**Pesticides in Honey Bee Colonies: 4 years of US National Honey Bee Disease Survey reveals real world exposure and associated morbidity risks.**

**Dennis vanEngelsdorp1\*‡, Kirsten S. Traynor1‡, Kathy Baylis5, Karen Rennich1, Eva Forsgren1,2, Robyn Rose3, Guyu Ye5, Grace Kunkel1, Shayne Madella1, , Dawn Lopez4, Jay Evans4, Jeffery Pettis4**

1Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park MD, 20742-4454, USA

2Department of Ecology, Swedish University of Agricultural Sciences, PO Box 7044, SE-75007 Uppsala, Sweden

3USDA Animal and Plant Health Inspection Service, 4700 River Road, Riverdale, MD, 20737, USA

4USDA ARS, Building 306, Beltsville Agricultural Research Center-East, Beltsville, MD, 20705, USA

5Deparment of Agricultural and Consumer Economics, University of Illinois, 1201 W. Gregory Dr., Urbana IL 61801

‡These co-authors contributed equally to this work and should both be considered first author.

\*Correspondence:

Dennis vanEngelsdorp dvane@umd.edu

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**ABSTRACT** The potential impacts of pesticides on honey bee health has been the focus of many recent research efforts. Over 4 years, we surveyed pesticide residues in stored pollen (bee bread), as well as covert and overt disease incidence as part of our US National Honey Bee Disease Survey (NHBDS). More than three-fourths (78.5%) of all samples (n=632) collected had at least one pesticide product detected, with an average of 3 compounds per contaminated sample. Of the 81 different products found, 28 of these co-occurred in samples with other pesticides at greater frequencies than expected by chance. Overall 6% (38 of 632) of the samples exceeded a Hazard Quotient (HQ) of 1,000, equivalent to 10% of a honey bees lethal dose (LD50), while 0.5% (n =3) exceeded a HQ of 10,000, suggesting honey bees would consume their LD50 during their nursing phase. While varroacides predominated (61.2%), fungicides were also fairly common, with at least one fungicide detected in 22% of samples (n = 142), and up to seven different fungicides detected in a single sample. The neonicotinoids were predominantly detected near maize and orange producing land, while fungicide detections were correlated with maize and specialty crop fields. We found fungicide prevalence was linked with increased *Nosema sp.* load, overt chalkbrood and covert black queen cell virus incidence, but a reduced incidence of DWV. Apiaries without *Varroa destructor* typically had residues from multiple varroacides, while samples with high varroa loads (10+ varroa per 100 bees) had significantly higher Hazard Quotient scores. When we compare disease incidence to landscape, controlling for season and region, we find significantly higher prevalence and levels of *Nosema sp.* in apiaries near corn during planting and near oranges, other treefruits and horticultural field crops, all of which are also associated with higher fungicide and neonicotinoid levels. We observe similar landscapes associated with higher incidence of black queen cell virus, iapv and abpv (?). Deformed wing virus was associated with winter wheat during planting, and berry fields. *Varroa destructor* is less clearly related to landscape factors. Both the mean number of pesticides and the mean number of fungicides detected per sample have increased over time, suggesting greater landscape saturation. This research is the most comprehensive assessment of real world pesticide exposures in US managed colonies to date. By linking exposure to colony morbidity measures it highlights areas of concern and should act as a guide for researches, policy makers and extension personnel dedicated to helping beekeepers keep their colonies healthy.

Pesticides play an important role in pollinator decline ([1-4](#_ENREF_1)), strongly effecting reproductive fitness of solitary and social bees. Though healthy honey bee colonies with a large reserve workforce are better able to withstand pesticide pressures ([5](#_ENREF_5), [6](#_ENREF_6)), complex interactions of pesticides with viruses, parasites and poor nutrition ([7-9](#_ENREF_7)) can weaken honey bee immune defenses and have been tied to colony declines ([2](#_ENREF_2), [4](#_ENREF_4), [10](#_ENREF_10)). The picture is further complicated by the toxic mix of pesticide residues entering the hive environment and colony food stream with 7.1 different pesticide residues detected on average in pollen samples ([11-13](#_ENREF_11)). Many of these pesticides have synergistic interactions ([12](#_ENREF_12), [14-17](#_ENREF_14)), making the risk to honey bee health difficult to untangle.

Honey bees provide critical pollination services valued at €153 billion worldwide ([18](#_ENREF_18)) and $17 billion in the US ([19](#_ENREF_19)). Despite increased attention, in the US, colony losses remain elevated since 2006 ([20-24](#_ENREF_20)) with recent increased colony mortality occurring during the summer months when colonies traditionally thrive ([25](#_ENREF_25), [26](#_ENREF_26)). Understanding and quantifying the risks of pesticides entering the hive environment is difficult ([3](#_ENREF_3), [27](#_ENREF_27)). Pesticide risk is currently determined via short-term acute toxicity tests on adult bees, which do not take synergistic, cumulative and sublethal effects into account. Little is known regarding the impacts of multiple pesticide residues, their synergistic interactions and the effects on different life stages of honey bees. Honey bees, like most other insects, rely on detoxification enzymes to metabolize foreign substances, primarily the cytochrome P450s. Decoding of the honey bee genome ([28](#_ENREF_28)) revealed a paucity of encoded P450s compared to other insects ([29](#_ENREF_29)). Honey bees’ reliance on a small number of enzymes to detoxify both natural and synthetic xenobiotics ([30](#_ENREF_30)) increases their risk, as exposure to multiple pesticides may overpower their detoxification system .

Honey bees collect pollen from plants and store it in cells near the brood nest. This stored pollen serves the colony as their sole protein source and is consumed primarily by young adult bees which convert the bee bread into proteinaceous glandular secretions fed to developing larvae ([31](#_ENREF_31)). During the active bee season, an individual worker will consume over 100 mg of pollen, predominantly during a 10-12 day window when bees activate their food producing glands and feed larvae ([32](#_ENREF_32), [33](#_ENREF_33)). Long lived winter bees consume 2 mg of pollen per day for general hive maintenance ([33](#_ENREF_33), [34](#_ENREF_34)) and can live over 120 days, thus consuming 240 mg of stored pollen during their lifespan. In addition to consumption, in-hive bees pack pollen into storage cells, though the exposure resulting from this activity is unknown. As described in the methods section below, the risk to individual workers from consuming contaminated food sources can be estimated using Hazard Quotients (HQ) that identify dose consumed divided by a screening benchmark of no adverse effects ([35](#_ENREF_35)).

Much of the recent honey bee related pesticide research has focused on the potential impacts of neonicotinoids ([36-42](#_ENREF_36)), a newer class of systemic water-soluble pesticides, which are taken up by the plant and translocated throughout all tissues, including the nectar and pollen of treated crops. Neonicotinoids are the most widely used pesticide class worldwide, worth $1.52 billion Euros in 2008 ([43](#_ENREF_43)). Except for the tiny share of organic maize planted, virtually all of the 97.6 million acres of maize seed planted in the US ([44](#_ENREF_44)) are coated with neonicotinoid insecticides as well as a variety of fungicides including Captan, Metalaxyl, Mefenoxam, Fludioxonil, Mefenoxam and the Strobilurins ([45](#_ENREF_45), [46](#_ENREF_46)). The most commonly used neonicotinoids for maize seed are clothianidin and thiamethoxam; the latter is metabolized to clothianidin in the insect ([47](#_ENREF_47)). Honey bees cannot distinguish neonicotinoid laced food sources and preferentially consume neonicotinoid contaminated sugar syrup in the lab ([48](#_ENREF_48)).

Fungicides are another widely used class of pesticides, routinely used on the vast majority of fruit and vegetable crops. As with neonicotinoids, most fungicides are applied as prophylactic treatments (i.e. seed coating or foliar application) or at the first appearance of symptoms. Fungicides are applied at least once per growing season to more than 80% of all agricultural crops, with 108 million pounds applied annually ([49](#_ENREF_49)), a market worth $8.9 billion in 2005 ([50](#_ENREF_50)). Because of their low toxicity to adult pollinators, fungicides, unlike insecticides, are often applied during bloom. The three staple crops of corn, soy and wheat are increasingly treated with fungicides to prevent disease (See supplemental text for details).

The aptly named *Varroa destructor* remains the most damaging pest of honey bee colonies ([51-54](#_ENREF_51)), which beekeepers control via application of a variety of varroacides. Applied directly into the hive, most varroacide products only kill phoretic *Varroa*. Varroa mites have evolved to become resistant to the two most commonly applied varroacides (coumaphos and fluvalinate) ([55-57](#_ENREF_55)), and residues from these lipophilic products continue to persist in the hive matrix even years after their use was discontinued ([13](#_ENREF_13)).

Previous reports on pesticide contaminants entering the honey bee colony matrix did not systematically survey wide geographical regions of the United States, but rather biasedly surveyed colonies that were reportedly suffering ill health ([13](#_ENREF_13)). Here we report on the overall pesticide exposure to bee colonies in their stored protein food source from a subset of colonies surveyed for the National Honey Bee Disease Survey (NHBDS) (Traynor et al, in press). Bee bread samples (n = 632) were collected from apiaries in 34 US States and Puerto Rico between 2011 and 2014 that had also been inspected for overt disease conditions and sampled for *Varroa*, *Nosema*, and viral load. Pesticide residues were used to calculate HQ scores, which summarized the hazard risk from both individual and multiple residues within a sample. These HQ scores were then correlated to several measures of colony morbidity. We then identified the agricultural crops surrounding the geocoded apiaries at the time the sample was taken to detect which landscapes were most strongly correlated with certain pesticide residues.

Materials & Methods:

Pollen was collected and pooled from eight colonies in a single apiary per sample. Briefly a disposable wooden stir stick was used to remove recently stored pollen from at least 4 cells per colony. Removed pollen from all eight colonies was placed in a single 15 ml centrifuge tube ([58](#_ENREF_58)). Samples were frozen after collection until ten apiaries were sampled in the state and then all samples shipped to the USDA Beltsville Bee Lab in Beltsville, MD where they were stored at -80°C. Samples were shipped on ice to the USDA-AMS National Science Laboratory in Gastonia NC for multi-pesticide residue analysis. Samples were extracted and analyzed for 171 pesticides and associated degradates at the ppb level as described in Mullin et al. (2010).

*Hazard Quotient:*

The Hazard Quotient (HQ) for stored pollen in each apiary was calculated as described by Stoner and Eitzer ([59](#_ENREF_59)), and here we expanded it to calculate additive risk from multiple residues. Briefly, the risk of available pollen to a bee was estimated as the sum of all pesticide residue concentrations in ppb divided by their respective LD50 in µg/bee for each residue in a given sample. This approach provides an estimate of the frequency of 50% lethal dose equivalents for bees that are present in the stored pollen. Actual exposure from bee bread depends on individual consumption and contact rates. Residue detections are measured in µg/kg (ppb) divided by an LD50 in µg/bee (see supplemental text and table S1 for details). A bee consumes at least its average body weight in pollen during its lifespan (~100 mg), so the HQ that would result in a 50% kill dose is 1,000,000 mg/100 mg = 10,000—assuming that toxic effects are cumulative, additive and not synergistic or antagonistic.

Another common way to consider risk is to use “a no-adverse effects” threshold. By including a safety factor of 1/10th, as done by the European Food Safety Authority for setting a limit of concern for bee residues ([60](#_ENREF_60" \o "European Food Safety Authority, 2013 #306)) and done by the EPA for setting food pesticide tolerances, then a HQ threshold of 1,000 would correspond with potential for some sublethal effects. A score of 1,000 corresponds to a bee consuming 1% of their LD50 daily, which adds up to 10% of their LD50 during the 10 day nursing phase if the pesticides are not detoxified. To determine risk to pollinators, the US EPA currently uses the more lenient 0.4 x LD50 exposure as its risk quotient (RQ) level of concern ([61](#_ENREF_61" \o "EPA, 2014 #314)) and limits assessments to testing of a single active ingredient consumed as a single dose. Thus only pesticides with a score above 40,000 would equate to the EPA 0.4 RQ score, indicating that bees would be exposed to 40% of their LD50 in a single day. The EPA does not currently use cumulative exposure in their risk assessment, however, if they did, then a nurse bee exposed to pollen with a HQ of 4,000 would consume 0.4 x LD50 in her 10 day nursing phase stage.

*Analyses:* Statistical analysis was conducted using JMP® 11.0.0 (SAS, Cary, NC). Comparisons across years, months, and interactions were analyzed with multifactorial ANOVA for HQ scores and pesticide residues and if significant, differences among groups were identified with Student T-tests. Mean results are reported with S.E. indicated. State, month and year were discarded as factors unless specifically indicated in the analysis. Relative Risk calculations were calculated after ([62](#_ENREF_62)).

We spatially join the USDA Animal and Plant Health Inspection Services (APHIS) Survey of Honey Bee Pests and Disease with NASS Cropscape data using the geographic coordinates of the apiaries and year the sample was taken. Not all NHBDS samples (ref Traynor et al.) were sampled for pollen residue; only 676 samples have pollen sample results. The NASS Cropscape Data Layer (CDL) is a raster land-cover layer with geo-referenced and crop-specific information using satellite images for continental U.S. at a resolution of 30 meters squared per pixel (USDA NASS n.d.). Since there is no crop information for samples in Hawaii, Guam and Puerto Rico, we exclude these areas from our analysis. We then map the sampled non-migratory apiaries onto cropscape data to determine landscapes and crops grown within a 2-mile radius of each apiary, as this is the vicinity in which bees typically do most of their foraging (Eckert, 1933). We then compared the percentage of the two mile buffer area occupied by each landscape for apiaries testing positive versus negative for fungicides, herbicides, insecticides, and neonicotinoids.

We then use a multivariate regression to estimate which landscapes are associated with higher loads and incendence of Nosema sp., Varroa destructor and multiple bee viruses. Here we use the broader sample of 1877 geocoded apiary observations from APHIS, not only those tested for residue. We consider both the cropped area in the 2-mile radius of the apiary as well as whether the sample was collected during the planting of the crop to account for the potential higher exposure to seed treatments during planting, particularly by air seeders (REF). Specifically, we considered the landscape use in the month prior to sample collection based on average state planting dates dates from USDA (REF). These dates are only available for major field crops, but these crops comprise the vast amount of agricultural acreage near apiaries and are more likely planted using air seeders. In this analysis, we control for season, USDA region and year of the sample. We also control for the minimum average temperature and average precipitation at the location and during the month of the sample, and whether the bees are used specifically for crop pollination.

Results:

Of the 632 bee bread samples analyzed, up to 13 different pesticide residues were detected in a sample with a mean of 2.37 ± 0.10 pesticides per sample (Fig. 1). No pesticides were detected in 21.5% of the samples (n = 136). When these are excluded from the analysis, the mean number of residues detected per sample is 3.02 ± 0.11.

Across all samples analyzed, the number of pesticide residues detected varied by year (F3,628 = 2.72, p = 0.044), with 2014 having significantly higher mean pesticide detections than 2013 and 2011-2012 not different from either group. When the pesticide free samples are excluded, then 2014 has higher mean pesticide residues compared to all other years (Fig. 2, F3,492 = 2.96, p = 0.032). The number of residues detected also varied by month, peaking in March with 5.86 ± 0.5 per sample (Fig 3). Mean pesticide residues decreased in late summer, rising slightly again in November and December. This late fall peak was due predominantly to increased varroacide applications, which were significantly higher than July-September (F10,621= 6.69, p < 0.0001). Throughout the sample set 1,497 pesticide detections were made, representing 81 of the 175 different active ingredients and their metabolites analyzed (Table 1), while 94 were never detected (Table S1). Beekeeper applied varroacides were the most common, followed by insecticides and fungicides (Fig. S1), while neonicotinoids were rarely detected.

Four of the five most prevalent residues detected were from beekeeper applied *Varroa*  treatments: fluvalinate (45.3%), coumaphos (34.46%), the breakdown product from amitraz 2,4 Dimethylphenyl formamide, abbreviated as DMPF (18.0%), and thymol (15.8%). Only the insecticide chlorpyrifos was detected more frequently than thymol, found in 16.3% of samples (See Table 1).

*Hazard Quotients:*

To better understand the potential risk of pesticide residues , we calculated hazard quotients (HQ) for individual pesticide residues. A score above 10,000 indicates that honey bees will consume more than their LD50 during their 10 day nursing phase. Only the very toxic insecticide prallethrin exceeded this threshold in 3 of 11 positive samples (Table 1). A HQ score above 1,000 indicates that honey bees are at risk of suffering sublethal effects. Overall 35 samples (5.5%) had individual pesticides that contributed more than 1,000 points to the HQ; these included the insecticides: bifenthrin, carbaryl, chlorpyrifos, cyfluthrin, fenpropathrin, the aforementioned prallethrin and permethrin, the neonicotinoids: clothianidin, imidacloprid and thiamethoxam, and the varroacide coumaphos (Table 1).

Since we analyzed the individual pesticide residues in bee bread samples, we also calculated the HQ from the multiple residues detected in our single aggregate apiary level sample by summing the risk from all detected residues. This calculation assumes that HQ risks are additive and not synergistic or antagonistic. Overall 6.0% of samples (n = 38) exceeded the 1,000 pt safety threshold, and only 0.47% (n = 3) exceeded 10,000 pts (Table 1). These high risk samples all originated in Oregon and were contaminated with prallethrin (209-800 ppb). Due to these high risk samples, Oregon had a mean HQ (for all years sampled) of 5,670.0 ± 466.8, higher than all other states (Fig. 4, F34,597 = 4.64, p < 0.0001), followed by Nebraska with an HQ of 1,617.5 ± 466.8 due primarily to neonicotinoids, then FL, MA, WV, GA, OH, LA and MT with a mean range of 390.6 to 831.0 ± up to 466.8.

*Varroacides:*

The most commonly detected class of pesticide was varroacides, with at least one varroacide detected in 65.7% of samples (n = 415). When detected, varroacides contributed a mean of 20.2 points to the HQ, exhibiting a HQ range from 0.04 to 1,189.7, with 2 samples contributing 500+ points due to coumaphos detected at 6,260 ppb and DMPF from Amitraz detected at 12,700 ppb. Thymol is considered non-toxic to bees (LD50 = 975); as such it contributed little to most HQ scores although it was detected at more than 1,000 ppb in 31 samples (Table 1) and at over 10,000 pbb in 7 samples.

*Insecticides:*

Insecticides were the next most common pesticide class, with at least one insecticide detected in 35.3% of samples (n = 223), adding a mean HQ score of 703.5 with a range from 0.02 to 29,629.6. Bifenthrin, Carbaryl, Chlorpyrifos, Cyfluthrin, Fenpropathrin, Permethrin, and Prallethrin each contributed more than 1,000 points to the HQ in at least one sample (Table 1).

*Neonicotinoids:*

In spite of the widespread use of the neonicotinoid class of insecticide, only 5.2% of samples (n = 33) were contaminated with detectable levels (for limits of detection (LOD) see Table 1 and S1) of neonicotinoid pesticide residues. These samples came predominantly from Nebraska in the corn belt, and specialty crop states (LA, FL, NJ and NY). The majority of positive samples (n = 26) had one neonicotinoid residue, while all the remaining neonicotinoid contaminated samples had two different neonicotinoid residues. The neonicotinoids found in samples included acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam (Table 1). Clothianidin and imidacloprid contributed more than 1,000 points to the HQ in at least one sample (Table 1). Of the clothianidin contaminated samples, 60% (n = 6) were from Nebraska. Five of those samples also contained thiamethoxam. Thus of the 10 pollen samples analyzed from Nebraska, only 40% were free of neonicotinoids, while 50% had two different neonicotinoid residues, resulting in a mean of 1.1 neonicotinoids detected per sample, higher than any other state (Fig. 5, F35,596=7.05, p < 0.0001). For the imidacloprid contaminated samples, 42.9% came from Florida, while 21.4% came from California and New Jersey. Florida had the next highest neonicotinoid mean of 0.375 detections per sample, significantly higher than all other surveyed states except Louisiana.

*Fungicides:*

Fungicides were also very common, detected in 22.5% of samples (n = 142). In all samples, mean fungicide detection was 0.39 (95% CI = 0.32-0.47). When the fungicide free samples were excluded, the mean fungicide residues detected per sample was 1.74 (95% CI = 1.52-1.96). The number of fungicides detected varied by year (Fig. 6, F3,628 = 8.23, p < 0.0001), increasing from a mean of 0.23 in 2011 to a mean of 1.02 in 2014 across all samples. Multiple fungicides were frequently detected in a single sample; the maximum number of fungicides detected increased annually from four fungicides in 2011 to seven fungicides in 2014. Altogether 12.0% (n = 17) of the fungicide positive samples contained four or more fungicides, with the majority (n = 11) collected from colonies in California. Fungicide residues varied by month (F10,621 = 8.27, p < 0.0001), peaking in March (mean = 1.57 ± 0.20) and April (mean = 1.19 ± 0.16), when the number of fungicides detected per sample were significantly higher than all other sampling months. Fungicides are considered relatively safe for adult bees and thus have high LD50s, contributing few points to the HQ score. The HQ contributed by fungicides varied by year, peaking in 2014 (Fig 6, top).

Despite being low contributors to the HQ score compared to other pesticide classes, elevated fungicide HQ scores have been linked to declines in wild bees ([63](#_ENREF_63)), bumble bees ([6](#_ENREF_6)) and imminent colony mortality of honey bees (see migratory paper), especially if they contribute more than five points to the HQ. In this survey, 16% of fungicide positive samples (n = 23) contributed at least five points to the HQ, including Azoxystrobin (1), Boscalid (3), Captan (1), Cabendazim (1), Chlorothalonil (5), Cyprodinil (1), Fenbuconazole (2), Fenhexamid (1), Pyraclostrobin (2), THPI (4) and Triticonazole (2). Even though they exhibit low HQ scores, the volume of fungicide residues in ppb for some commonly applied fungicides is high. Of the 21 different fungicides found in our samples, eight were detected at above 1,000 ppb in at least one sample (Table 1). THPI detected at 7,060 ppb was the highest fungicide residue detected, exceeding all other residues except the beekeeper applied varroacides thymol detected at 55,000 ppb in one sample and the amitraz residual DMPF detected at 12,700 ppb in a different sample.

*Herbicides:*

Herbicide detections were low compared to other pesticide classes, making up 6.9% of all pesticide detections. No herbicide contributed more than 1,000 points to the HQ; only Metolachlor was detected above 1,000 ppb in 2 of 6 samples testing positive for the product (Table 1).

*Morbitidy and residue level associations:*

Apiary inspectors who collected the bee bread samples inspected colonies for overt symptoms of common honey bee diseases. Only one disease, the fungal disease Chalkbrood (*Ascosphaera apis*), was found at prevelance levels high enough to conduct meanginful comparsions between symptomatic and non-symptomatic disease (Traynor *et al* in press). Fungicide HQ was elevated in samples collected from apairies with at least one colony positive for chalkbrood (HQFung = 1.76 ± 0.6) compared to samples taken from apiaires with no evidence of infection (HQFung = 0.56 ± 0.21; F1,466 = 3.86, p = 0.050).

The majority of bee bread samples analyzed for pesticide residues also had corresponding samples of adult honey bees taken to measure *Varroa* infestation per 100 bees and *Nosema* prevalence and load (n = 625). Stored pollen collected from apiaries with undetectable *Varroa* containedon average 1.71 ± 0.15 different varroacides, higher than all other groups (Fig. 7, top row). While the mean points contributed to the HQ by these varroacides was below 40 for all infestation levels, varroacides contributed significantly more points to colonies with zero varroa than in colonies with detectable levels of varroa infestation (Fig. 7, bottom row).

We compared the total HQ contributed from all pesticide residues detected in bee bread across different *Varroa* infestation levels and found it elevated in apiaries that had 10-25 varroa per 100 bees, where the mean HQ (898.2 ± 172.6), was 3x higher than all other groups (Fig. 8, top row). The main contributor to this elevated HQ score in the colonies with mite infestation levels of 10-25 mites per 100 bees were insecticides, contributing on average 95% of the HQ points to the samples total HQ score (F4,613=3.81, p = 0.005). However, these elevated HQ levels were not due to increased numbers of different pesticides. In fact apiaries with lower *Varroa* levels had greater pesticide residue diversity in their bee bread (Fig. 8, bottom row). This pattern of higher residue diversity in samples taken from apiaries with zero mite loads, was consistent across many pesticide classes including fungicides (F4,613 = 4.42, p = 0.0016), herbicides (F4,613 = 8.83, p < 0.0001), and varroacides (F4,613 = 7.99, p < 0.0001), but not insecticides (F4,613 = 1.76, p = 0.1359), including neonicotinoids (F4,613 = 1.86, p = 0.1165), which remained below 1 product detected per sample for insecticides and 0.25 for neonicotinoids across all varroa infestation groupings (Fig. 9).

The HQ score from all pesticides did not differ between sample groups by their *Nosema* spore load, nor did it differ for any of the individual pesticide classes. However, the mean number of different pesticide residues detected differed between samples collected from apiaries grouped by different Nosema threshold levels; apiaries with bees free of *Nosema* had 30% fewer pesticide residues compared to apiaries with bees with detectable Nosemainfections. A similar pattern was seen with fungicides, while varroacide residues had a similar but non-significant trend (p < 0.10). Neonicotinoids, in contrast, did not vary across *Nosema* levels (Fig. 10).

*Geospatial Analysis:*

The top ten land cover types located within 2 miles of apiaries for which we sampled pollen are listed in Table X and illustrated in figures 1-4. For all four classes of pesticide, we find that those apiaries without detected residues are surrounded by more forest, grassland and shrubland (in the surrounding 2 mile (3.2 km) diameter). Conversely, we find that apiaries with detected residues are surrounded by a larger amount of field crops (corn, soybeans and spring wheat in particular) and by four to seven times more area of specialty crops. While the acres of specialty crops may be small, covering only 4% of the nearby landscape, more than half of the apiaries sampled are within 2 miles of some specialty crops (322 of 602). Over 80 percent of the sampled apiaries were within 2 miles of corn, and more than 2/3rds of the apiaries were near soybean, winter wheat, alfalfa and hay.

Apiaries where no fungicides were detected had a greater proportion of their surrounding landscape covered in natural areas. Specifically, they had higher amount of nearby forest, grassland and shrubland when compared to apiaries in which fungicides were detected (25% vs 20% for forested uplands, p=0.03, 17% versus 13.5% for grassland, p=0.04 and 5.6 versus 1% for shrubland, p<0.01). Conversely, apiaries in which fungicides were detected had a greater proportion of their surrounding landscape covered with corn, soybean and spring wheat when compared to fungicide free apiaries (10% versus 8% for corn, p=0.08, 5.6% versus 8% for soybeans, p=0.01, and 1.2% versus 0.6% for spring wheat, p=0.07). While comparatively small in overall acreage, we find that apiaries with fungicides are on average surrounded by almost six times as much area of specialty crops than those apiaries where fungicide residues are not detected. Of the top 30 specialty crops, 14 have significantly more average area around contaminated apiaries (p<0.1 in all cases), and only 6 specialty crops have more area around uncontaminated apiaries, although none of these differences are statistically different from zero (for more details, please see SI). Specifically, we find that several tree crops including almonds, walnuts, pecans, apples, plums and cherries have significantly more area near apiaries where fungicides are found (p<0.01).

We repeat this same analysis for insecticides, and again find that apiaries where no insecticides are found tend to be near a larger area of forested upland and shrubland than the apiaries where insecticide residue was detected (p-stats of < 0.01 and 0.09 respectively). Conversely, apiaries where insecticides are found are near more soybean acres (p=0.08). They are also near four times as many specialty crop acres than their uncontaminated counterparts. Specifically, we observe higher average acres of almonds, citrus, apples and fruit such as watermelon, cranberries and grapes. In total, 13 of the 30 largest specialty crops have statistically significantly more area around apiaries where insecticides are found (p<0.1).

We next compare apiaries where neonicotinoids residue was present to those where no neonicotinoid residue was detected. Apiaries where neonicotinoid residues were detected were surrounded by less forested uplands (24% to 12%, p<0.01), and more corn acres (12.3% versus 8.4%, p=0.06) compared to apiaries where no neonicotinoids were detected. Apiaries where neonicotinoids were found were near five times as many specialty crop acres. In particular, neonicotinoid detections occurred more commonly in apiaries located near orange groves as compared to apiaries not near orange groves (5% compared to 0.06%, p<0.01). Neonicotinoids are often used against the Asian citrus psyllid which spreads the bacterial disease associated with citrus greening. Neonicotinoids were also associated with higher apple and cranberry acreages.

Last, we consider herbicide residues, and find that those apiaries with herbicide residues are near more forest, grassland and shrubland, and near less corn and soybeans (p-stat of <0.01for forest, 0.06 for grassland, 0.04 for shrubland, <0.01 for corn and 0.03 for soybeans). Apiaries where herbicide residue was detected are near almost seven times more specialty crop area, and 6 of the top 30 specialty crops are significantly larger near contaminated apiaries (almonds, walnuts, pecans, plums, tomatoes and greens).

***Cropscape and disease prevalence***

We next run multivariate regressions to explore what cropscapes are associated with disease prevalence. For *Nosema sp*., we find that a ten percent increase of nearby hay acres in a 2-mile radius of the apiary increases the amount of *Nosema* by about 0.6 percent, and increases the probability of the colony having *Nosema sp.* by three percent. Corn overall slightly decrease the amount of *Nosema sp.*, during those months when corn is being planted, a ten percent increase in nearby corn acres are associated with a one percent increase in *Nosema sp.* and a four percent probability of having the disease. The amount of specialty crop acreage is highly significantly associated with an increase in the probability of *Nosema sp.* an increase in the amount of *Nosema sp.* detected, with a ten percent increase in citrus associated with a nearly six percent increase in the probability of *Nosema sp.* and 1.3% increase in the amount of *Nosema sp.*. Similarly, a ten percent larger treefruit and horticulture area nearby were associated with a half a percent increase in the amount of *Nosema sp.* and a two percent increase in disease incidence (for horticulture). Last, a ten percent increase in acres of berries (a combination of strawberries, cranberries, blueberries and caneberries) is associated with an four percent increase in incidence. Given that the timing and location of sample collection was not random, we control for USDA region, season and year.

Varroa destructor was less clearly associated with landscape. A ten percent increase in almond acres was associated with a two percent increase in the number of mites and a ten percent increase in developed land was associated with a four percent increase in the number of mites (p<0.1 for almonds and p<0.01 for developed land).

We also compared the incidence of viruses to surrounding landscape and found that Acute Bee Paralysis and Israeli Acute Bee Paralysis Virus followed similar patterns as Nosema. The incidence of both viruses was found to be higher near corn acres during planting, with a 10 percent increase in acres generating an 11 and 16 percent increase in the probability of the two diseases. Israeli Acute Bee Paralysis is also more likely near larger acres of winter wheat during planting (a ten percent increase in acres associated with a 13 percent increase in incidence) and near a larger acres of oranges (a ten percent increase in acres associated with a 11 percent increase in incidence). Black queen cell virus was associated with larger soybean acres during planting (a ten percent increase in acres associated with a 15 percent increase in incidence) and acres of berries (strawberries, caneberries, blueberries and cranberries) and horticulture (a ten percent increase in acres associated with a 6 percent and 4 percent increase in incidence, respectively).

Deformed Wing Virus incidence was associated with hay acres, winter wheat during planting and berries (a ten percent increase in acres associated with a 5 percent, 8 percent and ten percent increase in incidence respectively). The incidence of Chalkbrood was associated with treefruit (ten percent increase in acres associated with an 11 percent increase in incidence while the Kashmiri Bee Virus was associated with soybean acres during planting, alfalfa acres during planting, treefuit and developed land (a ten percent increase in acres associated with a 35, 15 10 and 53 percent increase in incidence).

***Pesticide residues and viral prevalence:***

A relative risk analysis was conducted to highlight relationships between the presence of an individual class of pesticides and the prevalence of viral diseases in adult bees collected from those sampled colonies (Table 2). This was then further refined by examining the relative risk of individual pesticides with viral diseases (Table 3).The presence of any pesticide in bee bread was found to reduce the likelihood of Chronic bee paralysis virus (CBPV) prevalence (RR = 0.68, 95% CI = 0.5-0.91) in the bees collected from the same apiary by about 30%. This trend was probably driven by the fact that most pesticide detections in bee bread samples were varroacides, and the presence of varroacides in bee bread was related to 50% reduction in CBPV prevalence, with the presence of two varroacides Amitraz (DMPF) and Fluvalinate associated with an 84% and 45% reduction in CBPV respectively. While only trending towards significance, CBPV was found at lower rates in apiaries that contained thymol (RR = 0.15 or an 85% reduction). These reduced rates of disease may be the result of product effect on viral replication or host immune response; however, a more probable explanation is that bees with viruses were more likely to die in the presence of varroacides, and so reduced the virus prevalence load in the colony ([64](#_ENREF_64)).

Black queen cell virus (BQCV) was found 2.36 (95% CI = 1.00-5.62) times more often in apiaries with detectable levels of all fungicides as a group, but was not significantly elevated or reduced by any fungicide specifically. The presence of the varroacide Fenyproximate was associated with a 69% reduction in BQCV incidence (RR = 0.31, 95% CI = 0.12-0.82).

Israeli acute paralysis virus (IAPV) was found more often in samples that were collected in apiaries whose bee bread contained varroacides generally (RR =1.21, 95% CI = 1.02-1.43) and fluvalinate specifically (RR = 1.31. 95% CI = 1.04-1.65). The fungicide Captan also increased the risk of IAPV presence by a factor of 7.73 (95% CI = 1.10-56.90).

Lake Sinai Virus 2 (LSV-2) was also seen at higher levels in apiaries testing positive for varroacides generally (RR = 1.36, 95% CI = 1.07-1.72) and Amitraz (RR = 2.38, 95% CI = 1.23-4.60), Coumaphous (RR= 1.68, 95% CI = 1.07-2.62) and Fluvalinate (RR = 1.37, 95% CI =1.03-1.83) specifically. While the detection of non-varrocide insecticides was related to a decreased incidence of LSV-2 generally (RR = 0.66, 95% CI = 0.43-1.00), the presence of the organophosphate Methamidophos was found to increase the prevalence of LSV-2 by a factor of 6.32 (95% CI = 1.44-27.6). The fungicide Cyprodinil was also found associated with an increase in LSV-2 prevalence (RR = 3.64, 95% CI = 1.10-12.06).

The prevalence of Deformed wing virus (DWV) was consistently reduced by the presence of different products, including the varroacide Amitraz breakdown product DMPF (RR = 0.58, 95% CI = 0.38-0.88) and Fluvalinate (RR = 0.74, 95% CI = 0.61-0.92); the fungicides Boscalid (RR = 0.26), Cyprodinil (RR = 0.33, 95% CI = 0.13-0.81), Pyroclostrobin (RR = 0.27, 95% CI = 0.11-0.63) and Pyrimethanil (RR = 0.11 , 95% CI = 0.032-0.38); and the herbicides Oxyfluorfen (RR = 0.16, 95% CI = 0.03-0.81) and Pendimethalin (RR = 0.39, 95% CI =0.18-0.88). Excluding the relationship with varroacides, which is possibly explained by the effect these pesticides have on the viruses’ vector *Varroa*, the consistent finding of reduced prevalence of the nearly ubiquitous DWV, with the presence of certain fungicides and herbicides suggests these products may be preventing DWV replication or transmission, or cause DWV infected bees to die more quickly, thus lowering viral load by preferentially removing infected individuals

While Kashmiri bee virus (KBV) is associated with *Varroa*, unlike DWV this virus was found to have increased prevalence in bees sampled from apiaries in which Coumaphos (RR = 1.44, 95% CI = 1.06-1.94) and Fluvalinate (RR = 1.38, 95% CI = 1.11-1.74) was detected. The virus was also found at markedly higher rates in apiaries that had detectable exposures to the fungicide Captan (RR = 8.43, 95% CI = 1.20-58.80) and the herbicide Tebuthiuron (RR = 25.31, 95% CI = 2.67-239.57).

Acute bee paralysis virus (ABPV) was found in apiaries with Fluvalinate exposure at lower rates than expected by chance (RR = 0.73, 95% CI = 0.56-0.96), while this virus was 6.33 times more likely to be present in apiaries that had exposure to the organophosphate insecticide Phosmet.

***Pesticide combinations with potential synergistic interactions:***

Previous research has shown synergistic enhancement of the toxicity of pyrethroids, when combined with EBI fungicides ([16](#_ENREF_16)). Specifically the pyrethroid cyhalothrin exhibits increased synergy when combined with an EBI fungicide ([65](#_ENREF_65)), a combination found in 22.6% of the samples contaminated with cyhalothrin. All together pyrethroids were found with an EBI fungicide (DMI: SBI Class I) in 14 of our samples: Bifenthrin (3x), Cyfluthrin (2x), Cyhalothrin (8x), and Esfenvalerate (1x).

EBI fungicides obtain their fungicidal activity by disrupting ergosterol biosynthesis via cytochrome P450 inhibition and can inhibit insect P450s used for detoxification of insecticides. Spray tank mixtures frequently include both classes of pesticides, so concurrent detection of insecticides and fungicides in a single sample was common (see Table 6). The fungicide Cyprodinil was detected in 25 different pollen samples; more than half (n = 13) also contained the insecticide Chlorpyrifos. The fungicide Chlorothalonil was also frequently detected with the insecticide Chlorpyrifos (41.2%, n = 7 of 17), as was the fungicide Carbendazim (40.0%, n = 8 of 20).

We examined all pesticide residues that appeared in at least 10 samples for prevalent combinations. The two most commonly co-detected pesticide residues were the varroacides coumaphos and fluvalinate, appearing together in 136 samples. Many pesticides do not appear as frequently in the samples, yet when they do, they are more likely to co-occur with another pesticide (Table S2). The most prevalent combination detected was the neonicotinoid Clothianidin, which always appeared with the herbicide Atrazine (n = 10); both are frequently applied to maize. Of the 10 detections of the broad spectrum fungicide pyrimethanil (Tradename Scala®), nine also contained the fungicide Boscalid, eight contained the fungicide Cyprodinil, and seven contained the fungicide Pyraclostrobin. Pyrimethanil positive samples were only detected in 2013 & 2014 sampling years with a max of 7 and a mean of 4.7 different fungicide residues, highlighting the high prevalence of fungicide combinations. Samples contaminated with the fungicide Tebuconazole (n = 14) contained the insecticide Chlorpyrifos 71.4% of the time, significantly higher than expected.

Beekeeper applied varroacides appeared together more frequently than can be expected by chance: fenpyroximate, DMPF, and coumaphos respectively appeared with fluvalinate, 80.7%, 66.1% and 63.6% of the time. Excluding beekeeper applied varroacides and the family of Endosulfans, the next most prevalent co-occurring pesticide residues were the fungicide Boscalid with the fungicide Pyraclostrobin, detected together 62.5%, a fungicide combination found in the BASF product Pristine. Boscalid (tradename Endura®) with the fungicide Cyprodinil (tradename Vanguard®) were detected together 54.2%, two fungicides commonly applied to almond, grapes and strawberries. Although the fungicide fludioxonil was only detected in two different samples, it always appeared with cyprodinil, a fungicide combination sold under the tradename Switch®. The neonicotinoid Imidacloprid frequently co-occurred with the insecticides Chlorpyrifos (35.7%), Bifenthrin (28.6%), Cyhalothrin (28.6%), Fenpropathrin (28.6%), and the fungicide Azoxystrobin (28.6%). When prevalent combinations are examined by class (Table S2), insecticides including IGR and neonicotinoids frequently co-occurred with other insecticides (n = 34, prevalence range = 6.8-81.8%); fungicide combinations with other fungicides were frequent (n = 30, prevalence range = 10.7-90.0%); the same held for varroacides (n = 18, prevalence range = 10.0-80.7%); herbicides rarely appear together (n = 3, prevalence range = 10.3-20.7%).

Discussion:

We investigated pesticide contamination of the honey bee colony food stream by examining contaminants of the stored pollen, a colony’s only source of protein. An individual colony consumes approximately 13-18 kg of pollen annually ([32](#_ENREF_32)) and pollen has been reported as contaminated with the greatest numbers of different pesticide residues in the hive ([13](#_ENREF_13), [66](#_ENREF_66)). This national survey of pesticide residues in stored pollen, also known as bee bread, highlights that “normal” apiaries have an average of 2.37 pesticide residues (Fig. 1), a striking contrast to the 7.1 residues detected in bee bread in Mullin et al ([13](#_ENREF_13)) which biasedly sampled overtly sick colonies. The maximum number of pesticides we detected in a single pooled sample is 13 different pesticides, about one-third as many compared to the previous report of 31 from sick and failing colonies ([13](#_ENREF_13)). The majority (51%) of the pesticides we detected were beekeeper applied varroacides, not surprisingly as varroacides are applied directly into the hive, often multiple times per year. Many (coumaphos, fluvalinate, fenproxyimate and thymol) are long-term treatments that remain in the hive for one to six weeks, leaching into lipophilic wax, from where the varroacides can then migrate into the bee bread. US pesticide contamination peaks in the spring; similar to the peaks reported in Italy ([67](#_ENREF_67)) and France ([68](#_ENREF_68)).

Much attention has been given to the impact of the most widely used pesticide class—the systemic neonicotinoids—on honey bee health ([3](#_ENREF_3), [7](#_ENREF_7), [36](#_ENREF_36), [38](#_ENREF_38), [40](#_ENREF_40), [42](#_ENREF_42), [69-71](#_ENREF_69)). These water-soluble systemic pesticides worth $2.62 billion US ([43](#_ENREF_43)) were relatively rare in bee bread, appearing in 5.2% of samples. Our results are in line with previous studies that found limited neonicotinoids in the hive matrix (Migratory paper, ([13](#_ENREF_13), [68](#_ENREF_68)). Imidacloprid, the most commonly applied neonicotinoid with 5,450 tons estimated to have been sold worldwide in 2008 ([72](#_ENREF_72)) appeared in 2.2% of our bee bread samples, similar to the 0.8% detection rate reported in Lambert et al. (2013) from hive entrance trapped pollen in Western France, yet significantly lower than the 12.1% detection rate found in trapped pollen collected in Connecticut ([27](#_ENREF_27)). Notably, neonicotonoid residue is associated with those apiaries near larger average corn and orange acres, two crops with high percent of neonicotinoid use. Further, neonicotinoid residue is generally found at the time of field crop planting. Analyzed with a LOD of 1 ppb, it is remarkable that such a widely used pesticide which translocates into the nectar and pollen of flowering plants ([73](#_ENREF_73)) does not appear more frequently in the stored bee bread if honey bees are collecting neonicotinoid contaminated pollen ([74](#_ENREF_74)). Perhaps pollen storage and the conversion into partially fermented bee bread reduce the neonicotinoid load found in trapped pollen, an avenue of research worth further study. Highly toxic neonicotinoids may induce sublethal effects on honey bee health at the maximum (clothianidin, imidacloprid, and thiacloprid) and mean (clothianidin) concentrations detected (Table 1). Samples from Nebraska (NE), in the heart of the US corn belt, had significantly more neonicotinoid residues than any other state. In these samples thiamethoxam frequently co-occured with clothianidin; the former can easily be cleaved to the latter after application on the plant or when ingested by insects ([47](#_ENREF_47)), making it difficult to determine if the NE corn fields were pretreated with thiamethoxam, some of which was then cleaved to clothianidin, or if different fields within foraging distance of the hives had been treated with the individual neonicotinoids. These two neonicotinoids only co-occurred in NE, though thiamethoxam was detected in 6 samples without clothianidin in other states.

Fungicide residues were frequently detected, with an increase in fungicide HQ and diversity per pooled sample over time (Fig. 6). Fungicide residue was associated with higher nearby areas of corn and soybeans, as compared to larger areas of forested uplands near those apiaries where no fungicide residue was detected. Considered relatively harmless due to their low acute toxicity to adult honey bees, multiple fungicides were detected at more than 1,000 ppb (1 ppm), including chlorothalonil (4.9 ppm), cyprodinil (2.8 ppm), fenbucanazole (3.47 ppm), pyraclstrobin (1.07 ppm), and THPI (7.06 ppm). Only varroacides (Thymol = 55.8 ppm, DMPF = 12.7 ppm) were detected at higher concentrations than THPI. While occasional high contamination rates from in-hive applied varroacides are foreseeable, such high rates of fungicide contamination in the colony’s food stream are disconcerting. High fungicide residues exceeding in-hive applied varroacides were reported in a European study, with Boscalid, Captan, Iprodione detected at a maximum of 1.3, 1.5 and 1.9 ppm respectively ([75](#_ENREF_75)). The association between high fungicide residues and the fungal disease chalkbrood detected suggests that fungicide contamination from agricultural fields promotes the causative fungus Ascosphaera apis inside the hive. Similar co-occurrence of fungicides and chalkbrood incidences were reported in Yoder et al. (2013). A common symptom of stress, the increased incidence of chalkbrood may be indicative of nutritional stress caused by a disruption of the beneficial fungi and bacteria that co-inhabit the hive matrix ([76-79](#_ENREF_76)). Several other studies have raised concerns over the impacts of fungicides on pollinator health (Migratory paper, ([6](#_ENREF_6), [14](#_ENREF_14), [63](#_ENREF_63), [65](#_ENREF_65), [75](#_ENREF_75), [77](#_ENREF_77), [80-85](#_ENREF_80)), indicating a much needed reevaluation of fungicides as “bee safe”.

Numerous insecticides contributed more than 1,000 points to HQ scores (Table 1), indicating that honey bees ingest more than 10% of their LD50 from this contaminated protein source during their 10 day nursing phase, when honey bees consume up to 12 mg of stored bee bread per day ([32](#_ENREF_32)). These insecticides included bifenthrin, carbaryl, chlorpyrifos, cyfluthrin, fenpropathrin, permethrin, and prallethrin. Three of ten samples from Oregon were contaminated with high concentrations of highly toxic prallethrin (Concentration = 209-800 ppb; LD50 = 0.027), an insecticide used to kill wasps and approved for aerial spraying of mosquitos ([86](#_ENREF_86)). An additional sample from Ohio (132 ppb) and two from West Virginia (92-321 ppb) had high concentrations of prallethrin. Mosquito transmitted West Nile Virus continues to be an issue in all three of these states, indicating that colonies may be picking up prallethrin contaminated water or subject to spray drift. Insectides were more likely to be detected in apiaries within 2 miles of

Tank mixtures of insecticides with various fungicides are common, reducing application costs and enhancing pest control. Pyrethroid toxicity is synergistically enhanced when combined with EBI fungicides, as both are detoxified by the cytochrome P-450 monooxygenase system ([16](#_ENREF_16), [65](#_ENREF_65)). Specifically the pyrethroid cyhalthrin was found in combination with an EBI (DMI: SBI Class I) fungicide eight times, in 19% of all the samples contaminated with cyhalthrin (n = 42). Pyrethroids, detected 123 times, were found together with this synergistic type of fungicides 11.4% of the time (n = 14), thus potential synergistic interactions should not be dismissed. Many pesticide combinations occur at much greater frequency than expected by chance (Tables 1 and S2) and these warrant further research to determine potential synergistic interactions and their impact on honey bee health.

While pesticide contamination of bee bread was not as dire as previously reported, the high rate and toxicity of some contaminants detected still warrant concern. Bees entomb pollen, especially when contaminated with high levels of fungicides ([87](#_ENREF_87)). When reared under pollen-deprived conditions, bees are smaller, forage earlier, die younger, and communicate food resources poorly, even when food becomes abundant ([79](#_ENREF_79)). Pesticides, especially fungicides may negatively impact the nutritional profile of bee bread, resulting in pollen stressed bees despite the presence of stored pollen.

Apiaries with high *Varroa* loads had significantly higher HQ scores, due predominantly to insecticide contamination, suggesting that pesticides play an important yet poorly understood role in the escalation of this parasite. It may be that colonies with high insecticide loads lose a large percentage of their worker force, concentrating the population of mites in the colony on the remaining bees.

Overall, while only 6% of all samples exceeded our 1,000 point HQ threshold, the trend toward greater pesticide contamination and increased pesticide residues is of concern. The HQ model described only accounts for additive effects and can’t estimate the risk from potential synergistic interactions. As such, it may be conservative estimate of actual risks to honey bee health. This survey had some inherent limitations due to funding constraints, with samples often collected in a single state as a cluster. The pesticide analysis of samples cost over US $220,000, which doesn’t include costs for kit preparation, collecting, shipping, and storage. Ideally in a national survey the same apiaries would be sampled at multiple times throughout the year in all states, but such a study would require a budget of $3 million to measure pesticide exposure and its impacts on honey bee colony health over time. Despite the inherent limitations of the current study, we find an association between high pesticide exposure and increased disease (chalkbrood and viral infection) and elevated parasite pressure (varroa and nosema), confirming that multiple stress factors interact to reduce colony health. Increased focus on the potential synergistic interactions of frequently detected pesticide combinations and pesticide/parasite/disease interactions are urgently needed. In light of the current results, pesticide regulations that focus predominantly on the acute toxicity of a single active ingredient may not encompass true risks to pollinator health.

References

1. Anonymous (2015) Entomology: The bee-all and end-all. *Nature* 521(7552):S57-S59.

2. Goulson D, Nicholls E, Botias C, & Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347(6229):1435-+.

3. Sanchez-Bayo F & Goka K (2014) Pesticide Residues and Bees – A Risk Assessment. *PLoS ONE* 9(4):e94482.

4. Doublet V, Labarussias M, de Miranda JR, Moritz RFA, & Paxton RJ (2015) Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environmental Microbiology* 17(4):969-983.

5. Rundlof M*, et al.* (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* 521(7550):77-80.

6. Bernauer OM, Gaines-Day HR, & Steffan SA (2015) Colonies of Bumble Bees (Bombus impatiens) Produce Fewer Workers, Less Bee Biomass, and Have Smaller Mother Queens Following Fungicide Exposure. *Insects* 6:478-488.

7. Alaux C*, et al.* (2010) Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera). *Environmental Microbiology* 12(3):774-782.

8. vanEngelsdorp D, Tarpy DR, Lengerich EJ, & Pettis JS (2013) Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. *Preventive Veterinary Medicine* 108(2–3):225-233.

9. Francis RM, Nielsen SL, & Kryger P (2013) Varroa-Virus Interaction in Collapsing Honey Bee Colonies. *PLoS ONE* 8(3).

10. Becher MA, Osborne JL, Thorbek P, Kennedy PJ, & Grimm V (2013) REVIEW: Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models. *Journal of Applied Ecology* 50(4):868-880.

11. Bogdanov S (2006) Contaminants of bee products. *Apidologie* 37(1):1-18.

12. Johnson RM, Ellis MD, Mullin CA, & Frazier M (2010) Pesticides and honey bee toxicity - USA. *Apidologie* 41(3):312-331.

13. Mullin CA*, et al.* (2010) High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. *PLoS ONE* 5(3).

14. Johnson RM, Dahlgren L, Siegfried BD, & Ellis MD (2013) Acaricide, Fungicide and Drug Interactions in Honey Bees (Apis mellifera). *PLoS ONE* 8(1).

15. Thompson H & Wilkins S (2003) Assessment of the synergy and repellency of pyrethroid/fungicide mixtures. *Bulletin of Insectology* 56(1):131-134.

16. Schmuck R, Stadler T, & Schmidt HW (2003) Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (Apis mellifera L, Hymenoptera). *Pest Management Science* 59(3):279-286.

17. Vidau C*, et al.* (2011) Exposure to Sublethal Doses of Fipronil and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by Nosema ceranae. *PLoS ONE* 6(6).

18. Gallai N, Salles J-M, Settele J, & Vaissiere BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* 68(3):810-821.

19. Calderone NW (2012) Insect Pollinated Crops, Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992–2009. *PLoS ONE* 7(5):e37235.

20. Vanengelsdorp D, Underwood R, Caron D, & Hayes J (2007) An estimate of managed colony losses in the winter of 2006-2007: A report commissioned by the apiary inspectors of America. *American Bee Journal* 147(7):599-603.

21. vanEngelsdorp D, Hayes J, Underwood RM, & Pettis JS (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. *Journal of Apicultural Research* 49(1):7-14.

22. vanEngelsdorp D, Hayes J, Underwood RM, Caron D, & Pettis J (2011) A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. *Journal of Apicultural Research* 50(1):1-10.

23. vanEngelsdorp D*, et al.* (2012) A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership. *J. Apic. Res.* 51(1):115-124.

24. Spleen AM*, et al.* (2013) A national survey of managed honey bee 2011-12 winter colony losses in the United States: results from the Bee Informed Partnership. *J. Apic. Res.* 52(2).

25. Steinhauer NA*, et al.* (2014) A national survey of managed honey bee 2012-2013 annual colony losses in the USA: results from the Bee Informed Partnership. *J. Apic. Res.* 53(1):1-18.

26. Lee K*, et al.* (2015) A national survey of managed honey bee 2013–2014 annual colony losses in the USA. *Apidologie* 46(3):292-305.

27. Stoner KA & Eitzer BD (2013) Using a Hazard Quotient to Evaluate Pesticide Residues Detected in Pollen Trapped from Honey Bees (<italic>Apis mellifera</italic>) in Connecticut. *PLoS One* 8(10):e77550.

28. Weinstock GM*, et al.* (2006) Insights into social insects from the genome of the honeybee Apis mellifera. *Nature* 443(7114):931-949.

29. Claudianos C*, et al.* (2006) A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. *Insect Molecular Biology* 15(5):615-636.

30. Johnson RM*, et al.* (2012) Ecologically Appropriate Xenobiotics Induce Cytochrome P450s in Apis mellifera. *PLoS ONE* 7(2).

31. Crailsheim K (1992) The flow of jelly within a honeybee colony. *Journal of Comparative Physiology B* 162(8):681-689.

32. Crailsheim K*, et al.* (1992) Pollen consumption and utilization in worker honeybees (Apis mellifera carnica) - dependence of individual age and function. *Journal of Insect Physiology* 38(6):409-419.

33. Anonymous (2012) White Paper in Support of the Proposed Risk Assessment Process for Bees. (Office of Chemical Safety and Pollution Prevention, Washington, DC), p 275.

34. Crailsheim K*, et al.* (1993) Pollen utilization in non-breeding honeybees in winter. *Journal of Insect Physiology* 39(5):369-373.

35. EPA U (*Guidelines for Ecological Risk Assessment* (Agency EP).

36. Iwasa T, Motoyama N, Ambrose JT, & Roe RM (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera. *Crop Protection* 23(5):371-378.

37. Whitehorn PR, O’Connor S, Wackers FL, & Goulson D (2012) Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. *Science* 336(6079):351-352.

38. van der Sluijs JP*, et al.* (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. *Current Opinion in Environmental Sustainability* 5(3-4):293-305.

39. Sanchez-Bayo F (2014) The trouble with neonicotinoids. *Science* 346(6211):806-807.

40. Fairbrother A, Purdy J, Anderson T, & Fell R (2014) Risks of neonicotinoid insecticides to honeybees. *Environmental Toxicology and Chemistry* 33(4):719-731.

41. Simon-Delso N*, et al.* (2015) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research* 22(1):5-34.

42. Pisa LW*, et al.* (2015) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental Science and Pollution Research* 22(1):68-102.

43. Jeschke P, Nauen R, Schindler M, & Elbert A (2011) Overview of the Status and Global Strategy for Neonicotinoids. *Journal of agricultural and food chemistry* 59(7):2897-2908.

44. USDA-NASS (2013) U.S. Corn acreage up for the fifth straight year. (USDA National Agricultural Statistics Survey).

45. Lauer J (2005) Corn Seed Survival: The importance of seed fungicides and insecticides. *Wisconsin Crop Manager.* 12(11):75-76.

46. Solorzano CD & Malvick DK (2011) Effects of fungicide seed treatments on germination, population, and yield of maize grown from seed infected with fungal pathogens. *Field Crops Research* 122(3):173-178.

47. Nauen R, Ebbinghaus-Kintscher U, Salgado VL, & Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pesticide Biochemistry and Physiology* 76(2):55-69.

48. Kessler SC*, et al.* (2015) Bees prefer foods containing neonicotinoid pesticides. *Nature* 521(7550):74-76.

49. Gianessi L & Reigner N (2006) The importance of fungicides in US crop production. *Outlooks on Pest Management* 17(5):209-213.

50. Morton V & Staub T (2008) A Short History of Fungicides.

51. Bowen-Walker PL, Martin SJ, & Gunn A (1999) The transmission of deformed wing virus between honeybees (Apis mellifera L.) by the ectoparasitic mite Varroa jacobsoni Oud. *Journal of Invertebrate Pathology* 73(1):101-106.

52. Le Conte Y, Ellis M, & Ritter W (2010) Varroa mites and honey bee health: can Varroa explain part of the colony losses? *Apidologie* 41(3):353-363.

53. Mondet F, de Miranda JR, Kretzschmar A, Le Conte Y, & Mercer AR (2014) On the Front Line: Quantitative Virus Dynamics in Honeybee (Apis mellifera L.) Colonies along a New Expansion Front of the Parasite Varroa destructor. *Plos Pathogens* 10(8).

54. Oldroyd BP (2007) What's killing American honey Bees? *Plos Biology* 5(6):1195-1199.

55. Milani N (1995) THE RESISTANCE OF VARROA-JACOBSONI OUD TO PYRETHROIDS - A LABORATORY ASSAY. *Apidologie* 26(5):415-429.

56. Elzen PJ*, et al.* (1998) Fluvalinate resistance in Varroa jacobsoni from several geographic locations. *American Bee Journal* 138(9):674-676.

57. Milani N (1999) The resistance of Varroa jacobsoni Oud. to acaricides. *Apidologie* 30(2-3):229-234.

58. APHIS (*Sampling Pollen for Pesticide Residue*, (Service AaPHI).

59. Stoner KA & Eitzer BD (2013) Using a Hazard Quotient to Evaluate Pesticide Residues Detected in Pollen Trapped from Honey Bees (*Apis mellifera*) in Connecticut. *PLoS ONE* 8(10):e77550.

60. European Food Safety Authority (2013) *EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees).* (EFSA Journal ), (Authority EFS).

61. EPA (2014) Guidance for Assessing Pesticide Risks to Bees. (Environmental Protection Agency, Washington, D.C. 20460), p 59.

62. vanEngelsdorp D*, et al.* (2013) Standard epidemiological methods to understand and improve Apis mellifera health. *J. Apic. Res.* 52(4).

63. Park MG, Blitzer EJ, Gibbs J, Losey JE, & Danforth BN (2015) Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proc. R. Soc. B* 282(1809).

64. Martin SJ (2001) The role of Varroa and viral pathogens in the collapse of honeybee colonies: a modelling approach. *Journal of Applied Ecology* 38(5):1082-1093.

65. Pilling ED & Jepson PC (1993) Synergism between between EBl Fungicides and a Pyrethroid Insecticide in the Honeybee (*Apis mellifera*). *Pesticide Science* 39(4):293-297.

66. Chauzat M-P*, et al.* (2011) An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera Apidae) to monitor pesticide presence in continental France. *Environmental Toxicology and Chemistry* 30(1):103-111.

67. Ghini S*, et al.* (2004) Occurrence and distribution of pesticides in the province of Bologna, Italy, using honeybees as bioindicators. *Archives of Environmental Contamination and Toxicology* 47(4):479-488.

68. Lambert O*, et al.* (2013) Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of Western France. *PLoS ONE* 8(6):e67007.

69. Cresswell JE*, et al.* (2012) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). *Zoology* 115(6):365-371.

70. Matsumoto T (2013) Reduction in homing flights in the honey bee Apis mellifera after a sublethal dose of neonicotinoid insecticides. *Bulletin of Insectology* 66(1):1-9.

71. Pohorecka K*, et al.* (2013) EFFECTS OF EXPOSURE OF HONEY BEE COLONIES TO NEONICOTINOID SEED-TREATED MAIZE CROPS. *Journal of Apicultural Science* 57(2):199-208.

72. Pollack P (2011) *Fine Chemicals: The Industry and the Business.* (Wiley-Interscience, Hoboken, N.J.).

73. Bonmatin JM*, et al.* (2005) Quantification of imidacloprid uptake in maize crops. *Journal of agricultural and food chemistry* 53(13):5336-5341.

74. Chauzat MP*, et al.* (2006) Pesticides, pollen and honey bees

Les pesticides, le pollen et les abeilles. *Phytoma* (594):40-45.

75. Simon-Delso N*, et al.* (2014) Honeybee Colony Disorder in Crop Areas: The Role of Pesticides and Viruses. *PLoS ONE* 9(7).

76. Anderson KE*, et al.* (2013) Microbial Ecology of the Hive and Pollination Landscape: Bacterial Associates from Floral Nectar, the Alimentary Tract and Stored Food of Honey Bees (*Apis mellifera*). *PLoS ONE* 8(12):e83125.

77. Yoder JA*, et al.* (2013) Fungicide contamination reduces beneficial fungi in bee bread based on an area-wide field study in honey bee, Apis mellifera, colonies. *Journal of toxicology and environmental health. Part A* 76(10):587-600.

78. Saraiva MA*, et al.* (2015) Relationship between honeybee nutrition and their microbial communities. *Antonie van Leeuwenhoek*.

79. Scofield HN & Mattila HR (2015) Honey Bee Workers That Are Pollen Stressed as Larvae Become Poor Foragers and Waggle Dancers as Adults. *PLoS ONE* 10(4):e0121731.

80. Vandame R & Belzunces LP (1998) Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation. *Neuroscience Letters* 251(1):57-60.

81. Mussen EC, Lopez JE, & Peng CYS (2004) Effects of selected fungicides on growth and development of larval honey bees, Apis mellifera L. (Hymenoptera : Apidae). *Environmental Entomology* 33(5):1151-1154.

82. Ladurner E, Bosch J, Kemp WP, & Maini S (2005) Assessing delayed and acute toxicity of five formulated fungicides to Osmia lignaria Say and Apis mellifera. *Apidologie* 36(3):449-460.

83. Johnson RM & Percel EG (2013) Effect of a Fungicide and Spray Adjuvant on Queen-Rearing Success in Honey Bees (Hymenoptera: Apidae). *Journal of Economic Entomology* 106(5):1952-1957.

84. Elston C, Thompson HM, & Walters KFA (2013) Sub-lethal effects of thiamethoxam, a neonicotinoid pesticide, and propiconazole, a DMI fungicide, on colony initiation in bumblebee (Bombus terrestris) micro-colonies. *Apidologie* 44(5):563-574.

85. Pettis JS*, et al.* (2013) Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae. *PLoS ONE* 8(7).

86. EPA U (2003) *Prallethrin: Human Health Risk Assessment for the Public Health Use of Mosquito Adulticides Containing Prallethrin*, (United States Environmental Protection Agency HED).

87. vanEngelsdorp D*, et al.* (2009) "Entombed Pollen": A new condition in honey bee colonies associated with increased risk of colony mortality. *Journal of Invertebrate Pathology* 101(2):147-149.