stranded RNA viruses including: Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Israeli acute bee paralysis virus (IAPV), Kashmir bee virus (KBV), Deformed wing virus (DWV), Kakugo virus (KV), *Varroa destructor* virus-1 (VDV-1), Sacbrood virus (SBV), Slow bee paralysis virus (SBPV), Cloudy wing virus (CWV), Big Sioux River virus (BSRV), Aphid lethal paralysis virus (strain Brookings) (ALPV), Chronic bee paralysis virus (CBPV) (reviewed in [15,17*,28*]), the

Lake Sinai viruses (LSV) [21], and Bee macula-like virus (BeeMLV) [30]. In addition, one double-stranded DNA virus, *Apis mellifera* filamentous virus (AmFv) has been isolated from honey bees [31]. The majority of bee-infecting viruses were originally discovered and characterized in honey bees, likely since they are the most investigated species. Detection of these viruses in other arthropods indicates that origin of discovery does not necessarily reflect host-range, host–pathogen evolution, or directionality of inter-species transmission (i.e., ABPV, IAPV, DWV, BQCV, SBV, SBPV, LSV and VdMLV) [32,33,34,35,36,37]. Bee viruses are transmitted both vertically and horizontally [38], including between and among co-foraging wild and managed bee populations [32,39,40]. Viruses are also transmitted by *Varroa destructor* mites, which also support replication of a subset of these viruses [41,42,43,44]. Honey bee virus infections may cause deformities, paralysis, death, or remain asymptomatic [15]. The severity of virus infection is influenced by numerous factors that impact bee health, including genetic composition of both host and virus, immune response, synergistic and/or antagonistic pathogenic infections, microbial composition, nutritional status, and agrochemical exposure [15,27,28,45,46,47]. The focus of this review is to highlight recent studies on the abiotic and biotic factors that affect bee virus replication and pathogenicity.

Bee health, nutrition, habitat, and colony management

Bees obtain nutrients from nectar and pollen, and adequate nutrition is important for proper immune system function (reviewed in [48]). Though there have been few quantitative assessments of the relationship between nutritional status and pathogen burden ([49] and reviewed in [47]), several studies suggest that insufficient protein and low-diversity diets negatively impact bees' ability to defend against pathogens [49,50,51]. In laboratory-based studies, naturally DWV-infected honey bees that were fed a protein-free sucrose-syrup diet had significantly higher DWV levels compared to bees fed either pollen or a protein-supplement [50]. Intriguingly, the pollen-fed group had reduced DWV virus load by day four of the trial, whereas the protein supplement fed group exhibited reduced virus load several days later [50]. While an adequate amount of protein is important, a diverse pollen diet, as opposed to monofloral pollen or additional protein, enhanced adult bee immunocompetence (i.e., haemocyte concentration, fat body mass, and phenoloxidase and glucose oxidase activities) [49]. Together these studies suggest that while protein is important, the source of this protein is also critical to proper immune function. Similarly, bees fed honey, which consists of 30–45% fructose, 24–40% glucose, 0.1–4.8% disaccharides including sucrose, and minute amounts of micronutrients and amino acids, exhibited increased expression in more genes involved in detoxification,

immunity, aromatic amino acid metabolism, and oxidation and reduction, as compared to bees fed either sucrose or high fructose corn syrup [51,52]. Together, these studies indicate that proper nutrition (i.e., adequate protein and carbohydrates) and natural and diverse food sources (i.e., nectar and pollen) enhance bee immune function. However, the mechanisms and gene regulatory pathways involved in nutrition-dependent immunocompetence require further characterization. Future studies should employ both cage-studies, which provide a well-controlled environment to investigate individual bee responses and facilitate standardization of multiple variables (e.g., pathogen dose), and colony level studies. A more thorough understanding of the role of diet on bee health is important, as it is common for beekeepers to provide supplemental feed when natural sources are scarce. Overall, these studies indicate that managing landscapes to enhance floral, and therefore nutritional diversity will benefit the health of both managed and wild bee populations.

While floral resources are essential to bee health, flowers also serve as a hub for pathogen transmission and agrochemical exposure [32,33,40]. The most well documented intra- and inter-species transmissible bee pathogens are RNA viruses [32,33,39,53,54,55]. Transmission of these viruses is thought to be associated with bee foraging activities, as BQCV, SBV, and DWV have been detected in honey bee collected pollen [32,40]. In addition, inter-species transmission was demonstrated experimentally in greenhouse studies in which IAPV was transmitted from honey bees to bumble bees and vice versa [32]. Phylogenetic analyses of virus genome sequences (i.e., BQCV, DWV, and IAPV) obtained from foraging honey bees, pollen pellets, and non-*Apis* hymenopteran, including solitary bees, wasps, and bumble bees, did not cluster by host, providing further evidence of inter-species transmission [32]. In addition, IAPV was detected in non-*Apis* hymenopteran species collected from sites near IAPV-infected honey bee colonies, whereas wild hymenopterans obtained from areas proximal to honey bees that were not infected with IAPV were also IAPV-negative [32]. Likewise, recent evaluation of the viruses associated with sympatric honey bee and bumble bee populations in Great Britain and the Isle of Man indicated they were infected with similar strains of DWV and VDV [39], and BQCV, DWV, ABPV, SBPV, and SBV were detected in both honey bees and bumble bees in the same geographic area, though viral prevalence and abundance varied by species [33]. Based on modeling data, it was suggested that the directionality of DWV transmission was from honey bees to bumble bees, since DWV was more prevalent and abundant in honey bees than in bumble bees where ranges overlapped [39]. This relationship was reversed for ABPV and SBV, which were more prevalent in bumble bees than in honey bees where ranges overlapped [33]. Although viruses

are shared between honey bees and bumble bees, there have been very few studies that have investigated the role of viruses on bumble bee health, as most efforts have focused on the role of eukaryotic pathogens on bumble bee health [53^{••},56,57,58,59]. Additional epidemiologic studies are required to better understand pathogen transmission between and within wild and managed bee populations, since the dynamics of transmission will likely vary across different geographies and be influenced by the local abundance of particular bee species, pathogen prevalence, and anthropogenic factors including land use [48,60[•]] and agrochemical exposure [61^{••},62^{••}]. Proximity to urbanization and colony management have been linked to increased pathogen pressure on honey bees [60[•]]. A study of feral and non-commercially managed honey bee colonies across an urbanization gradient determined that feral bees were more immunocompetent (as indicated by approximately two-fold increased expression of four immune genes after challenge) than managed bees, that urbanization positively correlated with greater *Nosema ceranae* and BQCV prevalence, and that management was positively correlated with higher prevalence of both *Nosema apis* and *Nosema ceranae* [60[•]].

Impact of agrochemical exposure on virus replication and pathogenesis

Bee health is influenced by a variety of environmental factors including exposure to agrochemicals. Agrochemicals, including pesticides, herbicides, and fungicides are used widely across a range of landscapes (e.g., agricultural, non-agricultural, wild, managed, and residential), as well as within managed honey bee colonies. Agrochemical exposure sometimes results in acute bee losses, as well as sublethal toxicity, therefore there is much concern regarding the role of pesticides, particularly neonicotinoids, in bee declines (reviewed in [62^{••},63,64]). Although, the latest insecticide formulations may pose less of a threat to bee health as compared to previous formulations [62^{••},64,65]. Compared to other insects, honey bees have a reduced repertoire of genes involved in detoxification [66], and at least one study indicated that bees prefer neonicotinoid-containing food [67[•]]; these studies underscore the importance of further examining the risks associated with agrochemical exposure. Many studies have found that insecticides, including neonicotinoids, negatively impact bee health ([18[•],68,69[•],70,71,72[•],73^{••},74,75,76] and reviewed in [62^{••}]). However, several studies determined that typical field exposure levels are below known toxicity thresholds [77,78,79]; specifically, oral administration of imidacloprid at 5 ppb [77,78] or contact with thiacloprid at doses below 6 µg/bee (approximately 50 ppm) [80] had no observable negative impact.

The majority of studies investigating the effects of agrochemicals on bee health have focused on neonicotinoids (reviewed in [62^{••}]). Several studies suggest that

exposure to these chemicals increases pathogen abundance [18[•],76,81]. Specifically, full sized honey bee colonies exposed to imidacloprid (2 or 20 ppb in pollen patties) had greater levels of *Nosema ceranae* than unexposed colonies [18[•]]. Likewise, exposing bees to imidacloprid and clothianidin topically (0, 10, 20, and 30 ng per bee, which corresponds to approximately 83, 167, and 450 ppm) and orally (0.1, 1.0, and 10 ppb) resulted in a dose-dependent increase of DWV levels [73^{••}]. Similarly, sublethal, though not necessarily field-relevant, doses of thiacloprid (0.1 ppm in larval food) increased BQCV titers and larval mortality [76]. This indicates that agrochemical exposure and viral infection synergistically harm larvae, though negative impacts were not observed in adults [76].

There are numerous other (non-neonicotinoid) agrochemicals that are utilized in both agricultural and non-agricultural settings that have received less attention and scientific investigation [82], though they may impact pathogen abundance and bee health. Chlorpyrifos, an organophosphate, and Pristine[®], a fungicide composed of boscalid and pyraclostrobin used during almond bloom, negatively affected queen health [70]. Chlorpyrifos decreased queen emergence and increased DWV abundance, but not prevalence, in queens relative to the nurse bees tending them [70]. Colonies treated with chlorpyrifos and Pristine[®] had decreased queen emergence, but viral prevalence or abundance was not affected relative to chlorpyrifos alone [47]. In contrast, reduced queen emergence was not found when colonies in isolated swarm boxes were fed pollen treated with Pristine[®] or Pristine[®] with an adjuvant, whereas colonies treated with diflubenzuron, an insect growth regulator, had a significant reduction on queen survival [83].

In addition to agrochemical exposure from foraging (i.e., nectar and pollen) and via food sources (i.e., bee bread and royal jelly), honey bees are also exposed to agrochemicals within the colony (e.g., antibiotics and miticides). Beekeepers routinely utilize the fungicide Fumagillan-B[®] to reduce levels of *Nosema apis* and *Nosema ceranae*, and acaricides (e.g., tau-fluvalinate, thymol, coumaphos, formic acid, and amitraz) to reduce *Varroa destructor* mite infestation [61^{••},84]. While *Varroa* is one of many biotic factors contributing to colony losses [85], high *Varroa* levels, above the threshold of >3 mites per 100 bees [86], are associated with increased pathogen load [17[•],87,88^{••}] (reviewed in [89]). Furthermore, mites serve as a mechanism for pathogen transmission between colonies (reviewed in [90]). Unfortunately, some research suggests that acaricides may reduce honey bee immunocompetence [91]. Bees with compromised immune responses would be expected to harbor greater pathogen loads, though acaricide treated bees exhibited variable levels of pathogens [88^{••},91]. In contrast, acaricide treatment did not affect pathogen loads in colonies with low mite pressure [91]. However, bees obtained from colonies

that were treated with thymol and coumaphos exhibited significantly reduced expression of two immune genes (DSC37 and BASK) [91]. Interestingly, tau-fluvalinate application resulted in increased DWV levels in adult honey bees and *Varroa*, BQCV in mite-infested pupae, and SBV in pupae not infested with mites [88**]. Importantly, tau-fluvalinate treated colonies showed long-term reduction in DWV and DWV-associated symptoms, and thus demonstrated that proper acaricide use is important, and may be effective in controlling DWV levels [88**]. The relationships between mite levels, acaricide exposure, viral abundance, and bee immune gene expression are complex and variable. However, in one study, increased expression of honey bee immune genes (*relish*, *PGRP-S1*, *hymenoptaecin*, *apidaecin*, *defensin*, and *PPOAct*) corresponded to decreased mite reproduction [92**]. In addition, this study found no evidence that *Varroa* negatively impacted honey bee immunocompetence [92**]. The majority of colonies in North America are infested with *Varroa* mites [90] and most managed honey bees in North America are continuously exposed to acaricides in wax [61**]. Therefore, a better understanding of the impact of these stressors on bee health is an important area of ongoing research.

For many agrochemical formulations a lethal dose 50 (LD₅₀) and/or exposure threshold for bees at various stages of development is not known and even less information is available regarding synergistic interactions of these chemicals in bees [62**,93,94]. Regardless, research and public opinion have resulted in bans on the use of clothianidin, thiamethoxam, and imidacloprid in the EU [95] and have resulted in additional US EPA regulations on new registrations for neonicotinoids [96]. The evidence to date suggests that bee colony losses are not solely dependent upon agrochemicals, but are likely a result of a combination of factors. Agrochemicals can pose problems, but they are often required for large-scale production of agricultural crops, and their proper use as part of an integrated pest management strategy (IPM) often results in low to no levels of exposure in field settings [77,78,79]. These studies and increased prophylactic usage of neonicotinoids (i.e., as treated seeds with no other purchase options), underscore the importance of continued research on the effects of agrochemicals on bee health. In addition, loss of forage due to herbicide use in many landscapes impacts the availability of quality forage for all bee species (reviewed in [78]).

Bee microbiome

Bee-associated microbes are not limited to pathogens, but also include commensal microbes ([97,98] and reviewed in [46*]). The best characterized commensal microbes of bees are honey bee gut associated bacteria, including eight bacterial phylotypes predominantly in the *Proteobacteria*, *Firmicutes*, and *Actinobacteria* phyla ([99,100] and reviewed in [46*]). The relationship between the gut microbiome and viruses has been characterized in

mammals (reviewed in [101]) and in solitary insects, including fruit flies and mosquitoes. In fruit flies and mosquito spp., several strains of the bacteria *Wolbachia* reduce RNA virus replication and plasmodium infection [102,103]. *Wolbachia* 16S rRNA sequences have been detected in different subspecies of *Apis mellifera* samples from southern Africa [104] and Germany [105], and in five species of European bumble bees [110], but the potential influence of *Wolbachia* on virus infections in bees has not been studied. Recent findings suggest that *Parasaccharibacter apiumin* may improve larval survival [106], and enhance defense against *Nosema* [107], but the potential effects of this bacteria on virus replication is not known. Bee microbiome research has primarily focused on the benefits of these microbes to bee health, but not all bee-associated bacteria are beneficial; some may be opportunistic pathogens (e.g., *F. perrara*) [108,109], whereas others (i.e., *Paenibacillus larvae* and *Melissococcus plutonius*) are pathogenic. The relationship between the bee bacteriome and virome, as well as the effects of both on bee health require further characterization.

Summary

Bee pollinators inhabit a range of environments including wild, agricultural, and urban landscapes. In these diverse settings, multiple factors including pathogens, nutrient availability, agrochemical exposure, and the microbiome converge to affect bee health. These factors affect bee immunocompetence and virus replication and pathogenicity. Furthermore, land and pollinator management practices impact bee health and may result in increased pathogen pressure on bees. Managing landscapes to enhance floral diversity will benefit the health of both commercial and wild bee populations. Floral resources are not only important to bee health, but also serve as sites of pathogen transmission and agrochemical exposure. Agrochemicals, including those used within honey bee colonies, seem to impact disease severity, though the processes involved require further elucidation. Lastly, the emerging field of insect microbiome research presents exciting avenues of inquiry, including how the bacteriome and virome interact at the host-level. Better understanding of bee biology, the factors that influence bee health, pathogen transmission, and immune mechanisms will result in the development of management practices that support pollinator health.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

The Flenniken Laboratory is supported by the United States Department of Agriculture National Institute of Food and Agriculture, Agriculture and Food Research Initiative (USDA-NIFA-AFRI) Program, Montana Department of Agriculture Specialty Crop Block Grant Program, the National Institutes of Health IDeA Program COBRE grant GM110732, National Science Foundation EPSCoR NSF-IIA-1443108, Hatch Multistate

Funding (NC-1173), Project *Apis m.*, the Montana State Beekeepers Association, Montana State University, and the Montana State University Agricultural Experiment Station. Laura M. Brutscher is supported by the Project *Apis m.*-Costco Honey Bee Biology Fellowship. We would like to thank members of the Flenniken laboratory (Katie Daughenbaugh, Emma Garcia, Jenna Severson, Cayley Faurot-Daniels, and Elisa Boyd) for reviewing this manuscript prior to publication.

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