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| Environmental Factors of Honeybee Health |
| Do NEONICOTINOIDS MATTER? |

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**Abstract**

Since 2006, beekeepers around the world have reported unusually high rates of honeybee colony loss. In the last two years, attention has turned to neonicotinoids as a potential factor in honeybee disease and mortality. While fatal at high doses, neonicotinoids are primarily used as seed treatments, and researchers debate whether and how honeybees are exposed to these pesticides in regular conditions in the field at sufficient doses to do harm. We combine unique geocoded data on pollen samples across 40 US states over the past 4 years with crop data to ask where and when we observe evidence of neonicotinoid exposure in the hive, and what effect that exposure has on honey bee health. We find that neonicotinoids are largely found in hives near neonicotinoid-treated crops during planting, and that colonies with neonicotinoid contamination have higher levels of nosema, a virus associated with colony loss. We find no evidence of an effect of neonicotninoids on mites.

**Introduction**

Honey bees are valuable to global agriculture. A third of all food is dependent on pollinators. In the United States in particular, this pollination service adds approximately $15 billion in crop value every year (USDA 2013). In addition, harvested honey produced by operations with 5 or more colonies was worth approximately $287 million in 2012 (USDA NASS 2013), not including other byproducts including beeswax and bee pollen. But honey bees are under treat. Beekeepers have lost around 30 % of their colonies each winter since 2006/07. (National Honey Bee Health Stakeholders Conference 2012). . Even though the winter losses from 2013 to 2014 are among one of the lowest in eight years, two-thirds (66%) of beekeepers still exceeded their acceptable loss rate of 19% (Lee, et al. 2014). Added to these winter losses, beekeepers are now reporting record summer losses of 40% for last year (REF). Recent policy attention has turned to neonicotinoids as a potential source of colony decline. In this paper we ask whether we the presence of neonicotinoid-treated crops increases the probability of neonicotinoids found in the hive, and whether those contaminated colonies have higher levels of pests known to contribute to colony loss.

While the causes of the decline are still not completely understood, parasites such as Varroa mites (Guzman-Novoa, et al. 2010) and Nosema (Higes, et al. 2008) are known contributors to colony loss. Varroa destructor, a virus-transmitting parasite that was introduced to the United States in the mid-1980s, has been identified as the most detrimental pest for honey bees. Nosema is a gut parasite that harms bee colonies (USDA 2013). Other factors of honey bee loss include colony management practices, and poor nutrition, (Lee, et al.2014). Honey bee habitat is also decreasing with the expansion of modern agriculture and clearing of natural areas. These trends result in diminished food availability and nutritional diversity for both wild and managed bees. This trend is troubling because research has found that honey bee colonies near greater areas of open land sustain fewer colonies losses and produced more honey compared with colonies located near a greater portion of developed land (Naug and Dhruba 2009). Another study found that plant diversity from natural areas is essential for maintaining large enough bee populations to pollinate cultivated crop (Kremen, Williams and Thorp 2002). Research on Britain and the Netherlands has also found a link between decreases in the plants that bees pollinate and decreases in the bee population (Biesmeijer, et al. 2006).

Recently, attention has turned to pesticides as a possible contributor to colony decline. In particular, a class of nicotine-derived pesticides, neonicotinoids (neonics), including Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Thiacloprid and Thiamethoxam, have been implicated as a cause of bee deaths. Neonics were introduced in the late 1990s to replace the more toxic mass spraying of organophosphate and pyrethoid pesticides (Entine 2013). Most neonicotinoid pesticides protect plants from insects and are water-soluble and slowly break down in the environment (Hunt, 2012). In the United States, neonicotinoids are currently used on about 95 percent of corn and canola crops, the majority of cotton, sorghum and sugar beets, about half of all soybeans, and a vast majority of fruit, vegetable and grain crops (Grossman, 2013). In particular, the use of Clothianidin on corn in Iowa alone has almost doubled between 2011 and 2013 (USGS 2014).With the popular adoption of neonicotinoid seed treatments in current farming practices, there is a growing concern that neonics’ potential negative impact on bees might harm the world’s food production and supply. Therefore, the European Union had declared a 2-year ban on three neonicotinoids (Clothianidin, Imidacloprid and Thiametoxam) in 2013 as a precautionary action (European Commission 2013). While similar calls for a ban exist in the United States, the U.S., however, chose to continue the use of neonicotinoids due to a lack of proof of their harmful effects.

Unlike previous work that uses lab and field experiments to explore the relation between neonicotinoid seed treatments and honeybee health, we use a large number of samples collected from 40 U.S. states over 4 years to first ask whether we observe evidence of neonicotinoid exposure in a real world crop setting and ask which nearby crops are correlated with evidence of this exposure. In addition, we ask whether neonicotinoid contamination in the hive is correlated with higher disease loads.

**Literature Review**

With the EU recently banning the use of some neonicotinoids due to their suspected effect on honeybee heath, calls are increasing in the United States for a similar regulatory response. However, scientific experiments under lab conditions have suggested conflicting results regarding whether and how honeybees are exposed to the pesticide in regular field conditions.

Some researchers suggest that under typical crop conditions, bees are not exposed to high enough doses of neonicotinoids to cause health concerns. According to research conducted by Dr. Gus Lorenzo from University of Arkansas, neonicotinoids are not expressed in the reproductive part of corn, soy, or cotton plants in high enough levels to harm honey bee health (Lorenzo 2014). In fact, no neonicotinoids were detected in cotton and soy flowers. He therefore concludes that neonicotinoid seed treatments are not harmful to bees in terms of exposure to contaminated nectar and pollen. Using 2 groups of 8 honeybee colonies, Faucon et al. demonstrate that bees’ chronic exposure during the spring and summer to crops treated by neonicotinoids at the highest recommended rate does not affect the mortality of overwinterized colonies (Faucon et al. 2005, Cutler and Scott-Dupree 2007). The United Kingdom Department of Environment, Food and Rural Affairs compiled evidence on neonicotinoid exposure to honey bees and concluded that neonicotinoids do not harm bees under normal circumstances and that laboratory studies on the sub-lethal level of neonicotinoids created extreme situations that are not applicable to real world conditions (United Kingdom Department of Environment, Food and Rural Affairs 2013).

In contrast, many researchers and beekeepers argue that bees are exposed to neonicotinoids and that neonicotinoids have a negative impact on honeybee health. Using the liquid chromatography-tandem mass spectrometry (LC/MS-MS) analysis, Dr. Krupke has found that bees’ exposure to neonicotinoid compounds happen in several ways throughout the foraging period, especially during the planting season of treated maize (Krupke, 2012). Dr. Greg Hunt finds an extremely high concentration of Clothiandin and Thiamethoxam in talc, which is a seed treater that helps with seed flow during planting with an air seeder and improves seed spacing. A gram of talc containing 1.0% Clothianidin could theoretically kill a million bees if they ingest it, and could threaten about half as many bees if they come into contact with the dust (Laurino et al. 2011; Tremolada et al.2010). He thus concludes that bees may be exposed to a sub-lethal level of pesticides throughout the growing season even though the greatest danger occurs during planting. The popular adoption of neonicotinoid seed treatments which are persistent in plants makes it very difficult for bees to avoid exposure to these toxic chemicals. A controversial study by Dr. Chensheng Lu from Harvard University suggests that even sub-lethal exposure to neonicotinoids would impair honey bee winterization and thus lead to colony loss (Lu, et al. 2014). Even though Lu claimed that he had replicated CCD, there was not any support from prominent entomologists. Instead, several entomologists have argued that his sample size was too small to reach a conclusion and that he might have killed the bees himself by overdosing them in a cold winter (Entine 2014, Helman 2013).

Recent articles have also addressed concerns over the potential negative impact of neonicotinoids on bees. Paul Towers, from the Pesticide Action Network said that even though the amount of pesticide in the pollen of neonic-treated plants might be too small to kill bees, it was enough to disorient and reduce the ability for them to get food and communicate (Charles, 2013). Even though Bayer CropScience, the biggest seller for neonicotinoid pesticides claimed that neonics have been proven safe by most studies, they are taking precautionary action to work on a new system for planting corn that will reduce neonic release (Charles, 2013). Studies have also shown that the negative effect of neonicotinoids are not limited to bees; they harm birds, mammals, worms and aquatic insects as well (Thomson, 2014). [Add the new articles somewhere]

**Data and Methods**

For this study, we merge the USDA Animal and Plant Health Inspection Services (APHIS) Survey of Honey Bee Pests and Disease with NASS Cropscape data by geographic coordinates and year. We specifically focus on those colonies that are not migratory to ensure that the nearby cropscape appropriately represents the landscape for the time the sample was taken (Holt 2014). We consider apiaries as migratory if beekeepers list their operations as migratory or pollination and non-migratory otherwise. However, if the type of operation is listed as both stationary and pollination, we consider this apiary as non-migratory as well.

The USDA APHIS conducts the Honey Bee Pest and Disease Survey as a means of identifying pests, pathogens, and disease affecting honey bees in the United States. This data set contains information on apiary samples collected from 2009 to 2014 throughout the United States. Forty states with 2552 samples are in the data set. In each sampled apiary, at least 8 colonies are tested for a number of diseases and pests. Not all samples are tested for pollen residue; only 676 samples have pollen sample results. Since there is no crop information for samples in Hawaii, we exclude these areas from our analysis as well. Along with excluding migratory colonies, this leaves us with a sample of …..

We use Varroa mites and Nosema parasite loads as indicators of bee health and explore what environmental factors contribute to a higher prevalence of these diseases. The loads of both morbidity factors are continuous and log normally distributed, thus we include their natural log forms as variables of interest. Using geocoded data, we plot the raster density maps for Varroa mites and Nosema parasites for non-migratory apiaries with pollen results and find a correlation between the detection of neonics and higher levels of diseases (see Appendix… the map I made for GIS class). This suggests that bees’ exposure to neonics may potentially contribute to higher disease levels.

To estimate the degree of neonicotinoid exposure, we first identify the crops are traditionally seed treated with neonicotinoids, including corn, soy, cotton, canola, sorghum, barley, rice and wheat. Then we map the sampled non-migratory apiaries in APHIS onto NASS cropscape data determine the crops grown within a 2-mile radius of each apiary. The resolution of these data is set at 30 meters squared per pixel (USDA NASS n.d.). We extract the crop area within two miles of each apiary as this is vicinity in which bees typically do most of their foraging (Eckert, 1933). Honey bees will travel further if necessary but will conserve energy by foraging locally when possible (Eckert 1933). Therefore, this two mile area, which comprises over 8,000 acres, provides the best estimate of the crops and landscape that bees would interact with during their foraging. We then calculate the percentage of the two mile buffer area occupied by each crop with the assumption that a linear relationship exists between changes in treated crop area and morbidity loads.

With geographic coordinates for non-migratory apiaries, we also extract potential forage and weather data within the 2-mile buffers from Vegscape and Oregon State’s Prism database. USDA NASS provides data about on the vegetation cover of the United States over the period from 2000- 2014. For this study, we use data on the Normalized Difference Vegetation Index (NDVI), which measures the density of vegetation within pixels representing 15 acres of landscape (Mueller and Minchenkov 2013). Data on the NDVI is provided on a daily, weekly and biweekly basis. We use apiaries’ locations as well as sample collection time, and obtained the biweekly NDVI data, which provides information about the average NDVI within a 16 day window.

Both weather and water availability are important determinants of honey bee health as well. Cold weather is commonly associated with increased stress on bees because the bees will not venture out of the hive if temperatures are below 8 degrees Celsius, reducing their food intake (British Columbia Ministry of Agriculture 2012). We extract minimum average temperature as well as the total precipitation in the month the sample was collected. The data provided by Prism are supplied by calendar month, so for all sample taken on or before the 14th of the month, we use the weather data for the previous calendar month. For samples taken on the 15th or later, we use the month of the collection.

To control for the timing of exposure to pesticides and more abundant nectar sources, we collect information on the time of planting and blooming for neonics-treated crops. NASS collects agricultural plant timing data for select crops in some states. In this data set, we have information on the planting percentage by month for the United States each year. Corn, soy, cotton, canola, rice, sorghum, barley and spring wheat are planted in the spring. Winter wheat is planted in the fall. Most spring planting occurs between April and June. Fall planting occurs between September and November. Due to a lack of information, we estimate the planting window for canola is from April 20 to June 10 every year (Canola Council of Canada, 2013). Information on bloom timing of honeybee forage plants is provided on HoneyBeeNet, which not only lists the plants that bees frequently forage within each region within each state, but also whether each plant is significant nectar source or not (Nickeson, 2010).

Many of the honey bee forage crops fall into the natural area landscape category, so we consider the entire area to be in bloom if at least one of the forage crops is in bloom within the natural area category. Bloom timing for neonic-treated crops are also included in the data set to estimate pesticide exposure from pollen. Spring wheat, winter wheat, barley and rice are not considered as forage crops for honey bees and thus are missing bloom timing information from HoneyBeeNet. These crops are wind-pollinated are not adapted to attract pollinators. However, honey bees can consume pollen from these crops (Burlew 2013).

To control for region fixed effects, we use USDA census regions. These regions are selected to increase comparability with studies on overwintering losses and to isolate regionally cropping patterns. [Will add some summary stats on crops in each region later]

For our analysis, we first use a logit regression to ask what factors are associated with finding neonicotinoid contamination in the hive. Second, we use a multivariate regression to estimate the effect of neonicotinoid contamination on nosema and varroa loads. We use several specifications, first with no fixed effects, then with fixed effects for region and year. Then we include an increasing number of controls, such as forage availability and weather. In the first stage, we compare those apiaries that are near neonicotinoid-treated crops whose samples are taken during planting, to other apiaries near neonicotinoid-treated crops whose samples are taken other times of year, and to apiaries who are not near neonicotinoid-treated crops. For the second analysis, using the fixed effects, we compare disease outcomes of those apiaries were neonics are found to apiaries tested in the same region, in the same year, during the same time of year.

**Results**

First Stage – Factors associated with neonicotinoid contamination

The first stage examines whether an apiary with a large share of neonic-treated crops within the foraging radius would have higher probability to being contaminated by neonics during certain times of the year. In other words, we ask which time of the year do we observe neonicotinoid exposure in the apiary, and does this timing align with planting or blooming time of neonicotinoid-treated crops. We aggregated the percent area of all 9 commonly neonicotinoid-treated crops and interacted these numbers with planting and bloom time. Planting time and bloom time are both dummy variables indicating whether any treated crops within the 2-mile radius are being planted or in bloom on the date of the sample collection.

All the specifications below show strong evidence that the share of treated crops nearby during planting time positively contributes to the likelihood of apiaries to be contaminated by neonicotinoids. When we control for year fixed effects and region fixed effects, the coefficients on the interaction between neonicotinoid-treated crops during planting time increases, and the model fit improves. When we controlled for bloom time, we observe that, if anything, shares of treated crop nearby during bloom time are associated with a decrease in the probability that apiaries are contaminated by neonicodinoids. NDVI, an indicator of nearby vegetation and thus natural forace, is negatively correlated with the likelihood of observing neonicotninoids in the apiary, albeit this effect is not statistically significant. Precipitation and minimum temperature are slightly positively correlated with the probability of contamination, but the results are not significant either.



Second Stage – Disease Levels

In the second stage, we examine whether being contaminated by neonicotinoids is associated with an increase in disease loads. We run two sets of specifications for the commonly identified diseases: Nosema and Varroa. For Nosema, we first run a simple regression with binary variable indicating the contamination status of apiaries and month quadratic time trend to capture the fact that nosema is often highest earlier in the year. Then, we include year and region fixed effects and other control variables. According to the results, when foraging and weather are controlled for, an apiary that is contaminated by neonics tends to have about 0.41 million spores per bee higher in Nosema loads than one that is not contaminated. Since Nosema is one of the indicators for bee health, this result suggests that apiaries contaminated by neonics tend to have higher morbidity rates.

We ran the same regressions with mites loads as the outcome variable. When year and region fixed effects are controlled for, results suggest an insignificantly negative correlation between neonic contamination and mites loads.





**Conclusions**

Many regions are considering taking severe measures to reduce the use of neonicotinoids because of their hypothesized negative effect on honey bees. The scientific evidence behind this presumed associated is mixed. One debate in the literature is whether honey bees are exposed to neonics, which are primarily used as a seed treatment, in a regular agricultural setting. Two possible mechanisms of exposure are through the talc used along with the seed treatment to facilitate planting by air seeders, and from neonic-treated crop nectar. Second, there is a debate about whether these potentially low-levels of neonic-exposure are sufficient to affect honey bee health. Most work to date has focused on lab based or small field trials. To our knowledge, ours is the first paper to use a geographically-diverse set of data collected from commercial apiaries to ask whether we find evidence of the effect of neonicotinoid-treated crops in a real-world setting.

Using pesticide load and health data for 358 geocoded apiaries across 40 states, we first ask whether neonicotinoid contamination is associated with proximity to neonicotinoid-treated crops during planting or bloom time. Second, we ask whether those apiaries where neonicotinoids are found have higher levels of nosema or varroa, where both pests are strongly associated with colony loss. We find that apiaries sampled during the time that nearby neonicotinoid-treated crops are being planted are more likely to be contaminated by neonics, implying that even in real-world settings, honey bees may be exposed to neonics. Second, we find that those apiaries with neonicotinoid residue have higher levels of nosema, but not significantly different levels of varroa mites. Our work complements earlier smaller scale field studies that show a relation between low-level of neonicotinoid exposure and an increase in the level of nosema.

Because we use observational data, we cannot rule out all other factors that may affect both neonicotinoid contamination and disease. For example, most neonicotinoid-treated crops are planted using air seeders, so perhaps the dust generated from planting decreases honey bee health, and not neonics per se. Further, we cannot rule out that colonies located near neonicotinoid-treated crops that are tested during planting are different in some unobservable way than other colonies. Thus, our results should be treated as suggestive evidence, not necessarily proving a causal relation.

**References**

**Appendix**