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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. 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. In this paper, we first explore whether being located near neonicotinoid treated crops would increase honeybee morbidity, and then examine if other environmental factors including forage availability and weather would affect bee health. [Finding]

**Introduction**

Honey bees are valuable assets for the United States and the world. A third of all food is depended 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 managed honey bee operations with 5 or more colonies was worth approximately $287 million in 2012 (USDA NASS 2013), and other byproducts including beeswax and bee pollen have greatly improved people’s everyday life. However, the availability of managed honey bees has fallen and is under threat from colony loss. The Report on the National Honey Bee Health Stakeholders Conference in 2012 shows that the supply of bees has declined from about 6 million colonies in 1947 to 4 million in 1970, to 2.5 million in 2012. In particular, since 2006, when unexplained losses of honey bee colonies began to be reported as Colony Collapse Disorder (CCD), beekeepers have lost 30-90% of their honey bees every winter (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).

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 to honey bees. Nosema is a gut parasite that harms bee colonies (USDA 2013). Other factors including colony management practices, poor nutrition, pests and some socio-economic factors are also potential contributors to bee loss (Lee, et al.2014). The expansion of modern agriculture has led to more mono-cropping and clearing of natural areas. These trends result in diminished food availability and nutritional diversity for wild and managed bees. This 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 area 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 several 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 limited 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 examine which nearby crops are correlated with evidence of this exposure. In addition, we ask whether we observe any evidence that proximity to neonic-treated crops is correlated with higher disease loads. Our goal is to examine what the environmental factors of bee death are and whether the exposure to neonic-treated crops is one of them.

**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 findings suggest 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 from Purdue University 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 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). According to research by USGS, neonics have been found in surface water throughout the Midwest, where corn and soybean production are most prevalent (USGS 2014). This contamination in waterways might translate into a deteriorated heath of other creatures as well. [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; 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.

We use Varroa mites and Nosema parasite as indicators of bee health and explore what environmental factors would contribute to higher prevalence in 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. Following previous entomology literature, we add one to Varroa loads before the log transformation in order to retain zero observations (Holt, 2014). Nothing is added to Nosema load values before the transformation because half of the samples do not contain Nosema. 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 would potentially contribute to more death.

In order 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 composes over 8,000 acres provides the best estimate of the crops that bees would forage on.

We define crop area using to different strategies. First, we 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. The second strategy is to use natural log transformations of the crop areas plus the crop area pixel size. This method assumes that the marginal effects from increases of crop area at low level of crop acreages are higher than at high levels of crop acreage. Adding the pixel size helps insure that the observations of crop areas of zero do not drop out of the analysis. Both of these strategies are employed because real world relation between neonicotinoids and the morbidity factors is unknown. Therefore, there exist insufficient theoretical assumptions to guide a decision as to which area definition is more accurate.

With geographic coordinates for non-migratory apiaries, we also extract 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 used 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 used 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 (British Columbia Ministry of Agriculture 2012), reducing their food intake. Therefore, these weather variables are important indicators of bee stress as well as feeding opportunities, which people in the beekeeping community think are correlated with honey bee health. We extracted minimum average temperature as well as the total precipitation in the month the sample was collected. The data provided by Prism is supplied by calendar month, so for all sample taken on or before the 14th of the month, we used the weather data for the previous calendar month. For samples taken on the 15th or later, we used the month of the collection.

To control for the timing of exposures to pesticides and more abundant nectar sources, we collect information on the plant time and bloom time for neonics-treated crops. NASS collects agricultural plant timing data for select crops in some states. In this data set, there is 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]